Cryptorchidism (testicular maldescent) is one of the most common congenital malformations in males. It is associated with a fourfold increase in testicular cancer risk and, left untreated, may lead to low sperm counts and infertility (1, 2). Prevalence estimates vary depending on the case definition, age at ascertainment, and method of identifying cases. Prospective studies in which investigators examined infants directly have yielded cryptorchidism prevalences of 2–9 percent at birth, 0.7–2 percent in infant boys 3 months of age, and less than 1 percent in boys 1 year of age (3–7). Prevalence varies by country; Denmark’s prevalences, which are at least twice as high as those of its neighbors, are among the highest in the world (4, 8).

Although testicular descent is thought to be under hormonal control, the exact hormones and mechanisms governing descent are uncertain (9). The causes of cryptorchidism are also unknown, but events that increase fetal exposure to estrogens may play a role (10, 11). In particular, disruption of the normal androgen-estrogen balance in the fetal environment, by mechanisms that either lower endogenous androgen levels or increase the bioavailability and/or transplacental transfer of endogenous estradiol, is currently believed to contribute to cryptorchidism (2, 12, 13).

Alpha-fetoprotein (AFP) is produced initially by the yolk sac and later by the fetal liver. Although suspected to be involved in embryonic and fetal development, its biologic
roles have not been fully elucidated (14–16). Estradiol can induce a conformational change in AFP, exposing a growth-inhibitory epitope that inhibits estrogen-sensitive growth and possibly other estrogen-dependent processes, suggesting that AFP may modulate fetal responses to estrogens (17–19).

The fetus excretes AFP into the amniotic fluid, after which it is reingested and eventually degraded by the fetal liver (14). However, AFP also crosses the placenta and enters the maternal circulation, where it can be measured using an immunoassay (14, 20). Malformations that decrease the integrity of natural barriers between the fetus and the amniotic fluid (e.g., neural tube defects, omphalocele, gastroschisis, certain renal defects) and therefore increase the transfer of fetal substances into the amniotic fluid are associated with elevated maternal serum AFP levels (14). In contrast, chromosomal abnormalities are associated with decreased maternal serum AFP levels, for reasons that are unclear (14). Assessment of maternal serum AFP levels is included in prenatal testing protocols in many countries. Levels more than twice or less than half the median AFP value for a given gestational week signal the need for additional testing to rule out the above-mentioned abnormalities.

Since a disruption of the androgen-estrogen balance may contribute to cryptorchidism and since AFP may affect fetal responses to estrogens, we explored whether AFP levels (as reflected by maternal serum AFP levels in gestational weeks 14–22) were associated with the risk of cryptorchidism. We assembled a cohort of boys born to women participating in a Danish maternal serum AFP screening program and obtained outcome and covariate data from Denmark’s national health registries.

MATERIALS AND METHODS

Data sources

In 1980, a maternal serum AFP screening program was introduced in three well-defined areas in Denmark (South Jutland County, the city of Kolding, and the portion of the Copenhagen metropolitan area served by Hvidovre Hospital and the National Hospital of Denmark) (21). All women receiving antenatal care in these areas were offered screening. Information recorded at the time of screening included the woman’s national personal identification number, date of birth, the date of her last menstrual period, maternal serum AFP level, test date, and gestational age at the time of testing. Gestational age estimates were based on ultrasound examination information, when available; otherwise, the date of the last menstrual period was used in gestational age calculations.

All residents of Denmark are registered in the Danish Civil Registration System (CRS). Updated daily, the CRS assigns each resident a unique personal identification number, records demographic information, and tracks vital status. Information from various sources, including Danish population-based registries, can be linked via the personal identification number. We linked the maternal serum AFP screening program database with information from the CRS and three national registries. Using information from the

CRS and the National Birth Registry, we identified screening program participants who delivered liveborn male singletons, determined each boy’s birth weight and gestational age at birth, and tracked his vital status during our follow-up period. We collected information on cryptorchidism, other congenital malformations, chromosomal abnormalities, and surgeries from the National Discharge Registry, which contains all discharge diagnoses for inpatients discharged from Danish hospitals since 1977, as well as hospital outpatient diagnoses from 1995 onward. Additional information on malformations identified at birth was available through the National Malformations Registry, which operated between 1983 and 1994 and supplemented information being collected by the National Birth and Discharge registries. We obtained information on the women’s previous births from the CRS and the National Birth Registry.

Study cohort, follow-up, and case definition

We restricted our study to the liveborn singleton sons of Danish women who participated in the maternal serum AFP screening program between March 1, 1980, and June 30, 1994, and lived in a screening program area at the time of delivery (n = 28,412). We further restricted our cohort to the sons of women who had a known gestational age at the time of screening, were screened between gestational weeks 14 and 22, and had a nonzero, nonmissing serum AFP measurement (n = 27,320). We also excluded 1,902 boys with noncryptorchidism congenital malformations and/or chromosomal abnormalities (International Classification of Diseases (ICD), Eighth Revision, codes 740.00–759.99, excluding codes 752.10–752.19; ICD, Tenth Revision, codes Q00.0–99.9, excluding codes Q53.0–53.9 and code Q55.5). Although some of these boys had major malformations, many others had minor abnormalities (e.g., tear duct anomalies) not normally included in malformation statistics, such that the prevalence of malformations and chromosomal abnormalities in our cohort (7 percent) was higher than the generally accepted population prevalence of 3–5 percent. While excluding boys with minor malformations might have been unnecessary, the literature concerning relations among cryptorchidism, other malformations, and AFP is sparse, and we opted to exclude all malformations, no matter how minor. Of these 1,902 boys, 106 also had cryptorchidism.

The boys were followed from birth until the first of the following events: 1) cryptorchidism diagnosis; 2) death; 3) emigration from Denmark; 4) designated “missing person” in the CRS; or 5) December 31, 2001 (the end of follow-up). A boy was defined as cryptorchid if there was record of at least one cryptorchidism diagnosis code (ICD, Eighth Revision, codes 752.10–752.19; ICD, Tenth Revision, codes C62.0, Q53.0–53.9, and Q55.5) after 6 months of age or, if all diagnoses were made before 6 months of age, if there was record of at least one cryptorchidism diagnosis code and at least one code for orchidopexy (Nordic Medico-Statistical Committee Classification of Surgical Procedures (NCSG) codes 55640 and KFH10) or cryptorchidism repair surgery (NCSG code KFH00). No boy had cryptorchidism repair surgery (NCSG code KFH00) without a cryptorchidism diagnosis code. Boys recorded as having undergone
Cryptorchidism diagnosis were not considered to have cryptorchidism, as orchidopexy can be indicated for other diagnoses.

Statistical analysis

We standardized individual maternal serum AFP values for test year and gestational week at the time of testing by dividing each by the appropriate year- and gestational week-specific median maternal serum AFP value, yielding multiples of the median. Median maternal serum AFP values were based on serum AFP measurements from all women tested in a given year and gestational week. If there were fewer than 50 measurements for a given year-gestational week combination, we calculated the median using measurements from all women tested in the relevant gestational week over a 5-year period centered on the year of interest. If there were fewer than 50 measurements in the 5-year period, the median value was based on all women tested in that particular gestational week, irrespective of test year. The multiples of the median were then grouped into six categories defined a priori: <0.50, 0.50–0.74, 0.75–1.24 (the reference group), 1.25–1.99, 2.00–2.49, and ≥2.50.

All analyses were performed using SAS, version 9.1, software (SAS Institute, Inc., Cary, North Carolina). We evaluated the potential association between maternal serum AFP level (represented by multiples of the median categories) in gestational weeks 14–22 and cryptorchidism in the ensuing male singletons using log-linear binomial regression. Birth year (1-year categories, except 1980–1982 and 1993–1994) was included in all multivariate models. In addition, we evaluated the following potential confounders, all treated as categorical variables in our analyses: birth weight, gestational age at birth, weight for gestational age at birth, maternal parity, maternal age at the cohort child’s birth, and maternal age at first birth. Variables whose inclusion in a model containing maternal serum AFP level and birth year changed the risk ratio estimate for at least one AFP category by 10 percent or more were considered confounders and were retained in multivariate models. We also explored the use of splines to control for birth weight; however, replacing the categorical variable with splines changed the results very little, suggesting that control for confounding by birth weight using a categorical variable was adequate.

We tested for a linear trend in the maternal serum AFP category estimates by calculating the median of the values in each AFP category and entering the category median as a continuous term in the regression model. We evaluated the goodness of fit of the linear trend model by use of a likelihood ratio test that compared the trend model and the categorical multiples of the median model.

We conducted additional analyses to assess the robustness of the results to the case definition, to our decision to ignore any potential correlation between children with the same mother, and to the exclusion of boys with congenital abnormalities from the cohort. Because boys born earlier in the study period had more opportunity to be diagnosed with cryptorchidism than did boys born later, we also evaluated the extent to which variable length of follow-up might have biased our results by 1) stratifying our cohort by birth period (1980–1984, 1985–1989, 1990–1994) and looking for differences over time in the relation between maternal serum AFP level and cryptorchidism risk; and 2) conducting additional analyses using Poisson regression, which, unlike log-linear binomial regression, takes length of follow-up into account in addition to potential confounders.

RESULTS

Of the 25,418 boys in the study cohort, 663 (2.6 percent) were diagnosed with cryptorchidism during the follow-up period. Of these, 83 (12.5 percent) were diagnosed before 2 years of age, 345 (52.0 percent) by age 5 years, and 596 (89.9 percent) by age 10 years. The latest diagnosis was made at 15 years of age (one boy).

Associations between the risk of cryptorchidism and birth and maternal characteristics are presented in table 1. Compared with being born at 40 weeks’ gestation, being born very prematurely (gestational age: ≤32 weeks) was associated with a more than twofold increase in cryptorchidism risk. Gestation durations of 33–37 weeks were also associated with increased risk, but these associations were weaker. Birth weights of less than 3,000 g were associated with increased cryptorchidism risk, compared with birth weights of 3,000–3,499 g; the association with birth weights of less than 2,000 g was particularly strong (≥146 percent increase in risk). Being small for gestational age (weight for gestational age: <10th percentile) was also associated with an increased risk of cryptorchidism, with the smallest boys (<2.5th percentile) being 2.29 times as likely to have cryptorchidism as boys in the 50th–74th weight-for-gestational-age percentiles. Being born to a mother with one or more previous livebirths slightly decreased the risk of cryptorchidism, compared with being born to a mother with no previous livebirths. Risk also differed by birth year, with boys from early birth cohorts being at higher risk than were boys born later in the study period.

Cryptorchidism prevalence increased with increasing maternal serum AFP levels (table 2). Unadjusted risk ratios suggested that, compared with boys with maternal serum AFP levels within 25 percent of the median, boys with very low maternal serum AFP levels (<0.5 times the median) had a 30 percent (95 percent confidence interval [CI]: –21, 60) reduced risk of cryptorchidism, whereas boys with very high maternal serum AFP levels (≥2.5 times the median) had a 99 percent (95 percent CI: 20, 229) greater risk. Moderate maternal serum AFP levels were only weakly associated with cryptorchidism risk, if at all (table 2).

Of our list of potential confounders, only birth weight caused AFP risk ratio estimates to change by 10 percent or more when included in a model containing maternal serum AFP level and birth year. Because low birth weight and cryptorchidism might be caused by the same mechanism and adjustment for birth weight might therefore be inappropriate, we present multivariate results with and without adjustment for birth weight (table 2). Controlling for both birth year and birth weight weakened the association between very high maternal serum AFP levels and cryptorchidism risk.
Including gestational age at birth or weight for gestational age in models containing maternal serum AFP level and birth year attenuated the risk ratio estimate for AFP levels greater than or equal to 2.5 times the median somewhat, but not sufficiently to meet our confounding criteria (gestational age in model: risk ratio (RR) = 1.77, 95 percent CI: 1.07, 2.94; weight for gestational age in model: RR = 1.76, 95 percent CI: 1.06, 2.91). Adding either variable to models already adjusting for birth weight and birth year did not affect the analytical results (data not shown).

**Trend analyses**

The unadjusted risk ratios suggested a trend of increasing cryptorchidism risk with increasing maternal serum AFP level (table 2). The suggestion of a linear trend persisted after adjustment for birth year and birth weight (table 2). Tests for linear trend were statistically significant or borderline significant ($p = 0.006$ with no adjustment for confounding and $p = 0.05$ with adjustment for birth year and birth weight), and goodness-of-fit tests indicated that use of linear trend models was acceptable.

To help us evaluate whether it might be more appropriate to consider the relation between maternal serum AFP level and cryptorchidism risk using a linear trend model, with maternal serum AFP multiples of the median treated as a continuous variable, we plotted risk ratios for subdivisions of our six original multiples of the median categories; the risk ratios were adjusted for birth year and birth weight (figure 1). Risk ratios for moderate multiples of the median categories (i.e., 0.70–1.70) all lay near the null (RRs = 0.89–1.07), suggesting that the proposed linear trend might be driven by the effect of extreme multiples of the median (i.e., <0.70 or >1.70). Because figure 1 seemed more indicative of a threshold effect than of a true linear relation between maternal serum AFP level and cryptorchidism risk, we judged it most appropriate to continue to treat maternal serum AFP multiples of the median as a categorical variable, allowing cryptorchidism risk to vary in nonlinear fashion between categories.

**Additional analyses**

To make our case group more homogeneous and to improve the likelihood that only true cases of cryptorchidism were included, we excluded cases who had not had their cryptorchidism surgically repaired ($n = 263$) and repeated our analyses. This exclusion affected our results very little; after adjustment for birth year and birth weight, the effect of very low maternal serum AFP levels (<0.5 times the median) on cryptorchidism risk was slightly attenuated (RR = 0.74, 95 percent CI: 0.38, 1.45), while the effects of high and very high maternal serum AFP levels (2–2.49 and ≥2.5 times the median, respectively) were slightly strengthened (RR = 1.14, 95 percent CI: 0.66, 2.00 and RR = 1.86, 95 percent CI: 1.02, 3.40, respectively). To evaluate the possibility that within-family correlation might have affected our results, we restricted a separate set of analyses to the first child enrolled for each mother (612 cases of cryptorchidism, 22,571 boys in total). Once again, the exclusions affected the results only slightly (RR$_{AFP <0.5 \times \text{the median}} = 0.76, 95$ percent CI: 0.44, 1.32; RR$_{AFP 2–2.49 \times \text{the median}} = 1.15, 95$ percent CI: 0.63, 1.82; RR$_{AFP \geq 2.5 \times \text{the median}} = 1.53, 95$ percent CI: 0.89, 2.65; all RRs adjusted for birth year and birth weight), suggesting that ignoring potential correlation between children with the same mother was likely acceptable.

When we repeated our analyses including all 1,902 boys with congenital malformations and/or chromosomal abnormalities, the AFP risk ratio estimates changed little (<10 percent) in both unadjusted and adjusted analyses. However, with the inclusion of these additional boys, the risk ratio for AFP levels greater than or equal to 2.5 times the median remained statistically significant after adjustment for birth year and birth weight (RR = 1.72, 95 percent CI: 1.10, 2.71).

The relation between maternal serum AFP levels and cryptorchidism risk did not appear to vary by birth period (data not shown). In addition, results from Poisson models were very similar to results from log-linear binomial models adjusting for the same confounders (data not shown), suggesting that differences in length of follow-up did not affect our results.

**DISCUSSION**

We examined the association between maternal serum AFP levels in gestational weeks 14–22 and the risk of isolated cryptorchidism in 25,418 boys born to mothers participating in a Danish maternal serum AFP screening program. After adjusting for confounders, we found that very high maternal serum AFP levels (≥2.5 times the median) were associated with a 53–90 percent increased risk of cryptorchidism. Very low maternal serum AFP levels (<0.5 times the median) were associated with a 32–49 percent decrease in risk, but these risk ratios were accompanied by wide confidence intervals that included the null.

No other studies have examined the relation between maternal serum AFP levels early in pregnancy and cryptorchidism risk. A single case-control study (199 cases) of third trimester maternal hormone levels and cryptorchidism found no difference between mean AFP levels in mothers of cases and controls (22). However, the use of group means may have masked differences in the proportions of mothers of cases and mothers of controls with extreme (very high or very low) AFP levels. Alternatively, extreme AFP levels may be associated with increased cryptorchidism risk only at specific times during gestation.

The observed association between high maternal serum AFP levels and increased cryptorchidism risk can be explained in two ways. It is unlikely that AFP production increases in response to cryptorchidism; increases in maternal serum AFP levels associated with other malformations result from increased amounts of AFP reaching the maternal circulation, rather than from increased fetal AFP production. Nevertheless, high maternal serum AFP levels may truly reflect high fetal production of AFP; high AFP levels could result from natural (nonpathogenic) high production by the fetus or from fetal regulatory problems leading to overproduction of AFP. Since AFP interacts with estrogens, excess AFP in the fetal environment could play a role in the biologic events producing cryptorchidism by either...
affecting the androgen-estrogen balance directly or exacer-
bating the effects of an existing balance disruption. Alter-
natively, elevated maternal serum AFP levels may not reflect 
high fetal levels but rather may simply be a marker for 
placental dysfunction, some aspect of which contributes to 
cryptorchidism. For example, increased transplacental dif-
fusion stemming from such dysfunction could result in in-
creased amounts of AFP being transferred from fetus to

table continue
of which can reflect or contribute to placental dysfunction, and between preeclampsia and cryptorchidism risk (24).

We constructed our case definition and inclusion criteria to yield, as nearly as possible, a homogeneous group of true cases of isolated cryptorchidism. Spontaneous testicular descent is common in the first 3 months of life (up to 6 months in premature boys), occurring more infrequently thereafter and very rarely after 1 year of age (5, 7). To allow time for spontaneous descent, we accepted cryptorchidism diagnoses made before 6 months of age only if accompanied by repair surgery or a confirmatory diagnosis registered more than 6 months after birth. Boys with suspected but unconfirmed cryptorchidism were not included as cases. Additionally, we restricted our cohort to singletons because the etiology of cryptorchidism in singletons and twins may differ. Furthermore, maternal serum AFP levels from twin pregnancies are twice those measured at the same point during singleton pregnancies (25, 26), making comparison of maternal serum AFP levels from singleton and twin pregnancies problematic.

Because extreme maternal serum AFP values often signal the presence of congenital malformations or chromosomal abnormalities in the fetus, a disproportionate percentage of women with such values undergo induced abortions. Consequently, sons of women with extreme serum AFP values, particularly boys with congenital abnormalities, were less likely to survive to birth than were sons of women with midrange serum AFP values. Certain congenital abnormalities are associated with extreme maternal serum AFP levels, and many abnormalities are thought to be associated with cryptorchidism (14, 24, 27–31). To help mitigate potential selection bias due to differential rates of induced abortion for congenital abnormalities by maternal serum AFP level, we excluded boys with chromosomal abnormalities and/or congenital malformations other than cryptorchidism from the study population. Excluding these boys also allowed us to focus on isolated cryptorchidism, which may differ etiologically from cryptorchidism found in association with other abnormalities. However, this exclusion had little effect on our results.

The adverse consequences of cryptorchidism for later male reproductive health (and the resulting importance of early diagnosis and treatment) have been widely appreciated for only the last 10–15 years. While the Danish Pediatric

### TABLE 1. Continued

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of boys</th>
<th>Cases</th>
<th>Risk ratio*</th>
<th>95% confidence interval</th>
<th>p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal parity (no. of prior births)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>13,354</td>
<td>371</td>
<td>2.8</td>
<td>1 Referent</td>
<td>0.20</td>
</tr>
<tr>
<td>1</td>
<td>8,767</td>
<td>212</td>
<td>2.4</td>
<td>0.87 0.74, 1.03</td>
<td></td>
</tr>
<tr>
<td>≥2</td>
<td>3,297</td>
<td>80</td>
<td>2.4</td>
<td>0.87 0.69, 1.11</td>
<td></td>
</tr>
<tr>
<td>Maternal age (years) at cohort child’s birth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15–19</td>
<td>554</td>
<td>12</td>
<td>2.2</td>
<td>0.78 0.44, 1.39</td>
<td>0.65</td>
</tr>
<tr>
<td>20–24</td>
<td>6,141</td>
<td>171</td>
<td>2.8</td>
<td>1 Referent</td>
<td></td>
</tr>
<tr>
<td>25–29</td>
<td>10,755</td>
<td>263</td>
<td>2.5</td>
<td>0.88 0.73, 1.06</td>
<td></td>
</tr>
<tr>
<td>30–34</td>
<td>6,032</td>
<td>160</td>
<td>2.7</td>
<td>0.95 0.77, 1.18</td>
<td></td>
</tr>
<tr>
<td>35–39</td>
<td>1,695</td>
<td>49</td>
<td>2.9</td>
<td>1.04 0.76, 1.42</td>
<td></td>
</tr>
<tr>
<td>≥40</td>
<td>241</td>
<td>8</td>
<td>3.3</td>
<td>1.19 0.59, 2.39</td>
<td></td>
</tr>
<tr>
<td>Maternal age (years) at first birth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12–19</td>
<td>1,990</td>
<td>53</td>
<td>2.7</td>
<td>1.02 0.76, 1.36</td>
<td>0.92</td>
</tr>
<tr>
<td>20–24</td>
<td>10,297</td>
<td>270</td>
<td>2.6</td>
<td>1 Referent</td>
<td></td>
</tr>
<tr>
<td>25–29</td>
<td>9,876</td>
<td>249</td>
<td>2.5</td>
<td>0.96 0.81, 1.14</td>
<td></td>
</tr>
<tr>
<td>30–34</td>
<td>2,749</td>
<td>78</td>
<td>2.8</td>
<td>1.08 0.84, 1.39</td>
<td></td>
</tr>
<tr>
<td>≥35</td>
<td>506</td>
<td>13</td>
<td>2.6</td>
<td>0.98 0.57, 1.70</td>
<td></td>
</tr>
<tr>
<td>Birth year‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1980–1984</td>
<td>3,075</td>
<td>109</td>
<td>3.5</td>
<td>1.57 1.25, 1.97</td>
<td>0.0004</td>
</tr>
<tr>
<td>1985–1989</td>
<td>12,375</td>
<td>329</td>
<td>2.7</td>
<td>1.18 1.00, 1.39</td>
<td></td>
</tr>
<tr>
<td>1990–1994</td>
<td>9,968</td>
<td>225</td>
<td>2.3</td>
<td>1 Referent</td>
<td></td>
</tr>
</tbody>
</table>

* From log-linear binomial regression models.
† p value associated with a two-sided test of homogeneity.
‡ For the sake of compactness, in this table we present birth year information in 5-year categories. However, when adjusting for birth year in our analyses, we use 1-year categories (with the exception of 1980–1982 and 1993–1994, which are aggregated).
Society now aims to have all cryptorchidism identified and treated by 2–4 years of age (32), cryptorchidism was historically viewed by many physicians and midwives as a minor defect, the repair of which could wait until late childhood or adolescence. Consequently, although cryptorchidism is a congenital defect that is observable at birth and the diagnosis can be confirmed within the first year of life, during our study period (1980–1994) it often went undiagnosed or unreported until later in childhood, as is apparent from the range and distribution of ages at diagnosis in our cohort.

Variability in age at diagnosis could have been cause for concern for two reasons. First, cryptorchidism is most reliably identified in the period after spontaneous testicular descent is likely to have occurred but before the cremaster reflex has strengthened to the point that distinguishing between cryptorchidism and retractile testes becomes a challenge, that is, between approximately 6 months and 1–2 years of age. However, only 12.5 percent of our cases were identified before age 2 years, presenting the possibility that some of our cases had retractile testes or other conditions, rather than true cryptorchidism. To improve the likelihood that only boys with true cryptorchidism were included as cases, we repeated our analyses excluding boys who had not undergone repair surgery, on the assumption that cryptorchidism would eventually have been repaired. This exclusion did not materially affect our results. Second, since

### TABLE 2. Risk ratios for cryptorchidism by maternal serum alpha-fetoprotein multiples of the median category, with and without adjustment for covariates, in a cohort of Danish boys whose mothers participated in a maternal serum alpha-fetoprotein screening program between March 1, 1980, and June 30, 1994*

<table>
<thead>
<tr>
<th>Maternal serum alpha-fetoprotein multiples of the median category</th>
<th>No. of boys</th>
<th>Cases</th>
<th>No.</th>
<th>%</th>
<th>Risk ratio</th>
<th>95% confidence interval</th>
<th>Risk ratio</th>
<th>95% confidence interval</th>
<th>Risk ratio</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.50</td>
<td>722</td>
<td>13</td>
<td>1.8</td>
<td>0.70</td>
<td>0.40, 1.21</td>
<td>0.67</td>
<td>0.39, 1.16</td>
<td>0.68</td>
<td>0.40, 1.19</td>
<td></td>
</tr>
<tr>
<td>0.50–0.74</td>
<td>4,520</td>
<td>110</td>
<td>2.4</td>
<td>0.95</td>
<td>0.77, 1.17</td>
<td>0.93</td>
<td>0.76, 1.16</td>
<td>0.96</td>
<td>0.77, 1.18</td>
<td></td>
</tr>
<tr>
<td>0.75–1.24</td>
<td>13,278</td>
<td>341</td>
<td>2.6</td>
<td>1.08</td>
<td>0.90, 1.29</td>
<td>1.07</td>
<td>0.89, 1.29</td>
<td>1.04</td>
<td>0.86, 1.25</td>
<td></td>
</tr>
<tr>
<td>1.25–1.99</td>
<td>5,970</td>
<td>165</td>
<td>2.8</td>
<td>1.17</td>
<td>0.74, 1.84</td>
<td>1.13</td>
<td>0.72, 1.78</td>
<td>1.06</td>
<td>0.67, 1.68</td>
<td></td>
</tr>
<tr>
<td>2.00–2.49</td>
<td>634</td>
<td>19</td>
<td>3.0</td>
<td>1.99</td>
<td>1.20, 3.29</td>
<td>1.90</td>
<td>1.15, 3.14</td>
<td>1.63</td>
<td>0.98, 2.72</td>
<td></td>
</tr>
<tr>
<td>≥2.50</td>
<td>294</td>
<td>15</td>
<td>5.1</td>
<td>1.99</td>
<td>1.20, 3.29</td>
<td>1.90</td>
<td>1.15, 3.14</td>
<td>1.63</td>
<td>0.98, 2.72</td>
<td></td>
</tr>
<tr>
<td>Linear trend</td>
<td>25,418</td>
<td>663</td>
<td>1.29</td>
<td>1.08, 1.54</td>
<td>1.28</td>
<td>1.07, 1.53</td>
<td>1.20</td>
<td>1.00, 1.44</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Results from log-linear binomial regression models.

### FIGURE 1. Risk ratios for cryptorchidism by maternal serum alpha-fetoprotein (AFP) multiples of the median category, adjusted for birth weight and birth year, Denmark, 1980–2001. The plotted maternal serum AFP values are the medians of the following maternal serum AFP multiples of the median categories: <0.50, 0.50–0.64, 0.65–0.74, 0.75–0.94, 0.95–1.04 (referent group), 1.05–1.24, 1.25–1.39, 1.40–1.59, 1.60–1.79, 1.80–1.99, 2.00–2.19, 2.20–2.49, and ≥2.50. The risk ratios were estimated using a log-linear binomial model.
cryptorchidism in our cohort was often diagnosed later in childhood, cohort children born early in the study period had more opportunity to be diagnosed with cryptorchidism than did children born later. However, the observed associations between maternal serum AFP levels and cryptorchidism risk persisted after adjustment for birth year. Furthermore, results from additional log-linear binomial analyses stratified by birth cohort and Poisson regression analyses accounting for length of follow-up were very similar to those from our main log-linear binomial models, suggesting that variable length of follow-up did not bias our results.

Using registry data required that cases be diagnosed and registered in the National Discharge Registry to come to our attention. Mild cryptorchidism that escaped detection and unreported cryptorchidism diagnosed outside the hospital setting would theoretically not be registered. However, we believe that most cryptorchidism cases were probably diagnosed and reported eventually (and therefore registered), particularly toward the end of the study period, and therefore there was little miscategorization of case status due to unregistered diagnoses. Furthermore, we expect that any such miscategorization would be independent of maternal serum AFP level, biasing the results toward the null, if at all.

Epidemiologic research in Denmark is facilitated by the CRS and a large selection of population-based health registries. The former allows for linkage of data from multiple sources and tracking of study subjects, while the latter permit researchers to identify most, if not all, cases of the outcome of interest diagnosed and/or treated at hospitals in Denmark. Use of registry data also greatly reduces the possibility of recall bias and miscategorization of covariate information. Using data from three registries linked with information from a population-based screening program, we showed that very high maternal serum AFP levels in gestational weeks 14–22 were associated with an increased risk of cryptorchidism in male offspring, compared with median maternal serum AFP levels; there was also the suggestion that very low maternal serum AFP levels might be associated with a reduced risk of cryptorchidism. These findings were very robust, indicating that the role of AFP in events leading to cryptorchidism should be considered further.

ACKNOWLEDGMENTS

This work was supported by grant 2107-04-0004 from the Danish Medical Research Foundation.
Conflict of interest: none declared.

REFERENCES


