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Maternal Age At Birth Delivery, Birth Order And Secondary Sex Ratio In The Old Order Amish Of Lancaster County

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MATERNAL AGE AT BIRTH DELIVERY, BIRTH ORDER AND SECONDARY SEX RATIO IN THE OLD ORDER AMISH OF LANCASTER COUNTY

A Thesis Presented

by

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ABSTRACT

MATERNAL AGE AT BIRTH DELIVERY, BIRTH ORDER AND SECONDARY SEX RATIO IN THE OLD ORDER AMISH OF LANCASTER COUNTY

MAY 2013

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Background: Theoretically, human males produce equal numbers of X-bearing and Y-bearing spermatozoa. Based on this theory, equal numbers of female and male fetuses should be produced and one would expect an equal number of males to females. However, the observation that more boys were born than girls was noted in the late 1660's. For every 100 girls born, 106 boys were born, representing a secondary sex ratio (SSR) of 1.06:1. Recent studies suggest the secondary sex ratio (SSR) is declining in industrialized countries. SSR is proposed as a sentinel for reproductive health. Declining SSR may reflect environmental factors or other influences of reproductive outcomes. **Methods:** We conducted a retrospective cohort study evaluating the association between maternal age, birth order and SSR in the Old Order Amish (OOA), a homogenous sub-group with large family sizes. We used data from the Anabaptist Genealogy Database consisting of records for live births from 1696-2003. We used t-tests to compare mean maternal age and birth order by offspring sex, ANOVA to evaluate whether SSR has changed over time, and logistic regression for multivariable models. We evaluated clustering of SSR within families using random effects models and likelihood ratio tests of random effects. **Results**: Maternal age was not associated with SSR (OR=1.003 [95% CI, 0.995-1.010), even after adjusting for birth order (AOR=1.000 [95% CI, 0.989-1.012). Similarly, we did not find an association between birth order and SSR in both unadjusted models (OR=1.007 [95% CI, 0.991-

1.022), and those adjusted for maternal age (AOR= 1.006 [95% CI, 0.982-1.032]). The proportion of male births varied, however, there was no significant secular trend in male births between 1696 -1900. Lastly, we found a significant random effect (P<0.05), which may provide indication that having male births is heritable in families. **Conclusions:** Neither maternal age nor birth order is associated with the sex of an offspring. These findings suggest that decreases in SSR are unrelated to demographic factors, and rather may be related to other factors such as environmental exposures or other xenobiotic chemicals. These results may be relevant in providing information to the leading indicators to the decline in SSR.

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CHAPTER 1

INTRODUCTION

In reproductive theory, human males produce equal numbers of X-bearing and Y-bearing spermatozoa (1). If this theory holds true, then equal numbers of female and male fetuses should be produced, therefore one would expect a theoretically equal 1:1 ratio of male to female due to genetics (2). However, in the late 1660s it was observed that more boys are born than girls (104 – 107 boys for every 100 girls, that is, a ratio of 1.06:1), which has since become a normalized proportion of male to female births (3). This secondary sex ratio (SSR) of 1.06:1 has been declining over the past 5 decades in many industrialized countries, particularly in Europe and the United States (2, 4). In fact, the proportion of male births in several European countries declined from 51.6 percent in 1950 to 51.3 percent in 1994 (5, 6) and similar declines have been reported in the both the U.S and Canada (4) .

The decline observed has potential implications for reproductive health. SSR is a sentinel for reproductive health and a declining SSR may reflect environmental factors or other influences of reproductive outcomes. Understanding the factors that impact SSR may help in understanding reproductive outcomes such as trends in infant mortality and morbidity. The decline also has sociodemographic implications. A higher rate of female births has potential threats to population stability by limiting the availability of mates if there are disproportionately more women than men, thus altering age at marriage and childbearing (1). Additionally, males have higher infant mortality rates than females, therefore, in order to have a balanced 1:1 male to female mating there need to be more male births.

There have been several proposed contributing risk factors leading to the decline in sex ratio, including: 1) biological factors such as sperm characteristics, levels of maternal hormone at

time of conception, and immunological interaction between mother and embryo; 2) behavioral factors such as physical stress and smoking; 3) environmental factors such as radiation and trace elements in water; and 4) social and demographic factors such as lower socioeconomic status, higher paternal age, maternal age and higher birth order ranks (1) . Our factors of interest are maternal age, defined as the age of the mother at birth delivery, and birth order which is the child rank in order to that of other siblings (1) .

The physiological mechanisms relating maternal age to SSR are unclear, however, prior studies suggest variations in physiology of the female reproductive system and variation in prenatal death and stillbirths by maternal age (1, 7) . Some studies suggest that higher maternal age serves as a stressor to the female reproductive system during pregnancies due to physical aging. Additionally, there is evidence that male embryos are more vulnerable to such stressors in the early development stages putting them at a higher risk of early intrauterine death compared to female embryos (8) . These factors suggest that women of older maternal age are more likely to have intrauterine death of male embryos because of their physiological condition.

Similarly, the physiological mechanisms between birth order and SSR are also unclear, but may be due to genetic factors that increase the likelihood of some families having more boys than girls, and also gender preferences within families (5, 7) . Studies also suggest that because the female reproductive system is typically in better physiological condition prior to first births than after multiple births, multiparous women have more fetal deaths and stillbirths compared to primiparous women (9) .

Previous epidemiologic studies of maternal age and SSR are extensive and have found either weak negative associations suggesting a higher SSR, i.e. more male births for younger mothers and thus a lower SSR for older mothers, or no association (1, 7, 8) . Studies

investigating the relationship between birth order and SSR are more consistent and many have found statistically significant negative associations suggesting that births of lower rank have higher proportions of males than those of higher ranks (1, 4, 7) . However, these studies were done using datasets such as national vital statistics and lack large enough family sizes to estimate independent effects of maternal age and birth order. Additionally, multivariate approaches to tease apart relations of SSR with maternal age and birth order are limited. To address these issues, we utilized the Old Order Amish (OOA) as our study population.

The OOA are a group that is well suited for this study due their unique characteristics. They are an isolated religious group known for their conservative lifestyles, characterized by large family sizes, reproductive isolation and high inbreeding (10, 11) . Their close-knit family units have not changed much over the years, and there has been little variability in socioeconomic status and day-to-day activities. Among OOA women, sedentary life-style, cigarette smoking, alcohol consumption and use of contraceptives are very rare (10) . Prior studies of influences of SSR may have been confounded by possible factors such as maternal smoking, maternal alcohol consumption and maternal weight, which may affect reproductive outcomes (3) . Our study using the OOA provides a measure of control for confounding by design. The wide range of maternal age and birth order allows contributes to the ability for assessment of independent relations with SSR. Additionally, the linked data of the OOA from the extensive and multi-generational record keeping, along with their large family sizes allows for consideration of family effects.

CHAPTER 2

METHODS

Study Design and Population

We conducted a retrospective cohort study to evaluate the association between maternal age, birth order and SSR using the data from the Anabaptist Genealogical Database (AGD). The AGD includes information on the Old Order Amish and Mennonite cohorts that are both subgroups of the Anabaptist faith residing in Lancaster County, Pennsylvania, who were born between the mid-1600 and 2003 (10) . The AGD is a merger of 3 sources of genealogy books (12) , and includes the first and last names, gender, address, birth and death dates of individuals whose information was largely gathered from historical accounts and church records. However, names of children who were stillborn or those who died as a neonate may be missing from the database (9).

The first source consisted of *Fisher Family History*, a genealogy book that gives the most complete account for the "contemporary" Amish in Lancaster, and had over 55,636 individuals organized by family units (10, 12) . The second source is the *Amish and Amish Mennonite Genealogies,* which focused on individuals born before 1870. This source included data on 30,853 individuals who were not entirely OOA, but were all members of the more general Anabaptist faith (12). Lastly, the third source was a merger of a large computerized Anabaptist genealogy maintained by Mr. James Hostetler, a genealogist in Richmond, Virginia (12) .

The AGD was initially designed as a convenient genealogy database for gene mapping research on rare disorders in the Anabaptist population, which was conducted between 1996 and 2001 by researchers at the National Institutes of Health (NIH) (12) . Genealogies have shown to be a valid method for linking parent-child relationships (12). Use of genealogy is also the most

feasible means of obtaining data on maternal age at delivery in this population, particularly for those living between the 16th and 19th centuries. Researchers have used the AGD to detect the causative genes for Mckusick-Kaufman syndrome (13), and other genetic syndromes. Hsueh et al. evaluated the relationship of diabetes (14) and obesity and blood pressure (15) . In assessing Type II diabetes, Hsueh et al. found that Type II diabetes in the OOA was phenotypically similar to that of Caucasians in the NHANES III study population in the US, although the observed prevalence in the OOA was approximately half of that in the NHANES III Caucasians (95% CI 0.23-0.84) (13) .

For the present study we excluded those births post the year 1900 from our analyses due to recruitment and participation of OOA women in current research including breast cancer studies. For this reason, SSR in the AGD at this time point is highly skewed and is not truly representative of the SSR in the OOA. As a result, our data includes the OOA of Lancaster County living between 1652 and 1900.

Exposure and Outcome Assessments

Both maternal age and birth order are included in the AGD. Birth dates of mothers, recorded in the genealogy were linked to birth dates of each child to calculate mothers' age at the time of each birth (10) . We assessed maternal age both as a continuous and categorical variable. Birth order was assessed by linking each child's last name and birth date to determine the order of sib ship. Similarly, we assessed birth order as continuous and as a categorical variable. SSR was determined by proportion male in our analyses and was assessed as a binary variable (male [1] vs. female [0]).

Statistical Analysis

All statistical analyses were completed using SAS (version 9.3) software. In order to describe our study population, we first obtained summary characteristics of the OOA. To evaluate whether the distribution of offspring sex varies by maternal age or by birth order we used t-tests to compare mean maternal age and mean birth order by male and female births. To assess whether SSR has changed overtime within the OOA, we first created birth cohorts by categorizing years of birth into deciles. We then used ANOVA to compare the mean proportion of male births by birth cohort, conditional on maternal age and birth order, and Tukey's post-hoc test for pairwise comparisons of sex ratio between each pair of birth cohorts. Logistic regression was used for multivariable models of the association between maternal age, birth order and the likelihood of having a male birth as odds ratios (OR) and the associated 95% confidence intervals (CI) were determined. We also evaluated clustering of SSR within families using a random effects model including a maternal effect. Statistical significance was assessed by fitting models with and without the random effect, and using a likelihood-ratio test to test the effect of the maternal effect. Tests were considered significant at the $p<0.05$ levels.

CHAPTER 3

RESULTS

Summary characteristics of the OOA Cohort

There were a total of 15,989 births recorded in the Lancaster County subset of the AGD between the years 1652 and 2003. However, after excluding those births >1900 there were a total of 6689 births as shown in Table 1. In this sub-set of births between 1696 and 1900, there were more male births (51.3%) than female births (48.7%) recorded in the database. The majority of births (51.3%) were recorded between the years1850 and 1900. The average maternal age at birth was 30.5 years (SD = 6.7) and the average number of children within the OOA families was 7.7 children (SD = 3.6).

SSR distribution is unrelated to maternal age and birth order within the OOA Cohort

Table 2 shows the results of our bivariate analyses. In evaluating the distribution of offspring sex, we observed that the distribution of male births and female births did not significantly vary with the mean maternal age (p>0.05). Likewise, the distributions of male and female births were not significantly different with mean birth order (p>0.05) (Table 2).

SSR exhibits no clear secular trend overtime within the OOA Cohort

In evaluating whether SSR has changed overtime we observed that the proportion of male births did not significantly vary by birth cohort in both unadjusted analysis, and also conditional on maternal age and birth order (Figure1.) The percentage male increased from 43% (95% CI= 39% - 48%) in 1696 to 51% (95% CI= 47% - 55%) in 1815. It declined from 52% (48% - 56%) in 1841 to 46% (42% - 49%) in 1860 and then increased from 46% (42% - 49%) in 1860 to 51% (47% - 54%) in 1882. Subsequently, the male proportion then declined to 46% in 1900. However, no clear trend was apparent in these comparisons, and after conducting post-hoc tests

we observed that there was no statistically significant pairwise difference between any two birth cohorts (p>0.05).

SSR is unrelated to maternal age and birth order independently

Under the assumption of a linear relationship between maternal age and SSR, we first assessed maternal age as a continuous variable. We found that maternal age was not significantly associated with the likelihood of having male births even after adjusting for birth order (adjusted (A)OR =1.000, 95% CI = 0.989-1.012) which is shown in Table 3a. Similarly, under the assumption of linearity we assessed the relationship between birth order and SSR, using birth order as a continuous variable. We found that birth order was not significantly associated with the likelihood of having male births in both unadjusted (OR= 1.007, 95% CI = 0.991-1.022) and adjusted (AOR=1.006, 95% CI = 0.982-1.032) models.

We then modeled the relationship between maternal age and SSR by categorizing maternal age into deciles as displayed in Table 3b. After categorizing maternal age we found that maternal age was not statistical significantly associated with the odds of having male births across all age groups even after adjusting for birth order. Likewise, after categorizing birth order into nine groups we observed that birth order was not associated with SSR across birth order ranks (Table 3b)(Figure 2).

There is significant heritability of SSR based on random effects models

Finally, random effect models were run to evaluate clustering of SSR within families. Inclusion of a random effect corresponding to maternal ID was observed to significantly improve model fit based on likelihood ratio chi-square tests we observed that the maternal effect improved the fit of our model (P<0.05) (data not shown). Similar results were observed in unadjusted models, as well as those adjusted for maternal age and birth order.

CHAPTER 4

DISCUSSION

In our dataset of the Old Order Amish of Lancaster County living between 1696 and 1900 we did not find associations of maternal age or birth order with SSR. Results were null in unadjusted and adjusted models. Similarly, we did not observe a trend in the proportion of male births from 1696 – 1900. However, we did find a significant maternal clustering effect in evaluating whether having male births is heritable in families.

To our knowledge there are no previous studies that have assessed the relationships between maternal age, birth order and SSR using the AGD, where specific effects of maternal age and birth order within large families can be assessed. However despite our unique cohort, our findings are consistent with most previous studies that have taken a multivariate approach in their assessment using datasets taken from medical birth registries and national vital statistics.

Novitski and Kimball evaluated the relationship between paternal age, maternal age, birth order and SSR by conducting a population-based retrospective cohort (16) . The authors used all live births in the United States collected by the office of vital statistics in the year 1955, (n=3,645,750). The authors found no significant association between paternal age, maternal age and SSR, but found a significant interaction term with paternal age and birth order, though interpretation of this latter observation is unclear. Similarly, Rueness et al. conducted a population-based retrospective cohort study using all live and stillbirths in Norway during the years 1967 – 2006, (n=2,206,040) (8) . They used data collected from the Medical Birth Registry of Norway and found that there was no statistically significant association between maternal age and SSR across all age strata where maternal age ≥ 45 had an OR=1.06, 95% CI: 0.96 – 1.16 and maternal age ≤ 19 had an OR= 1.01, 95% CI: 0.99 – 1.02) (8) which is similar to our findings.

We had intriguing findings suggesting that the likelihood of having male births may be heritable. Few prior studies have evaluated heritability of sex ratio. The Behavior Genetic Study used data from the National Longitudinal Survey of Youth (NLSY) 1994, a national survey that contains family information including the sex of each respondent's biological child (n=15000 children) (17) . The study assessed the correlation of the proportion of boys (n=1281) by degree of relatedness among women comprising siblings, cousins and twins. The authors found no significant correlation with proportion of male births with genetic relatedness and concluded that their data did not provide support for a genetic influence on sex composition within families. However, the authors also noted that their data is limited by a small sample size (only1281 male births) due to attrition (17) . Additionally, their analyses were limited to only immediate family members.

Strengths of our study include large family sizes and a wide range of maternal age (ranging from ages 14 – 55 years), which is ideal for SSR studies. Additionally, we were able to evaluate secular trends in the proportion of male births over time because of the large amount of available historical birth data, comprising two centuries of records (1696 -1900).

Our study is also subjected to several limitations. Our study consisted of only 6689 births, which is a small sample size as compared to those of several other studies. Although the AGD contains data on paternal relationships, we did not evaluate the effects of paternal age on SSR due to missing data. We are unsure that stillbirths were recorded in the genealogy and may have misclassified stillbirths as live births; therefore the observed SSR could be inaccurate. This would represent nondifferential misclassification error unless the likelihood of stillbirth were related to offspring sex and maternal age and/or birth order. It is also possible that errors could have been made in translating dates of births from the genealogy books to the computerized

database, thus making mothers appear to be older or younger than they really were at birth delivery. This nondifferential misclassification would bias our results towards the null, thereby reducing magnitudes of our effect estimates of the association of maternal age and SSR. Selection bias is possible if inclusion/exclusion in the birth records were related to both infant sex and maternal age and/or birth order. For example, if younger mothers who had more boys than girls were more likely to be recorded in the genealogy than older mothers with fewer boys, our results would be biased towards the null. However, due to the nature of this study and its use of birth records collected for non-study purposes, we find this bias unlikely. Additionally, use of the OOA as our study population reduced the possibility of confounding by restriction. As described in our methods, the Amish are an isolated group with little variability in diet, behavior and lifestyle factors, and limited exposure to environmental factors.

CHAPTER 5

CONCLUSIONS

We found no associations of maternal age or birth order with SSR in our data. Our findings suggest that neither maternal age nor parity determines the sex of the offspring. Larger studies may help to further evaluate these relationships. However, the absence of relations of maternal age and parity with offspring sex suggests that any observed declines in SSR is likely to be due to factors such as environmental exposures rather than demographic shifts in the population. Our study found a significant maternal clustering effect indicating that having male births may be heritable in families. We recommend that larger studies using a similar cohort are needed to further investigate this relationship.

Table 1. Descriptive Characteristics of members of the Lancaster County OOA born between 1696 and 1900 (n=6689)

Characteristics	
Sex N(**%**)	
Male	3432 (51.3)
Female	3257 (48.7)
Year of Birth	
(range)	1696 - 1900
Mean*(SD)*	1846 (35.8)
Time Range (N%)	
<1700	1 (.01)
1700 - 1749	43 (0.6)
1750 - 1849	3217 (48.1)
>1850	3428 (51.3)
Maternal age at Birth	
Mean(*SD*)	30.5(6.7)
(range)	(14 - 55)
Birth Order (rank no.)	
Mean *(SD)*	5.1 (3.1)
(range)	(1-17)
Family Characteristics (N)	2259
Number of Children (per couple)	
Mean(*SD*)	7.7(3.6)
(range)	(1 - 20)
Number of Daughters (per couple)	
Mean(*SD*)	3.8(2.3)
(range)	(0 - 13)
Number of Sons (per couple)	
Mean(SD)	3.9(2.4)
(range)	(0 - 14)

Table 2. Distribution of Sex by Maternal Age and Birth Order among members of the Lancaster County OOA born between 1696 and 1900 (n=6689)

	Male Birth (N= 3432)	Female Birth (N=3257)	*p*-value*
Maternal Age Mean (*SD*)	30.51 (6.7)	30.39 (6.6)	0.484
Birth Order Mean (*SD*)	5.15 (3.2)	5.09 (3.1)	0.393

**p*-value determined by two sample t-test as described in the text

Table 3a. Multivariate Analyses of Maternal Age and Birth Order and the Odds of having a Male Birth in the members of the Lancaster County OOA born between 1696 and 1900 (n=6689)

	OR (95% CI)	*AOR (95% CI)**
Maternal Age (years)	1.003 (0.995-1.010)	1.000 (0.989-1.012)
Birth Order (rank no.)	1.007(0.991-1.022)	1.006 (0.982-1.032)

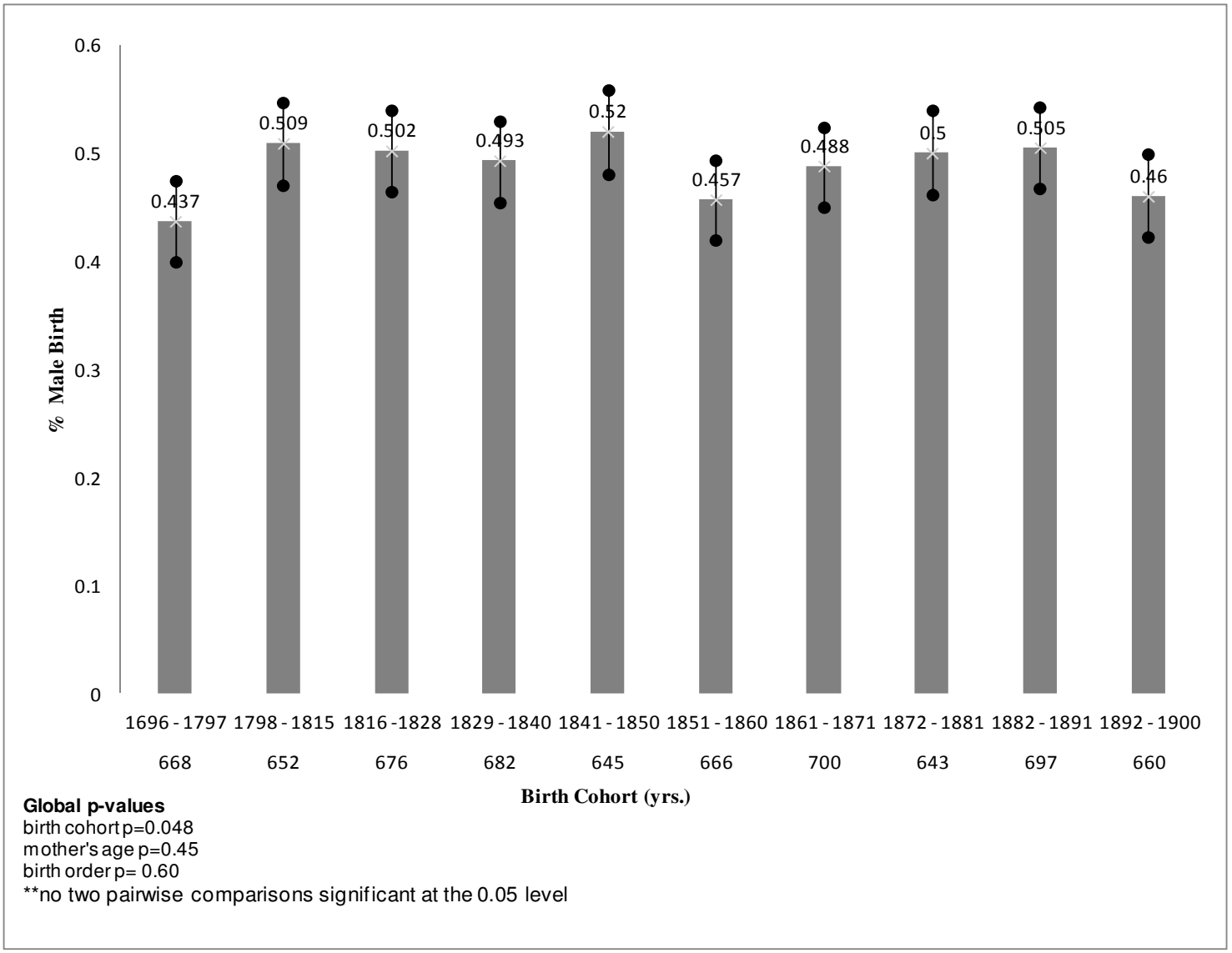
*Adjusted for maternal age and birth order alternatively

Table 3b. Logistic Regression of Maternal Age and Birth Order and the Odds of Having a Male Birth in the members of the Lancaster County OOA born between 1696 and 1900 (n=6689)

	Crude OR (95% CI)	*AOR (95% CI)**		*Crude OR (95% CI)*	*AOR (95% CI)**
Maternal Age (yrs.)			**Birth Order (rank no.)**		
14 - 21	0.931 (0.744-1.164)	1.005 (0.748-1.352)	1	*Referent*	*Referent*
22 - 24	1.007 (0.830-1.222)	1.009 (0.846-1.428)	2	0.938 (0.773-1.138)	0.923 (0.757-1.125)
25 - 25	0.952 (0.738-1.227)	1.051 (0.777-1.422)	3	1.057 (0.870-1.284)	1.035 (0.839-1.276)
26 - 27	1.008 (0.819-1.242)	1.121 (0.865-1.452)	4	0.856 (0.703-1.044)	0.842 (0.674-1.052)
28 - 29	0.956 (0.775-1.178)	1.073 (0.836-1.378)	5	0.879 (0.741-1.044)	1.008 (0.797-1.274)
30 - 32	0.913 (0.751-1.109)	1.014 (0.807-1.273)	6	0.854 (0.695-1.051)	0.850 (0.664-1.089)
33 - 34	1.010 (0.812-1.255)	1.110 (0.875-1.406)	7	1.043 (0.841-1.293)	1.038 (0.799-1.349)
35 - 36	0.990 (0.790-1.241)	1.063 (0.838-1.347)	8	0.865 (0.637-1.159)	0.888 (0.672-1.174)
37 - 39	1.040 (0.845-1.280)	1.078 (0.873-1.332)	≥9	1.104 (0.919-1.326)	1.121 (0.864-1.454)
40 - 55	*Referent*	*Referent*			

*Adjusted for maternal age and birth order alternatively

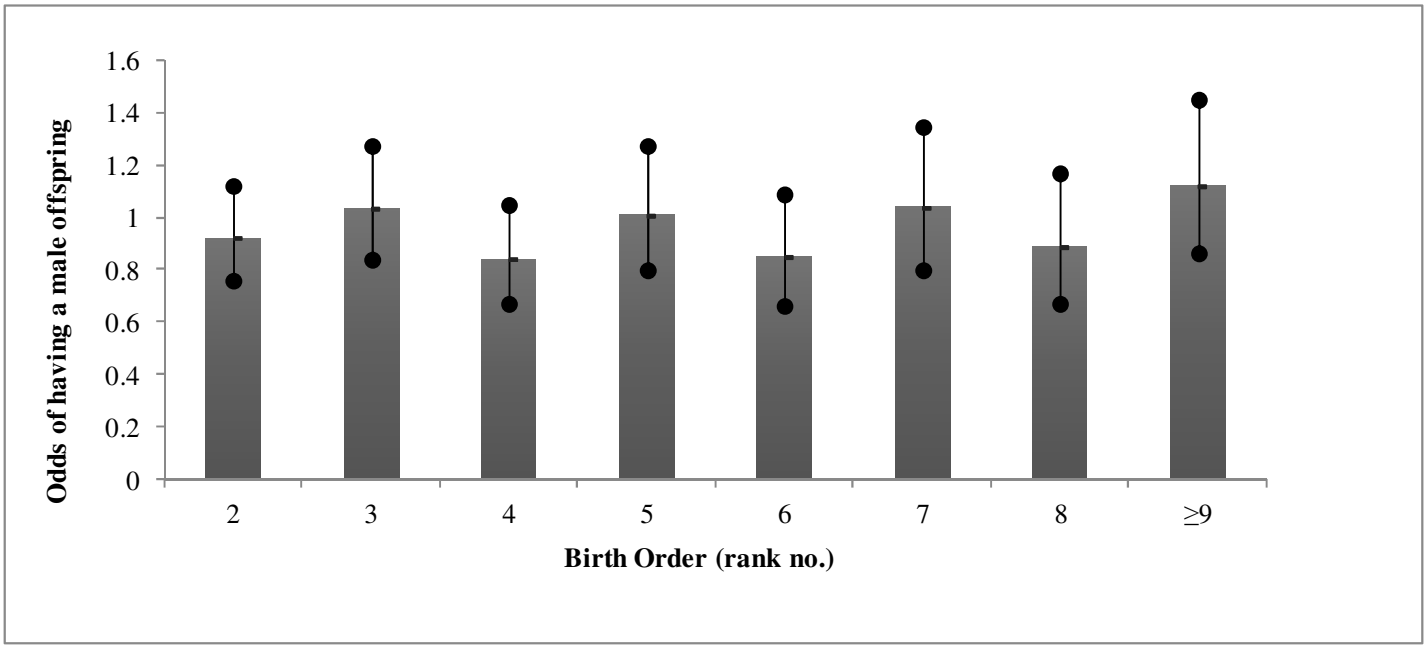
Figure 1: Distribution of Male Births By Birth Cohort Conditional on Mother's Age and Birth Order



**p-value* determined by ANOVA as described in text

Figure1. Distribution of Male Births by Birth Cohort (decile) Conditional on Mother's Age and Birth Order in the members of the Lancaster County OOA born between 1696 and 1900 (n=6689)

Figure 2: Odds of Having a Male Offspring by Birth Order Conditional on Mother's Age



*Adjusted for Maternal age at delivery

Figure 2. Odds of Having a Male Offspring Conditional on Mother's Age in the members of the Lancaster County OOA born between 1696 and 1900 (n=6689)

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