A systematic review and meta-analysis of perinatal variables in relation to the risk of testicular cancer—experiences of the mother

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Background We undertook a systematic review and meta-analysis of perinatal variables in relation to testicular cancer risk, with a specific focus upon characteristics of the mother.

Methods EMBASE, PubMed, Scopus and Web of Science databases were searched using sensitive search strategies. Meta-analysis was undertaken using STATA 10.

Results A total of 5865 references were retrieved, of which 67 met the inclusion criteria and contributed data to at least one perinatal analysis. Random effects meta-analysis found maternal bleeding during pregnancy [odds ratio (OR) 1.33, 95% confidence interval (CI) 1.02–1.73], birth order (primiparous vs not, 1.08, 95% CI 1.01–1.16; second vs first, OR 0.94, 95% CI 0.88–0.99; third vs first, OR 0.91, 95% CI 0.83–1.01; fourth vs first, OR 0.80, 95% CI 0.69–0.94) and sibship size (2 vs 1, OR 0.93, 95% CI 0.75–1.15; 3 vs 1, OR 0.89, 95% CI 0.74–1.07; 4 vs 1, OR 0.75, 95% CI 0.62–0.90) to be associated with testicular cancer risk. Meta-analyses that produced summary estimates which indicated no association included maternal age, maternal nausea, maternal hypertension, pre-eclampsia, breech delivery and caesarean section. Meta-regression provided evidence that continent of study is important in the relationship between caesarean section and testicular cancer (P = 0.035), and a meta-analysis restricted to the three studies from the USA was suggestive of association (OR 1.67, 95% CI 1.07–2.56).

Conclusions This systematic review and meta-analysis has found evidence for associations of maternal bleeding, birth order, sibship size and possibly caesarean section with risk of testicular cancer.

Keywords Epidemiology, meta-analysis, pregnancy, review, systematic, testicular neoplasms

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Introduction

Testicular cancer is a malignancy with an unusual incidence pattern, rising rapidly through adolescence and peaking during young adulthood.\textsuperscript{1,2} This pattern, combined with the age at which the precursor lesion, carcinoma in situ, is thought to arise,\textsuperscript{3-5} has prompted theories of a very early pathogenesis, the initiation of which is likely to be prenatal.\textsuperscript{6-8}

In many predominantly Caucasian countries of the developed world the incidence of testicular cancer has increased over the past 40 years,\textsuperscript{9-11} which has spurred further research into its pathogenesis. However, the only risk factors consistently associated with testicular cancer are cryptorchidism, prior history of testicular cancer and family history of testicular cancer.\textsuperscript{12} The possibility that in utero and early life exposures are important has prompted investigation of proxy measures of pre- and perinatal environmental exposures, but the results of such studies are often inconsistent,\textsuperscript{13} a problem exacerbated by small sample sizes and multiple testing. Therefore, we undertook a systematic review and meta-analysis of pre- and perinatal variables in relation to the risk of testicular cancer with a specific focus upon maternal characteristics.

Methods

The literature databases Scopus (Elsevier B.V., Amsterdam, The Netherlands; 1823–2008), EMBASE (Elsevier B.V., Amsterdam, The Netherlands; 1974–2008), PubMed (National Center for Biotechnology Information, US National Institutes of Health, USA; 1950–2008) and Web of Science (Thomson Reuters, New York, USA; 1900–2008) were searched using highly sensitive search strategies that incorporated a vast array of terms for many perinatal variables and testicular cancer (copies of these search strategies are available on request). Final electronic searches, which had no restriction on language, were conducted on 24 November 2008. Articles from these searches were pooled and managed using Endnote X2.\textsuperscript{14}

Titles, abstracts and keywords were independently reviewed as needed for selection of potentially relevant references by two individuals (M.B.C. and M.P.M.). The full text was retrieved of any reference that gave any indication that it might contain data on at least one perinatal variable and testicular cancer or if it was a review article of testicular cancer (copies of these search strategies are available on request). Citations of retrieved articles were checked for references that may have been missed or absent from the databases utilized. Cases had to be identified as testicular cancer cases and the age range could not be restricted to or include infantile testicular cancers. There were no stringent criteria for controls but, if a study had more than one control group, the preference order was population, neighbourhood, hospital and cancer. Inclusion criteria for categorical variable analyses, such as maternal bleeding or maternal nausea, stipulated that the study had to be a cohort or case–control in design and provide tabulated numbers of cases and controls that were and were not exposed. Similar criteria were applied to continuous variable analyses but the data had to be tabulated into at least three categories of exposure or the study needed to provide the number, mean and standard deviation of the variable for the case and control groups. This data format enables per unit log odds ratios (ORs) and standard errors of the log ORs to be estimated for continuous, normally distributed variables, using methods previously described.\textsuperscript{15} Authors of references which alluded to, but did not provide, data that met the inclusion criteria necessary for analysis were contacted in a request for supplementary information. If a manuscript and author of a study could not provide data to enable calculation of a log OR and standard error of the log OR but provided an estimate of risk that was minimally adjusted (e.g. adjusted only for age), then this was included in the analysis. If the population bases of two or more studies were judged to have overlapped considerably, the preference for retention in the analysis was for: cohort studies over case–control studies, given no discrepancy in the number of categories of the variable available for analysis; larger studies over smaller studies, given studies of the same design and risk estimates with the lowest error, given studies of the same design and similar size. The two latter criteria were used to select among multiple manuscripts of the same study. Studies that were adjudged to have a small likelihood of geo-temporal overlap in their base populations were retained in analyses. Studies that met the inclusion criteria for an analysis had data extracted into Microsoft Excel, which was subsequently checked twice for consistency. These data were then imported into STATA 10\textsuperscript{16} for statistical analysis.

Statistical analysis

For categorical variable analyses, unadjusted log ORs and standard errors of the log OR were calculated for each study using either logistic regression or, for dichotomous variables, the direct approach of the meta-analysis command (metatran) in STATA. Ninety-five percent confidence intervals (CIs) were estimated using the Woolf method.\textsuperscript{17} For continuous variable analyses, methods previously described were used to estimate per unit log ORs and standard errors of the log ORs.\textsuperscript{15} For dichotomous analyses with zero-count cells, 0.5 was added to each cell for analysis via STATA’s metan command. Random effects meta-analyses were conducted as the primary analysis to allow for variation in true associations across studies. $I^2$ was used as the estimate of heterogeneity, which is the percentage of total variation in risk estimates attributable to genuine variation rather than sampling error.\textsuperscript{19} To assess meta-analytic assumptions and
explore the relation between precision and magnitude of association, funnel plots were generated with Egger’s test; an arbitrary but conservative $P$-value $<0.1$ was used to judge asymmetry. The influence of each individual study in a meta-analysis was investigated by omitting each study in turn and re-estimating the summary estimate using STATA’s metaninf command. Meta-analyses using a fixed effects model were also conducted as an additional measure of sensitivity. Meta-regression was conducted using the variables: continent of study, data ascertainment (self-report; registry/medical record) and study design (cohort; case–control), which were specified $a$ priori. Given the number of meta-analyses undertaken and space restrictions of publication, these additional analyses will only be mentioned if they produced a $P$-value below the arbitrary threshold of $0.05$ or if they were deemed necessary for comprehensive interpretation.

**Results**

There were a total of 5865 articles after duplicates had been deleted. Of these, 358 articles had their full text retrieved, the citations of which were checked for any articles that may have been missed or which were absent in the databases utilized. A further 118 articles were subsequently identified and retrieved, giving a total full text article count of 476. Authors of 41 of these studies were contacted in a request for supplementary information, 33 of whom replied with 13 providing additional unpublished data. In total there were 67 articles that met the inclusion criteria, each of which was included in at least one perinatal analysis.

This article details the analyses of variables pertaining mainly to the mother as opposed to the son. Specifically, it shows meta-analyses of the variables: maternal age, maternal nausea, maternal hypertension, pre-eclampsia, maternal bleeding, breech birth, caesarean section, birth order and sibship size. Table 1 details the studies included in each meta-analysis and those excluded due to large geotemporal overlap or being additional reports of a study already included in the meta-analysis. The results of the random effects meta-analyses are shown in Figure 1. Similar results were derived using fixed effects models (Supplementary Figure 1 available as supplementary data at *IJE* online), thus only the random effects analyses are discussed and presented herein.

Maternal age, analysed as both a continuous (per year: OR 1.00, 95% CI 0.99–1.01, $I^2 = 81$%; Supplementary Figure 2 available as supplementary data at *IJE* online) and categorical variable (low: OR 0.97, 95% CI 0.85–1.10, $I^2 = 66$%, Supplementary Figure 3 available as supplementary data at *IJE* online; high: OR 1.02, 95% CI 0.95–1.11, $I^2 = 15$%, Supplementary Figure 4 available as supplementary data at *IJE* online), did not provide evidence for an association with risk of testicular cancer. The substantial heterogeneity was not explained by meta-regression of variables specified $a$ priori.

The meta-analysis of maternal nausea included 12 studies that generated a summary estimate of 1.15 (95% CI 0.92–1.45, $I^2 = 55$%) (Supplementary Figure 5 available as supplementary data at *IJE* online). Eleven of the studies included were based on self-report data, which made it impossible to assess the effect of recall bias on the results. Aschim et al. provided the only study that utilized medical records, the unadjusted risk estimate of which was 1.63 (95% CI 0.84–3.15). Assessment of recall bias was also hampered in the analysis of maternal hypertension, the summary OR of which was 1.25 (95% CI 0.97–1.62, $I^2 = 0$%) (Supplementary Figure 6 available as supplementary data at *IJE* online). Only two of the eight were record/registry-based, and combination of their estimates in a random effects model provided a lower estimate (OR 1.10, 95% CI 0.71–1.70) to that of the analysis of all eight studies. In contrast, exclusion of one of the eight studies from the maternal hypertension meta-analysis produced a higher estimate, which further supported the likelihood of a true association between maternal hypertension and testicular cancer (OR 1.55, 95% CI 1.11–2.16, $I^2 = 0$%).

Eight studies provided data for the meta-analysis of pre-eclampsia that ultimately provided a null estimate (Supplementary Figure 7 available as supplementary data at *IJE* online). Egger’s test provided a $P$-value of 0.007 for publication bias, which was also evidenced by the pattern of the funnel plot (not shown).

Maternal bleeding was found to increase the risk of testicular cancer by $\sim 33\%$ (OR 1.33, 95% CI 1.02–1.73, $I^2 = 33$%; Figure 2). There was, however, evidence of publication bias on both visual inspection of the funnel plot (not shown) and from the result of Egger’s test ($P = 0.035$). Only one study included in this meta-analysis was record/registry-based and this provided an OR of 1.02 (95% CI 0.71–1.46).

Neither breech birth (Supplementary Figure 8 available as supplementary data at *IJE* online) nor caesarean section (Supplementary Figure 9 available as supplementary data at *IJE* online) appeared to be associated with risk of testicular cancer when compared with normal vaginal delivery. From the sensitivity analysis of caesarean section it was noted that exclusion of one study provided a summary estimate that indicated the possibility of an association (OR 1.50, 95% CI 1.05–2.14) and reduced the heterogeneity to 0%. In addition, meta-regression of continent of study provided a $P$-value of 0.035, and an analysis restricted to the three studies from the USA produced a summary risk estimate of 1.67 (95% CI 1.07–2.56, $I^2 = 0$%).
Table 1  Studies included and excluded from each meta-analysis

<table>
<thead>
<tr>
<th>Analytic variable</th>
<th>Included</th>
<th>Excluded</th>
<th>Geo-temporal overlap</th>
<th>Same study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maternal age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td>(23, 32, 37–48)</td>
<td>(24, 49, 50) due to (32)</td>
<td>(51) due to (43); (52) due to (23)</td>
<td></td>
</tr>
<tr>
<td>Categorical</td>
<td></td>
<td></td>
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<tr>
<td>Low vs referent</td>
<td>(23, 37–41, 43–48, 50, 53)</td>
<td>(24, 32, 49) due to (53)</td>
<td>(51) due to (43); (52) due to (23); (49) due to (50)</td>
<td></td>
</tr>
<tr>
<td>High vs referent</td>
<td>(23, 32, 37–41, 43–48)</td>
<td>(24, 49, 50) due to (32)</td>
<td>(51) due to (43); (52) due to (23); (49) due to (50)</td>
<td></td>
</tr>
<tr>
<td>Maternal nausea</td>
<td>(23, 37, 38, 40, 44, 46, 48, 51, 54–57)</td>
<td>(58) due to (55)</td>
<td></td>
<td></td>
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<tr>
<td>Maternal hypertension</td>
<td>(23, 24, 37, 40, 54, 56, 58, 59)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Pre-eclampsia</td>
<td>(23, 37, 40, 46, 50, 51, 53, 54)</td>
<td>(24) due to (50)</td>
<td>(52) due to (23); (49) due to (50)</td>
<td></td>
</tr>
<tr>
<td>Maternal bleeding</td>
<td>(23, 37, 38, 40, 46, 48, 51, 54, 58–60)</td>
<td></td>
<td>(52) due to (23)</td>
<td></td>
</tr>
<tr>
<td>Breech birth</td>
<td>(37, 38, 50–52, 54)</td>
<td></td>
<td>(49) due to (50)</td>
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<tr>
<td>Caesarean section</td>
<td>(23, 37, 38, 50, 51, 54–56)</td>
<td></td>
<td>(52) due to (23); (49) due to (50)</td>
<td></td>
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<tr>
<td><strong>Birth order</strong></td>
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<td></td>
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</tr>
<tr>
<td>Primiparous vs not primiparous</td>
<td>(23, 37–41, 43–48, 50, 53–56, 61–63)</td>
<td>(58) due to (55); (24, 32) due to (53)</td>
<td>(51) due to (43); (52) due to (23); (49) due to (50)</td>
<td></td>
</tr>
<tr>
<td>Categoricala</td>
<td>(23, 32, 37–40, 43–47, 61–63)</td>
<td>(24, 49, 50, 53) due to (32)</td>
<td>(51) due to (43); (52) due to (23)</td>
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<tr>
<td><strong>Sibship size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Categorical</td>
<td>(32, 37, 39, 43, 46, 47, 62, 64)</td>
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</table>

aIn the categorical analysis of birth order, Heimdahl is only included in second vs first and not third vs first or fourth vs first as these data were not available.
Figure 1  Forest plot of each variable's random effects meta-analytic summary estimate of association with testicular cancer

Figure 2  Forest plot of maternal bleeding and testicular cancer meta-analysis
Birth order was found to be inversely associated with risk of testicular cancer (Figure 1). The dichotomous analysis of primiparous versus not primiparous gave a summary estimate of 1.08 (95% CI 1.01–1.16, $I^2 = 28\%$, Figure 3). In addition, the risk of testicular cancer decreased further with increasing birth order (Figure 4).

Sibship size showed a similar pattern to that of birth order and this may be expected given the correlation between these two variables. The effect estimates decline as sibship size increases (Figure 5).

Discussion

In the 16 meta-analyses of nine variables presented herein, we have found evidence that maternal bleeding, birth order and sibship size are associated with risk of testicular cancer. This exercise compounds our knowledge of testicular cancer aetiology through systematic and statistical evaluation of all published studies investigating maternal pre- and perinatal characteristics.

The association with maternal bleeding is intriguing but not without contention, given the evidence of publication bias and the availability and inclusion of only a single study which used record/registry data. Most cases of maternal bleeding during pregnancy presumably derive from a vascular disruption of the maternal–fetus interface. Although interpretation of this association may be difficult, given the heterogeneity in exposure due to varied causes of presentation,25 bleeding is generally the result of a developmental aberration, such as aggressive implantation or abnormal placental differentiation, which may be hypothesized to disrupt fetal development and increase risk for a prenatal, stem-cell progenitor of testicular cancer. An analysis of a large cohort of women having borne a son with abnormal bleeding during pregnancy may be helpful in further testing this association and elucidating its underlying causes.

The meta-analyses of birth order and sibship size evidenced an inverse relationship with risk of testicular cancer. The underlying causes of association with these variables could theoretically be due to altered in utero hormonal milieu,26–29 delayed exposure to a communicable agent,30,31 parental sub-fertility32 and socio-economic status, although the latter hypothesis has been discredited through analysis.32,33 Identifying whether it is solely birth order or sibship size that confers decreased testicular cancer risk or whether it is a combination of these two variables may help discriminate between these hypotheses. Disentangling the relationships between these two variables and
testicular cancer has proved difficult due to their correlation and this analysis could not attempt such due to the lack of access to the full dataset of each study. However, the study by Richiardi et al. was large enough to permit an analysis stratified by both birth order and sibship size. The authors found that both variables retained an equally strong inverse association with testicular cancer risk, which led them to speculate parental sub-fertility as the underlying causal factor.

The benefits of undertaking this systematic review and meta-analysis lie not just in those associations that have been reinforced; information has also been produced for those variables that have been found to be null in relation to testicular cancer risk. The summary estimates, forest plots, heterogeneity and funnel plots of the meta-analyses of maternal age at birth provide consistent evidence that this variable does not affect the neonate’s risk of developing testicular cancer. Maternal age has long presented an intriguing hypothesis, insofar as it is associated with various pre-natal developmental abnormalities as well as brain and breast cancers.35 The evidence presented here with regards to testicular cancer, however, is unequivocal to a conclusion of no association, although it is important to note that these analyses are not restricted to primiparous women, thus interpreting this analysis of maternal age as a proxy for factors that also vary with parity, such as hormonal milieu, may lack validity.

Severe nausea and/or vomiting during pregnancy is thought to be associated with a hormonal perturbation that could theoretically affect prenatal development and subsequent risk of testicular cancer. However, from our meta-analysis of all available data there was little evidence of association between this exposure and testicular cancer risk.

The summary estimate for maternal hypertension was slightly indicative of an association with testicular cancer and became more so when a single study was excluded.24 However, this study does not appear to be inherently different in any aspect relative to the others being combined, thus the association after its exclusion is possibly a false-positive result due to...

**Figure 4** Forest plot of birth order (categorical) and testicular cancer meta-analyses
multiple testing; sensitivity analyses of putative meta-analytic associations often find a combination of studies that simultaneously strengthen the result and decrease the heterogeneity, this being especially so when the study contributes a null estimate (OR 0.90, 95% CI 0.59–1.36) with a relatively large weight (39%, Supplementary Figure 6 available as supplementary data at IJE online). Given these caveats, the summary estimate still indicated an elevated risk, and future studies may wish to pursue this hypothesis further. In addition, the study by Pettersson et al. found ‘mild’ hypertension to be a risk factor and ‘severe’ hypertension to be protective for testicular cancer. We were unable to conduct such analyses, as all of the other studies included in this meta-analysis had coded hypertension dichotomously, making elucidation of severity impossible. Lastly, if any association between hypertension and testicular cancer does exist, it is likely to be mediated through hypertension directly rather than pre-eclampsia (or proteinuria), as the summary estimate for this meta-analysis was indicative to no association.

The meta-analysis of breech birth provided no evidence of association with risk of testicular cancer when compared with vaginal delivery. It is unfortunate that reasons for caesarean section are often not known in these studies, as this is the preferred mode of delivery for babies in breech position; such information could provide added power for an analysis of breech position up until delivery. It is possible that breech birth only increases risk of non-seminoma, but further analysis of any such relationship was not possible in this systematic review as the case group is rarely stratified by histology.

The analysis of caesarean section did provide tentative evidence for association, especially when restricted to studies based in the USA, an analysis prompted by meta-regression of continent of study. Although such data mining activities were specified a priori, they remain prone to the caveats of multiple testing and reduced statistical power, increasing the chances of a type I error. However, it remains possible that there is geographic variation in the association of caesarean section and testicular cancer risk, and further scrutiny of a possible relationship is warranted.

The main limitation of the meta-analyses presented is that estimates are unadjusted or minimally adjusted. Although this ensures that study-specific
estimates are derived from similar statistical models, the caveat of confounding remains. However, for the majority of variables assessed, it remains unclear which covariates would impact the summary estimate sufficiently to justify their inclusion in the model. This is because: few of the independent variables assessed appear to share an association with testicular cancer; there is rarely concurrence of a maximally adjusted statistical model for a variable across studies; and the independent variables that do share an association with testicular cancer are likely to be proxies for an underlying causal exposure, which further complicates the issue. Another limitation is that we cannot exclude recall bias from our analyses, although meta-regressions of study design (cohort; case-control) and data ascertainment (self-report; registry/medical record) had little effect on the summary estimates obtained.

The strength of this article is that it is the first meta-analytic review of maternal age, maternal nausea, maternal hypertension, maternal bleeding, pre-eclampsia, breech birth, caesarean section, birth order and sibship size in relation to testicular cancer. Moreover, the systematic literature searches are supplemented with additional unpublished data, without which some studies would have been precluded from analysis. Therefore, the summary estimates represent a methodical synopsis of the entire published literature.

In conclusion, this systematic review and meta-analysis has found associations of maternal bleeding, birth order, sibship size and possibly caesarean section with risk of testicular cancer. Further investigation of these variables and the underlying causes of their association with testicular cancer pathogenesis are warranted.

**KEY MESSAGES**

- The aetiology of testicular cancer remains largely elusive although initiation of pathogenesis is thought to have a prenatal origin.
- Results of testicular cancer studies are often inconsistent, a problem exacerbated by small sample size and multiple testing.
- Through systematic review and meta-analysis we find associations of maternal bleeding, birth order, sibship size and possibly caesarean section with risk of testicular cancer.

**References**


**Supplementary data**

Supplementary data are available at *IJE* online.

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**Conflict of interest:** None declared.
PERINATAL VARIABLES AND TESTICULAR CANCER


