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ORIGINAL ARTICLE *Andrology*

Average sperm count remains unchanged despite reduction in maternal smoking: results from a large cross-sectional study with annual investigations over 21 years

L. Priskorn[1,2], L. Nordkap[1,2], A.K. Bang[1,2], M. Krause[1,2], S.A. Holmboe[1,2], D.L. Egeberg Palme[1,2], S.B. Winge[1,2], N. Mørup[1,2], E. Carlsen[1,3], U.N. Joensen[1,4], M. Blomberg Jensen[1,2], K.M. Main[1,2], A. Juul[1,2], N.E. Skakkebaek[1,2], T.K. Jensen[1,2,5], and N. Jørgensen*[1,2]

[1]Department of Growth and Reproduction, Rigshospitalet, University of Copenhagen, Blegdamsvej 9, Copenhagen 2100, Denmark [2]International Center for Research and Research Training in Endocrine Disruption of Male Reproduction and Child Health (EDMaRC), Rigshospitalet, University of Copenhagen, Blegdamsvej 9, Copenhagen 2100, Denmark [3]Fertility Clinic, Rigshospitalet, University of Copenhagen, Blegdamsvej 9, Copenhagen 2100, Denmark [4]Department of Urology, Rigshospitalet, University of Copenhagen, Blegdamsvej 9, Copenhagen 2100, Denmark [5]Department of Environmental Medicine, University of Southern Denmark, J. B. Winsløws Vej 17, Odense 5000, Denmark

*Correspondence address. Department of Growth and Reproduction, Rigshospitalet, University of Copenhagen, Copenhagen 2100, Denmark. E-mail: Niels.Joergensen@regionh.dk

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STUDY QUESTION: How are temporal trends in lifestyle factors, including exposure to maternal smoking *in utero*, associated to semen quality in young men from the general population?

SUMMARY ANSWER: Exposure to maternal smoking was associated with lower sperm counts but no overall increase in sperm counts was observed during the study period despite a decrease in this exposure.

WHAT IS KNOWN ALREADY: Meta-analyses suggest a continuous decline in semen quality but few studies have investigated temporal trends in unselected populations recruited and analysed with the same protocol over a long period and none have studied simultaneous trends in lifestyle factors.

STUDY DESIGN, SIZE, DURATION: Cross-sectional population-based study including ~300 participants per year (total number = 6386) between 1996 and 2016.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The study is based on men from the Greater Copenhagen area, Denmark, with a median age of 19 years, and unselected with regard to fertility status and semen quality. The men delivered a semen sample, had a blood sample drawn and a physical examination performed and answered a comprehensive questionnaire, including information on lifestyle and the mother's pregnancy. Temporal trends in semen quality and lifestyle were illustrated graphically, and trends in semen parameters and the impact of prenatal and current lifestyle factors were explored in multiple regression analyses.

MAIN RESULTS AND THE ROLE OF CHANCE: Throughout the study period, 35% of the men had low semen quality. Overall, there were no persistent temporal trends in semen quality, testicular volume or levels of follicle-stimulating hormone over the 21 years studied. The men's alcohol intake was lowest between 2011 and 2016, whereas BMI, use of medication and smoking showed no clear temporal trends. Parental age increased, and exposure *in utero* to maternal smoking declined from 40% among men investigated in 1996–2000 to 18% among men investigated in 2011–2016. Exposure to maternal smoking was associated with lower sperm counts but no overall increase in sperm counts was observed despite the decrease in this exposure.

LIMITATIONS, REASONS FOR CAUTION: Information of current and prenatal lifestyle was obtained by self-report, and the men delivered only one semen sample each.

WIDER IMPLICATIONS OF THE FINDINGS: The significant decline in *in utero* exposure to maternal smoking, which was not reflected in an overall improvement of semen quality at the population level, suggest that other unknown adverse factors may maintain the low semen quality among Danish men.

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Introduction

Semen quality and temporal trends in semen quality have received much focus during the last 25 years (Carlsen *et al.*, 1992; Levine *et al.*, 2017). In 1996, we initiated a programme with annual cross-sectional studies of more than 300 young Danish men, unselected with regard to fertility status and semen quality, to determine semen quality and associated lifestyle factors. We previously reported a small increase in sperm concentration from a median of 43 million/mL (1996–2000) to 48 million/mL (2006–2010), but the median levels were still considerably lower than among men from infertile couples in the 1940s (Jørgensen *et al.*, 2012). In parallel, the incidence of other male reproductive disorders, such as testicular cancer, seems to have increased (Skakkebaek *et al.*, 2001, 2016), and testosterone levels appear to have declined over the last decades (Andersson *et al.*, 2007; Travison *et al.*, 2007; Perheentupa and Ma, 2013). Some of these alterations, including poor semen quality, have been suggested to be interrelated and may be due to testicular dysgenesis as development of the male reproductive system *in utero* may be particularly sensitive to foetal exposures (Skakkebaek *et al.*, 2001, 2016).

In utero exposure to maternal smoking has in epidemiological studies consistently been associated with a reduction in sperm counts of 20–50% and reduced chances of achieving a pregnancy (Storgaard *et al.*, 2003; Jensen *et al.*, 1998, 2004a, 2005; Ramlau-Hansen *et al.*, 2007; Virtanen *et al.*, 2017). Smoking, also during pregnancy, has been frequent among Danish women (Wisborg *et al.*, 1998; Euro-Peristat Project with SCPE and EUROCAT, 2013; Nordic Council of Ministers, 2015). But along with a general decline in smoking, the proportion of Danish women smoking during pregnancy has declined from 34% in 1989 to 16% in 2005 (Wisborg *et al.*, 1998; Statens Insitut for Folkesundhed, 2007; Egebjerg Jensen *et al.*, 2008), which could be expected to result in an improved semen quality in the birth cohorts most recently reaching adulthood. Environmental exposures and lifestyle during adulthood, such as tobacco and marijuana smoking, BMI and diet, have also previously been associated with impaired spermatogenesis (Jensen *et al.*, 2004a, 2004b, 2010, 2013a, 2013b, 2014; Joensen *et al.*, 2009; Lassen *et al.*, 2014; Gundersen *et al.*, 2015; Priskorn *et al.*, 2016; Nordkap *et al.*, 2016). Thus, testicular function, including semen quality, is established *in utero* but can subsequently be affected by post-natal environmental exposures and lifestyle. However, the impact of prenatal and adult lifestyle factors on temporal trends in semen quality at a population level has not previously been studied.

In this study we analyse temporal trends in various prenatal and adult lifestyle factors, including *in utero* exposure to maternal smoking, and the association to semen quality between 1996 and 2016 among more than 6000 young Danish men recruited using the same protocol during a 21-year period.

Materials and Methods

Study population

In Denmark, all young men, except those with severe chronic illness, are required to undergo a physical examination at age 18–25 years to determine their fitness for military service. During this compulsory physical examination we approached the draftees, representing the general population, without considering whether they were declared fit for military service or not, and invited them to participate in our study. Those who consented were given an appointment for examination at the Department of Growth and Reproduction at Rigshospitalet (Copenhagen, Denmark).

A total of 7012 men participated from 1996 to 2016. Inclusion criteria for the present publication were that both the man himself and his mother were born and raised in Denmark and that the man lived in the Greater Copenhagen area, which was the case for 6413 men. Furthermore, 27 men were excluded due to current use of anabolic steroids (self-reported or indicated from blood sample), leaving 6386 men eligible for analyses (1996–2000 $N = 1339$; 2001–2005 $N = 2254$; 2006–2010 $N = 1274$ and 2011–2016 $N = 1519$).

Ethical approval

Ethical approval was obtained from the local ethical committee (journal no. H-KF-289428) and all participants gave informed consent.

Study setup

All participants completed a questionnaire prior to the day of study participation, at which they delivered a semen sample, had a blood sample drawn for assessment of FSH using a time-resolved immunofluorometric assay (Delfia Wallac, Turku, Finland), and had a physical examination. The

examination included assessment of testis size by orchidometry and ultrasonography, evaluation of gynaecomastia by palpation, and the men's weight and height were measured and BMI calculated (kg/m^2). Participants were compensated for their time (DKK 500 ≈ US$ 85). Participation over time varied between 19 and 31%, with an average of 24%. A detailed description of the study methods and design has been published previously (Jørgensen *et al.*, 2002, 2012).

Semen analysis

All men provided a semen sample by masturbation in a room close to the semen laboratory and the period of ejaculation abstinence was recorded. The men had been asked to abstain from ejaculation for at least 48 h before sampling but were still included if abstinence time was shorter. The semen sample was kept at 37°C until analysis. This was from 1996 performed in accordance with the current World Health Organization (WHO) guidelines (World Health Organization, 2010). In short, semen volume was assessed by weighing, sperm concentration was determined using a Bürker-Türk haemocytometer and the total sperm count was calculated (semen volume × sperm concentration). For assessment of sperm motility, two drops of well-mixed semen were placed on a glass slide and examined with phase-contrast microscopy; classifying the spermatozoa as progressive motile, non-progressive motile or immotile. Fixed and Papanicolaou stained morphology slides were evaluated according to 'Krüger's strict criteria' (Menkveld *et al.*, 1990). For all assessments, counts were done in duplicates and the average was used. The semen laboratory coordinated and participated in an external quality control programme for sperm concentration from the beginning of the study where all participating centres every month received five undiluted, fresh, preserved semen samples from normal donors. The results did not indicate any trends over time in the assessment in our laboratory and accordingly, no adjustment of the results according to the quality control has been implemented (Jørgensen *et al.*, 2002, 2012; Fernandez *et al.*, 2012). The laboratory was in 2015 accredited by national authorities with the semen analysis methods that have been used throughout the study period

Questionnaire

All participants completed a questionnaire, covering demographics, lifestyle (e.g. smoking, use of medication and alcohol intake during the week prior to study participation) and reproductive and general health as previously described (for details see (Jørgensen *et al.*, 2012; Nordkap *et al.*, 2016; Priskorn *et al.*, 2016)). Furthermore, a part of the questionnaire covered the time before and during pregnancy and the young men were encouraged to consult with their mothers when filling in this information (e.g. parental age, fertility treatment, maternal and paternal smoking during pregnancy, and birth weight) (Jensen *et al.*, 2004a). Parental smoking was assessed based on the crude questions: 'Did your mother smoke while she was pregnant with you?' and 'Did your father smoke while your mother was pregnant with you?' The content of the questionnaire was changed during the study period as new information on potentially relevant factors emerged, and some questions have been slightly rephrased for clarity. The focus of this publication is data which can be compared throughout the whole or most of the study period. Data on some included variables were, however, not available until 2001 (Table I). Responses were reviewed by the investigators, face-to-face with the participant, to clarify missing or ambiguous information.

Statistical analyses

Descriptive statistics (medians and 5–95 percentiles or frequencies) were calculated on data from the questionnaire, physical examination and semen parameters. Descriptions were made for the total population and for four periods to reveal differences over time (1996–2000, 2001–2005, 2006–2010 and 2011–2016) (Jørgensen *et al.*, 2012). Differences between periods were tested using chi-square test for categorical variables and Kruskal–Wallis test for continuous variables. Temporal trends in sperm counts, sperm morphology and motility, FSH and testicular volume, as well as pre- and post-natal factors were illustrated graphically, and Spearman's rank-order correlation for the inverse association between sperm concentration and FSH was calculated. First, the temporal trends in semen parameters were investigated in linear regression models without considering a potential impact of parallel changes in lifestyle factors, and thus, adjusted analyses took into account only age and period of abstinence and for motility analyses also time between delivery of semen sample and start of motility analysis. To meet model assumptions of normally distributed residuals and homoscedasticity, semen parameters were transformed to achieve the best fit; evaluated by inspecting residual plots (for details see Supplementary Table S2). To elucidate a potential effect of bias due to self-selection into the study of men concerned about their semen quality and a change in such a bias over time, we repeated the analyses after excluding all men reporting any reproductive health issues prior to study participation, such as cryptorchidism at birth, testicular torsion, varicocele, sexually transmitted diseases or phimosis (see Supplementary Table S1).

We also categorized the men according to semen quality as recently described (Damsgaard *et al.*, 2016), as a deficit in one semen characteristic can hamper the man's fertility chances, even when the other semen characteristics are normal. Low semen quality was defined as sperm concentration <15 million/mL, and/or progressive sperm motility <32%, and/or normal sperm morphology <4%; high semen quality was defined as sperm concentration >40 million/mL, and progressive sperm motility >50%, and normal sperm morphology >9%; whereas all other men were grouped as having an intermediate semen quality. Temporal trend in this categorical semen variable was tested using the Mantel–Haenszel test for trend. In sensitivity analyses, low semen quality was instead defined as semen parameters lower than the reference levels of the fifth edition of the WHO manual (World Health Organization, 2010) and high semen quality as semen parameters above or equal to reference levels of the fourth edition of the WHO manual (World Health Organization, 1999), while all other men were defined as having intermediate semen quality.

The impact of prenatal and current lifestyle factors (Table I) on the observed trends was explored by adding these factors one at a time as covariates in the multiple linear regression analyses described above and comparing effect estimates for period with and without these covariates in the model.

All *P*-values were two-sided and a *P*-value <0.05 was considered statistically significant. Statistical analyses were performed using PASW GradPack V.22.0 (SPSS Inc.).

Results

Temporal trends in semen quality

Overall, the study population was comparable over time (Table I and Supplementary Table SI). The median semen parameters for the entire population were 44 million/mL for sperm concentration, 140 million for total sperm count, 6.5% morphologically normal spermatozoa and 60% progressive motile spermatozoa (Supplementary Table SII). Overall, there were no persistent trends in sperm counts over the 21 years studied. Differences in semen parameters over the study period were small, and similar in unadjusted and adjusted models (Supplementary Table SII). Seven percentage of the men reported cryptorchidism at birth with varying frequencies between periods, but the percentage of men without known previous reproductive health issues was around 80% during the entire period (Supplementary Table SI). Repeating regression analyses for trends in semen parameters in this group yielded results similar to

Table I Physical characteristics and self-reported information of participants, cross-sectionally investigated 1996–2016.

	N with data	Period					*P*-value for difference[c]
		1996–2016 (*N* = 6386)	1996–2000 (*N* = 1339)	2001–2005 (*N* = 2254)	2006–2010 (*N* = 1274)	2011–2016 (*N* = 1519)	
Continuous variables		Median (5–95 percentile)					
Age (years)	6359	19.0 (18.4–21.7)	19.0 (18.5–22.4)	18.9 (18.4–21.3)	19.0 (18.4–21.8)	19.0 (18.4–21.8)	<0.001
Height (m)	6325	1.81 (1.71–1.92)	1.81 (1.71–1.92)	1.81 (1.70–1.91)	1.82 (1.71–1.93)	1.82 (1.72–1.94)	<0.001
Weight (kg)	6343	73.6 (59.3–95.6)	73.6 (59.0–97.5)	73.3 (59.2–97.0)	74.1 (60.1–96.0)	73.7 (59.1–93.4)	0.3
BMI (kg/m^2)	6300	22.3 (18.6–28.6)	22.4 (18.8–28.9)	22.4 (18.7–29.0)	22.4 (18.7–28.6)	22.1 (18.2–27.5)	0.01
Cigarettes daily, smokers only	2676	10.0 (0.2–20.0)	10.0 (1.0–20.0)	10.0 (1.0–20.0)	8.0 (1.0–20.0)	4.0 (0.03–20.0)	<0.001
Alcohol consumption (units/week)[a]	6163	10 (0–38)	11 (0–38)	11 (0–37)	12 (0–42)	7 (0–33)	<0.001
Testis size, mean, palpation (mL)	6243	20 (13–28)	20 (12–28)	20 (13–28)	23 (14–29)	20 (12–28)	<0.001
Testis size, mean, ultrasound (mL)	6088	14 (9–22)	15 (9–24)	14 (9–21)	14 (9–21)	13 (8–20)	<0.001
Ejaculation abstinence (h)	6364	62 (33–148)	63 (35–168)	62 (38–135)	63 (37–134)	61 (37–134)	0.001
Time until motility analysis (min)	6313	35 (15–80)	40 (25–90)	33 (20–67)	40 (15–90)	30 (10–70)	<0.001
Mother's age at birth (years)	4659	28 (21–37)	–	27 (20–36)	29 (21–37)	30 (22–38)	<0.001
Father's age at birth (years)	4647	31 (23–42)	–	30 (22–41)	31 (23–42)	32 (24–42)	<0.001
Categorical variables		%					
Cigarette smokers	6314	43.6	42.5	39.1	45.4	49.6	<0.001
Mother smoked during pregnancy	5927	33.2	40.0	40.7	30.9	17.8	<0.001
Father smoked while mother pregnant	4558	46.7	–	52.9	48.6	36.1	<0.001
Medication last 3 months[b]	6321	12.7	14.6	9.7	15.1	13.5	<0.001
Presence of gynaecomastia	6188	2.8	2.2	2.4	3.2	3.1	0.17
Born after fertility treatment	3006	4.6	–	5.5	2.7	5.2	0.006
Birth weight <2500 g	4975	7.0	5.6	9.4	6.5	5.1	<0.001

[a]Sum of intake of beer, wine and strong alcohol during the week prior to participation in the study. [b]Taken any medication for at least a week 3 months prior to participation in the study. [c]*P*-value for comparison of results between study periods. Kruskal–Wallis test has been used for continuous variables and chi-square test for categorical variables.

results for the whole population (Supplementary Table SII). Semen parameters, FSH and testis volume exhibited a large variation between individuals but with unchanged distributions across examination years (Fig. 1). In periods exhibiting slightly higher sperm concentration, FSH levels tended to be correspondingly lower with a Spearman correlation of −0.2 ($P < 0.001$) (Supplementary Fig. S1).

The proportion of men with oligozoospermia (sperm concentration <15 million/mL) was ~16% (13–20%) with no trend over time ($P = 0.4$). Overall, 35% (30–44%) of the men had low semen quality based on the combined semen parameters data and only 22% (12–25%) had high semen quality (Fig. 2), also with no time trend ($P = 0.5$). In sensitivity analyses, using other thresholds for the definition of low, intermediate and high semen, distributions changed but were still stable over time.

Temporal trends in pre- and post-natal factors

Figure 3 illustrates trends in sperm concentration and total sperm count between 1996 and 2016 together with changes in relevant prenatal and adult lifestyle factors. The median BMI remained stable around 22.3 kg/m^2, as did the proportion of men who had taken any medication for at least a week during the three months prior to study participation (13%). The proportion of smokers differed slightly between periods without any clear trend, but among the smokers, the number of cigarettes per day decreased from a median of 10 per day in 1996–2000 to 4 in 2011–2016. Alcohol consumption was lower in 2011–2016 with a median weekly intake of 7 units compared to 11–12 units in the previous periods. Maternal age at son's birth increased from 27 years for men studied in 2001–2005 to 30 years in 2011–2016, and paternal age increased from 30 years to 32 years. Both maternal and paternal smoking during the prenatal period decreased considerably during the study period: 40% of the men investigated in 1996–2000 had been exposed *in utero* to maternal smoking compared to 18% of the men investigated in 2011-2016 and correspondingly for paternal smoking, which declined from 53% in 2001–2005 to 36% in 2011–2016. Overall, 7% reported to be born with low birthweight with some variation between periods (Table I and Fig. 3).

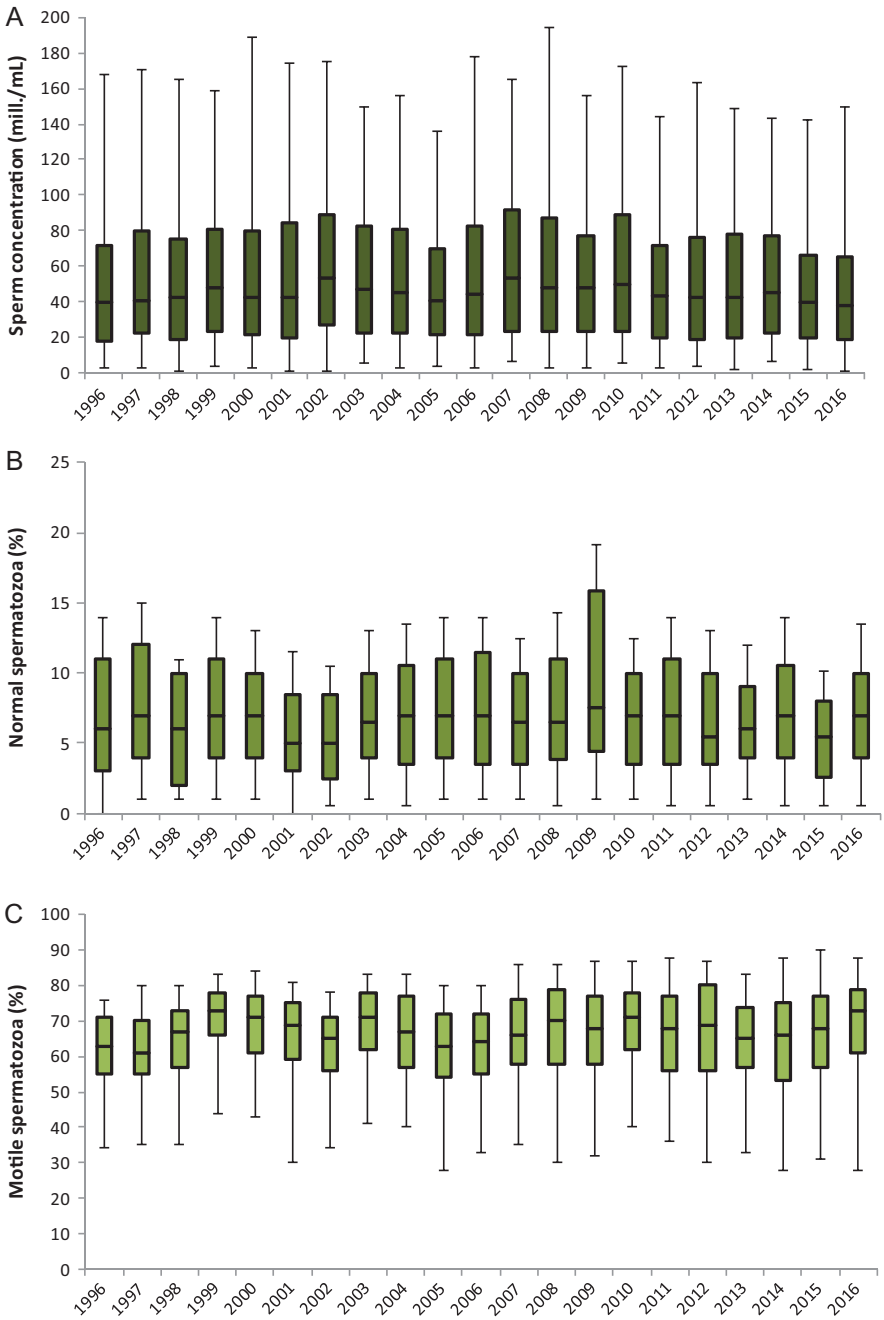

Figure 1 Markers of testicular function, (**A**) sperm concentration, (**B**) morphologically normal spermatozoa, (**C**) motile spermatozoa, (**D**) FSH and (**E**) testicular volume assessed by ultrasound, in young men from the general population, cross-sectionally investigated 1996–2016. Coloured bars show the 25–75th percentiles with median and whiskers show the 5–95th percentiles.

The association between trends in prenatal and adult lifestyle factors and semen quality

When a participating man's current smoking was included in the analyses of trends in semen quality along with parental smoking during the prenatal period, only maternal smoking was significantly associated with sperm concentration and total sperm count, while there were no associations to sperm motility or morphology (Fig. 4). The difference in sperm concentration depending on exposure to smoking *in utero* was 6 million/mL with no modifying effect of study period (analysed as an interaction between period and maternal smoking as well as the association between smoking and sperm concentration stratified on period). Due to the decline in the percentage of mothers smoking during pregnancy (Table I and Fig. 3), when adjusting the trend analyses for maternal smoking, the overall difference in sperm concentration between the periods 1996–2000 and 2011–2016 increased slightly

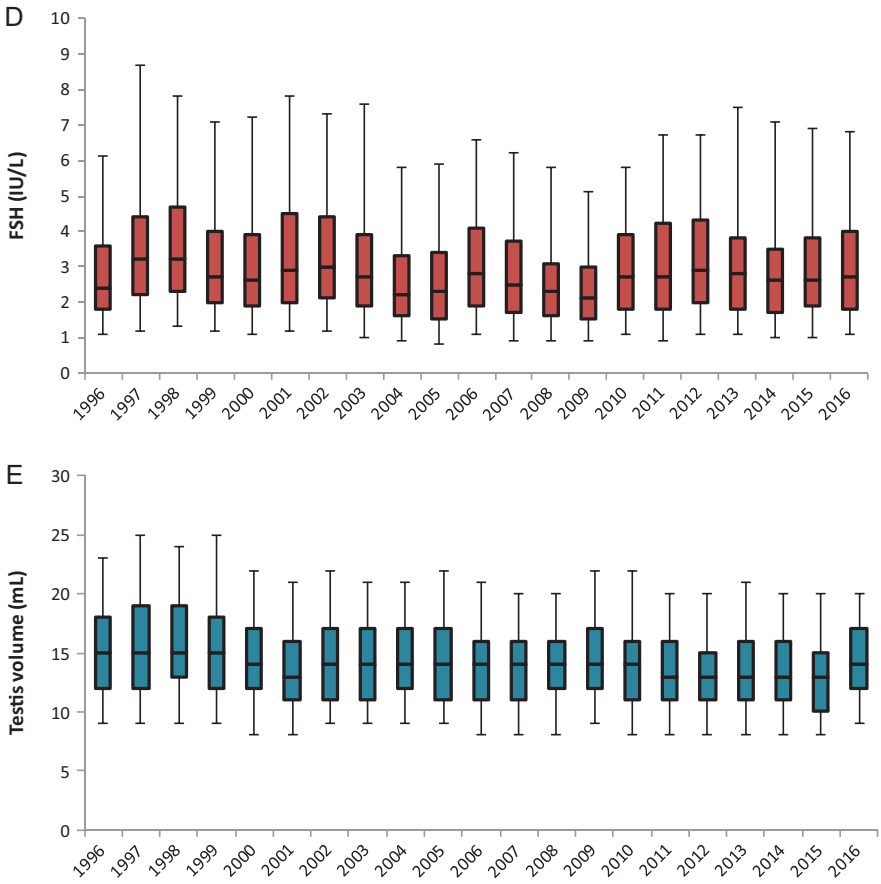

Figure 1 *Continued*

from 2 million/mL ($P = 0.4$) without adjustment to 3 million/mL ($P = 0.051$) (Fig. 4).

Adjusting for adult lifestyle factors or parental age at birth did not change the time trends in sperm concentration (data not shown). Fertility treatment and low birthweight were associated with a lower sperm concentration in trend analyses (data not shown). However, both were rare and cannot explain the unchanged trends in sperm counts.

Discussion

We report that during a 21-year period approximately one-third of young Danish men had low semen quality, defined according to WHO's reference values, which remained unchanged despite a significant decrease of the proportion of mothers smoking during pregnancy. No consistent trends in BMI, men's own smoking, alcohol intake or use of medication were observed.

Low semen quality among men from the general population has been reported in many other countries, but these studies include a short time period and do not provide data on long-term time trends (Jørgensen *et al.*, 2001, 2002, 2006; Paasch *et al.*, 2008). In line with our results, stable semen quality was observed in a comparable Swedish population between 2000 and 2010 (Axelsson *et al.*, 2011), while recent declines in sperm concentration have been reported in Finnish, Spanish and French men from the general population (Jørgensen *et al.*, 2011; Mendiola *et al.*, 2013; Rolland *et al.*, 2013). Whereas the trends in semen quality have been widely debated, solid data exists on trends in testicular cancer, which is associated to semen quality—both within the individual man and at a population level (Jørgensen *et al.*, 2011; Skakkebaek *et al.*, 2001, 2016). Overall, these data show increasing or high but stabilizing incidence rates for testicular cancer in many European countries and the US, supporting the hypothesis of adverse trends in male reproductive health, which may have reached a plateau in some countries, including Denmark (Skakkebaek *et al.*, 2016). In contrast to prior studies focusing solely on trends in semen quality, we have also investigated trends in prenatal and adult factors that may influence semen quality trends. Many exposures (e.g. smoking, maternal smoking, psychological stress, general health, caffeine and alcohol intake, and BMI) have been investigated in subgroups of the men included in this study and have been associated to semen quality (Joensen *et al.*, 2009; Jensen *et al.*, 2004a, 2004b, 2010, 2013a, 2013b, 2014; Lassen *et al.*, 2014; Gundersen *et al.*, 2015; Nordkap *et al.*, 2016; Priskorn *et al.*, 2016). Most of the included lifestyle factors, which we had data on for a longer period, e.g. BMI, overall showed no or minor changes over time. Thus, these factors should not influence the observed trends. However, parental age increased and the proportion of parents smoking during the prenatal period decreased during the years studied. In line with results from a previous study (Priskorn *et al.*, 2014), we did not observe a

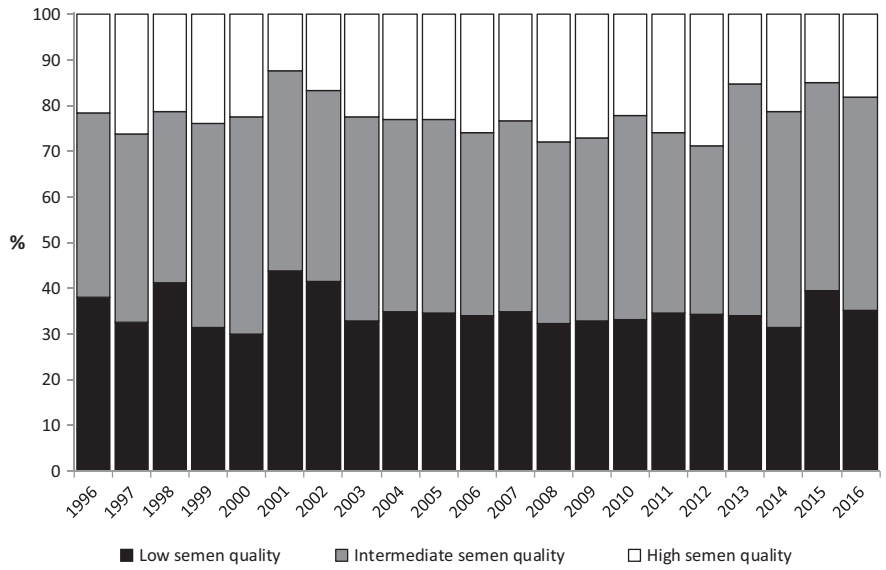

Figure 2 Frequency of low, intermediate and high semen quality in young men from the general population, cross-sectionally investigated 1996–2016. Semen quality categories are based on unadjusted values of sperm concentration, percentage of morphologically normal spermatozoa, and percentage of motile spermatozoa. Low semen quality was defined as sperm concentration <15 million/mL, and/or sperm motility <32%, and/or normal sperm morphology <4%; high semen quality was defined as sperm concentration >40 million/mL, and sperm motility >50%, and normal sperm morphology >9%; whereas all other men were grouped as having intermediate semen quality (Damsgaard *et al.*, 2016).

negative association between the observed increased parental age and semen quality in the male offspring. Consistent with other studies, reporting adverse effects of *in utero* exposure to maternal smoking on semen quality, maternal smoking in our data was associated to lower sperm counts, and a minor effect of the decrease in maternal smoking was reflected in our data. However, despite a more than 50% reduction in this exposure, no improvement in semen quality on the population level was observed in the most recent years (Storgaard *et al.*, 2003; Jensen *et al.*, 2004a, 2005; Ramlau-Hansen *et al.*, 2007). Our data suggests that without a decline in *in utero* exposure to maternal smoking, the average semen quality of young men of today may have decreased slightly instead of remaining stable. This study therefore suggests that other unknown factors with adverse effects on semen quality may have counteracted the expected small benefit of decreased maternal smoking. However, it is important to remember that the variation in semen quality between individuals is large and that semen quality is influenced by a wide range of both pre- and post-natal factors (Sharpe, 2010, 2012). Thus, the variables included in the present study explain only a minor part of the variation.

Strength of this large study are that it was based on a large number of men from the general population who had been recruited from the same geographical area during the entire study period, and all semen samples were analysed in the same laboratory with the same assessment methods. We had a single semen 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 in evaluating population trends in a large group of men (Stokes-Riner *et al.*, 2007). Our findings of low, unchanged sperm counts were strongly supported by unchanged testicular volume and FSH levels during the entire study period (Jensen *et al.*, 1997; Bahk *et al.*, 2010; Hart *et al.*, 2015). Importantly, testicular ultrasonography and FSH measurements were carried out in the same department using the same methods throughout the period. The men participating in the study were not different from non-participants regarding place or year of birth, although slightly better educated (Andersen *et al.*, 2000; Gundersen *et al.*, 2015). We believe that participants represent the general population regarding reproductive function as they have essentially no knowledge of their fertility potential and concentrations of reproductive hormones did not differ between participants and the 79% of non-participants who in a sub-study agreed to have a blood sample drawn (Andersen *et al.*, 2000). Furthermore, the proportion of men with known prior reproductive health issues was unchanged during the study period, suggesting that there was no change in a potential recruitment bias. Besides, trend analyses showed similar results after excluding this group of men with pre-existing conditions. However, the proportion of men reporting to have had or been treated for cryptorchidism varied between study periods, which may be due to changes in the phrasing of this question over time. It is a limitation that the information on maternal and paternal smoking relies on retrospectively collected self-reported data, which could bias the results due to misclassification of these exposures. There are no directly comparable data, but the trend in the frequency of mother's smoking in this population resembles that reported from other sources covering part of the same period but at a slightly lower level (Wisborg *et al.*, 1998; Egebjerg Jensen *et al.*, 2008), which could suggest some underreporting. Furthermore, the observed association between maternal smoking during pregnancy and semen quality in the son is lower than what has been reported in other studies where adverse effects for instance have been reported when mothers smoked more than 10 cigarettes per day but not at lower levels (Storgaard *et al.*, 2003). Thus, the weaker association could be due to the rather crude

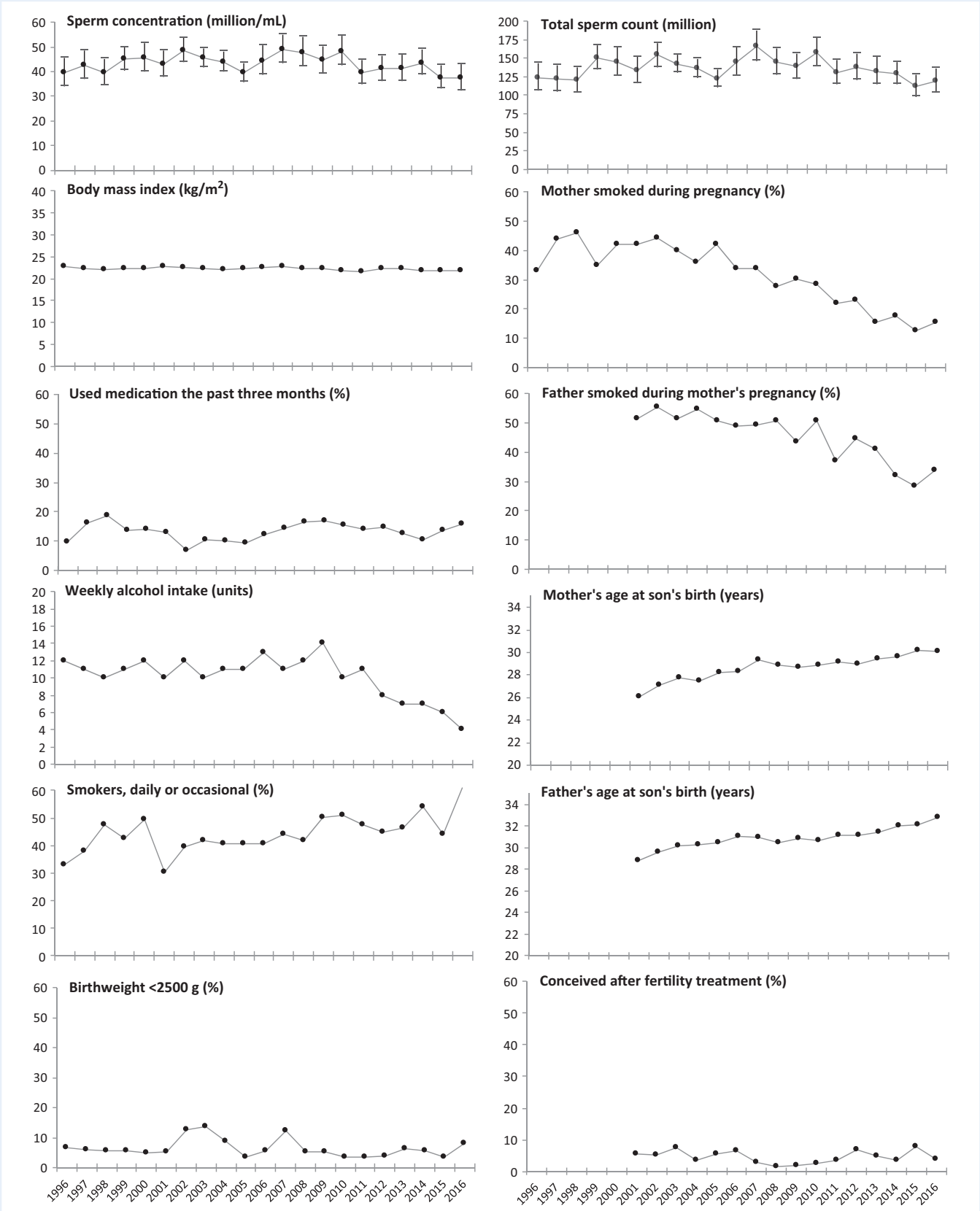

Figure 3 Sperm counts and lifestyle factors in young men from the general population, cross-sectionally investigated 1996–2016. Trends in sperm concentration and total sperm count are illustrated as geometric means ± 2 standard error of the mean. Trends in BMI and parental age are illustrated as medians and trends in all other variables are presented as percentages.

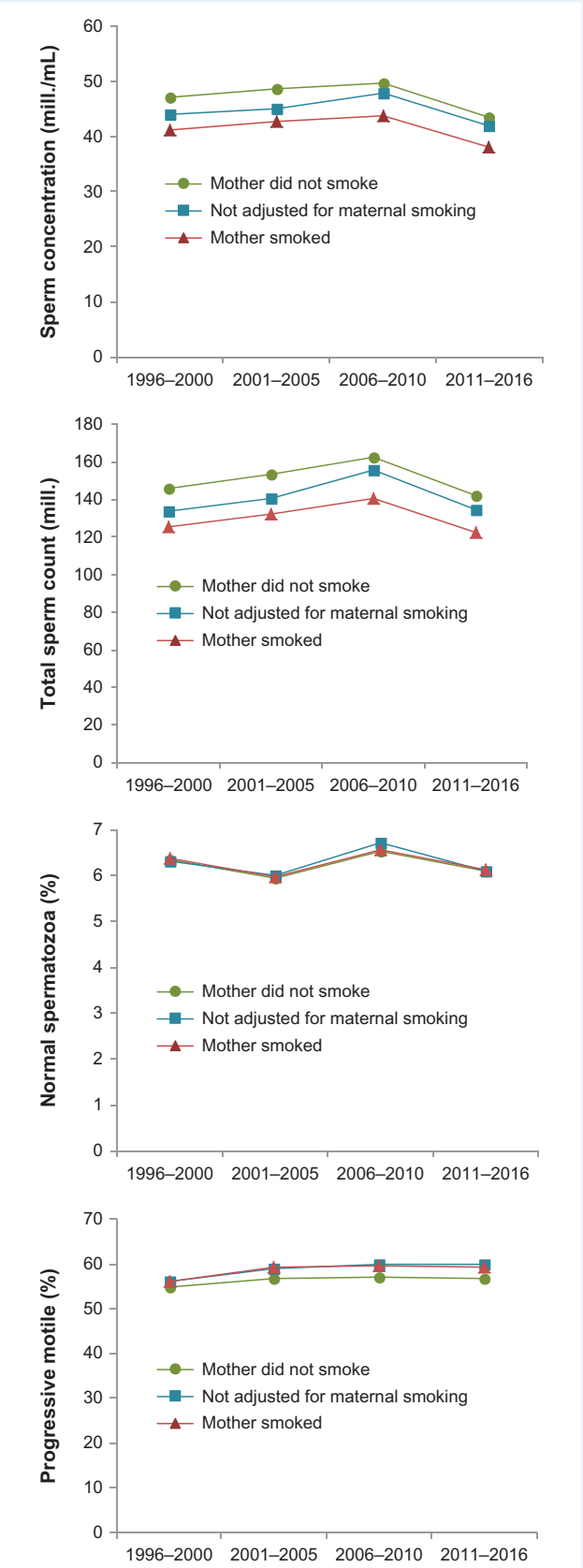

Figure 4 Impact of maternal smoking on semen quality and trends in semen quality in young men from the general population, cross-sectionally investigated 1996–2016. All semen parameters are adjusted and standardized to age = 19 years. Sperm concentration, total sperm count and total morphologically normal count furthermore adjusted for and standardized to period of abstinence = 72 h, and motility furthermore adjusted for and standardized to time between delivery of semen sample and start of motility analysis = 30 min.

assessment of maternal smoking (yes/no) not enabling us to stratify analyses on the amount smoked which could dilute the observed association as could potential underreporting.

The consistent large proportion of young men with low semen quality is of concern. The definition of low semen quality used in this paper was based on the WHO reference ranges for individual semen parameters (World Health Organization, 2010). A lower reference level based on the fifth percentile as suggested by the WHO may not discriminate sufficiently as studies suggest that chances of achieving a pregnancy are reduced already at higher levels (Bonde *et al.*, 1998; Guzick *et al.*, 2001; Slama *et al.*, 2002). Thus, we included the intermediate category, where one or more semen parameters were above the WHO levels but still not optimal according to the literature. Poor semen quality has been suggested to be part of the explanation for low fertility rates in Denmark and many other countries, and the high need for assisted reproductive techniques (Lassen *et al.*, 2012; Skakkebaek *et al.*, 2016). Besides being associated to fertility chances, poor semen quality is also associated to increased morbidity and mortality later in life (Jensen *et al.*, 2009; Eisenberg *et al.*, 2014, 2015; Latif *et al.*, 2017).

In conclusion, semen quality was persistently low during our annual studies of more than 6000 young Danish men examined during a 21-year period with unchanged inclusion and analysis methods with 35% having a sub-optimal semen quality. This was observed despite a large decline in men exposed *in utero* to maternal smoking. Poor semen quality may have long-term consequences not only for fertility but also for health later in life. Our study suggests that focus is needed on preventing other adverse factors besides maternal smoking.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

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Author's roles

Substantial contribution to conception and design: L.P., N.J., N.E.S. and T.K.J. Data acquisition: L.P., A.K.B., L.N., M.K., S.A.H., D.L.E.P., S.B.W., N.M., E.C., U.N.J., M.B.J. and N.J. Data analysis: L.P. and N.J. Data interpretation: All authors. Drafting the article: L.P. and N.J. Revising the article critically for important intellectual content: All authors. Final approval of the article: All authors.

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Conflict of interest

Authors have nothing to declare. None of the funders had any role in the study design, collection, analysis or interpretation of data, writing of the article or publication decisions.

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