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Alcohol binge drinking during pregnancy and cryptorchidism

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BACKGROUND: Recent studies have suggested gestational weeks 8–14 as a time window of particular importance to the intrauterine development of the male genitalia, and prenatal exposure to alcohol is under suspicion as a risk factor for cryptorchidism. We examined if prenatal exposure to alcohol, and especially binge drinking, during the suggested programming window is associated with an increased risk of cryptorchidism.

METHODS: The authors used data on 41 268 live born singleton boys of mothers who were enrolled into the Danish National Birth Cohort in 1996–2002. During early childhood, 1598 cases of cryptorchidism were identified and 398 of these were orchiopexy verified. Maternal alcohol consumption including number and timing of binge drinking episodes was assessed in two computer-assisted telephone interviews around gestational weeks 17 and 32. Adjusted hazard ratios (HRs) of cryptorchidism were estimated by Cox regression.

RESULTS: Average weekly alcohol consumption as well as frequency of binge drinking at any time during pregnancy was not associated with risk of cryptorchidism. Binge drinking in gestational weeks 7–15 was associated with a slightly increased risk of cryptorchidism with adjusted HRs between 1.03 and 1.66.

CONCLUSION: Prenatal exposure to alcohol—measured as average intake as well as frequency and timing of binge drinking—was not associated with cryptorchidism. Our findings, however, do not rule out that binge drinking during the suggested male programming window may increase the risk of cryptorchidism.

Key words: alcohol drinking / congenital abnormalities / cryptorchidism / prenatal exposure / delayed effects

Although cryptorchidism (undescended testis) is the most common abnormality in newborn boys worldwide, the aetiology remains largely unknown (Virtanen and Toppari, 2008). In Denmark, prevalences of 4–9% among newborn boys and of 1–2% in 1-year-old boys have been reported (Boisen *et al*., 2004). Adverse maternal lifestyle and environmental exposures during pregnancy are suspected to interfere with the normal testicular descent and increase the risk of cryptorchidism (Skakkebaek *et al*., 2001; Virtanen and Toppari, 2008). During pregnancy, there are several critical stages in the development of the testes: during gestational weeks 7–8, the genital ridge and testes begin to form; around weeks 10–14, the transabdominal migration begins and lasts until weeks 20–23 and during weeks 26–35 of gestation, the testis moves from the groin through the inguinal canal to the scrotum, i.e. the transinguinal descent (Klonisch *et al*., 2004).

Studies on rats have suggested the existence of a time window where normal androgen action is crucial and programmes future male genitalia development including testicular descent (Welsh *et al*., 2008). In humans, the late transinguinal testicular descent is androgen dependent and is the descending process most commonly affected in cryptorchid boys (Amann and Veeramachaneni, 2007). It is hypothesized that androgen action around weeks 8–14 of gestation programmes the transinguinal descent in weeks 26–35, and thus the susceptible time period is much earlier than previously assumed (Welsh *et al*., 2008). The existence of such a programming window in humans, however, remains to be shown.

The association between alcohol consumption during pregnancy and risk of cryptorchidism has been addressed in three recent studies (Damgaard *et al*., 2007; Jensen *et al*., 2007a; Mongraw-Chaffin *et al*., 2008). One of the studies found average weekly alcohol intake to be highly associated with transient cryptorchidism and also

indicated that binge drinking may be associated with an increased risk (Damgaard et al., 2007). In a previous study, we found some indication for an association between binge drinking and cryptorchidism (Jensen et al., 2007a). However, none of the previous studies had information on the timing of the binge episodes. Binge drinking in early pregnancy is still frequent in many countries (Olsen et al., 1989; Strandberg-Larsen et al., 2008), and in Denmark 25–50% of pregnancies experience at least one episode (Kesmodel et al., 2003; Strandberg-Larsen et al., 2008). The duration of binge episodes is most often limited to a few hours and the short half-life of ethanol makes the fetal exposure very limited in time.

In this study, we examined if prenatal exposure to binge drinking, and especially exposure during the suggested programming window, is associated with an increased risk of cryptorchidism.

Methods

Study population

The study is based on the Danish National Birth Cohort (DNBC), which is a population-based cohort of children born by women who were pregnant during 1996–2002 and intended to carry their pregnancy to term (Olsen et al., 2001). Pregnant women were invited to participate by their general practitioner at their first antenatal visit. Around 50% of all practitioners in Denmark participated and around 60% of the invited women consented to be interviewed by telephone about exposures during pregnancy and developmental information on their child. The interviews occurred around gestational weeks 17 (quartiles: 14–20), 32 (quartiles: 30–34) and at 6 (quartiles: 5–6) and 18 (quartiles: 18–19) months of age. English translations of the interviews are available at www.bsmb.dk. All Regional Science Ethics Committees in Denmark approved the DNBC and before we initiated this study we obtained approval from the Danish Data Protection Board.

Of all enrolled women ($n = 100\,418$), we limited our analysis to women who gave birth to a live born singleton boy and completed the first interview ($n = 44\,481$). The mother–boy pairs with incomplete information for the following factors were excluded: binge drinking ($n = 163$), gestational age ($n = 441$), a discrepancy between self-report and the information recorded in the National Hospital Discharge Registry on gestational age of >1 week ($n = 2228$), boy not identifiable in the Danish Civil Registration System ($n = 3$), household occupational status ($n = 99$), parity ($n = 27$), time to pregnancy and infertility treatment ($n = 97$), smoking habits during pregnancy ($n = 47$), diabetes ($n = 66$) and average alcohol consumption ($n = 42$). The remaining 41 268 mother–boy pairs were eligible for the analyses.

Exposure assessment

In the first interview, 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, each of which corresponds to approximately 12 g of alcohol. The weekly intake of these alcoholic beverages was added up to a total. Women, who reported to consume alcohol, but less than one drink per week, were assigned a numeric value of 1/2 drink per week. Average alcohol consumption was categorized into non-drinkers, 1/2 –$1_{1/2}$, 2–$3_{1/2}$ and 4 or more drinks per week.

In the first and second interviews, women were also asked how many times and when they had consumed five or more alcoholic drinks on a single occasion (binge drinking) since the onset of pregnancy, defined as the first day of the last menstrual period. We used the information from the first interview to quantify exposure to binge drinking from onset of pregnancy to the time of the first interview. If the women provided information on binge drinking in the second interview, we used this to quantify exposure in the period between the two interviews. We categorized the number of binge episodes as 0, 1, 2 and 3+. In accordance with the assumed critical stages of the testes development and considering that binge drinking is more common in the early phase of pregnancy, we categorized, before doing any analyses, the timing of binge drinking as 0 versus ≥1 episodes in the following time windows: the preconceptional period (weeks 1–2), the period of fertilization and implantation (weeks 3–4), the early embryonic period (weeks 5–6), the late embryonic period when the testes begins to form (weeks 7–9), the transabdominal migration of the testes (weeks 10–15) and the mid-fetal phase (weeks 16–25), respectively.

Of the binge-drinking mothers, 8% had not reported timing for at least one of their binge episodes. Instead of excluding these mother–boy pairs, we estimated when the episodes had occurred by means of multiple imputation. Based on the data with complete information, we created a probability distribution of timing of binge drinking. This probability distribution was made dependent on whether the mothers recognized their pregnancy before week 4 of pregnancy, in weeks 4–7, in weeks 8–12 or after week 12, or did not recall the week of recognition. We used information on time of interview or any other binge drinking episodes to define for every undated binge episode a possible time period in which the episode could have occurred. For example, if a woman reported two binge episodes and reported timing for the first episode, but did not recall the timing for the second, the possible time period for the second binge episode was defined to be between the first binge episode and the time of interview. The imputation procedure for missing information on timing was repeated five times and the reported estimates are the average estimates and standard errors.

Covariates

We decided *a priori* to adjust for the following potential cofounders: maternal age in years when giving birth (<25, 25–29, 30–34, 35–39 and 40+) (McGlynn et al., 2006), household occupational status (higher grade professionals, middle-grade professionals, skilled workers, unskilled workers, students and unemployed for more than 1 year), parity before the index boy (0, 1 and 2+) (Jones et al., 1998; Akre et al., 1999), time to pregnancy and infertility treatment (unplanned, 0–5, 6–12, 13+ months without fertility treatment and received treatment) (McGlynn et al., 2006; Jensen et al., 2007b), smoking during pregnancy (none, stopped during pregnancy, 1–10 and 11+ cigarettes per day) (Jensen et al., 2007b) and self-reported information on diabetes mellitus (yes versus no) (Virtanen et al., 2006).

Case ascertainment

Information on cryptorchidism, other congenital malformations and surgical operations on boys in the cohort was obtained from the Danish Hospital Discharge Register, which contains information on all in-patient as well as outpatient diagnoses and performed surgical operations. In this study, we use three end-points with increasing diagnostic specificity: first, boys whose mothers in the third and/or fourth interview stated that at least one of the testes was not in the scrotum and/or boys having a cryptorchidism diagnosis (ICD10-codes: Q53, Q531, Q531A, Q532, Q532A and Q539); second, boys recorded with a cryptorchidism diagnosis and third, boys with a diagnosis and who also underwent orchiopexy (codes KKFH00, KKFH01 and KKFH10 in the Nordic Classification of Surgical Procedures). Orchiopexy indicates that the cryptorchidism condition is persisting and thus requires surgery, whereas maternal report and/or diagnosis of cryptorchidism will also cover transient cases of cryptorchidism where the testis occasionally descend spontaneously and therefore do not necessarily require any further clinical actions.

Statistical methods

Cryptorchidism is considered a congenital malformation but not all cases are identified at the time of birth. Some will be diagnosed and treated during childhood. The boys in the DNBC had neither reached an age where all hospitalized cryptorchidism cases are identified, nor did they have the same age at end of follow up—the youngest boy was $3_{1/2}$ years and the oldest 9 years. To account for this, we estimated crude and adjusted hazard ratios (HRs) by means of Cox-regression models with boy's age as the time variable. The boys entered the analyses at birth and were followed until their age at the first diagnosis (if the case status was based on maternal report, their first birthday), death, emigration out of Denmark or end of follow-up (31 December 2006), whichever came first. We used the robust sandwich estimate for the covariance matrix to account for the dependency between 1297 boys who had a sibling in the study. The analyses were performed by use of SAS software, version 9.1.

We analysed average intake and binge drinking in separate models as well as in a combined model and made separate analyses for each outcome. The number of weeks with information on binge drinking was included in the strata statement in the Cox-regression models, including the number of binge episodes, in order to account for the fact that women had information on binge drinking from different spans of pregnancy, because the gestational week of interviewing varied and we only used information from the second interview for some mothers ($n = 37\,388$). We analysed timing of binge drinking by including the different time periods into one model, and we examined if inclusion of overall number of binge episodes and average intake, respectively, changed the HRs.

We performed sensitivity analyses to check how resistant our results were to changes in the analytical approach. First, birthweight, gestational age and other congenital malformations are important risk factors for cryptorchidism (Jones *et al*., 1998; Akre *et al*., 1999; Weidner *et al*., 1999), and we have assumed these to be intermediary to the association between alcohol and cryptorchidism instead of confounders. However, some may argue that these factors reflect the presence of confounders at baseline that causes these outcomes and we, therefore, evaluated whether inclusion of these variables in the multiple regressions changed the estimates. Second, we restricted the populations to women with an average intake of a maximum of 7 drinks per week ($n = 41\,209$) and subsequently to women with a maximum of 5 binge drinking episodes ($n = 41\,046$) to exclude women who may be alcoholics. Third, as another attempt to avoid the dependency between boys of the same mother, we restricted the analyses to the first-born boy ($n = 39\,971$). Fourth, not all of the mothers participated in the post-natal interviews, and we restricted the population to mothers who had either answered the third or the fourth interview ($n = 37\,292$). Fifth, in order to check if our results were sensitive to the imputation procedure, we restricted the time-specific analyses to women with complete information on timing ($n = 40\,392$). Finally, pregnancy is a selective event that often occurs several times and selective forces operate before and during pregnancy. To avoid behaviour modification bias that arises from a change in women's drinking behaviour related to difficulties in obtaining a pregnancy (Weinberg *et al*., 1994; Joffe *et al*., 2005), and confounding from previous adverse pregnancy outcomes (Olsen, 1994), we restricted the population to first-time pregnant mothers who had conceived within 6 months of trying ($n = 9960$).

Results

Altogether, 1598 boys (3.9%) were either recorded in the Danish Hospital Discharge Register with a cryptorchidism diagnosis or the mother reported at age 6 or 18 months that at least one of the testes were not in the scrotum. Of these, 355 boys were diagnosed with cryptorchidism but had no maternal report. Of the 810 boys diagnosed with cryptorchidism, 398 (49%) had an orchiopexy. Of the mothers, 26% had experienced at least one binge drinking episode, 45% reported weekly intake of at least half a drink, but only 2% had an average weekly intake of four drinks or more. The binge drinking mothers were more often unintended pregnant, while mothers who had been treated for infertility more often completely abstained from alcohol. Further population characteristics are shown in Table I.

In the entire cohort, average weekly alcohol intake was associated with decreased hazards of cryptorchidism in a dose-dependent manner and this was seen for all three end-points. However, no clear inverse association was seen when restricting the data to first-time pregnancies that were conceived within 6 months of trying (Table II).

In the entire cohort, exposure to one or two binge episodes during pregnancy was associated with HRs below one when compared with boys not exposed to binge drinking. The decreased HRs were no longer present when the analyses were restricted to first-time pregnancies that were conceived within 6 months of trying, except for boys who underwent orchiopexy (Table III). Amongst the pregnancies achieved within 6 months, boys exposed to three or more binge episodes had HRs of 1.51 (95% confidence interval (CI): 0.95, 2.41) for diagnosis of cryptorchidism and 1.82 (95% CI: 0.97, 3.37) for orchiopexy compared with unexposed boys (Table III).

Data from the entire cohort did not support our prior hypothesis of special vulnerability to binge drinking in weeks 7–15, which is the suggested programming window for the end-point. The HRs of binge drinking in weeks 3–4 and 16–25, respectively, were below one when compared with boys unexposed in the same time span (data not shown). In the analyses restricted to first-born boys conceived within 6 months of trying, we saw no indication of reduced hazards of cryptorchidism, and binge drinking in weeks 7–9 and 10–15 was associated with HRs of cryptorchidism above one (HR between 1.03 and 1.66 depending on the type of cases considered), but the precision of these estimates was low (Table IV).

Adjustment for potential confounders, birthweight, gestational age and congenital malformations had no or very limited influence on the estimates. None of the sensitivity analyses, except restricting the cohort to first-time pregnant mothers who had conceived within 6 months of trying, influenced our conclusions.

Discussion

We found no overall support for prenatal alcohol exposure as a risk factor for cryptorchidism, but our findings bring limited support to the hypothesis that binge drinking during different stages of the testis development may be harmful. Our results based on the entire cohort point towards a protective effect of alcohol, but we consider this to be attributable to self-selection into the cohort, behaviour modification and change of exposures related to knowledge from earlier pregnancies. Self-selection bias could be present because participation may easily be associated with both alcohol intake and factors directly associated with cryptorchidism, such as time to pregnancy or prior congenital malformations. Likewise, because of the

Table I Maternal characteristics of the study population and characteristics according to alcohol consumption during pregnancy, the Danish National Birth Cohort 1996–2002

	Total population *n* (%)	Median age (5; 95 percentile)	Primi-parous (%)	Unplanned pregnancy (%)	Infertility treated (%)	Non-smokers (%)	Diabetes mellitus (%)	Middle or higher grade professionals (%)
Total population	41 268 (100)	30 (23; 37)	46.9	11.3	5.8	74.5	0.4	55.1
Average alcohol intake (drinks per week)								
0	22 757 (55.1)	29 (23; 37)	49.6	12	6.3	73.8	0.5	50.8
$\frac{1}{2}$–$1\frac{1}{2}$	13 750 (33.3)	30 (24; 37)	45.2	10	5.1	76.9	0.4	59.4
2–$3\frac{1}{2}$	3885 (9.4)	31 (25; 38)	40.2	11	5.1	72.3	0.2	63.5
4+	876 (2.1)	33 (25; 40)	32.6	14.6	4.7	62.6	0.2	61.3
Number of binge episodes								
0	30 567 (74.1)	30 (23; 37)	43.3	10	6.8	77.5	0.4	55.2
1	6669 (16.2)	29 (23; 37)	54	12.4	3.1	70.3	0.4	55.5
2	2401 (5.8)	29 (23; 37)	62.3	17.1	2.9	62.4	0.4	53.7
3+	1631 (3.9)	30 (23; 38)	62.1	21	1.9	53.6	0.4	53.7
Timing of binge episodes[a]								
Weeks 1–2	3431 (8.3)	29 (23; 37)	62.8	15.2	3	64.9	0.2	56.8
Weeks 3–4	6018 (14.6)	29 (23; 37)	62	14.4	2.5	66.8	0.2	56.8
Weeks 5–6	2096 (5.1)	29 (22; 37)	59	18	2.4	62.1	0.4	53.7
Weeks 7–9	839 (2.0)	29 (22; 38)	53.8	25.3	2.7	54.6	0.8	44
Weeks 10–15	706 (1.8)	30 (22; 38)	40.7	21.1	3.7	52.8	1	42.8
Weeks 16–25	780 (2.1)	30 (22; 39)	38.3	18.1	3.2	52.7	0.8	40.8

[a]Percentages for the total population does not add to one hundred because not all were binge drinkers and adding up the number of binge drinkers during each time window will exceed the total number of binge drinkers, because some women reported binge drinking in more than one of the time windows.

Table II **HR and 95% CI for three definitions of cryptorchidism according to average weekly alcohol intake during pregnancy in the Danish National Birth Cohort 1996–2002.**

Average alcohol intake (drinks per week)	Entire cohort (n = 41 268)				Restricted cohort (n = 9960)[a]			
	Case type/number of cases (%)	Crude HR	Adjusted HR[b]	95% CI	Case type/number of cases (%)	Crude HR	Adjusted HR[b]	95% CI
	Maternal report/diagnosis of cryptorchidism[c]	HR	HR	95% CI	Maternal report/diagnosis of cryptorchidism[c]	HR	HR	95% CI
0	943 (4.1)	1	1	Reference	243 (4.2)	1	1	Reference
$\frac{1}{2}$–1$\frac{1}{2}$	500 (3.6)	0.87	0.89	0.79, 0.99	121 (3.7)	0.88	0.91	0.73, 1.14
2–3$\frac{1}{2}$	132 (3.4)	0.82	0.82	0.69, 0.99	38 (4.9)	1.18	1.21	0.86, 1.71
4+	23 (2.6)	0.63	0.63	0.41, 0.94	3 (2.4); 3 (2.6)	0.57	0.54	0.17, 1.67
	Diagnosis of cryptorchidism[d]	HR	HR	95% CI	Diagnosis of cryptorchidism[d]	HR	HR	95% CI
0	468 (2.1)	1	1	Reference	127 (2.2)	1	1	Reference
$\frac{1}{2}$-1$\frac{1}{2}$	261 (1.9)	0.92	0.93	0.80, 1.08	70 (2.1)	0.98	1.01	0.75, 1.36
2–3$\frac{1}{2}$	70 (1.8)	0.87	0.87	0.68, 1.12	23 (3.0)	1.37	1.39	0.88, 2.19
4+	11 (1.3)	0.61	0.58	0.32, 1.06	1 (0.8)	0.36	0.32	0.05, 2.29
	Orchiopexy[e]	HR	HR	95% CI	Orchiopexy[e]	HR	HR	95% CI
0	234 (1.0)	1	1	Reference	64 (1.1)	1	1	Reference
$\frac{1}{2}$–1$\frac{1}{2}$	126 (0.9)	0.89	0.91	0.73, 1.13	34 (1.0)	0.94	0.99	0.65, 1.49
2–3$\frac{1}{2}$	31 (0.8)	0.77	0.79	0.54, 1.15	8 (1.0)	0.94	0.98	0.47, 2.08
4+	7 (0.8)	0.77	0.77	0.36, 1.63	0 (0.0)	–	–	–

[a]9960 first-born boys of mothers who conceived within 6 months of trying.
[b]Adjusted for maternal age, parity, time to pregnancy and infertility treatment, self-reported diabetes mellitus, smoking habits during pregnancy and occupational status in the household.
[c]Maternal report at age 6 or 18 moths and/or a cryptorchidism diagnosis in the National Hospital Discharge Registry (ICD-10 codes: Q53, Q531, Q531A, Q532, Q532A and Q539).
[d]A cryptorchidism diagnosis in the National Hospital Discharge Registry.
[e]A cryptorchidism diagnosis and an orchiopexy procedure in the National Hospital Discharge Registry (Nordic Classification of Surgical Procedures codes: KKFH00, KKFH01 and KKFH10).

Table III HR and 95% CI for three definitions of cryptorchidism according to number of binge drinking episodes during pregnancy in the Danish National Birth Cohort 1996–2002

Number of binge episodes	Entire cohort (n = 41 268)				Restricted cohort (n = 9960)[a]			
	Case type/number of cases (%)	Crude HR	Adjusted HR[b]	95% CI	Case type/number of cases (%)	Crude HR	Adjusted HR[b]	95% CI
	Maternal report/diagnosis of cryptorchidism[c]	HR	HR	95% CI	Maternal report/diagnosis of cryptorchidism[c]	HR	HR	95% CI
0	1209 (4.0)	1	1	Reference	260 (4.0)	1	1	Reference
1	239 (3.6)	0.9	0.88	0.77, 1.02	80 (4.0)	1.00	1.00	0.78, 1.29
2	85 (3.5)	0.89	0.85	0.70, 1.06	36 (4.2)	1.05	1.05	0.74, 1.49
3+	65 (4.1)	0.99	0.94	0.73, 1.21	29 (5.1)	1.24	1.22	0.83, 1.79
	Diagnosis of cryptorchidism[d]	HR	HR	95% CI	Diagnosis of cryptorchidism[d]	HR	HR	95% CI
0	621 (2.0)	1	1	Reference	143 (2.2)	1	1	Reference
1	118 (1.8)	0.86	0.83	0.68, 1.01	41 (2.0)	0.93	0.93	0.66, 1.32
2	39 (1.6)	0.79	0.73	0.53, 1.01	17 (2.0)	0.89	0.9	0.54, 1.49
3+	32 (2.0)	0.94	0.85	0.60, 1.21	20 (3.5)	1.56	1.51	0.95, 2.41
	Orchiopexy[e]	HR	HR	95% CI	Orchiopexy[e]	HR	HR	95% CI
0	316 (1.0)	1	1	Reference	72 (1.1)	1	1	Reference
1	52 (0.8)	0.74	0.7	0.52, 0.94	16 (0.8)	0.72	0.72	0.42, 1.24
2	14 (0.6)	0.56	0.5	0.29, 0.85	6 (0.7)	0.63	0.62	0.27, 1.45
3+	16 (1.0)	0.93	0.81	0.49, 1.34	12 (2.1)	1.84	1.82	0.97, 3.37

[a]9960 first-born boys of mothers who conceived within 6 months of trying.
[b]Adjusted for maternal age, parity, time to pregnancy and infertility treatment, self-reported diabetes mellitus, smoking habits during pregnancy and occupational status in the household.
[c]Maternal report at age 6 or 18 moths and/or a cryptorchidism diagnosis in the National Hospital Discharge Registry (ICD-10 codes: Q53, Q531, Q531A, Q532, Q532A and Q539).
[d]A cryptorchidism diagnosis in the National Hospital Discharge Registry.
[e]A cryptorchidism diagnosis and an orchiopexy procedure in the National Hospital Discharge Registry (Nordic Classification of Surgical Procedures codes: KKFH00, KKFH01 and KKFH10).

Table IV HR and 95% CI for cryptorchidism according to prenatal exposure to binge drinking in specific time periods of pregnancy among 9960 first-born boys of mothers, who conceived within 6 months of trying, enrolled in the Danish National Birth Cohort 1996–2002

Timing	Maternal report/diagnosis of cryptorchidism[a]					Diagnosis of cryptorchidism[b]					Orchiopexy[c]				
	Cases (%)	Crude		Adjusted[d]		Cases (%)	Crude		Adjusted[d]		Cases (%)	Crude		Adjusted[d]	
		HR	95% CI	HR	95% CI		HR	95% CI	HR	95% CI		HR	95% CI	HR	95% CI
1–2 weeks															
0	352 (4.0)	1	Reference			193 (2.2)	1	Reference			98 (1.1)	1	Reference		
1+	53 (4.3)	1.04	0.77, 1.40	1.04	0.77, 1.41	28 (2.3)	0.99	0.66, 1.50	1.00	0.66, 1.52	8 (0.6)	0.53	0.25, 1.11	0.54	0.26, 1.14
3–4 weeks															
0	318 (4.1)	1	Reference			170 (2.2)	1	Reference			82 (1.0)	1	Reference		
1+	87 (4.1)	0.97	0.76, 1.24	0.98	0.76, 1.25	51 (2.4)	1.06	0.76, 1.48	1.06	0.76, 1.47	24 (1.1)	1.12	0.70, 1.80	1.12	0.70, 1.79
5–6 weeks															
0	375 (4.0)	1	Reference			205 (2.2)	1	Reference			97 (1.0)	1	Reference		
1+	30 (4.4)	1.02	0.70, 1.48	1.02	0.70, 1.48	16 (2.3)	1.01	0.61, 1.68	1.00	0.60, 1.68	9 (1.3)	1.2	0.60, 2.39	1.19	0.60, 2.39
7–9 weeks															
0	390 (4.0)	1	Reference			215 (2.2)	1	Reference			102 (1.1)	1	Reference		
1+	15 (6.2)	1.45	0.87, 2.41	1.38	0.83, 2.30	6 (2.4)	1.1	0.49, 2.45	1.03	0.47, 2.27	4 (1.6)	1.42	0.54, 3.75	1.35	0.52, 3.52
10–15 weeks															
0	383 (4.0)	1	Reference			209 (2.2)	1	Reference			102 (1.1)	1	Reference		
1+	10 (7.2)	1.76	0.91, 3.42	1.66	0.84, 3.31	4 (2.9)	1.38	0.45, 4.29	1.3	0.44, 3.79	2 (1.7)	1.51	0.35, 6.58	1.47	0.35, 6.12
No information[e]	12 (4.6)					8 (3.1)					2 (0.8)				
16–25 weeks															
0	363 (5.0)	1	Reference			195 (2.7)	1	Reference			99 (1.4)	1	Reference		
1+	4 (2.8)	0.55	0.18, 1.66	0.52	0.17, 1.57	1 (1.1)	0.41	0.06, 2.96	0.37	0.05, 2.71	1 (1.1)	0.78	0.10, 6.01	0.73	0.09, 5.67
No information[e]	38 (4.2)					25 (2.8)					6 (0.7)				

[a]Maternal report at age 6 or 18 moths and/or a cryptorchidism diagnosis in the National Discharge Registry (ICD-10 codes: Q53, Q531, Q531A, Q532, Q532A and Q539).
[b]A cryptorchidism diagnosis in the National Hospital Discharge Registry.
[c]An orchiopexy procedure in the National Hospital Discharge Registry (Nordic Classification of Surgical Procedures codes: KKFH00, KKFH01 and KKFH10).
[d]Adjusted for maternal age, parity, time to pregnancy and infertility treatment, self-reported diabetes mellitus, smoking habits during pregnancy and occupational status in the household.
[e]No information was available because the latest interview was answered before the last week in the time window.

widespread consensus that alcohol consumption during pregnancy brings harm to the fetus, it is very likely that women who have experienced difficulties in obtaining their pregnancy or have a history of previous negative pregnancy outcomes will abstain from alcohol more often than women without these experiences. To overcome these potential sources of bias, we restricted all analyses to a cohort of first-time pregnancies that were conceived within 6 months of trying. In this subcohort, the precision of estimates was relatively low, but we observed no 'protective effect' of alcohol, and binge drinking three or more times was associated with increased HRs of cryptorchidism. The finding of increased HRs in weeks 7–9 and 10–15 is intriguing, in spite of the low precision, due to the strong *a priori* hypothesis of a male programming window in these weeks. Some may argue that the restriction to a subcohort of women without any past pregnancy experience to make use of when they decide to avoid alcohol or drink in the index pregnancy is a too conservative way to account for previous reproductive experience. However, adjustments for parity and time to pregnancy/infertility treatment did not influence the results, but the restriction did, which points toward a residual confounding that cannot be controlled without restriction. Therefore, we consider the estimates based on the first-time pregnancies to be the most valid for the association between alcohol and cryptorchidism.

Whether pregnant women are able to provide accurate information on timing in pregnancy may seem questionable because the exact time of fertilization is unknown and it is difficult to recall week-by-week behaviours. However, it has been argued that Danish pregnant women are very concerned about binge drinking and therefore know exactly when in pregnancy they drank high amounts of alcohol on one occasion (Kesmodel and Schioler Kesmodel, 2002; Kesmodel and Frydenberg, 2004). Recall of binge drinking is affected by the length of the recall period, and a short recall period results in a higher proportion of binge drinkers (Strandberg-Larsen *et al.*, 2006). In the DNBC, we have relatively short recall periods, because data are collected around gestational weeks 14–20, and again at around week 32. Interviews have been shown to be a reliable method to obtain information on alcohol consumption in pregnant Danish women (Kesmodel and Olsen, 2001), and the questions on binge drinking have proven to yield valid and reliable information, and are the only validated method for the collection of data on timing of binge drinking (Kesmodel and Frydenberg, 2004; Kesmodel, 2001). The validity of maternal report of cryptorchidism, registered diagnoses and orchiopexy procedures has to our knowledge never been established. We presented three types of end-points with increasing diagnostic specificity and decreasing sensitivity, and the diagnostic specificity of cases both diagnosed with and operated for cryptorchidism is expected to be almost 100%.

Recently, three studies with prospective exposure measurements have investigated the association between prenatal alcohol exposure and cryptorchidism (Damgaard *et al.*, 2007; Jensen *et al.*, 2007a; Mongraw-Chaffin *et al.*, 2008). Damgaard *et al.* (2007) reported a markedly increased risk of transient cryptorchidism following a moderate weekly alcohol intake of five or more drinks, but women with a much higher intake may also carry the risk. They also indicated that binge drinking (one or more episodes) may be associated with a modestly increased risk of cryptorchidism. On the contrary, Jensen *et al.* (2007a) reported no adverse effect (a protective effect, if anything) of average weekly alcohol consumption on persistent cryptorchidism, but also indicated a modestly increased risk of cryptorchidism among binge drinkers. Mongraw-Chaffin *et al.* (2008) supported the finding of no association between average intake and persistent cryptorchidism, but they did not investigate binge drinking. Our study corroborates the findings of Jensen *et al.* and Mongraw-Chaffin *et al.* on persistent cryptorchidism, and the contrasting findings of Damgaard *et al.* may be explained by differences in phenotype, since most of their cases were transient with spontaneous descent within 3 months. Our study is the first one able to take the timing of binge episodes into consideration, and it has more power than the previously conducted studies, making it possible to restrict the study to first-time pregnant mothers with a time to pregnancy of less than 6 months. Interestingly, in the restricted material, we saw elevated cryptorchidism risk only with exposure in the time window that we *a priori* considered most important. The modest risk elevations presented by Damgaard *et al.* and Jensen *et al.*, looking at exposure during the entire pregnancy, is to be expected if binge drinking is only hazardous in weeks 7–15 because exposure misclassification tends to attenuate associations (Kesmodel, 2001; Jensen *et al.*, 2007a).

A recent study suggested that sufficient androgen action in the male programming window (weeks 8–14) is crucial for future masculinization, including testicular descent (Welsh *et al.*, 2008). Studies of pregnant women have shown that alcohol intake during pregnancy increases the estrogen level and decreases the testosterone level (Petridou *et al.*, 1992; Wuu *et al.*, 2002; Stevens *et al.*, 2005; Nagata *et al.*, 2007). Therefore, reduced androgen action is a possible mechanistic explanation of our findings.

In conclusion, we found no overall support for prenatal alcohol exposure as a risk factor for cryptorchidism. Our findings bring some support to the hypothesis that binge drinking during gestational weeks 8–14 may increase the risk of cryptorchidism, but the hypothesis needs to be evaluated in an independent data source.

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