**IN UTERO EXPOSURE OF ALCOHOL AND PUBERTY IN SONS. A FOLLOW-UP STUDY OF A PREGNANCY COHORT.**

<table>
<thead>
<tr>
<th>Journal:</th>
<th>BMJ Open</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID:</td>
<td>bmjopen-2013-004467</td>
</tr>
<tr>
<td>Article Type:</td>
<td>Research</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>18-Nov-2013</td>
</tr>
</tbody>
</table>
| Complete List of Authors: | Håkonsen, Linn Berger; Department of Public Health, Section for Epidemiology, Aarhus University  
Brath-Lund, Mette Louise; Danish Ramazzini Center, Department of Occupational Medicine, Aarhus University Hospital, Denmark  
Hounsgaard, Marie Louise; Department of Public Health, Section for Epidemiology, Aarhus University  
Olsen, Jørn; Department of Public Health, Section for Epidemiology, Aarhus University  
Ernst, Andreas; Department of Public Health, Section for Epidemiology, Aarhus University  
Thulstrup, Ane Marie; Danish Ramazzini Center, Department of Occupational Medicine, Aarhus University Hospital, Denmark  
Bech, Bodil; Department of Public Health, Section for Epidemiology, Aarhus University  
Ramlau-Hansen, Cecilia ; Institute of Public Health, Department of Epidemiology |
| **Primary Subject Heading**: | Paediatrics |
| Secondary Subject Heading: | Epidemiology, Obstetrics and gynaecology, Reproductive medicine, Sexual health |
| Keywords: | EPIDEMIOLOGY, Community child health < PAEDIATRICS, REPRODUCTIVE MEDICINE |
IN UTERO EXPOSURE OF ALCOHOL AND PUBERTY IN SONS.
A FOLLOW-UP STUDY OF A PREGNANCY COHORT.

Corresponding author: Linn Berger Håkonsen, Department of Public Health, Section for Epidemiology, Aarhus University, Denmark, E-mail: linnhaak@rm.dk

Linn Berger Håkonsen\textsuperscript{a}, Mette Louise Brath-Lund\textsuperscript{b}, Marie Louise Hounsgaard\textsuperscript{b}, Jørn Olsen, Ph.D\textsuperscript{a}, Andreas Ernst\textsuperscript{a}, Ane Marie Thulstrup, Ph.D\textsuperscript{b}, Bodil Hammer Bech, Ph.D\textsuperscript{a}, Cecilia Høst Ramlau-Hansen, Ph.D\textsuperscript{a}

\textsuperscript{a}Department of Public Health, Section for Epidemiology, Aarhus University, Denmark
\textsuperscript{b}Danish Ramazzini Center, Department of Occupational Medicine, Aarhus University Hospital, Denmark.

Running title: Maternal alcohol consumption and puberty in sons.

\textit{Key words: Prenatal exposure, Pregnancy, Alcohol drinking, Life style, Puberty}

Word count: 2813

Number of tables: 3
ABSTRACT

Objectives: Epidemiological studies have raised concern about the reproductive consequences of in utero exposure to alcohol. Exposure to maternal lifestyle factors have been associated to altered pubertal development, but the impact of prenatal alcohol exposure on male puberty is unknown. Thus, the objective was to explore whether prenatal alcohol exposure alters pubertal development in boys.

Setting: Follow up study of a Danish pregnancy cohort.

Participants: Sons (N = 2,522) of women who were enrolled in a Danish pregnancy cohort in 1984 - 1987.

Primary and secondary outcome measures: Indicators of pubertal development, assessed by age at first nocturnal emission, voice break, acne and regular shaving.

Results: We found a tendency towards a later age at first nocturnal emission and voice break following in utero exposure to binge drinking. Sons exposed to ≥5 binge drinking episodes during pregnancy experienced their first nocturnal emission 7.3 months [95% CI: -2.8, 17.4] later and voice break 4.9 months [95% CI: -0.6, 10.4] later than unexposed sons. Results on average weekly alcohol consumption were in the same direction, but, differences were smaller and also statistically insignificant.

Conclusions: We found no strong support for the hypothesis that in utero exposure to weekly alcohol consumption is a risk factor for altered pubertal development. Although a tendency towards a delayed pubertal development among sons exposed to binge drinking during fetal life was observed, studies with prospectively collected data on pubertal development are needed to explore this further.
Strengths and limitations of this study

Strengths of this study

- Large pregnancy cohort with a rather high participation rate (60%)
- Prospectively collected data on maternal alcohol consumption
- High prevalence of women drinking alcohol during pregnancy
- Ability to study dose-response effects
- “State of the art” statistical methods

Limitations of this study

- Self-reported data on pubertal development
- Lack of valid indicators of pubertal development in boys
- A relatively large amount of missing data on the indicators of pubertal development.
- Risk of uncontrolled or residual confounding
INTRODUCTION

Alcohol consumption during pregnancy has been associated with adverse pregnancy outcomes (1;2) and health complications for the exposed children.(3) Despite these well-established risks, alcohol consumption is still common during pregnancy in many countries (4;5) and is, thus, one of the major modifiable causes of adverse pregnancy outcomes in the Western world.

Interest in male reproductive consequences of prenatal exposure to alcohol has grown recently, as focus on causes of subfecundity has intensified worldwide. In males, one study reported an association between prenatal alcohol exposure and cryptorchidism,(6) a congenital malformation that may predispose to impaired semen quality, whereas other studies have shown inconsistent results.(7-9) In adult life, indications of reduced semen quality among exposed sons have been reported.(10)

A few studies have investigated the association between maternal alcohol consumption during pregnancy and pubertal development in girls. One of these observed later age of menarche in a small group of heavily exposed girls,(11) but two more recent studies did not support this finding.(12;13) Shrestha et al. also assessed binge drinking episodes during pregnancy, but found no effects on timing of menarche, neither of binge drinking nor weekly alcohol consumption.(13)

In boys, studies on risk factors for altered pubertal development are sparse. It is well-established that the variability in puberty depends on genetic factors, ethnicity and nutritional conditions,(14) but in utero exposures may also have an early
“programming” role. It has been indicated that male pubertal development may be accelerated following in utero exposure to cigarette smoking. (15-17)

This novel study aims to explore whether prenatal alcohol exposure alters pubertal development in boys.

**METHODS**

This study is based on data from the pregnancy cohort “Healthy habits for two” (18) established in 1984 – 1987 in the municipalities of Aalborg and Odense, Denmark. The cohort included 11,980 pregnant women (87% of all invited) who, at a routine visit at the midwife around 36th gestational week, completed a questionnaire concerning lifestyle, demographic and health-related characteristics before and during pregnancy, including questions on consumption of alcoholic beverages during pregnancy. The pregnant women were all Danish citizens and since only 1% of Danish women aged 20 - 34 years were immigrants or descendants from immigrants during 1984 - 1987, the participants were most likely Caucasians. (19) Of the 11,980 pregnant women, 11,144 delivered live born singletons. Of these, 5,716 were boys. In 2005, 5142 sons (between 18 and 21 years of age), who were alive and living in Denmark were identified in the Danish Civil Registration System and invited to complete an internet-based questionnaire. A total of 2,810 (55%) sons responded.

**Exposure assessment**

In the questionnaire completed by the pregnant women around the 36th gestational week, the women were asked about their average weekly intake of beer, wine and spirits while being pregnant. One drink was defined as one bottle of beer (0.33 l), one
glass of wine, or one glass of spirits. We calculated the weekly intake of these alcoholic beverages for each woman. The average maternal alcohol intake during pregnancy was categorised in four groups: 0, 0.5 – 1.5, 2.0 – 4.0 or > 4.0 drinks/week. Further, the women were asked how many times they had consumed eight or more alcoholic drinks on a single occasion (defined as binge drinking) while being pregnant with the following pre-determined response categories: 0, 1 – 4, 5 – 9, 10 – 19, or > 20 times. We formed three groups according to number of binge drinking episodes; 0, 1 – 4 and ≥5 times during pregnancy.

Assessment of pubertal development in sons

In the follow-up questionnaire administered to the sons in 2005, four questions concerning different indicators of pubertal development were asked: ‘Have you had acne?’, ‘Has your voice broken?’, ‘Have you started to shave regularly?’ and ‘Have you had your first nocturnal emission?’. If they answered “yes”, they were also asked to provide the age in years and months at which the event first occurred. We converted the month into a fraction of a year and added years, to create a continuous outcome variable for each of the four events.

Covariates

Potential confounders were identified a priori: Maternal age in years (continuous), maternal pre-pregnancy body mass index (BMI) (<18.5, 18.5 – 24.9 and >24.9 kg/m²), maternal smoking during pregnancy (smoker, former smoker (stopped before pregnancy) and non-smoker), maternal chronic diseases (diabetes mellitus, epilepsy, arthrosis, heart disease, cancer, psychiatric disorders, allergy or other chronic diseases, combined into one variable: present versus not present), municipality of
residence at the time of delivery (urban areas versus rural areas), family socio-economic status based on the highest ranking of job description or academic background between parents at the time of pregnancy (white collar workers, blue collar workers and unemployed or students) and co-habitation status of the parents at birth (mother living with the father of the child versus mother not living with the father of the child).

**Statistical analyses**

**Missing information**

The number (%) of participants who gave information on age of the four outcome variables were: Acne: 1,804 (64%), voice break: 1,696 (60%), regular shaving: 2,128 (76%) and first nocturnal emission: 924 (33%). About three-quarter of these only provided age in years. Information on maternal average weekly alcohol consumption was complete and there were only 4 (0.1%) missing values on binge drinking. Further, the level of missing values in covariates varied from 0 to 6.9% (Table I). Unexposed sons had more missing values in the pubertal events compared with sons exposed to alcohol during pregnancy. However, differences were rather small.

Since complete case analysis can lead to biased estimates and limited power, we addressed the missing data problem by using multiple imputations, which yield unbiased and more precise estimates if data are missing at random.(20;21) Briefly, the multiple imputations model is an approach that creates several \( m > 1 \) different imputed data sets, based on other known subject characteristics from the whole data set. The \( m \) complete data sets are then analysed and the results are combined by use of the so-called Rubin’s rule, thus producing a single set of inference that include the variability associated with the missing data.
Prior to multiple imputations, we excluded sons who had not at least provided an age in years for one of the four events (n = 288). We performed the multiple imputations using an interval regression imputation model (100 imputed datasets) with interval censoring of the indicators of pubertal events. The following variables were included in the main imputation model: age at first nocturnal emission, age at acne, age at voice break, age at regular shaving, maternal pre-pregnancy BMI, maternal cigarette smoking, maternal age at delivery, alcohol consumption during pregnancy, chronic diseases of the mother, municipality of residence at the time of delivery, family’s socio-economic status and co-habitation of the parents. We performed different imputation models, including 1) a higher number of imputed datasets, m = 120, 2) only exposure and outcome variables, and 3) more covariates than in the main imputation model. We then compared the results to check for consistency, i.e. the sensitivity of the results to the choice of model used for imputations.

Data analyses

Data on age of the four indicators of pubertal development were approximately normally distributed. Thus, we calculated the mean ages with 95% confidence intervals (CI) for each of the pubertal events. We estimated partial correlation coefficients between the four indicators of pubertal development adjusted for the covariates described above. Further, we performed multiple linear regression analyses with maternal alcohol consumption during pregnancy, with both average weekly intake and number of binge drinking episodes as the explanatory variable in separate models. We adjusted for the potential confounders mentioned above. Moreover, we estimated trends using average maternal alcohol consumption and binge drinking as continuous variables. Since 2.1% of the women contributed to the cohort with more
than one child, we applied robust standard errors in the adjusted analyses to account for clustering.

We also performed sensitivity analyses. Firstly, we repeated the multiple regression analyses using different multiple imputation models, to check for consistency of the chosen imputation model, as noted before. Secondly, we performed restricted analyses based on the participants who reported at least age in years at all event (complete case analyses). All statistical analyses were performed using Stata 12 software (Stata Corporation, College Station, TX).

RESULTS

The cohort included 2,810 men, but after exclusion of 288 men due to no information on the pubertal events, the final study population constituted 2,522 participants. The excluded men did not differ from the responders with regard to in utero exposure to alcohol.

Approximately 84% of the mothers consumed alcohol on a weekly basis during pregnancy and about 9% reported having >4 drinks of alcohol per week. In total, 16% of the mothers had experienced at least one binge drinking episode during pregnancy, and 2% had ≥5 binge drinking episodes.

In table I, the characteristics of the pregnant women by average weekly alcohol consumption and number of binge drinking episodes are presented. Women with a high average weekly alcohol consumption were on average older and had a lower BMI than women with no alcohol intake. Further, women consuming alcohol during pregnancy had higher socioeconomic status and were more likely to live in urban areas, compared with abstainers. Women binge drinking ≥5 times during pregnancy
were more often cigarette smokers, were more likely to live in urban areas and not live with the father of the child.

The crude mean (95% CI) ages of the four indicators of pubertal development among all participants were: Acne: 14.6 [14.5, 14.7] years, voice break: 14.5 [14.5, 14.6] years, start of regular shaving: 17.2 [17.2, 17.3] years and first nocturnal emission 14.8 [14.7, 14.9] years. The adjusted correlation coefficients between the four pubertal milestones varied between 0.30 and 0.60 (Table II).

We observed a tendency towards a higher age at all four indicators of pubertal development with higher weekly average levels of alcohol intake; however, the differences were small and not statistically significant (Table III). Compared with unexposed sons, sons exposed to >4.0 drinks per week during fetal life were 0.30 [95% CI: -0.19, 0.80] years older at first nocturnal emission, corresponding to 3.6 [95% CI: -2.3, 9.6] months. Regarding binge drinking during pregnancy, we observed a somewhat stronger indication of an older age at all four indicators of pubertal development than seen for weekly average alcohol consumption. Sons exposed to ≥5 binge drinking episodes during pregnancy experienced their first nocturnal emission 7.3 [95% CI: -2.8, 17.4] months later and voice break 4.9 [95% CI: -0.6, 10.4] months later than unexposed. Differences between the groups for the other pubertal milestones were smaller.

We repeated all analyses based on four alternative imputation models and found essentially the same results as those presented in Table III (data not shown). For the restricted analyses (complete cases), results were in the same direction (data not shown).
DISCUSSION

We found no strong evidence for an association between maternal alcohol consumption during pregnancy and pubertal development among sons, but our results provide some indication that binge drinking during pregnancy may be associated with timing of pubertal development.

Our findings of slightly delaying, if any, influence of alcohol consumption during pregnancy on pubertal development in boys are consistent with results from experimental studies. Exposure to ethanol in utero has been linked to delayed sexual maturation in female rats (22;23) and, recently, prenatally exposed male rats also showed a delayed reproductive development and onset of spermatogenesis compared to unexposed.(24) Evidence from epidemiological studies in girls is, however, inconsistent. In a small preliminary investigation by Robe et al., a higher percentage of girls with late onset of menarche was observed among girls exposed to ≥ 2 drinks of alcohol per day.(11) Similar results were observed by Windham et al. when comparing highly exposed and low exposed girls, however, after adjusting for potential confounding factors the effect on age of menarche diminished.(12) This was corroborated by a recent study by Shrestha et al. (13) The results by Robe et al. may well have been confounded, and further, the discrepant findings could be due to differences in exposure levels. The levels of exposure in the study by Windham et al. and Shrestha et al. may have been too low to detect effects.

In this present study, we used prospectively collected information on alcohol intake from the mothers during pregnancy. This limits the risk of differential recall bias,
however, there is a considerable risk of non-differential recall bias. The prevalence of drinking in our study was high, since moderate alcohol intake during pregnancy was socially accepted in Denmark at the time of data collection. Any underreporting is probably non-differential, which most often induces bias towards the null hypothesis. We had the ability to study dose-response effects and further, we had a rather large proportion (8%) of mothers with an alcohol intake of >4.0 drinks per week. The analyses on binge drinking during pregnancy were, however, limited to few highly exposed sons (2%). When studying effects of prenatal alcohol exposure, one major challenge is to disentangle the toxic effects of alcohol from the underlying and possibly confounding factors associated with alcohol consumption.(25) It is well established that lifetime abstainers differ from drinkers on a number of demographic, lifestyle and socioeconomic characteristics.(26) Although, these differences might not be expected to be as comprehensive in a study population of pregnant women, the abstainers did differ from the drinkers in our data. Although, we controlled for various potential confounders, we cannot exclude residual or unmeasured confounding as an explanation for our findings.

Although the participation rate in the birth cohort was high (87%), there is a risk of selection bias related to attrition in this study, as only 55% of the sons participated in the follow-up in 2005. Another limitation is the relatively large amount of missing data in the cohort, especially concerning the four indicators of pubertal development. We addressed this by using multiple imputation models which provides more valid results than the complete case analysis if data are missing at random.(21) Also, we compared estimates from different imputation models.
The data on indicators of pubertal development were based on self-reports at the age of 18 to 21 years. One may argue that as the presence of acne or regular shaving may not occur for all men, these two events may not be good predictors of pubertal development. On the other hand, age at first nocturnal emission and voice break are considered valid indicators of pubertal development in boys, compared to age of menarche in girls.(27;28) Due to the recall time, varying between 1 and 12 years, we expect some misclassification, most likely non-differential, resulting in bias in the null direction.

Despite the high prevalence of alcohol consumption in this study, the number of sons exposed to binge drinking was low, and the results must therefore be interpreted with caution. There may well be a threshold in both time and levels, for the effect of alcohol on male pubertal development. It is plausible that a detrimental effect varies by gestational age producing different vulnerable time windows. However, in this study population, there was not sufficient power to study very high levels of exposure to alcohol during fetal life. Further, we only measured the average number of drinks per week or number of binge drinking episodes during the entire pregnancy and did not have data on the exact time of alcohol consumption. Therefore, we cannot distinguish between early or late exposure in pregnancy and this may mask potential effects of prenatal exposure to high levels of alcohol in vulnerable time windows.

The hypothesis that exposure to alcohol in fetal life delay pubertal development has biological plausibility. Differentiation and development of the male genitals begin around gestational week 7–8, and evidence from experimental studies suggests a window for “male programming”, occurring from week 8–14 of gestation, where
sufficient androgen levels are essential for normal development. (29-33) Alcohol readily passes the placental barrier, thereby directly affecting the developing foetal endocrine organs. (34) Alcohol intake during pregnancy has been shown to increase estrogen levels and decrease the testosterone levels in both maternal and umbilical blood (35-37), thus, the intrauterine hormonal milieu and consequently the fetal hormone balance may well be affected. (34;38) Although, the exact mechanisms are not well understood, the onset of puberty in boys is under control of the hypothalamic-pituitary-gonadal axis (39) and alterations of the fetal hormonal milieu and endocrine system may affect pubertal development later in life. (40)

Further research is needed to investigate the association between maternal alcohol consumption and pubertal development. Future studies would benefit from prospectively collected data on pubertal development by following the children during the years of puberty with clinical examinations, Tanner staging and assessment of changes in hormonal levels.

In summary, we found little evidence to support for the hypothesis that in utero exposure to weekly alcohol consumption is a strong risk factor for altered pubertal development, but we observed a tendency toward a delayed pubertal development among sons exposed to binge drinking during fetal life.
Reference List


(16) Ravnborg TL, Jensen TK, Andersson AM, Toppari J, Skakkebaek NE, Jorgensen N. Prenatal and adult exposures to smoking are associated with adverse effects on reproductive hormones, semen quality, final height and body mass index. Hum Reprod 2011 May;26(5):1000-11.


<table>
<thead>
<tr>
<th>Maternal weekly alcohol consumption during pregnancy</th>
<th>0 drinks/week</th>
<th>0.5 – 1.5 drinks/week</th>
<th>2.0 – 4.0 drinks/week</th>
<th>&gt; 4.0 drinks/week</th>
<th>Test for trend</th>
<th>PR value</th>
<th>Number (%) of missing values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (mean (SD))</td>
<td>26.3 (5.2)</td>
<td>27.7 (4.4)</td>
<td>28.8 (4.3)</td>
<td>29.3 (4.4)</td>
<td>&lt;0.001</td>
<td>0.49</td>
<td>3 (0.1)</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker, n (%)</td>
<td>174 (37.5)</td>
<td>524 (33.0)</td>
<td>176 (33.9)</td>
<td>82 (34.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smoker, n (%)</td>
<td>242 (52.2)</td>
<td>893 (56.4)</td>
<td>281 (54.0)</td>
<td>128 (54.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Past smoker, n (%)</td>
<td>48 (10.3)</td>
<td>168 (10.6)</td>
<td>63 (12.1)</td>
<td>26 (11.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-pregnancy body mass index (BMI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td>193 (6.9)</td>
<td></td>
</tr>
<tr>
<td>&lt;18.5 kg/m², n (%)</td>
<td>44 (10.4)</td>
<td>113 (7.6)</td>
<td>37 (7.7)</td>
<td>20 (8.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.5 – 24.9 kg/m², n (%)</td>
<td>303 (71.3)</td>
<td>1,198 (80.8)</td>
<td>404 (83.8)</td>
<td>193 (84.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;24.9 kg/m², n (%)</td>
<td>78 (18.3)</td>
<td>171 (11.6)</td>
<td>41 (8.5)</td>
<td>15 (6.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic diseases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.80</td>
<td>48 (1.7)</td>
<td></td>
</tr>
<tr>
<td>Yes, n (%)</td>
<td>74 (16.3)</td>
<td>265 (17.0)</td>
<td>96 (18.8)</td>
<td>36 (15.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No, n (%)</td>
<td>381 (83.7)</td>
<td>1,295 (83.2)</td>
<td>416 (81.2)</td>
<td>199 (84.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Municipality of residence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td>3 (0.1)</td>
<td></td>
</tr>
<tr>
<td>Urban areas, no (%)</td>
<td>269 (58.1)</td>
<td>1,018 (64.1)</td>
<td>359 (69.2)</td>
<td>169 (71.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural areas, no (%)</td>
<td>194 (41.9)</td>
<td>570 (35.9)</td>
<td>160 (30.8)</td>
<td>68 (28.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family’s socio-economic status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>White collar workers, n (%)</td>
<td>286 (61.6)</td>
<td>1,225 (77.1)</td>
<td>443 (85.2)</td>
<td>206 (86.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue collar workers, n (%)</td>
<td>135 (29.1)</td>
<td>303 (19.1)</td>
<td>59 (11.4)</td>
<td>22 (9.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unemployed or students, n (%)</td>
<td>43 (9.3)</td>
<td>61 (3.8)</td>
<td>18 (3.5)</td>
<td>9 (3.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohabitation status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.92</td>
<td>154 (5.5)</td>
<td></td>
</tr>
<tr>
<td>Parents living together, n (%)</td>
<td>423 (96.8)</td>
<td>1,466 (79.7)</td>
<td>488 (89.0)</td>
<td>219 (95.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parents not living together, n (%)</td>
<td>14 (3.2)</td>
<td>31 (2.1)</td>
<td>5 (1.0)</td>
<td>10 (4.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Number of maternal binge drinking episodes during pregnancy

<table>
<thead>
<tr>
<th></th>
<th>0 times</th>
<th>1 - 4 times</th>
<th>≥ 5 times</th>
<th>Test for trend</th>
<th>Number (%) of missing values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (mean (SD))</td>
<td>27.8 (4.6)</td>
<td>27.8 (4.4)</td>
<td>28.8 (5.1)</td>
<td>0.55</td>
<td>3 (0.1)</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td>5 (0.2)</td>
</tr>
<tr>
<td>Smoker, n (%)</td>
<td>745 (31.8)</td>
<td>178 (44.2)</td>
<td>32 (59.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smoker, n (%)</td>
<td>1,365 (58.2)</td>
<td>158 (39.2)</td>
<td>18 (33.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Past smoker, n (%)</td>
<td>234 (10.0)</td>
<td>67 (16.6)</td>
<td>4 (7.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-pregnancy body mass index (BMI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.38</td>
</tr>
<tr>
<td>&lt;18.5 kg/m², n (%)</td>
<td>183 (8.4)</td>
<td>27 (7.1)</td>
<td>4 (7.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.5 – 24.9 kg/m², n (%)</td>
<td>1,740 (79.8)</td>
<td>314 (82.9)</td>
<td>42 (77.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;24.9 kg/m², n (%)</td>
<td>258 (11.8)</td>
<td>38 (10.0)</td>
<td>8 (14.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic diseases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.57</td>
</tr>
<tr>
<td>Yes, n (%)</td>
<td>398 (17.2)</td>
<td>65 (16.5)</td>
<td>8 (14.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No, n (%)</td>
<td>1,911 (82.8)</td>
<td>330 (83.5)</td>
<td>46 (85.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Municipality of residence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>Urban areas, no (%)</td>
<td>1,498 (63.9)</td>
<td>276 (68.5)</td>
<td>41 (74.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural areas, no (%)</td>
<td>847 (36.1)</td>
<td>127 (31.5)</td>
<td>14 (25.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family’s socio-economic status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.07</td>
</tr>
<tr>
<td>White collar workers, n (%)</td>
<td>1,821 (77.6)</td>
<td>297 (73.7)</td>
<td>40 (72.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue collar workers, n (%)</td>
<td>423 (18.0)</td>
<td>83 (20.6)</td>
<td>12 (21.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unemployed or students, n (%)</td>
<td>104 (4.4)</td>
<td>23 (5.7)</td>
<td>3 (5.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohabitation status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Parents living together, n (%)</td>
<td>2,181 (98.1)</td>
<td>366 (96.8)</td>
<td>45 (90.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parents not living together, n (%)</td>
<td>43 (1.9)</td>
<td>12 (3.2)</td>
<td>5 (10.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table II. Adjusted\(^a\) correlation between age at indicators of pubertal development

<table>
<thead>
<tr>
<th></th>
<th>Acne</th>
<th>Voice break</th>
<th>Regular shaving</th>
<th>First nocturnal emission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acne</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Voice break</td>
<td>0.60</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular shaving</td>
<td>0.30</td>
<td>0.38</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>First nocturnal emission</td>
<td>0.34</td>
<td>0.40</td>
<td>0.30</td>
<td>1.00</td>
</tr>
</tbody>
</table>

\(^a\) Results are adjusted for maternal alcohol consumption during pregnancy, maternal age at delivery, maternal pre-pregnancy BMI, maternal smoking during pregnancy, maternal chronic diseases, municipality of residence at the time of delivery, family’s socio-economic and cohabitation status of the parents at birth
Table III. Age difference in years [mean [95% CI]] of indicators of pubertal development among 2,522 boys according to average weekly alcohol intake and binge drinking episodes during pregnancy

<table>
<thead>
<tr>
<th>Indicators of male pubertal development</th>
<th>First nocturnal emission</th>
<th>Acne</th>
<th>Voice break</th>
<th>Regular shaving</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Distribution (%)</td>
<td>Adjusted^ mean difference [95% CI]</td>
<td>Adjusted^ mean difference [95% CI]</td>
<td>Adjusted^ mean difference [95% CI]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mean difference [95% CI]</td>
<td>mean difference [95% CI]</td>
<td>mean difference [95% CI]</td>
</tr>
<tr>
<td>Average alcohol intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 drinks/week</td>
<td>16</td>
<td>reference</td>
<td>reference</td>
<td>reference</td>
</tr>
<tr>
<td>0.5 – 1.5 drinks/week</td>
<td>56</td>
<td>0.15 [-0.13, 0.43]</td>
<td>0.06 [-0.12, 0.24]</td>
<td>-0.01 [-0.19, 0.16]</td>
</tr>
<tr>
<td>2.0 – 4.0 drinks/week</td>
<td>19</td>
<td>0.25 [-0.12, 0.62]</td>
<td>0.09 [-0.13, 0.31]</td>
<td>0.11 [-0.10, 0.32]</td>
</tr>
<tr>
<td>&gt; 4.0 drinks/week</td>
<td>9</td>
<td>0.30 [-0.19, 0.80]</td>
<td>0.09 [-0.19, 0.37]</td>
<td>0.03 [-0.24, 0.31]</td>
</tr>
<tr>
<td>Test for trend, p-value</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.97</td>
<td>0.23</td>
<td>0.50</td>
</tr>
<tr>
<td>Binge drinking episodes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 times</td>
<td>84</td>
<td>reference</td>
<td>reference</td>
<td>reference</td>
</tr>
<tr>
<td>1 – 4 times</td>
<td>14</td>
<td>-0.09 [-0.39, 0.21]</td>
<td>-0.02 [-0.21, 0.17]</td>
<td>-0.07 [-0.24, 0.11]</td>
</tr>
<tr>
<td>≥ 5 times</td>
<td>2</td>
<td>0.61 [-0.23, 1.45]</td>
<td>0.06 [-0.42, 0.53]</td>
<td>0.41 [-0.05, 0.87]</td>
</tr>
<tr>
<td>Test for trend, p-value</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.36</td>
<td>0.95</td>
<td>0.45</td>
</tr>
</tbody>
</table>

^ Adjusted for maternal age at delivery, maternal pre-pregnancy BMI, maternal smoking during pregnancy, maternal chronic diseases, municipality of residence at the time of delivery, family’s socio-economic and cohabitation status of the parents at birth.
# STROBE Statement—checklist of items that should be included in reports of observational studies

<table>
<thead>
<tr>
<th>Item No</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Title and abstract</strong></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(a) Indicate the study’s design with a commonly used term in the title or the abstract - <em>this is included in the abstract</em></td>
</tr>
<tr>
<td></td>
<td>(b) Provide in the abstract an informative and balanced summary of what was done and what was found - <em>this is included in the abstract</em></td>
</tr>
<tr>
<td><strong>Introduction</strong></td>
<td>2</td>
</tr>
<tr>
<td>Background/rationale</td>
<td>Explain the scientific background and rationale for the investigation being reported - <em>this is included in the introduction</em></td>
</tr>
<tr>
<td>Objectives</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>State specific objectives, including any prespecified hypotheses - <em>this is included in the introduction</em></td>
</tr>
<tr>
<td><strong>Methods</strong></td>
<td>4</td>
</tr>
<tr>
<td>Study design</td>
<td>Present key elements of study design early in the paper - <em>this is included in the methods on page 5</em></td>
</tr>
<tr>
<td>Setting</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection - <em>this is included in the methods on page 5</em></td>
</tr>
<tr>
<td>Participants</td>
<td>6</td>
</tr>
</tbody>
</table>
| | (a) **Cohort study**—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up - *this is included in the methods on page 5-6*
| | **Case-control study**—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls  |
| | **Cross-sectional study**—Give the eligibility criteria, and the sources and methods of selection of participants |
| | (b) **Cohort study**—For matched studies, give matching criteria and number of exposed and unexposed  |
| | **Case-control study**—For matched studies, give matching criteria and the number of controls per case |
| Variables | 7  |
| | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable - *this is included in the methods on page 5-7* |
| Data sources/ measurement | 8*  |
| | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group - *this is included in the methods on page 5-7* |
| Bias | 9  |
| | Describe any efforts to address potential sources of bias - *this is included in the methods on page 7* |
| Study size | 10 |
| | Explain how the study size was arrived at - *this is included in the methods on page 5* |
| Quantitative variables | 11 |
| | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why |
| Statistical methods | 12 |
| | (a) Describe all statistical methods, including those used to control for confounding - *this is included in the methods on page 7-9* |
| | (b) Describe any methods used to examine subgroups and interactions - *this is included in the methods on page 7-9* |
(c) Explain how missing data were addressed
- this is included and explained in detail in the methods on page 7-8

(d) Cohort study—If applicable, explain how loss to follow-up was addressed

Case-control study—If applicable, explain how matching of cases and controls was addressed

Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy

(e) Describe any sensitivity analyses
- this is included in the methods on page 7-9

Continued on next page
Results

Participants 13*
(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
- this is explained on page 9
(b) Give reasons for non-participation at each stage
- this is explained on page 9
(c) Consider use of a flow diagram
- This was considered but not included in the manuscript. Participation and exclusions are explained in detail.

Descriptive data 14*
(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
- this is described on page 9-10 and also presented in table 1
(b) Indicate number of participants with missing data for each variable of interest
- this is described on page 7, and also presented in table 1
(c) Cohort study—Summarise follow-up time (eg, average and total amount)

Outcome data 15*
Cohort study—Report numbers of outcome events or summary measures over time
- this is described in the Methods.
Case-control study—Report numbers in each exposure category, or summary measures of exposure
Cross-sectional study—Report numbers of outcome events or summary measures

Main results 16
(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
- this is presented in the results
(b) Report category boundaries when continuous variables were categorized
- this is described and also presented in table 3.
(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
- Not relevant in this present result section

Other analyses 17
Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
This is presented in the results section

Discussion

Key results 18
Summarise key results with reference to study objectives
- This is described.

Limitations 19
Discuss limitations of the study, taking into account sources of potential bias or imprecision.
Discuss both direction and magnitude of any potential bias
- This is described in detail in the discussion.

Interpretation 20
Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
- This is described.

Generalisability 21
Discuss the generalisability (external validity) of the study results
- This is described.

Other information

Funding 22
Give the source of funding and the role of the funders for the present study and, if applicable,
for the original study on which the present article is based
- not relevant in this present study

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.
# IN UTERO EXPOSURE OF ALCOHOL AND PUBERTY IN BOYS. A PREGNANCY COHORT STUDY

<table>
<thead>
<tr>
<th>Journal:</th>
<th>BMJ Open</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID:</td>
<td>bmjopen-2013-004467.R1</td>
</tr>
<tr>
<td>Article Type:</td>
<td>Research</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>05-May-2014</td>
</tr>
</tbody>
</table>
| Complete List of Authors: | Håkonsen, Linn Berger; Department of Public Health, Section for Epidemiology, Aarhus University  
Brath-Lund, Mette Louise; Danish Ramazzini Center, Department of Occupational Medicine, Aarhus University Hospital, Denmark  
Hounsgaard, Marie Louise; Department of Public Health, Section for Epidemiology, Aarhus University  
Olsen, Jørn; Department of Public Health, Section for Epidemiology, Aarhus University  
Ernst, Andreas; Department of Public Health, Section for Epidemiology, Aarhus University  
Thulstrup, Ane Marie; Danish Ramazzini Center, Department of Occupational Medicine, Aarhus University Hospital, Denmark  
Bech, Bodil; Department of Public Health, Section for Epidemiology, Aarhus University  
Ramlau-Hansen, Cecilia; Institute of Public Health, Department of Epidemiology |
| <b>Primary Subject Heading</b>: | Paediatrics |
| Secondary Subject Heading: | Epidemiology, Obstetrics and gynaecology, Reproductive medicine, Sexual health |
| Keywords: | EPIDEMIOLOGY, Community child health < PAEDIATRICS, REPRODUCTIVE MEDICINE |
IN UTERO EXPOSURE OF ALCOHOL AND PUBERTY IN BOYS

A PREGNANCY COHORT STUDY

Corresponding author: Linn Berger Håkonsen, Department of Public Health, Section for Epidemiology, Aarhus University, Denmark, E-mail: linnhaak@rm.dk

Linn Berger Håkonsen\textsuperscript{a}, Mette Louise Brath-Lund\textsuperscript{b}, Marie Louise Hounsgaard\textsuperscript{b}, Jørn Olsen, Ph.D\textsuperscript{a}, Andreas Ernst\textsuperscript{a}, Ane Marie Thulstrup, Ph.D\textsuperscript{b}, Bodil Hammer Bech, Ph.D\textsuperscript{a}, Cecilia Høst Ramlau-Hansen, Ph.D\textsuperscript{a}

\textsuperscript{a}Department of Public Health, Section for Epidemiology, Aarhus University, Denmark

\textsuperscript{b}Danish Ramazzini Center, Department of Occupational Medicine, Aarhus University Hospital, Denmark.

Running title: Maternal alcohol consumption and puberty in sons.

Key words: Prenatal exposure, Pregnancy, Alcohol drinking, Life style, Puberty

Word count: 3007

Number of tables: 3
ABSTRACT

Objectives: Epidemiological studies have raised concern about the reproductive consequences of in utero exposure to alcohol. Maternal lifestyle factors have been associated to altered pubertal development, but the impact of prenatal alcohol exposure on male puberty is unknown. Thus, the objective was to explore whether prenatal alcohol exposure alters pubertal development in boys.

Setting: Follow-up of a Danish pregnancy cohort.

Participants: Sons (N = 2,522) of women who were enrolled in a Danish pregnancy cohort in 1984-1987.

Primary and secondary outcome measures: Indicators of pubertal development, assessed by age at first nocturnal emission, voice break, acne and regular shaving.

Results: We found a tendency towards a later age at first nocturnal emission and voice break following in utero exposure to binge drinking. Sons exposed to ≥5 binge drinking episodes during pregnancy experienced their first nocturnal emission 7.3 months [95% CI: -2.8, 17.4] later and voice break 4.9 months [95% CI: -0.6, 10.4] later than unexposed sons. Results on average weekly alcohol consumption were in the same direction, but, differences were smaller and not statistically significant.

Conclusions: We found no strong support for the hypothesis that in utero exposure to weekly alcohol consumption is a risk factor for altered pubertal development, but a tendency towards a delayed pubertal development among sons exposed to binge drinking during fetal life was observed. Longitudinal studies with data collected as the children go through puberty are needed to explore this further.
Strengths and limitations of this study

Strengths of this study

- Large pregnancy cohort with a rather high participation rate (55%)
- Prospectively collected data on maternal alcohol consumption
- Data with a large exposure contrast
- Ability to study dose-response effects
- “State of the art” statistical methods

Limitations of this study

- Self-reported data on pubertal development
- Lack of valid indicators of pubertal development in boys
- A relatively large amount of missing data on the indicators of pubertal development.
- Risk of uncontrolled or residual confounding
INTRODUCTION

Alcohol consumption during pregnancy has been associated with adverse pregnancy outcomes (1;2) and child morbidity. (3) Although controversy still exist regarding the safety of low levels (4) and specific timing of exposure, women are advised to abstain from alcohol when planning to conceive and throughout pregnancy. Yet, alcohol consumption is common during pregnancy in many countries (5;6) and is, thus, one of the major modifiable risk factors possibly affecting fetal growth.

Interest in male reproductive consequences of prenatal exposure to alcohol has grown recently, as focus on causes of subfecundity has intensified worldwide. In males, one study reported an association between prenatal alcohol exposure and cryptorchidism,(7) a congenital malformation that may predispose to impaired semen quality, whereas other studies have shown inconsistent results.(8-10) In adult life, indications of reduced semen quality among exposed sons have been reported.(11)

A few studies have investigated the association between maternal alcohol consumption during pregnancy and pubertal development in girls. One of these observed later age of menarche in a small group of heavily exposed girls,(12) but two more recent studies did not support this finding.(13;14) Shrestha et al. also assessed binge drinking episodes during pregnancy, but found no effects on timing of menarche, neither of binge drinking nor weekly alcohol consumption.(14)

In boys, studies on risk factors for altered pubertal development are sparse. It is well-established that the variability in onset of puberty depends on genetic factors, ethnicity and nutritional conditions,(15) but in utero exposures may also have an early
“programming” role. It has been indicated that male pubertal development may be accelerated following \textit{in utero} exposure to cigarette smoking (16-18) and maternal obesity.\textsuperscript{(19)}

This study aims to explore whether prenatal alcohol exposure alters pubertal development in boys.

\textbf{METHODS}

This study is based on data from the pregnancy cohort “Healthy habits for two” \textsuperscript{(20)} established in 1984 – 1987 in the municipalities of Aalborg and Odense, Denmark. The cohort included 11,980 pregnant women (87\% of all invited) who, at a routine visit to the midwife around 36\textsuperscript{th} gestational week, completed a questionnaire concerning lifestyle, demographic and health-related characteristics before and during pregnancy, including questions on consumption of alcoholic beverages during pregnancy. The pregnant women were all Danish citizens and since only 1\% of Danish women aged 20 - 34 years were immigrants or descendants from immigrants during 1984 - 1987, the participants were most likely Caucasians.\textsuperscript{(21)} Of the 11,980 pregnant women, 11,144 delivered live born singletons. Of these, 5,716 were boys. In 2005, 5142 sons (between 18 and 21 years of age), who were alive and living in Denmark were identified in the Danish Civil Registration System and invited to complete an internet-based questionnaire. A total of 2,810 (55\%) sons responded. The study was approved by the Danish Data Protection Agency (J.nr: 2011-41-6435).

\textbf{Exposure assessment}
In the questionnaire completed by the pregnant women around the 36th gestational week, the women were asked about their average weekly intake of beer, wine and spirits while being pregnant. One drink was defined as one bottle of beer (0.33 l), one glass of wine, or one glass of spirits. We calculated the weekly intake of these alcoholic beverages for each woman. The average maternal alcohol intake during pregnancy was categorised in four groups: 0, 0.5 – 1.5, 2.0 – 4.0 or > 4.0 drinks/week. Further, the women were asked how many times they had consumed eight or more alcoholic drinks on a single occasion (defined as binge drinking) while being pregnant with the following pre-determined response categories: 0, 1 – 4, 5 – 9, 10 – 19, or > 20 times. We formed three groups according to number of binge drinking episodes; 0, 1 – 4 and ≥5 times during pregnancy.

Assessment of pubertal development in sons

In the follow-up questionnaire administered to the sons in 2005, four questions concerning different indicators of pubertal development were asked: ‘Have you had acne?’ ‘Has your voice broken?’ ‘Have you started to shave regularly?’ and ‘Have you had your first nocturnal emission?’. If they answered “yes”, they were also asked to provide the age in years and months at which the event first occurred. We converted the month into a fraction of a year and added years, to create a continuous outcome variable for each of the four events.

Covariates

Potential confounders in the cohort data were identified a priori: Maternal age in years (continuous), maternal pre-pregnancy body mass index (BMI) (<18.5, 18.5 – 24.9 and >24.9 kg/m²), maternal smoking during pregnancy (smoker, former smoker
(stopped before pregnancy) and non-smoker), maternal chronic diseases (diabetes mellitus, epilepsy, arthritis, heart disease, cancer, psychiatric disorders, allergy or other chronic diseases, combined into one variable: present versus not present), municipality of residence at the time of delivery (urban areas versus rural areas), family socio-economic status based on the highest ranking of job description or academic background between parents at the time of pregnancy (white collar workers, blue collar workers and unemployed or students) and co-habitation status of the parents at birth (mother living with the father of the child versus mother not living with the father of the child).

**Statistical analyses**

**Missing information**

The number (%) of participants who gave information on age of the four outcome variables were: Acne: 1,804 (64%), voice break: 1,696 (60%), regular shaving: 2,128 (76%) and first nocturnal emission: 924 (33%). About three-quarter of these only provided age in years. Information on maternal average weekly alcohol consumption was complete and there were only 4 (0.1%) missing values on binge drinking. Further, the level of missing values in covariates varied from 0 to 6.9% (Table I). Unexposed sons had more missing values in the pubertal events compared with sons exposed to alcohol during pregnancy. However, differences were rather small.

Since complete case analysis can lead to biased estimates and limited power, we addressed the missing data problem by using multiple imputations, which often yield unbiased and more precise estimates if data are missing at random.(22;23) Briefly, the multiple imputations model is an approach that creates several \( m > 1 \) different imputed data sets, based on other known subject characteristics from the whole data...
set. The \( m \) complete data sets are then analysed and the results are combined by use of the so-called Rubin’s rule, thus producing a single set of inference that include the variability associated with the missing data.

Prior to multiple imputations, we excluded sons who had not provided an age in years for one of the four events (\( n = 288 \)). We performed the multiple imputations using an interval regression imputation model (100 imputed datasets) with interval censoring of the indicators of pubertal events. The following variables were included in the main imputation model: age at first nocturnal emission, age at acne, age at voice break, age at regular shaving, maternal pre-pregnancy BMI, maternal cigarette smoking, maternal age at delivery, alcohol consumption during pregnancy, chronic diseases of the mother, municipality of residence at the time of delivery, family’s socio-economic status and co-habitation of the parents. We performed different imputation models, including 1) a higher number of imputed datasets, \( m = 120 \), 2) only exposure and outcome variables, and 3) more covariates than in the main imputation model. We then compared the results to check for consistency, i.e. the sensitivity of the results to the choice of model used for imputations.

**Data analyses**

Data on age of the four indicators of pubertal development were symmetrically distributed. Thus, we calculated the mean ages with 95% confidence intervals (CI) for each of the pubertal events. We estimated partial correlation coefficients between the four indicators of pubertal development adjusted for the covariates described above. Further, we performed multiple linear regression analyses with maternal alcohol consumption during pregnancy, with both average weekly intake and number of binge drinking episodes as the explanatory variable in separate models. We adjusted for the
potential confounders mentioned above. Moreover, we estimated trends using average maternal alcohol consumption and binge drinking as continuous variables. Since 2.1% of the women contributed to the cohort with more than one child, we applied robust standard errors in the adjusted analyses to account for clustering.

We also performed sensitivity analyses. Firstly, we repeated the multiple regression analyses using different multiple imputation models, to check for consistency of the chosen imputation model, as noted before. Secondly, we performed restricted analyses based on the participants who reported at least age in years at all event (complete case analyses). Third, to test for interaction between maternal weekly alcohol consumption and maternal cigarette smoking, we fitted a multiple regression model testing for interaction among the complete cases. All statistical analyses were performed using Stata 12 software (Stata Corporation, College Station, TX).

RESULTS

The cohort included 2,810 men, but 288 men provided no information on the pubertal events and the final study population constituted 2,522 participants. The excluded men did not differ from the responders with regard to in utero exposure to alcohol.

Approximately 84% of the mothers consumed alcohol on a weekly basis during pregnancy and about 9% reported having >4 drinks of alcohol per week. In total, 16% of the mothers had experienced at least one binge drinking episode during pregnancy, and 2% had ≥5 binge drinking episodes.

In table I, the characteristics of the pregnant women by average weekly alcohol consumption and number of binge drinking episodes are presented. Women with a high average weekly alcohol consumption were on average older and had more binge
drinking episodes and a lower BMI than women with no alcohol intake. Further, women consuming alcohol during pregnancy had higher socioeconomic status and were more likely to live in urban areas, compared with abstainers. Women binge drinking ≥5 times during pregnancy did more often smoke, had a higher weekly alcohol intake during pregnancy and were more likely to live in urban areas and not live with the father of the child.

The crude mean (95% CI) ages of the four indicators of pubertal development among all participants were: Acne: 14.6 [14.5, 14.7] years, voice break: 14.5 [14.5, 14.6] years, start of regular shaving: 17.2 [17.2, 17.3] years and first nocturnal emission 14.8 [14.7, 14.9] years. The adjusted correlation coefficients between the four pubertal milestones varied between 0.30 and 0.60 (Table II).

We observed a tendency towards a higher age at all four indicators of pubertal development with higher weekly average levels of alcohol intake; however, the differences were small and not statistically significant (Table III). Compared with unexposed sons, sons exposed to >4.0 drinks per week during fetal life were 0.30 [95% CI: -0.19, 0.80] years older at first nocturnal emission, corresponding to 3.6 [95% CI: -2.3, 9.6] months. Regarding binge drinking during pregnancy, we observed a somewhat stronger indication of an older age at all four indicators of pubertal development than seen for weekly average alcohol consumption. Sons exposed to ≥5 binge drinking episodes during pregnancy experienced their first nocturnal emission 7.3 [95% CI: -2.8, 17.4] months later and voice break 4.9 [95% CI: -0.6, 10.4] months later than unexposed. Differences between the groups for the other pubertal milestones were smaller.
We repeated all analyses based on four alternative imputation models and found essentially the same results as those presented in Table III (data not shown). For the restricted analyses (complete cases), results were in the same direction (data not shown). Finally, in the interaction analyses, there was no indication of effect measure modification by maternal cigarette smoking (data not shown).

**DISCUSSION**

We found no strong evidence for an association between maternal alcohol consumption during pregnancy and pubertal development among sons, but our results indicated that binge drinking during pregnancy may be associated with later age of pubertal development in boys.

Our findings of slightly delaying pubertal development in boys are consistent with results from experimental studies. Exposure to ethanol *in utero* has been linked to delayed sexual maturation in female rats (24;25) and, recently, prenatally exposed male rats also showed a delayed reproductive development and onset of spermatogenesis compared to unexposed.(26) Thus, it is plausible that prenatal exposure to alcohol could affect onset of spermatogenesis in humans. Evidence from epidemiological studies on pubertal development in girls is, however, inconsistent. In a small preliminary investigation by Robe *et al.*, a higher percentage of girls with late onset of menarche was observed among girls exposed to ≥ 2 drinks of alcohol per day.(12) Similar results were observed by Windham *et al.* when comparing highly exposed and low exposed girls, however, after adjusting for potential confounding factors the effect on age of menarche diminished.(13) Further, Shrestha *et al.* (14) reported no association between in utero exposure to alcohol and age of menarche.
The results by Robe et al. may well have been confounded, but the discrepant findings could be due to differences in exposure levels. The levels of exposure in the study by Windham et al. and Shrestha et al. may have been too low to detect effects.

In this present study, we used prospectively collected information on alcohol intake from the mothers during pregnancy. This limits the risk of differential recall bias, however, there is a considerable risk of non-differential recall bias driving effect measures toward the null. The prevalence of drinking in our study was high, since moderate alcohol intake during pregnancy was socially accepted in Denmark at the time of data collection. We had the ability to study dose-response effects with a large exposure contrast and, further, we had a rather large proportion (8%) of mothers with an alcohol intake of >4.0 drinks per week. The analyses on binge drinking during pregnancy were, however, limited to few highly exposed sons (2%). When studying effects of prenatal alcohol exposure, one major challenge is to disentangle the toxic effects of alcohol from the underlying and possibly confounding factors associated with alcohol consumption. It is well established that lifetime abstainers differ from drinkers on a number of demographic, lifestyle and socioeconomic characteristics. Although, these differences might not be expected to be as comprehensive in a study population of pregnant women, the abstainers did differ from the drinkers in our data. Although, we controlled for various potential confounders, we cannot exclude residual or unmeasured confounding as an explanation for our findings.

Although the participation rate in the birth cohort was high (87%), there is a risk of selection bias related to attrition in this study, as only 55% of the sons participated in
the follow-up in 2005. Another limitation is the relatively large amount of missing data in the cohort, especially concerning the four indicators of pubertal development. We addressed this by using multiple imputation models which provides more valid results than the complete case analysis if data are missing at random. (23) Also, we compared estimates from different imputation models.

The data on indicators of pubertal development were based on self-reports at the age of 18 to 21 years. One may argue that the presence of acne or regular shaving may not occur for all men and these two events may therefore not be good predictors of pubertal development. On the other hand, age at first nocturnal emission and voice break are considered valid indicators of pubertal development in boys, comparably to age of menarche in girls. (29;30) Previous data from Danish boys have shown that the median age at first ejaculation (spermache) occurred at 13.4 years (31), and mean age at voice break were 14.0 years (32), which is slightly earlier than in the present study. This small discrepancy may well be explained by the recall time in our study which varied between 1 and 12 years. We expect some misclassification, yet most likely non-differential, resulting in bias in the null direction.

Despite the high prevalence of alcohol consumption in this study, the number of sons exposed to binge drinking was low, and the results must therefore be interpreted with caution. Even low alcohol intake during pregnancy lower maternal serum testosterone levels (33) which could interfere with both the intrauterine hormonal environment and the hormonal system in the developing fetus. However, there may well be a threshold for both dose and timing of exposure on the effect on male pubertal development. It is
plausible that a detrimental effect varies by gestational age and there may be more than one vulnerable time window.

In this study population, there was not sufficient information to study very high levels of exposure to alcohol during fetal life. Further, we only measured the average number of drinks per week or number of binge drinking episodes during the entire pregnancy and did not have data on the exact time of alcohol consumption. Therefore, we cannot distinguish between early or late exposure in pregnancy and this may mask potential effects of prenatal exposure to high levels of alcohol in vulnerable time windows.

It is biological plausible that exposure to alcohol in fetal life delay pubertal development. Differentiation and development of the male genitals begin around gestational week 7–8, and evidence from experimental studies suggests a window for “male programming”, occurring from week 8–14 of gestation, where sufficient androgen levels are essential for normal development.(34-38) Alcohol readily passes the placental barrier, thereby possibly affecting the endocrine organs of the developing foetus.(39) Alcohol intake during pregnancy has been shown to increase estrogen levels and decrease the testosterone levels in both maternal and umbilical blood (40-42), thus, the intrauterine hormonal milieu and consequently the fetal hormone balance may well be affected.(39;43) Although, the exact mechanisms are not well understood, the onset of puberty in boys is under control of the hypothalamic-pituitary-gonadal axis (44) and alterations of the fetal hormonal milieu and endocrine system may affect pubertal development later in life.(45)
Further research is needed to investigate the association between maternal alcohol consumption and pubertal development. Future studies would benefit from data collected on pubertal development by following the children during the years of puberty with clinical examinations, Tanner staging and assessment of changes in hormonal levels.

In summary, we found little evidence to support the hypothesis that in utero exposure to weekly alcohol consumption is a strong risk factor for altered pubertal development, but we observed a tendency toward a delayed pubertal development among sons exposed to maternal binge drinking during fetal life.
Contributorship Statement:

Linn Berger Håkonsen: Ms. Håkonsen contributed to design of the study, carried out the data analyses, contributed to data interpretation, drafted the manuscript and approved the final manuscript as submitted.

Mette Louise Brath-Lund: Ms. Brath-Lund contributed to data interpretation and drafting of the manuscript, critically reviewed the manuscript and approved the final manuscript as submitted.

Marie Louise Hounsgaard: Ms. Hounsgaard contributed to data analyses and data interpretation, critically reviewed the manuscript and approved the final manuscript as submitted.

Jørn Olsen: Dr. Olsen contributed to design of the study, acquisition of data, contributed to data analyses and data interpretation, critically reviewed the manuscript and approved the final manuscript as submitted.

Andreas Ernst: Mr. Ernst contributed to data interpretation, critically reviewed the manuscript and approved the final manuscript as submitted.

Ane Marie Thulstrup: Dr. Thulstrup contributed to data interpretation, critically reviewed the manuscript and approved the final manuscript as submitted.

Bodil Hammer Bech: Dr. Bech contributed to acquisition of data, data interpretation, critically reviewed the manuscript and approved the final manuscript as submitted.

Cecilia Høst Ramlau-Hansen: Dr. Ramlau-Hansen contributed to design of the study, acquisition of data, contributed to data analyses and data interpretation, critically reviewed the manuscript and approved the final manuscript as submitted.

Competing Interests: None

Data Sharing Statement: No additional data available

Reference List


IN UTERO EXPOSURE OF ALCOHOL AND PUBERTY IN BOYSSONS. A FOLLOW-UP STUDY OF A PREGNANCY COHORT STUDY.

Corresponding author: Linn Berger Håkonsen, Department of Public Health, Section for Epidemiology, Aarhus University, Denmark, E-mail: linnhaak@rm.dk

Linn Berger Håkonsen\textsuperscript{a}, Mette Louise Brath-Lund\textsuperscript{b}, Marie Louise Hounsgaard\textsuperscript{b}, Jørn Olsen, Ph.D\textsuperscript{a}, Andreas Ernst\textsuperscript{a}, Ane Marie Thulstrup, Ph.D\textsuperscript{b}, Bodil Hammer Bech, Ph.D\textsuperscript{a}, Cecilia Høst Ramlau-Hansen, Ph.D\textsuperscript{a}

\textsuperscript{a}Department of Public Health, Section for Epidemiology, Aarhus University, Denmark

\textsuperscript{b}Danish Ramazzini Center, Department of Occupational Medicine, Aarhus University Hospital, Denmark.

Running title: Maternal alcohol consumption and puberty in sons.

Key words: Prenatal exposure, Pregnancy, Alcohol drinking, Life style, Puberty

Word count: 30072813

Number of tables: 3
ABSTRACT

Objectives: Epidemiological studies have raised concern about the reproductive consequences of in utero exposure to alcohol. Exposure to maternal lifestyle factors have been associated to altered pubertal development, but the impact of prenatal alcohol exposure on male puberty is unknown. Thus, the objective was to explore whether prenatal alcohol exposure alters pubertal development in boys.

Setting: Follow-up study of a Danish pregnancy cohort.

Participants: Sons (N = 2,522) of women who were enrolled in a Danish pregnancy cohort in 1984 - 1987.

Primary and secondary outcome measures: Indicators of pubertal development, assessed by age at first nocturnal emission, voice break, acne and regular shaving.

Results: We found a tendency towards a later age at first nocturnal emission and voice break following in utero exposure to binge drinking. Sons exposed to ≥5 binge drinking episodes during pregnancy experienced their first nocturnal emission 7.3 months [95% CI: -2.8, 17.4] later and voice break 4.9 months [95% CI: -0.6, 10.4] later than unexposed sons. Results on average weekly alcohol consumption were in the same direction, but, differences were smaller and not statistically significant.

Conclusions: We found no strong support for the hypothesis that in utero exposure to weekly alcohol consumption is a risk factor for altered pubertal development. Although a tendency towards a delayed pubertal development among sons exposed to binge drinking during fetal life was observed, longitudinal studies with
prospectively collected data collected as the children go through puberty on pubertal development are needed to explore this further.

Strengths and limitations of this study

Strengths of this study

- Large pregnancy cohort with a rather high participation rate (55%)
- Prospectively collected data on maternal alcohol consumption
- Data with a large exposure contrast: High prevalence of women drinking alcohol during pregnancy
- Ability to study dose-response effects
- “State of the art” statistical methods

Limitations of this study

- Self-reported data on pubertal development
- Lack of valid indicators of pubertal development in boys
- A relatively large amount of missing data on the indicators of pubertal development.
- Risk of uncontrolled or residual confounding
INTRODUCTION

Alcohol consumption during pregnancy has been associated with adverse pregnancy outcomes (1,2) and health complications for the exposed children (3). Although controversy still exists regarding the safety of low levels (4) and specific timing of exposure, women are advised to abstain from alcohol when planning to conceive and throughout pregnancy. Yet, despite these well-established risks, alcohol consumption is still common during pregnancy in many countries (5,6) and is, thus, one of the major modifiable risk factors possibly affecting adverse pregnancy outcomes and fetal growth in the Western world.

Interest in male reproductive consequences of prenatal exposure to alcohol has grown recently, as focus on causes of subfecundity has intensified worldwide. In males, one study reported an association between prenatal alcohol exposure and cryptorchidism, a congenital malformation that may predispose to impaired semen quality, whereas other studies have shown inconsistent results (8-10). In
adult life, indications of reduced semen quality among exposed sons have been
reported.\(^{(11)}\)\(^{(10)}\)

A few studies have investigated the association between maternal alcohol
consumption during pregnancy and pubertal development in girls. One of these
observed later age of menarche in a small group of heavily exposed girls,\(^{(12)}\)\(^{(11)}\) but
two more recent studies did not support this finding.\(^{(13,14)}\)\(^{(12,13)}\) Shrestha \textit{et al.} also
assessed binge drinking episodes during pregnancy, but found no effects on timing of
menarche, neither of binge drinking nor weekly alcohol consumption.\(^{(14)}\)\(^{(13)}\)

In boys, studies on risk factors for altered pubertal development are sparse. It is well-
established that the variability in onset of puberty depends on genetic factors,
etnicity and nutritional conditions\(^{(15)}\)\(^{(14)}\) but \textit{in utero} exposures may also have an
early “programming” role. It has been indicated that male pubertal development may
be accelerated following \textit{in utero} exposure to cigarette smoking\(^{(16-18)}\)\(^{(15-17)}\) and
maternal obesity.\(^{(19)}\)

This study aims to explore whether prenatal alcohol exposure alters pubertal
development in boys.

\section*{METHODS}

This study is based on data from the pregnancy cohort “Healthy habits for two”\(^{(20)}\)\(^{(18)}\) established in 1984 – 1987 in the municipalities of Aalborg and Odense,
Denmark. The cohort included 11,980 pregnant women (87\% of all invited) who, at a
routine visit to the midwife around 36\textsuperscript{th} gestational week, completed a questionnaire
concerning lifestyle, demographic and health-related characteristics before and during pregnancy, including questions on consumption of alcoholic beverages during pregnancy. The pregnant women were all Danish citizens and since only 1% of Danish women aged 20 - 34 years were immigrants or descendants from immigrants during 1984 - 1987, the participants were most likely Caucasians.(21)(49) Of the 11,980 pregnant women, 11,144 delivered live born singletons. Of these, 5,716 were boys. In 2005, 5142 sons (between 18 and 21 years of age), who were alive and living in Denmark were identified in the Danish Civil Registration System and invited to complete an internet-based questionnaire. A total of 2,810 (55%) sons responded. The study was approved by the Danish Data Protection Agency (J.nr: 2011-41-6435).

Exposure assessment

In the questionnaire completed by the pregnant women around the 36th gestational week, the women were asked about their average weekly intake of beer, wine and spirits while being pregnant. One drink was defined as one bottle of beer (0.33 l), one glass of wine, or one glass of spirits. We calculated the weekly intake of these alcoholic beverages for each woman. The average maternal alcohol intake during pregnancy was categorised in four groups: 0, 0.5 – 1.5, 2.0 – 4.0 or > 4.0 drinks/week. Further, the women were asked how many times they had consumed eight or more alcoholic drinks on a single occasion (defined as binge drinking) while being pregnant with the following pre-determined response categories: 0, 1 – 4, 5 – 9, 10 – 19, or > 20 times. We formed three groups according to number of binge drinking episodes; 0, 1 – 4 and ≥5 times during pregnancy.
Assessment of pubertal development in sons

In the follow-up questionnaire administered to the sons in 2005, four questions concerning different indicators of pubertal development were asked: ‘Have you had acne?’, ‘Has your voice broken?’, ‘Have you started to shave regularly?’ and ‘Have you had your first nocturnal emission?’. If they answered “yes”, they were also asked to provide the age in years and months at which the event first occurred. We converted the month into a fraction of a year and added years, to create a continuous outcome variable for each of the four events.

Covariates

Potential confounders in the cohort data were identified a priori: Maternal age in years (continuous), maternal pre-pregnancy body mass index (BMI) (<18.5, 18.5 – 24.9 and >24.9 kg/m²), maternal smoking during pregnancy (smoker, former smoker (stopped before pregnancy) and non-smoker), maternal chronic diseases (diabetes mellitus, epilepsy, arthrosis, heart disease, cancer, psychiatric disorders, allergy or other chronic diseases, combined into one variable: present versus not present), municipality of residence at the time of delivery (urban areas versus rural areas), family socio-economic status based on the highest ranking of job description or academic background between parents at the time of pregnancy (white collar workers, blue collar workers and unemployed or students) and co-habitation status of the parents at birth (mother living with the father of the child versus mother not living with the father of the child).

Statistical analyses

Missing information
The number (%) of participants who gave information on age of the four outcome variables were: Acne: 1,804 (64%), voice break: 1,696 (60%), regular shaving: 2,128 (76%) and first nocturnal emission: 924 (33%). About three-quarter of these only provided age in years. Information on maternal average weekly alcohol consumption was complete and there were only 4 (0.1%) missing values on binge drinking. Further, the level of missing values in covariates varied from 0 to 6.9% (Table I). Unexposed sons had more missing values in the pubertal events compared with sons exposed to alcohol during pregnancy. However, differences were rather small.

Since complete case analysis can lead to biased estimates and limited power, we addressed the missing data problem by using multiple imputations, which often yield unbiased and more precise estimates if data are missing at random.\textsuperscript{(22;23)(20;21)} Briefly, the multiple imputations model is an approach that creates several ($m > 1$) different imputed data sets, based on other known subject characteristics from the whole data set. The $m$ complete data sets are then analysed and the results are combined by use of the so-called Rubin’s rule, thus producing a single set of inference that include the variability associated with the missing data.

Prior to multiple imputations, we excluded sons who had not at least provided an age in years for one of the four events ($n = 288$). We performed the multiple imputations using an interval regression imputation model (100 imputed datasets) with interval censoring of the indicators of pubertal events. The following variables were included in the main imputation model: age at first nocturnal emission, age at acne, age at voice break, age at regular shaving, maternal pre-pregnancy BMI, maternal cigarette smoking, maternal age at delivery, alcohol consumption during pregnancy, chronic diseases of the mother, municipality of residence at the time of delivery, family’s
socio-economic status and co-habitation of the parents. We performed different imputation models, including 1) a higher number of imputed datasets, \( m = 120 \), 2) only exposure and outcome variables, and 3) more covariates than in the main imputation model. We then compared the results to check for consistency, i.e. the sensitivity of the results to the choice of model used for imputations.

**Data analyses**

Data on age of the four indicators of pubertal development were approximately normally symmetrically distributed. Thus, we calculated the mean ages with 95% confidence intervals (CI) for each of the pubertal events. We estimated partial correlation coefficients between the four indicators of pubertal development adjusted for the covariates described above. Further, we performed multiple linear regression analyses with maternal alcohol consumption during pregnancy, with both average weekly intake and number of binge drinking episodes as the explanatory variable in separate models. We adjusted for the potential confounders mentioned above. Moreover, we estimated trends using average maternal alcohol consumption and binge drinking as continuous variables. Since 2.1% of the women contributed to the cohort with more than one child, we applied robust standard errors in the adjusted analyses to account for clustering.

We also performed sensitivity analyses. Firstly, we repeated the multiple regression analyses using different multiple imputation models, to check for consistency of the chosen imputation model, as noted before. Secondly, we performed restricted analyses based on the participants who reported at least age in years at all event (complete case analyses). Third, to test for interaction between maternal weekly alcohol consumption and maternal cigarette smoking, we fitted a multiple regression model testing for
interaction among the complete cases. All statistical analyses were performed using Stata 12 software (Stata Corporation, College Station, TX).

RESULTS

The cohort included 2,810 men, but after exclusion of 288 men due to provided no information on the pubertal events and, the final study population constituted 2,522 participants. The excluded men did not differ from the responders with regard to in utero exposure to alcohol.

Approximately 84% of the mothers consumed alcohol on a weekly basis during pregnancy and about 9% reported having >4 drinks of alcohol per week. In total, 16% of the mothers had experienced at least one binge drinking episode during pregnancy, and 2% had ≥5 binge drinking episodes.

In table I, the characteristics of the pregnant women by average weekly alcohol consumption and number of binge drinking episodes are presented. Women with a high average weekly alcohol consumption were on average older and had more binge drinking episodes and a lower BMI than women with no alcohol intake. Further, women consuming alcohol during pregnancy had higher socioeconomic status and were more likely to live in urban areas, compared with abstainers. Women binge drinking ≥5 times during pregnancy did more often smoke, had a higher weekly alcohol intake during pregnancy and were more often cigarette smokers, were more likely to live in urban areas and not live with the father of the child.

The crude mean (95% CI) ages of the four indicators of pubertal development among all participants were: Acne: 14.6 [14.5, 14.7] years, voice break: 14.5 [14.5, 14.6]
years, start of regular shaving: 17.2 [17.2, 17.3] years and first nocturnal emission
14.8 [14.7, 14.9] years. The adjusted correlation coefficients between the four
pubertal milestones varied between 0.30 and 0.60 (Table II).

We observed a tendency towards a higher age at all four indicators of pubertal
development with higher weekly average levels of alcohol intake; however, the
differences were small and not statistically significant (Table III). Compared with
unexposed sons, sons exposed to >4.0 drinks per week during fetal life were 0.30
[95% CI: -0.19, 0.80] years older at first nocturnal emission, corresponding to 3.6
[95% CI: -2.3, 9.6] months. Regarding binge drinking during pregnancy, we observed
a somewhat stronger indication of an older age at all four indicators of pubertal
development than seen for weekly average alcohol consumption. Sons exposed to ≥5
binge drinking episodes during pregnancy experienced their first nocturnal emission
7.3 [95% CI: -2.8, 17.4] months later and voice break 4.9 [95% CI: -0.6, 10.4] months
later than unexposed. Differences between the groups for the other pubertal
milestones were smaller.

We repeated all analyses based on four alternative imputation models and found
essentially the same results as those presented in Table III (data not shown). For the
restricted analyses (complete cases), results were in the same direction (data not
shown). Finally, in the interaction analyses, there was no indication of effect measure
modification by maternal cigarette smoking (data not shown).

DISCUSSION
We found no strong evidence for an association between maternal alcohol consumption during pregnancy and pubertal development among sons, but our results provide some indication that binge drinking during pregnancy may be associated with later age timing of pubertal development in boys.

Our findings of slightly delaying, if any, influence of alcohol consumption during pregnancy on pubertal development in boys are consistent with results from experimental studies. Exposure to ethanol in utero has been linked to delayed sexual maturation in female rats (24-25, 22-23) and, recently, prenatally exposed male rats also showed a delayed reproductive development and onset of spermatogenesis compared to unexposed (26, 24). Thus, it is plausible that prenatal exposure to alcohol could affect onset of spermatogenesis in humans. Evidence from epidemiological studies on pubertal development in girls is, however, inconsistent. In a small preliminary investigation by Robe et al., a higher percentage of girls with late onset of menarche was observed among girls exposed to ≥ 2 drinks of alcohol per day (12, 11). Similar results were observed by Windham et al. when comparing highly exposed and low exposed girls, however, after adjusting for potential confounding factors the effect on age of menarche diminished (13, 12). Further, Shrestha et al. (14) reported no association between in utero exposure to alcohol and age of menarche. This was corroborated by a recent study by Shrestha et al. (13). The results by Robe et al. may well have been confounded, and further, the discrepant findings could be due to differences in exposure levels. The levels of exposure in the study by Windham et al. and Shrestha et al. may have been too low to detect effects.
In this present study, we used prospectively collected information on alcohol intake from the mothers during pregnancy. This limits the risk of differential recall bias, however, there is a considerable risk of non-differential recall bias driving effect measures toward the null hypothesis. The prevalence of drinking in our study was high, since moderate alcohol intake during pregnancy was socially accepted in Denmark at the time of data collection. Any underreporting is probably non-differential, which most often induces bias towards the null hypothesis. We had the ability to study dose-response effects with a large exposure contrast and, further, we had a rather large proportion (8%) of mothers with an alcohol intake of >4.0 drinks per week. The analyses on binge drinking during pregnancy were, however, limited to few highly exposed sons (2%). When studying effects of prenatal alcohol exposure, one major challenge is to disentangle the toxic effects of alcohol from the underlying and possibly confounding factors associated with alcohol consumption. It is well established that lifetime abstainers differ from drinkers on a number of demographic, lifestyle and socioeconomic characteristics. Although, these differences might not be expected to be as comprehensive in a study population of pregnant women, the abstainers did differ from the drinkers in our data. Although, we controlled for various potential confounders, we cannot exclude residual or unmeasured confounding as an explanation for our findings.

Although the participation rate in the birth cohort was high (87%), there is a risk of selection bias related to attrition in this study, as only 55% of the sons participated in the follow-up in 2005. Another limitation is the relatively large amount of missing data in the cohort, especially concerning the four indicators of pubertal development. We addressed this by using multiple imputation models which provides more valid...
results than the complete case analysis if data are missing at random.\(^{(23)(24)}\) Also, we compared estimates from different imputation models.

The data on indicators of pubertal development were based on self-reports at the age of 18 to 21 years. One may argue that as the presence of acne or regular shaving may not occur for all men and these two events may therefore not be good predictors of pubertal development. On the other hand, age at first nocturnal emission and voice break are considered valid indicators of pubertal development in boys, comparable to age of menarche in girls.\(^{(29;30)(27;28)}\) Previous data from Danish boys have shown that the median age at first ejaculation (spermache) occurred at 13.4 years \(^{(31)}\), and mean age at voice break were 14.0 years \(^{(32)}\), which is slightly earlier than in the present study. This small discrepancy may well be explained by due to the recall time in our study which, varying between 1 and 12 years, we expect some misclassification, yet most likely non-differential, resulting in bias in the null direction.

Despite the high prevalence of alcohol consumption in this study, the number of sons exposed to binge drinking was low, and the results must therefore be interpreted with caution. Even low alcohol intake during pregnancy lower maternal serum testosterone levels \(^{(33)}\) which could interfere with both the intrauterine hormonal environment and the hormonal system in the developing fetus. However, there may well be a threshold for in both dose and timing of exposure and levels, on for the effect of alcohol on male pubertal development. It is plausible that a detrimental effect varies by gestational age, and there may be more than one producing different vulnerable time windows.
However, in this study population, there was not sufficient information power to study very high levels of exposure to alcohol during fetal life. Further, we only measured the average number of drinks per week or number of binge drinking episodes during the entire pregnancy and did not have data on the exact time of alcohol consumption. Therefore, we cannot distinguish between early or late exposure in pregnancy and this may mask potential effects of prenatal exposure to high levels of alcohol in vulnerable time windows.

It is biological plausible that exposure to alcohol in fetal life delay pubertal development has biological plausibility. Differentiation and development of the male genitals begin around gestational week 7–8, and evidence from experimental studies suggests a window for “male programming”, occurring from week 8–14 of gestation, where sufficient androgen levels are essential for normal development. Alcohol readily passes the placental barrier, thereby possibly affecting the endocrine organs of the developing foetal endocrine organs. Alcohol intake during pregnancy has been shown to increase estrogen levels and decrease the testosterone levels in both maternal and umbilical blood, thus, the intrauterine hormonal milieu and consequently the fetal hormone balance may well be affected. Although, the exact mechanisms are not well understood, the onset of puberty in boys is under control of the hypothalamic-pituitary-gonadal axis and alterations of the fetal hormonal milieu and endocrine system may affect pubertal development later in life.

Further research is needed to investigate the association between maternal alcohol consumption and pubertal development. Future studies would benefit from
prospectively-collected data on pubertal development by following the children during the years of puberty with clinical examinations, Tanner staging and assessment of changes in hormonal levels.

In summary, we found little evidence to support the hypothesis that in utero exposure to weekly alcohol consumption is a strong risk factor for altered pubertal development, but we observed a tendency toward a delayed pubertal development among sons exposed to maternal binge drinking during fetal life.
Table I. Maternal characteristics according to average weekly alcohol consumption and binge drinking episodes

<table>
<thead>
<tr>
<th>Maternal weekly alcohol consumption during pregnancy</th>
<th>0 drinks/week</th>
<th>0.5 - 1.5 drinks/week</th>
<th>2.0 - 4.0 drinks/week</th>
<th>&gt; 4.0 drinks/week</th>
<th>Test for trend</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (mean (SD))</td>
<td>26.3 (4.3)</td>
<td>25.7 (4.5)</td>
<td>26.8 (4.2)</td>
<td>29.4 (4.1)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Pre-pregnancy body mass index (BMI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White collar workers, n (%)</td>
<td>291 (44.2)</td>
<td>300 (45.3)</td>
<td>293 (46.0)</td>
<td>288 (44.4)</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Blue collar workers, n (%)</td>
<td>276 (45.8)</td>
<td>275 (54.7)</td>
<td>272 (54.0)</td>
<td>278 (55.6)</td>
<td>&lt;0.001</td>
<td>0.38</td>
</tr>
<tr>
<td>Unemployed or students, n (%)</td>
<td>203 (41.9)</td>
<td>209 (41.9)</td>
<td>206 (41.1)</td>
<td>207 (41.1)</td>
<td>0.92</td>
<td>0.68</td>
</tr>
<tr>
<td>Cohabitation status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parents living together, n (%)</td>
<td>303 (71.3)</td>
<td>302 (71.1)</td>
<td>305 (71.4)</td>
<td>307 (71.4)</td>
<td>&lt;0.001</td>
<td>0.09</td>
</tr>
<tr>
<td>Parents not living together, n (%)</td>
<td>303 (71.3)</td>
<td>302 (71.1)</td>
<td>305 (71.4)</td>
<td>307 (71.4)</td>
<td>&lt;0.001</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Table II. Adjusted correlation between age at indicators of pubertal development

<table>
<thead>
<tr>
<th>Acne</th>
<th>Voice break</th>
<th>Regular shaving</th>
<th>First nocturnal emission</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>1.00</td>
<td>0.34</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Results are adjusted for maternal alcohol consumption during pregnancy, maternal age at delivery, maternal pre-pregnancy BMI, smoking during pregnancy, maternal chronic diseases, municipality of residence at the time of delivery, family's socio-economic status and cohabitation status of the parents at birth.

Number of maternal binge drinking episodes during pregnancy

<table>
<thead>
<tr>
<th>Age, years (mean (SD))</th>
<th>0 times</th>
<th>1 - 4 times</th>
<th>≥ 5 times</th>
<th>Test for trend P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cigarette smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smoker, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal weekly alcohol consumption</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 drink/week</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 - 1.5 drinks/week</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0 - 4.0 drinks/week</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 4.0 drinks/week</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-pregnancy body mass index (BMI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18.5 kg/m², n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.5 - 24.9 kg/m², n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;24.9 kg/m², n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic diseases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Municipality of residence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban areas, no (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural areas, no (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family's socio-economic status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White collar workers, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue collar workers, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unemployed or students, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohabitation status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parents living together, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parents not living together, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P-values for trends and missing values for categorical variables.
Table III. Age difference in years [mean [95% CI]] of indicators of pubertal development among 2,522 boys according to average weekly alcohol intake and binge drinking episodes during pregnancy

<table>
<thead>
<tr>
<th>Distribution</th>
<th>Indicators of male pubertal development</th>
<th>Adjusted mean difference [95% CI]</th>
<th>Adjusted mean difference [95% CI]</th>
<th>Adjusted mean difference [95% CI]</th>
<th>Adjusted mean difference [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average alcohol intake</td>
<td>First nocturnal emission</td>
<td>Acne</td>
<td>Voice break</td>
<td>Regular shaving</td>
<td></td>
</tr>
<tr>
<td>0 drinks/week</td>
<td>16</td>
<td>reference</td>
<td>reference</td>
<td>reference</td>
<td>reference</td>
</tr>
<tr>
<td>0.5 – 1.5 drinks/week</td>
<td>56</td>
<td>0.15 [-0.13, 0.43]</td>
<td>0.06 [-0.12, 0.24]</td>
<td>-0.01 [-0.19, 0.16]</td>
<td>0.02 [-0.15, 0.19]</td>
</tr>
<tr>
<td>2.0 – 4.0 drinks/week</td>
<td>19</td>
<td>0.25 [-0.12, 0.62]</td>
<td>0.09 [-0.13, 0.31]</td>
<td>0.11 [-0.10, 0.32]</td>
<td>0.03 [-0.18, 0.23]</td>
</tr>
<tr>
<td>&gt; 4.0 drinks/week</td>
<td>9</td>
<td>0.30 [-0.19, 0.80]</td>
<td>0.09 [-0.19, 0.37]</td>
<td>0.03 [-0.24, 0.31]</td>
<td>0.08 [-0.16, 0.33]</td>
</tr>
<tr>
<td>Test for trend, p-value</td>
<td>0.97</td>
<td>0.23</td>
<td>0.50</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>Binge drinking episodes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 times</td>
<td>84</td>
<td>reference</td>
<td>reference</td>
<td>reference</td>
<td>reference</td>
</tr>
<tr>
<td>1 – 4 times</td>
<td>14</td>
<td>-0.09 [-0.39, 0.21]</td>
<td>-0.02 [-0.21, 0.17]</td>
<td>-0.07 [-0.24, 0.11]</td>
<td>0.03 [-0.14, 0.20]</td>
</tr>
<tr>
<td>≥ 5 times</td>
<td>2</td>
<td>0.61 [-0.23, 1.45]</td>
<td>0.06 [-0.42, 0.53]</td>
<td>0.41 [-0.05, 0.87]</td>
<td>0.17 [-0.20, 0.54]</td>
</tr>
<tr>
<td>Test for trend, p-value</td>
<td>0.36</td>
<td>0.95</td>
<td>0.45</td>
<td>0.37</td>
<td></td>
</tr>
</tbody>
</table>

* Adjusted for maternal age at delivery, maternal pre-pregnancy BMI, maternal smoking during pregnancy, maternal chronic diseases, municipality of residence at the time of delivery, family’s socio-economic and cohabitation status of the parents at birth.
Reference List


the male offspring: two decades of follow-up. Hum Reprod 2010 Sep;25(9):2340-5.


(17) Ravnborg TL, Jensen TK, Andersson AM, Toppari J, Skakkebaek NE, Jorgensen N. Prenatal and adult exposures to smoking are associated with adverse effects on reproductive hormones, semen quality, final height and body mass index. Hum Reprod 2011 May;26(5):1000-11.


Reference List


(16) Ravnborg TL, Jensen TK, Andersson AM, Toppari J, Skakkebaek NE, Jorgensen N. Prenatal and adult exposures to smoking are associated with adverse effects on reproductive hormones, semen quality, final height and body mass index. Hum Reprod 2011 May;26(5):1000-11.


Table 1. Maternal characteristics according to average weekly alcohol consumption and binge drinking episodes

<table>
<thead>
<tr>
<th>Maternal weekly alcohol consumption during pregnancy</th>
<th>0 drinks/week</th>
<th>0.5 – 1.5 drinks/week</th>
<th>2.0 – 4.0 drinks/week</th>
<th>&gt;4.0 drinks/week</th>
<th>Test for trend</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 464</td>
<td>n = 1,589</td>
<td>n = 520</td>
<td>n = 237</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years (mean (SD))</td>
<td>26.3 (5.2)</td>
<td>27.7 (4.4)</td>
<td>28.3 (4.3)</td>
<td>29.3 (4.4)</td>
<td>&lt;0.001</td>
<td>2.011</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>0.49</td>
<td>5.0 (2.2)</td>
<td>5.0 (2.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker, n (%)/Non-smoker, n (%)</td>
<td>174 (37.5)</td>
<td>524 (33.0)</td>
<td>176 (33.0)</td>
<td>83 (34.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Past smoker, n (%)</td>
<td>48 (10.3)</td>
<td>168 (10.6)</td>
<td>61 (12.1)</td>
<td>26 (11.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-pregnancy body mass index (BMI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td>10.1</td>
</tr>
<tr>
<td>&lt;18.5 kg/m², n (%)</td>
<td>44 (10.4)</td>
<td>113 (7.6)</td>
<td>40 (8.8)</td>
<td>19 (8.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.5 – 24.9 kg/m², n (%)</td>
<td>303 (71.3)</td>
<td>1,198 (80.8)</td>
<td>404 (84.6)</td>
<td>193 (84.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;24.9 kg/m², n (%)</td>
<td>78 (18.3)</td>
<td>171 (11.6)</td>
<td>41 (8.5)</td>
<td>15 (6.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic diseases</td>
<td>0.80</td>
<td>48 (1.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes, n (%)</td>
<td>24 (4.5)</td>
<td>265 (17.0)</td>
<td>96 (18.3)</td>
<td>36 (15.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No, n (%)</td>
<td>381 (85.7)</td>
<td>1,225 (83.0)</td>
<td>416 (81.2)</td>
<td>169 (84.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Municipality of residence</td>
<td>0.0004</td>
<td>2 (0.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban area, n (%)</td>
<td>269 (58.1)</td>
<td>1,018 (64.1)</td>
<td>350 (69.3)</td>
<td>160 (71.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural area, n (%)</td>
<td>194 (41.9)</td>
<td>520 (35.9)</td>
<td>160 (30.8)</td>
<td>68 (28.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family’s socio-economic status$</td>
<td>0.004</td>
<td>4 (0.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White collar worker, n (%)</td>
<td>266 (61.6)</td>
<td>1,233 (77.1)</td>
<td>443 (85.3)</td>
<td>206 (86.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue collar worker, n (%)</td>
<td>135 (29.1)</td>
<td>303 (19.1)</td>
<td>59 (11.4)</td>
<td>22 (9.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unemployed or student, n (%)</td>
<td>43 (9.3)</td>
<td>61 (3.8)</td>
<td>18 (3.5)</td>
<td>9 (3.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohabitation status</td>
<td>0.02</td>
<td>154 (5.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parents living together, n (%)</td>
<td>423 (96.8)</td>
<td>1,466 (97.9)</td>
<td>488 (99.0)</td>
<td>219 (95.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parents not living together, n (%)</td>
<td>14 (3.2)</td>
<td>31 (2.1)</td>
<td>5 (1.0)</td>
<td>10 (4.4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Number of maternal binge drinking episodes during pregnancy

<table>
<thead>
<tr>
<th>Number of maternal binge drinking episodes during pregnancy</th>
<th>0 times</th>
<th>1 – 4 times</th>
<th>≥5 times</th>
<th>Test for trend</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 2,348</td>
<td>n = 403</td>
<td>n = 55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years (mean (SD))</td>
<td>27.8 (4.6)</td>
<td>27.8 (4.4)</td>
<td>28.3 (4.1)</td>
<td>0.55</td>
<td>2.011</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>0.001</td>
<td>5.0 (2.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table II. Adjusted* correlation between age at indicators of pubertal development

<table>
<thead>
<tr>
<th></th>
<th>Acne</th>
<th>Voice break</th>
<th>Regular shaving</th>
<th>First nocturnal emission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acne</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Voice break</td>
<td>0.60</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular shaving</td>
<td>0.30</td>
<td>0.38</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>First nocturnal emission</td>
<td>0.34</td>
<td>0.40</td>
<td>0.30</td>
<td>1.00</td>
</tr>
</tbody>
</table>

*Results are adjusted for maternal alcohol consumption during pregnancy, maternal age at delivery, maternal pre-pregnancy BMI, maternal smoking during pregnancy, maternal chronic diseases, municipality of residence at the time of delivery, family's socio-economic and cohabitation status of the parents at birth.
Table III. Age difference in years (mean [95% CI]) of indicators of pubertal development among 2,522 boys, according to average weekly alcohol intake and binge drinking episodes during pregnancy.

<table>
<thead>
<tr>
<th>Indicators of male pubertal development</th>
<th>First nocturnal emission</th>
<th>Acne</th>
<th>Voice break</th>
<th>Regular shaving</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution</td>
<td>Adjusted mean</td>
<td>Adjusted mean</td>
<td>Adjusted mean</td>
<td>Adjusted mean</td>
</tr>
<tr>
<td>Adjusted mean difference</td>
<td>[95% CI]</td>
<td>[95% CI]</td>
<td>[95% CI]</td>
<td>[95% CI]</td>
</tr>
<tr>
<td>Average alcohol intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 drinks/week</td>
<td>16</td>
<td>reference</td>
<td>reference</td>
<td>reference</td>
</tr>
<tr>
<td>0.5 - 1.5 drinks/week</td>
<td>56</td>
<td>0.15 [0.01, 0.29]</td>
<td>0.06 [0.01, 0.21]</td>
<td>0.01 [0.01, 0.21]</td>
</tr>
<tr>
<td>2.0 - 4.0 drinks/week</td>
<td>19</td>
<td>0.25 [0.01, 0.49]</td>
<td>0.04 [0.01, 0.22]</td>
<td>0.11 [0.01, 0.22]</td>
</tr>
<tr>
<td>&gt; 4.0 drinks/week</td>
<td>9</td>
<td>0.30 [0.18, 0.41]</td>
<td>0.09 [0.18, 0.31]</td>
<td>0.03 [0.18, 0.31]</td>
</tr>
<tr>
<td>Test for trend p-value</td>
<td>0.97</td>
<td>0.73</td>
<td>0.59</td>
<td>0.30</td>
</tr>
<tr>
<td>Binge drinking episodes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 times</td>
<td>84</td>
<td>reference</td>
<td>reference</td>
<td>reference</td>
</tr>
<tr>
<td>1 - 4 times</td>
<td>14</td>
<td>0.09 [0.09, 0.21]</td>
<td>0.02 [0.01, 0.12]</td>
<td>0.07 [0.01, 0.21]</td>
</tr>
<tr>
<td>≥ 5 times</td>
<td>2</td>
<td>0.61 [0.33, 0.89]</td>
<td>0.06 [0.02, 0.63]</td>
<td>0.11 [0.05, 0.27]</td>
</tr>
<tr>
<td>Test for trend p-value</td>
<td>0.36</td>
<td>0.95</td>
<td>0.45</td>
<td>0.22</td>
</tr>
</tbody>
</table>
*Adjusted for maternal age at delivery, maternal pre-pregnancy BMI, maternal smoking during pregnancy, maternal chronic diseases, municipality of residence at the time of delivery, family’s socio-economic and cohabitation status of the parents at birth.
STROBE Statement—checklist of items that should be included in reports of observational studies

<table>
<thead>
<tr>
<th>Item No</th>
<th>Recommendation</th>
</tr>
</thead>
</table>
| Title and abstract | 1 (a) Indicate the study’s design with a commonly used term in the title or the abstract - this is included in the abstract  
(b) Provide in the abstract an informative and balanced summary of what was done and what was found - this is included in the abstract |
| Introduction | 2 Explain the scientific background and rationale for the investigation being reported - this is included in the introduction |
| Objectives | 3 State specific objectives, including any prespecified hypotheses - this is included in the introduction |
| Methods | 4 Present key elements of study design early in the paper - this is included in the methods on page 5 |
| Setting | 5 Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection - this is included in the methods on page 5 |
| Participants | 6 (a) Cohort study—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up - this is included in the methods on page 5  
Case-control study—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls  
Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants  
(b) Cohort study—For matched studies, give matching criteria and number of exposed and unexposed  
Case-control study—For matched studies, give matching criteria and the number of controls per case |
| Variables | 7 Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable - this is included in the methods on page 5 |
| Data sources/ measurement | 8* For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group - this is included in the methods on page 5 |
| Bias | 9 Describe any efforts to address potential sources of bias - this is included in the methods on page 7 |
| Study size | 10 Explain how the study size was arrived at - this is included in the methods on page 5 |
| Quantitative variables | 11 Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why |
| Statistical methods | 12 (a) Describe all statistical methods, including those used to control for confounding - this is included in the methods on page 5  
(b) Describe any methods used to examine subgroups and interactions - this is included in the methods on page 5 |
(c) Explain how missing data were addressed
- this is included and explained in detail in the methods on page 7-8

(d) Cohort study—If applicable, explain how loss to follow-up was addressed

Case-control study—If applicable, explain how matching of cases and controls was addressed

Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy

(g) Describe any sensitivity analyses
- this is included in the methods on page 7-9

Continued on next page
Results

Participants 13*
(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
- this is explained on page 9
(b) Give reasons for non-participation at each stage
- this is explained on page 9
(c) Consider use of a flow diagram
- This was considered but not included in the manuscript. Participation and exclusions are explained in detail.

Descriptive data 14*
(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
- this is described on page 9-10 and also presented in table 1
(b) Indicate number of participants with missing data for each variable of interest
- this is described on page 7, and also presented in table 1
(c) Cohort study—Summarise follow-up time (eg, average and total amount)

Outcome data 15*
Cohort study—Report numbers of outcome events or summary measures over time
- this is described in the Methods.
Case-control study—Report numbers in each exposure category, or summary measures of exposure
Cross-sectional study—Report numbers of outcome events or summary measures

Main results 16
(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
- this is presented in the results
(b) Report category boundaries when continuous variables were categorized
- this is described and also presented in table 3.
(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
- Not relevant in this present result section

Other analyses 17
Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
This is presented in the results section

Discussion

Key results 18
Summarise key results with reference to study objectives
- This is described.

Limitations 19
Discuss limitations of the study, taking into account sources of potential bias or imprecision.
Discuss both direction and magnitude of any potential bias
- This is described in detail in the discussion.

Interpretation 20
Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
- This is described.

Generalisability 21
Discuss the generalisability (external validity) of the study results
- This is described.

Other information

Funding 22
Give the source of funding and the role of the funders for the present study and, if applicable,
for the original study on which the present article is based
- not relevant in this present study

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

In utero exposure to alcohol and puberty in boys: a pregnancy cohort study

Linn Berger Håkonsen, Mette Louise Brath-Lund, Marie Louise Hounsgaard, Jørn Olsen, Andreas Ernst, Ane Marie Thulstrup, Bodil Hammer Bech and Cecilia Høst Ramla-Hansen

*BMJ Open* 2014 4:
doi: 10.1136/bmjopen-2013-004467

Updated information and services can be found at: http://bmjopen.bmj.com/content/4/6/e004467

These include:

**References**
This article cites 43 articles, 2 of which you can access for free at: http://bmjopen.bmj.com/content/4/6/e004467#BIBL

**Open Access**
This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 3.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/3.0/

**Email alerting service**
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Topic Collections**
Articles on similar topics can be found in the following collections

- Epidemiology (2176)
- Obgyn (353)
- Paediatrics (651)
- Sexual health (155)

**Notes**

To request permissions go to: http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to: http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to: http://group.bmj.com/subscribe/