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April Dawson Date

EPIDEMIOLOGY OF TWINNING IN A POPULATION-BASED SAMPLE OF LIVE BIRTHS, 1997-2007

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EPIDEMIOLOGY OF TWINNING IN A POPULATION-BASED SAMPLE OF LIVE BIRTHS, 1997-2007

By

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2004

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ABSTRACT

EPIDEMIOLOGY OF TWINNING IN A POPULATION-BASED SAMPLE OF LIVE BIRTHS, 1997-2007

By April Dawson

PURPOSE: Twin birth rates in the United States have increased 70% over the last three decades. Twinning is associated with significantly increased risk of infant and maternal morbidity and mortality. Although previous studies have examined risk factors for twinning, most are in populations that pre-date the widespread use of fertility treatments.

METHODS: We analyzed data from the National Birth Defects Prevention Study (NBDPS), an ongoing multi-center case control study of major birth defects in the United States. The study population included NBDPS control mothers, a random sample of mothers of liveborn infants without major birth defects in the study regions, who reported gestation of twin or singleton infants. We assessed associations of twinning among mothers that did not report any use of fertility treatments with maternal demographic characteristics and periconceptional exposures using logistic regression to estimate adjusted odds ratios (aOR) and 95% confidence intervals (CI).

RESULTS: Between October 1997 and December 2007, 227 (2.7%) of 8305 mothers reported gestation of twins. Among mothers who did not report any use of fertility treatments, 154 (1.9%) of 7936 mothers reported gestation of twins. Non-Hispanic black mothers were more likely to report twin gestation than non-Hispanic white mothers (aOR 1.68, 95% CI: 1.10-2.55) and mothers of twins were more likely to smoke during pregnancy (aOR 1.64, 95% CI: 1.10-2.45) than mothers of singletons. Mothers of twins were more likely to have two or more previous live births than no previous live births (aOR: 1.49, 95% CI: 0.95-2.32) and were less likely to report periconceptional oral contraceptive use (aOR 0.59, 95% CI: 0.33-1.05) than mothers of singletons. Factors such as maternal age, height, pre-pregnancy weight, education, annual household income, and periconceptional folic acid use were not statistically significant predictors of twinning among mothers who did not report any use of fertility treatments.

CONCLUSION: Factors such as parity, oral contraceptive use, maternal race/ethnicity, and tobacco smoking may have an impact on the frequency of twinning among women who do not use fertility treatments.

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INTRODUCTION

In the United States it is estimated that approximately 3% of all live births are multiples, with twins comprising 95% of those births (1). Twinning is associated with a number of pregnancy complications and adverse maternal and fetal outcomes. Women with twin pregnancies are at higher risk for pregnancy-induced hypertension, pre-eclampsia, and gestational diabetes than mothers with singleton pregnancies (2, 3). A study of pregnancy-related morbidity and mortality found that, compared to mothers of singletons, mothers of multiples had twice the risk of mortality related to the pregnancy and were also at increased risk for preterm labor and cesarean delivery (3). Studies have also shown that twin infants are at higher risk of restricted growth, low or very low birth weight, congenital malformations, and fetal and infant mortality (4-7). The risk of adverse outcomes due to twin gestation is particularly concerning in light of the 70 percent increase in the rate of twin births in the United States since 1980, during which time the rate has increased from 18.9 to 32.1 per 1000 births (1).

Dizygotic (DZ) twinning results from the ovulation and independent fertilization of two oocytes during the same menstrual cycle. DZ twins, also referred to as "fraternal" twins, are as genetically similar as siblings of different gestation, and may be same-sex (male-male or female-female) or opposite-sex (male-female) pairs (8). Monozygotic (MZ) twinning, in contrast, occurs when a single fertilized oocyte divides to form two separate embryos during early development. MZ twins, commonly called "identical" twins, are genotypically identical and are the same gender (2). Historically, MZ twinning has been thought to be a random event, with the rate of MZ twin births observed to be fairly static at approximately 4 per 1000 pregnancies (1,4). Therefore, the variability in the rate of twinning is thought to be a function of the variability in the rate of DZ twinning.

The increase in DZ twinning that has been observed since 1980 has been largely attributed to the advent of fertility treatments and increasing maternal age. Pregnancies achieved through the use of assisted reproductive technology (ART), fertility-enhancing procedures that involve the handling of both eggs and sperm or of embryos outside the body, are known to be at a higher risk of multiple births in comparison to naturally conceived pregnancies (9). A 2003 report estimated the twin birth rate to be 444.7 per 1000 live births among ART patients in the United States in 2000, with 13.6% of all multiple births in the U.S. attributable to ART (9). Fertility treatments, other than ART, such as the use of ovulation stimulating medications (i.e. clomiphene citrate), have also been shown to provide a significant contribution the rate of twin births, with one report indicating that 20.9% of twin births in the U.S. in 2000 were attributable to non-ART fertility treatments (9). Although fertility treatments have been implicated as the leading factor in the increase in twinning rates, nearly 60 percent of all twins are the result of natural conception (8).

Although subfertility is associated with older maternal age, advanced maternal age has also been observed to be a significant predictor for DZ twinning. The association between advancing maternal age and an increased rate of twinning was first observed in 1865 by Scottish obstetrician, Matthews Duncan (10). This observation was refined in 1891 to clarify that maternal age is associated with DZ twinning only, with no effect on MZ twinning (11). In 1970, M.G. Bulmer estimated that the rate of DZ twinning increased 300% between age 15 and age 37 (11). More recently, a retrospective analysis of 1980-

1999 birth data from the National Center for Health Statistics found larger increases in the rate of twinning among women 30-34, 35-39, and 40-44 (62%, 81%, and 110% respectively) compared to women 20-24 (27%) (12).

While the increases in the occurrence of DZ twinning has been largely attributed to increasing use of fertility treatments and increasing maternal age, the literature suggests that there are many other factors that affect the natural conception rate of DZ twins. Even in the absence of fertility treatments, a family history of twinning and increased parity has been shown to increase the risk of DZ twinning (4). A study in Italy found that women with a history of multiple births in first-degree relatives had more than twice the risk of conceiving DZ twins and 2.7 times the risk for conceiving MZ twins than those that did not (13). However, other studies have only been able to confirm a relationship between family history of twinning and DZ twinning (14,15).

Like maternal age, the association between higher parity and increased rates of twinning has been observed since the 1800s (10). Although parity is highly correlated with maternal age, increased rates of twinning have been observed among multiparous women, independent of maternal age and use of fertility treatments (10). Several studies using populations that predate the widespread use of fertility-enhancing therapies found a positive association between parity and frequency of DZ twinning (10, 16-18). However, other studies have not been able to replicate these results, with some finding no association between parity and DZ twinning, and one finding a negative association between parity and MZ twinning (13, 19).

Maternal body composition has often been proposed as a factor in twinning, with higher rates of twins observed among overweight and obese women who reported spontaneous conception of twins, particularly in opposite-sex twins as compared to both same-sex twins (regardless of zygosity) and singletons (20-22). However, the literature is less clear about the individual effects of maternal height and maternal pre-pregnancy weight, with some evidence that increased height is a risk factor for DZ twinning independent of maternal weight (16, 21, 22).

The rates of twinning vary geographically, with the highest rates worldwide reported in Nigeria and the lowest in Japan (16, 23). Contributing to this phenomenon are differences in the rate of DZ twinning among different racial and ethnic groups (20). In 1970, Bulmer noted that the rate of twins among blacks was twice the rate of twins among whites (8). However, more recent reports have indicated that the historical difference in twinning among white and black mothers has largely dissipated, but also noted a lower DZ twinning rate among Hispanics when compared to non-Hispanic whites (1, 4, 24). The most recent National Vital Statistics Report estimated the rate of twins among non-Hispanic white mothers to be 36.0 per 1,000 live births in 2006, 36.8 per 1,000 live births for non-Hispanic black mothers, and 21.8 per 1,000 live births for Hispanic mothers (1).

The geographical differences in twinning rates may also be related to environmental and socioeconomic factors. A 2009 study using data from Africa and the Caribbean found an inverse relationship between socioeconomic factors, such as wealth and living conditions, and the incidence of multiple births of unassisted conception (25). In contrast, a report from South Korea, found parents of twins to be at a higher socioeconomic status, as determined by completion of higher levels of education, in comparison to the parents of singletons, although this study did not control for use of assisted reproductive therapies (26).

Maternal smoking has also been associated with an increased risk of twinning. A recent study of a retrospective cohort from the Netherlands Twin Register, comparing mothers of MZ and DZ twins, found an association between any smoking during pregnancy and DZ twinning (22). However, other studies have only been able to replicate this finding among women who were heavy smokers (13, 27).

Periconceptional folic acid and oral contraceptive use have been studied as possible factors in DZ twinning. Folic acid supplementation is recommended for all women of childbearing age as it has been shown to reduce the risk of neural tube defects in offspring (28). In 1994, results from a randomized controlled trial of periconceptional multivitamin supplementation in Hungary indicated that the rate of multiple births was higher in women receiving multivitamins containing 0.8 mg of folic acid in comparison to women receiving only trace elements (29, 30). Following this report, a number of studies have been conducted to examine the association between multivitamin-containing folic acid supplementation and twinning, however most have found no association (31-33).

In the 1970's a slight decline in worldwide twinning rates over the preceding two decades was observed, with speculation that this decline was partly due to the increased use of oral contraception (34-36). However, the results of studies examining the association between the recent discontinuation of oral contraceptives and twinning have been widely variable, with some finding an increased risk of DZ or MZ twinning (37-39), some finding no association (35), and some finding a decreased risk of DZ or MZ twinning (40).

Although twinning is a phenomenon that has been studied extensively, much of the work involving risk factors for twinning of unassisted conception was conducted in populations that predate the modern ART era. Further, the results of many of these studies are limited due to small numbers or inadequate control of covariates. In the present study, we propose to use data from the control participants in the National Birth Defects Prevention Study to evaluate factors associated with twinning among mothers of children without major birth defects.

METHODS AND MATERIALS

Study Population

The National Birth Defects Prevention Study (NBDPS) is an ongoing multi-center case control study of major birth defects in the United States. The NBDPS is a collaborative effort of the Centers for Birth Defects Research and Prevention in Arkansas, California, Georgia, Iowa, Massachusetts, North Carolina, New Jersey, New York, Texas, and Utah. Study methods for recruitment of participants and data collection for the NBDPS have been described in detail elsewhere (40-42). Briefly, NBDPS cases include infants with major birth defects, identified through population-based surveillance systems in each state. NBDPS controls, live born infants with no major birth defects, are selected at random from birth certificates or birth hospital records. Only one infant per family is eligible for participation in the NBDPS. This analysis was limited to mothers of NBDPS controls, of which there were 8078 mothers of singleton infants and 227 mothers of a twin infant, with expected dates of delivery between October 1, 1997 and December 31, 2007. Mothers of higher order multiples were excluded from the analysis (n=23).

Exposures of Interest

For this study we were interested in examining maternal demographics and periconceptional exposures that are commonly cited in the literature as important predictors of twinning. Information on exposure was obtained by computer-assisted telephone interview, which consisted of questions detailing demographic information and exposures that occurred from three months prior to conception through the end of the pregnancy. Mothers were interviewed, in English or Spanish, between six weeks and 24 months after their expected date of delivery (EDD).

We defined fertility treatment use as any use of fertility-enhancing medications or procedures by either the mother or father, which included ART and non-ART treatments (yes, no). One specific non-ART treatment that we examined, individually, was the use of clomiphene citrate (yes, no). Maternal age at delivery was categorized as less than 25 years, 25-29, 30-34, 35-39, and greater than or equal to 40 years of age. Parity was categorized as no previous live births, one previous live birth, and more than one previous live birth. Maternal race/ethnicity was categorized as non-Hispanic white, non-Hispanic black, Hispanic, and other.

In order to assess the relationship between maternal body composition and twinning, we were interested in the independent effects of maternal height and maternal pre-pregnancy weight, in addition to examining the combined effects as represented by maternal pre-pregnancy body mass index. To maintain consistency with previous analyses of twinning and body composition, maternal height and maternal pre-pregnancy weight were categorized as quartiles (height: less than159, 159-163, 164-168, and greater than 168 cm; pre-pregnancy weight: less than 57, 57-64, 65-74, greater than 74 kg) (17-19). Pre-pregnancy body mass index was calculated by dividing maternal pre-pregnancy body weight (in kilograms) by height (in meters) squared and categorized as underweight (less than 18.5), normal weight (18.5-24.9), overweight (25-29.9), and obese (greater than or equal to 30) (43).

To assess the relationship between socioeconomic status and twinning, mother's education and household income were used as proxy measures. The mother's highest level of education ascertainment was categorized as less than high school graduate, high school graduate, and greater than high school. Mother's annual household income in the year prior to the pregnancy in question was categorized as less than $10,000, between $10,000 and $50,000, and greater than $50,000 per year.

Maternal periconceptional smoking was categorized as any report of smoking from the month prior to conception through the third month of pregnancy (yes, no). Oral contraceptive use was defined as any use during the month prior to conception through the infant's date of birth (yes, no). Folic acid use was defined as any use of a multivitamin containing folic acid during the month prior to conception through the first month of pregnancy (yes, no). Other variables that were considered as possible covariates in the analysis were the study site and year of expected date of delivery.

Outcome of Interest

The outcome of interest in this analysis was twinning, as compared to singleton gestation among mothers of live-born infants without any major birth defects. The primary method of ascertainment of information on plurality was the computer-assisted telephone interview. During the interview, mothers were asked, "In this pregnancy, how many babies were you carrying?" If necessary, a second question was asked, "Did you have a single baby, twins, or more babies?" A secondary source of information for plurality was the study infant's birth certificate or mother's medical record. In the event of discrepancies between the two sources regarding plurality, we deferred to the interview response in order to minimize underreporting of twin gestations in which early fetal demise of the co-twin occurred.

Analysis

Unadjusted odds ratios and corresponding 95 percent confidence intervals were calculated for all of the exposures of interest, to assess the relationship between each variable and twinning, regardless of conception method.

Because we were primarily interested in the factors associated with the unassisted conception of twins, mothers who received treatments for infertility, either assisted reproduction techniques or treatment with ovulation-inducing medication, were excluded from the adjusted analysis (73 mothers of twins, 296 mothers of singletons excluded). To calculate adjusted odds ratios and corresponding 95 percent confidence intervals for each exposure of interest, directed acyclic graphs (DAGs) were constructed to determine which variables would be considered as possible confounders (see Appendix). A multivariable logistic regression model was constructed for each exposure, including all possible confounders as determined by the DAG. Likelihood ratio tests were conducted to evaluate statistical interaction between the exposure of interest and potential confounders.

Finally, to assess factors associated with twinning among mothers that received assisted conception, descriptive statistics and unadjusted odds ratios with corresponding 95% confidence intervals were calculated for each exposure of interest. Due to the small sample size of mothers who reported use of fertility treatments, we were unable to estimated adjusted odds ratio. SAS version 9.2 (Cary, NC) was used to conduct all analyses. This project was reviewed and approved under the expedited review process of the Emory University Institutional Review Board.

RESULTS

Among the 8,341 mothers who completed the maternal interview and delivered a live-born infant with no major birth defects between October 1, 1997 and December 31, 2007, 99.9% (N=8,328) reported the plurality of the pregnancy in question. After excluding mothers of higher order multiples (n=23), 227 (2.7%) of 8305 mothers reported gestation of twins.

The demographic characteristics of mothers of control infants along with the respective unadjusted odds ratios are presented in Table 1. 32.2% of mothers of twins reported use of any fertility treatment compared to 3.7% of mothers of singletons. Use of clomiphene citrate was also higher among mothers of twins (8.4%) compared to mothers of singletons (1.6%). The proportion of twins was highest among mothers from Massachusetts (4.9%) and New Jersey (4.7%), and lowest in California (0.8%). Among all mothers of controls, the frequency of twinning increased with increasing maternal age: 58.6% of mothers of twins were age 30 years or greater, compared to 38.6% of mothers of singletons. Only 1.4% of Hispanic mothers reported twin gestation, compared to 3.2% of non-Hispanic white mothers, 3.5% of non-Hispanic black mothers, and 2.4% of mothers who reported other race/ethnicity. Mothers of twins were more likely to report use of a folic acid containing multivitamin use during the month prior to pregnancy through the first month of the pregnancy compared to mothers of singletons. Additionally, mothers who reported a household income greater than $50,000 were more likely to be mothers of twins than mothers who reported a household income between $10,000 and $50,000. The frequency of twinning was greatest among mothers that completed more than 12 years of education (Table 1). Chi square tests for trend were significant for maternal age at delivery and education (*p* <0.0001), but were not significant (*p* > 0.05) for maternal height, pre-pregnancy weight, pre-pregnancy body mass index, parity, household income, or year of expected date of delivery.

Of the 7,936 mothers who did not report any use of fertility treatments, 154 (1.9%) mothers reported twin gestation. Among mothers who did not report any use of fertility treatments and when controlling for study site, Non-Hispanic black mothers were more likely to deliver twins than non-Hispanic white mothers (OR: 1.68; 95% CI: 1.10-2.55). When controlling for maternal age, race/ethnicity, education, household income, parity, study site, and year of EDD, a significant association was observed between maternal periconceptional smoking and twinning (OR: 1.64; 95% CI: 1.10-2.45). Women aged 35 to 39 years were 1.51 times more likely to be mothers of twins than women aged 25 to 29 years, after controlling for maternal race/ethnicity, education, income, state of maternal residence and year of EDD (OR: 1.51; 95% CI: 0.91-2.50). An association between having more than one previous live birth and twinning was observed in the crude analysis (OR: 1.67; 95% CI: 1.19-2.49), however after controlling for maternal age, race/ethnicity, education, household income, and state of maternal residence, this association was observed to be attenuated and of only borderline statistical significance (OR: 1.49; 95% CI: 0.95-2.32). Oral contraceptive use was negatively associated with twinning, although the adjusted odds ratio was of borderline statistical significance (OR: 0.59; 95% CI: 0.33-1.05). No significant association was observed between twinning and body composition (height, weight, or BMI), education, household income, or folic acid containing multivitamin use among mothers who reported no use of fertility treatments (Table 2).

Among the 368 mothers who reported any use of fertility treatments, 73 (18.9%) reported twin gestation. We observed several crude associations with twinning among mothers who reported use of fertility treatments. In comparison to mothers age 25-29, maternal age at delivery of 30-34 (OR: 2.76, 95% CI: 1.27-6.02) and 35-39 (OR: 2.57, 95% CI: 1.09-6.04) was significantly associated with twinning. 84.7% of all mothers reporting fertility treatment use were non-Hispanic white, with the proportion of non-Hispanic white mothers slightly higher among mothers of twins (90.4%) than mothers of singletons (83.4%). Compared to mothers less than 159 cm tall, mothers 159-163 cm were significantly less likely to report twin gestation (OR: 0.39; 95% CI: 0.17-0.94), however, this trend did not continue for taller women. Additionally, a significant association was observed between having more than a high school education and twinning (OR: 3.08; 95% CI: 1.07-8.87). Compared to mothers with an annual household income between $10,000 and $50,000, mothers with an annual household income greater than $50,000 were 4.64 times more likely to report twin gestation (95% CI: 2.04-10.57). A significant association was also observed between folic acid use and twinning (OR 3.19; 95% CI: 1.11-9.12). No significant associations were observed between twinning and pre-pregnancy weight or body mass index, maternal smoking, oral contraceptive use, or state of maternal residence among mothers who reported any use of fertility treatments (Table 3).

Figure 1 depicts the distribution of maternal age at delivery by plurality and report of fertility treatments. The mean age for mothers that reported any use of fertility treatments was higher for both mothers of singletons (32.2 years) and mothers of twins (34.0 years) than for mothers who did not report any use of fertility treatments (27.8 and 29.0 years, respectively). The overall appearance of the distributions was more similar within the fertility treatment groups than within the plurality groups. A similar figure depicting maternal pre-pregnancy weight by plurality and report of fertility treatments supported that maternal pre-pregnancy weight did not appear to be meaningfully different between treatment groups or between mothers of twins and singletons (Figure 2).

DISCUSSION

Factors associated with twinning have been studied since the late 1800's. Following the advancement of fertility-enhancing therapies in the late 1970's, the focus of this work has shifted away from factors other than fertility treatments. For this study, we evaluated a number of demographic and periconceptional exposures among control mothers in a large population-based study and stratified by fertility treatment usage in order to identify risk factors for twinning in both groups.

We observed that twin pregnancies were a relatively common occurrence among control mothers in the National Birth Defects Prevention Study, with approximately 2.7% of mothers reporting gestation of twins. Among all control mothers, we observed a significantly higher frequency of twinning in Massachusetts participants compared to participants from other sites. However, this association is likely to be confounded by fertility treatment use, as a previous study found that use of assisted reproductive technologies was significantly higher in participants from Massachusetts compared to other study sites in this population (45). In addition, this association was not observed in analyses restricted to mothers reporting use of fertility treatments.

Among all control mothers, maternal age was found to be a significant predictor of twinning, with mothers 35 to 39 having the highest risk of twins. However, after stratifying by fertility treatment use this association was only significant for mothers that reported use of fertility treatments. A comparison of descriptive statistics for maternal age, stratified by fertility treatments and plurality, indicated that the distributions of maternal age were more similar within fertility treatment groups than within plurality groups (Figure 1). Based on these results, it appears that maternal age may be an important predictor of fertility treatment use, but may not be as important in twinning. Our findings are in contrast to previous studies that have found advanced maternal age to be significantly associated with twinning. This discrepancy with previously reported data may be the result of our outcome measure, which included all mothers of twins regardless of zygosity, as many of the previous studies found an association with twinning in mothers of DZ twins but not mothers of MZ twins (8, 11, 18, 19). Although our sample size was relatively large, there were small numbers of older mothers, which may have limited our ability to observe an association.

In the analysis of all control mothers, the associations observed for maternal pre-pregnancy weight, socioeconomic factors, race/ethnicity, were consistent with previous findings (4, 9, 12, 20, 26). However, because fertility treatment use is such a strong predictor of twinning in our analysis (OR: 12.51; 95% CI: 9.25-16.91), the interpretation of these findings is limited because many of these factors are also associated with use of fertility treatments.

Among mothers that did not report any use of fertility treatments, non-Hispanic black mothers were significantly more likely to report a twin pregnancy than non-Hispanic white mothers, which is consistent with previous reports that found twin birth rates in the United States were highest among non-Hispanic black women (1, 11).

Among mothers who did not report any use of fertility treatments, maternal pre-pregnancy weight, education, and household income were not significantly associated with twinning. In contrast, maternal education and household income were significantly associated with twinning in the unadjusted analysis of mothers reporting use of fertility treatments. However, we were unable to estimate adjusted odds ratio due to small sample size and it is likely that these unadjusted estimates are confounded by maternal age and race/ethnicity. Although fertility treatment use can be considered an intermediate variable in the causal path of many of our exposures of interest (see Appendix), these results underscore the need to stratify by fertility treatment use in order to estimate the associations of our exposures of interest among the majority of the population who do not use fertility treatments.

Among mothers who did not report any use of fertility treatments, mothers of twins were more likely to report two or more previous births and were more likely to report smoking during the period from one month prior to conception through the third month of pregnancy. These results are consistent with previous findings (10-11, 16-18, 22).

Oral contraceptive use from one month prior to conception through the infant's date of birth was observed to have a negative association with twinning in mothers who did not report any use of fertility treatments. This association is consistent with previous findings, supporting a decreased frequency in the conception of twins following recent discontinuation of oral contraceptives (34-36).

In unadjusted analyses, folic acid containing multivitamin use during pregnancy was observed to be significantly associated with twinning both among all control mothers and mothers reporting fertility treatment use. However, when we adjusted for several factors, including maternal age and race/ethnicity (for mothers not reporting use of fertility treatments), this association was no longer observed, which is consistent with previous studies (31-33).

This study is limited by the use of self-reported data from a retrospective maternal interview, so there is the possibility of exposure misclassification due to inaccurate recall.

There may also have been differential recall of exposures depending on use of fertility treatments; however this should not have substantially impacted our results as our adjusted analyses were restricted to mothers who did not report any use of fertility treatments. We were not able to verify the accuracy or validity of the responses for the periconceptional exposures, nor were we able to verify the use of fertility treatments. The women were interviewed up to 2 years after the birth of their child and the timing of the interviews varied between individuals. This analysis was limited to mothers that completed the maternal interview, which may have led to selection bias in this sample. However, a recent analysis found that maternal and infant characteristics of NBDPS control participants were generally similar to those of their base population (48).

We compared the plurality reported by the mothers in the interview with information recorded on the infant's birth certificate or mother's hospital record. These records indicated some discrepancies in plurality, with mothers reporting higher rates of twin gestation in the interviews than reflected in the vital record. Although studies have found a high degree of concordance between plurality reported by maternal surveys and on birth certificates, some variability does exist and may be due, in part, to early fetal demise of one of the co-twins (46).

Another limitation of this study was the inability to determine zygosity of the twin infants, as only one infant in each twin pair was eligible to be included in this study. This is an important consideration as many demographic factors and periconceptional exposures have only been observed to have associations with DZ twinning (10, 11, 22). Therefore, we would anticipate that the associations we have observed would have been stronger among DZ twins than among all twins. Future work will involve linking study participant records with birth certificate data for the co-twin. This will allow us to obtain information on the gender of the co-twin, and conduct analyses with opposite sex twins as a proxy for the dizygotic twins in this sample.

Strengths of this study include use of a population-based sample from ten different states across the United States, which provided an economically and demographically diverse sample. Although we did not have information on one important confounder, family history of twinning, through the use of a detailed questionnaire we were able to control for and assess a variety of factors associated with twinning. This analysis provides an analysis of demographic factors and periconceptional exposures among a modern sample of mothers that reported use of fertility treatments in addition to mothers that reported unassisted conception of twins. The literature is limited concerning demographic and periconceptional exposures associated with twinning among women who reported use of fertility treatments, and therefore even though our sample size was sufficient only to estimate crude associations, the NBDPS data allowed us to contribute to the twinning literature.

Although fertility treatment use was found to be the strongest predictor of twinning, this study suggests that use of fertility treatments is not the sole contributing factor in the dramatic increase in twinning that has been observed over the past three decades. Other factors such as parity, oral contraceptive use, maternal race/ethnicity, and tobacco smoking may have an impact and should be examined further. There also appear to be characteristics that may make women who receive fertility treatments more likely to have a twin gestation. Identifying these characteristics may help women make more informed decisions about their fertility treatment options, and may help physicians tailor their patient’s treatment plan, such as type of treatment or number of embryos to implant, in order to minimize the risk of a twin pregnancy.

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Table 1: Twinning among mothers of live-born infants with no major birth defects by demographic and lifestyle factors, National Birth Defects Prevention Study, 1997-2007.

Maternal characteristics	**Mothers of twins (n=227)**	**Mothers of singletons (n=8078)**	**Unadjusted odds ratio (95% Confidence interval)**
	N (%)	N (%)	
Age at Delivery (years)			
< 25	39 (17.2%)	2728 (33.8%)	**0.58 (0.38-0.88)**
25-29	55 (24.2%)	2228 (27.6%)	Referent
30-34	79 (34.8%)	2015 (24.9%)	**1.59 (1.12-2.25)**
35-39	47 (20.7%)	940 (11.6%)	**2.03 (1.36-3.01)**
≥ 40	7 (3.1%)	167 (2.1%)	1.69 (0.75-3.79)
Maternal race/ethnicity			
Non-Hispanic white	156 (68.7%)	4757 (58.9%)	Referent
Non-Hispanic black	32 (14.1%)	892 (11.0%)	1.09 (0.74-1.61)
Hispanic	27 (11.9%)	1869 (23.1%)	**0.44 (0.29-0.67)**
Other	12 (5.3%)	486 (6.0%)	0.75 (0.42-1.36)
Fertility treatment use (any)			
Yes	73 (32.2%)	295 (3.7%)	**12.51 (9.25-16.91)**
No	154 (67.8%)	7782 (96.3%)	Referent
Clomiphene citrate use			
Yes	19 (8.4%)*	129 (1.6%)	**7.44 (4.48-12.36)**
No	154 (67.8%)	7782 (96.3%)	Referent
Height (cm)			
< 159	52 (22.9%)	2039 (25.2%)	Referent
159-163	56 (24.7%)	2060 (25.5%)	1.07 (0.73-1.56)
164-168	53 (23.4%)	1836 (22.7%)	1.13 (0.77-1.67)
> 168	62 (27.3%)	1855 (23.0%)	1.31 (0.90-1.91)
Pre-pregnancy weight (kg)			
< 57	49 (21.6%)	2034 (25.2%)	Referent
57-64	56 (24.7%)	2286 (28.3%)	1.02 (0.69-1.50)
65-75	66 (29.1%)	1841 (22.8%)	**1.49 (1.02-2.17)**
> 75	52 (22.9%)	1852 (22.9%)	1.17 (0.79-1.73)
Pre-pregnancy body mass index (kg/m^2)			
Underweight (< 18.5)	10 (4.4%)	420 (5.2%)	0.87 (0.45-1.67)
Normal (18.5-24.9)	117 (51.5%)	4268 (52.8%)	Referent
Overweight (25-29.9)	55 (24.2%)	1765 (21.9%)	1.14 (0.82-1.57)
Overweight (≥ 30)	37 (16.3%)	1297 (16.1%)	1.04 (0.72-1.51)
Parity			
0	93 (41.0%)	3230 (40.0%)	1.03 (0.76-1.40)
1	75 (33.0%)	2687 (33.3%)	Referent
2+	59 (26.0%)	2160 (26.7%)	0.98 (0.69-1.38)

* Percentages may not sum to 100 due to missing values

Table 1 (continued): Twinning among mothers of live-born infants with no major birth defects by demographic and lifestyle factors, National Birth Defects Prevention Study, 1997-2007

Maternal characteristics	**Mothers of twins (n=227)**	**Mothers of singletons (n=8078)**	**Unadjusted odds ratio (95% Confidence interval)**
	N (%)	N (%)	
Education			
< High school graduate	22 (9.7%)	1401 (17.3%)	0.74 (0.44-1.24)
High school graduate	42 (18.5%)	1968 (24.4%)	Referent
> High school graduate	163 (71.8%)	4702 (58.2%)	**1.62 (1.15-2.29)**
Annual household income			
< $10,000	26 (11.5%*)	1439 (17.8%)	0.88 (0.56-1.39)
$10,000-$50,000	70 (30.8%)	3425 (42.4%)	Referent
> $50,000	113 (49.8%)	2543 (31.5%)	**2.17 (1.61-2.94)**
Smoking (B1-P3)[1]			
Yes	41 (18.1%)	1494 (18.5%)	0.99 (0.94-1.05)
No	186 (81.9%)	6580 (81.5%)	Referent
Oral contraceptive use (B1-DOIB)[2]			
Yes	26 (11.5%)	1096 (13.6%)	0.74 (0.45-1.22)
No	39 (17.8%)	1214 (15.0%)	Referent
Folic acid use (B1-P1)[3]			
Yes	142 (62.60%)	4102 (50.8%)	**1.62 (1.23-2.13)**
No	85 (37.4%)	3976 (49.2%)	Referent
Study site			
Arkansas	18 (7.9%)	1031 (12.8%)	0.62 (0.33-1.15)
California	8 (3.5%)	1005 (12.4%)	**0.28 (0.13-0.63)**
Georgia	24 (10.6%)	851 (10.5%)	Referent
Iowa	36 (15.9%)	890 (11.0%)	1.43 (085-2.43)
Massachusetts	51 (22.5%)	967 (12.0%)	**1.87 (1.12-3.06)**
New Jersey	27 (11.9%)	545 (6.8%)	1.76 (1.00-3.08)
New York	12 (5.3%)	704 (8.7%)	0.60 (0.30-1.22)
North Carolina	14 (6.2%)	552 (6.8%)	0.89 (0.46-1.75)
Texas	20 (8.8%)	943 (11.7%)	0.75 (0.41-1.37)
Utah	17 (7.5%)	590 (7.3%)	1.02 (0.54-1.92)

* Percentages may not sum to 100 due to missing values

[1] Any reported use 1 month prior to pregnancy through three months after conception

[2] Any reported use 1 month prior to pregnancy through the date of infant's birth

[3] Any reported use 1 month prior to pregnancy through one month after conception

Table 2: Twinning among mothers reporting no use of fertility treatments by demographic and lifestyle factors, National Birth Defects Prevention Study, 1997-2007

Maternal Characteristic	**Mothers of twins (n= 154)**	**Mothers of singletons (n=7782)**	**Unadjusted odds ratio (95% confidence interval)**	**Adjusted odds ratio (95% confidence interval)**
Age at delivery (years)				
< 25	39 (25.3%)	2710 (34.8%)	0.67 (0.44-2.34)	0.73[a] (0.46-1.17)
25-29	46 (29.9%)	2153 (27.7%)	Referent	Referent
30-34	39 (25.3%)	1896 (24.4%)	0.96 (0.63-1.48)	1.01 (0.64-1.58)
35-39	27 (17.5%)	876 (11.3%)	1.44 (0.89-2.34)	1.51 (0.91-2.50)
≥ 40	3 (2%)	147 (1.9%)	0.96 (0.29-3.11)	0.98 (0.30-3.21)
Race/ethnicity				
Non-Hispanic white	90 (58.4%)	4511 (57.9%)*	Referent	Referent
Non-Hispanic black	30 (19.5%)	880 (11.3%)	**1.71 (1.12-2.60)**	**1.68[b] (1.10-2.55)**
Hispanic	25 (16.2%)	1844 (23.7%)	0.68 (0.44-1.06)	0.67 (0.43-1.05)
Other	9 (5.8%)	477 (6.1%)	0.95 (0.47-1.89)	0.92 (0.46-1.85)
Height (cm)				
< 159	34 (22.1%)	1979 (25.4%)	Referent	Referent
159-163	47 (30.5%)	1985 (25.5%)	1.38 (0.88-2.15)	1.33[c] (0.85-2.08)
164-168	34 (22.1%)	1770 (22.7%)	1.12 (0.69-1.81)	1.07 (0.65-1.73)
> 168	35 (22.7%)	1763 (22.7%)	1.16 (0.72-1.86)	1.07 (0.65-1.75)
Pre-pregnancy weight (kg)				
< 57	35 (22.7%)	1985 (25.5%)	Referent	Referent
57-64	37 (24%)	2204 (28.8%)	0.95 (0.59-1.52)	0.91[d] (0.55-1.52)
65-75	43 (27.9%)	1774 (22.8%)	1.38 (0.88-2.16)	1.39 (0.85-2.29)
> 75	35 (22.7%)	1754 (22.5%)	1.13 (0.71-1.82)	1.13 (0.67-1.91)
Pre-pregnancy body mass index (kg/m^2)				
Underweight (<18.5)	7 (4.6%)	413 (5.3%)	0.90 (0.41-1.95)	1.14[e] (0.52-2.50)
Normal (18.5-24.9)	78 (50.7%)	4118 (52.9%)	Referent	Referent
Overweight (25-29.9)	34 (22.1%)	1697 (21.8%)	1.06 (0.70-1.59)	1.11 (0.73-1.69)
Obese (≥ 30)	27 (17.5%)	1229 (15.8%)	1.16 (0.75-1.81)	1.23 (0.78-1.95)

*Percentages may not add to 100 due to missing values
[a]Adjusted for maternal race/ethnicity, education level, annual household income, study site, year of EDD [b]Adjusted for study site [c]Adjusted for year of mother's birth, maternal race/ethnicity, study site [d]Adjusted for maternal age, race/ethnicity, height, education level, annual household income, parity, study site, year of EDD [e]Adjusted for maternal age, race/ethnicity, education level, annual household income, parity, study site, year of EDD

Table 2 (continued): Twinning among mothers reporting no use of fertility treatments by demographic and lifestyle factors, National Birth Defects Prevention Study, 1997-2007

Maternal Characteristic	**Mothers of twins (n= 154)**	**Mothers of singletons (n=7782)**	**Unadjusted odds ratio (95% confidence interval)**	**Adjusted odds ratio (95% confidence interval)**
Parity				
0	46 (29.9%)	3068 (39.4%)	Referent	Referent
1	55 (35.7%)	2593 (33.3%)	1.42 (0.95-2.10)	1.36[f] (0.89-2.07)
2+	53 (34.4%)	2120 (27.2%)	**1.67 (1.19-2.49)**	1.49 (0.95-2.32)
Education				
< High school graduate	21 (13.6%)	1392 (17.9%)	0.76 (0.45-1.31)	0.70[g] (0.38-1.27)
High school graduate	38 (24.7%)	1924 (24.7%)	Referent	Referent
> High school graduate	95 (61.7%)	4459 (57.3%)	1.08 (0.74-1.58)	0.86 (0.57-1.30)
Annual household income				
< $10,000	25 (16.2%)	1422 (18.3%)	0.93 (0.58-1.48)	1.10[h] (0.67-1.82)
$10,000-$50,000	63 (40.9%)	3332 (42.8%)	Referent	Referent
> $50,000	54 (35.1%)	2374 (30.5%)	1.20 (0.83-1.74)	0.96 (0.63-1.44)
Smoking (B1-P3)[1]				
Yes	38 (24.7%)	1466 (18.8%)	1.41 (0.97-2.04)	**1.64[e] (1.10-2.45)**
No	116 (75.3%)	6312 (81.1%)	Referent	Referent
Oral contraceptive use (B1-DOIB)[2]				
Yes	20 (13%)	1079 (13.9%)	0.62 (0.36-1.08)	0.59[f] (0.33-1.05)
No	36 (23.4%)	1206 (15.5%)	Referent	Referent
Folic acid use (B1-P1)[3]				
Yes	73 (47.4%)	3853 (49.5%)	0.92 (0.67-1.27)	0.83[e] (0.58-1.19)
No	81 (52.6%)	3929 (50.5%)	Referent	Referent

*Percentages may not add to 100 due to missing values
[1] Any reported use 1 month prior to pregnancy through three months after conception [2] Any reported use 1 month prior to pregnancy through the date of infant's birth [3] Any reported use 1 month prior to pregnancy through one month after conception
[e] Adjusted for maternal age, race/ethnicity, education level, annual household income, parity, study site, year of EDD [f] Adjusted for maternal age, race/ethnicity, education level, annual household income, study site [g] Adjusted for maternal age, race/ethnicity, annual household income, study site, year of EDD [h] Adjusted for maternal age, race/ethnicity, education level, study site, year of EDD

Table 3: Twinning among mothers reporting any use of fertility treatments by demographic and lifestyle factors, National Birth Defects Prevention Study, 1997-2007

Maternal Characteristic	**Mothers of twins (n= 73)**	**Mothers of singletons (n=295)**	**Unadjusted odds ratio (95% confidence interval)**
Age at delivery (years)			
< 25	---	18 (6.1%)	[1]
25-29	9 (12.3%)	74 (25.1%)	Referent
30-34	40 (54.8%)	119 (40.3%)	**2.76 (1.27-6.02)**
35-39	20 (27.4%)	64 (21.7%)	**2.57 (1.09-6.04)**
≥ 40	4 (5.5%)	20 (6.8%)	1.64 (0.46-5.89)
Maternal race/ethnicity			
Non-Hispanic white	66 (90.4%)	246 (83.4%)*	Referent
Non-Hispanic black	2 (2.7%)	12 (4.1%)	[1]
Hispanic	2 (2.7%)	24 (8.1%)	[1]
Other	3 (4.1%)	9 (3.1%)	1.24 (0.33-4.72)
Height (cm)			
< 159	18 (24.7%)	59 (20%)	Referent
159-163	9 (12.3%)	75 (25.4%)	**0.39 (0.17-0.94)**
164-168	19 (26%)	66 (22.4%)	0.94 (0.45-1.97)
> 168	27 (37%)	92 (31.2%)	0.96 (0.49-1.89)
Pre-pregnancy weight (kg)			
< 57	14 (19.2%)	49 (16.6%)	Referent
57-64	19 (26%)	81 (27.5%)	0.82 (0.38-1.78)
65-74	23 (31.5%)	67 (22.7%)	1.20 (0.56-2.57)
> 74	17 (23.3%)	98 (33.2%)	0.61 (0.28-1.33)
Pre-pregnancy body mass index (kg/m^2)			
Underweight (<18.5)	3 (4.1%)	7 (2.4%)	1.64 (0.41-6.63)
Normal (18.5-24.9)	39 (53.4%)	149 (50.5%)	Referent
Overweight (25-29.9)	21 (28.8%)	68 (23.1%)	1.18 (0.65-2.16)
Obese (≥ 30)	10 (13.7%)	68 (23.1%)	0.56 (0.27-1.19)
Parity			
0	47 (64.4%)	162 (54.9%)	Referent
1	20 (27.4%)	93 (31.5%)	0.74 (0.41-1.33)
2+	6 (8.2%)	40 (13.6%)	0.52 (0.21-1.29)

* Percentages may not sum to 100 due to missing values

[1] OR not calculated for cell size ≤ 2

Table 3 (continued): Twinning among mothers reporting any use of fertility treatments by demographic and lifestyle factors, National Birth Defects Prevention Study, 1997-2007

Maternal Characteristic	**Mothers of twins (n= 73)**	**Mothers of singletons (n=295)**	**Unadjusted odds ratio (95% confidence interval)**
Education			
Less than high school graduate	1 (1.4%)	8 (2.7%)	[1]
High school graduate	4 (5.4%)	44 (14.9%)	Referent
More than high school graduate	68 (93.2%)	243 (82.4%)	**3.08 (1.07-8.87)**
Annual household income			
< $10,000	1 (1.4%)*	17 (5.8%)	[1]
$10,000-$50,000	7 (9.6%)	93 (31.5%)	Referent
> $50,000	59 (80.8%)	169 (57.3%)	**4.64 (2.04-10.57)**
Smoking (B1-P3)[2]			
Yes	3 (4.1%)	28 (9.5%)	0.41 (0.12-1.38)
No	70 (95.9%)	267 (90.5%)	Referent
Oral contraceptive use (B1-DOIB)[3]			
Yes	6 (8.2%)	17 (5.8%)	0.94 (0.19-4.76)
No	3 (4.1%)	8 (2.7%)	Referent
Folic acid use (B1-P1)[4]			
Yes	69 (94.5%)	249 (84.4%)	**3.19 (1.11-9.12)**
No	4 (5.5%)	46 (15.6%)	Referent
Study site			
Arkansas	3 (4.1%)	36 (12.2%)	0.35 (0.08-1.52)
California	----	19 (6.4%)	[1]
Georgia	6 (8.2%)	25 (8.5%)	Referent
Iowa	12 (16.4%)	50 (17.0%)	1.00 (0.34-2.98)
Massachusetts	30 (41.1%)	55 (18.6%)	2.27 (0.84-6.15)
New Jersey	11 (15.1%)	15 (5.1%)	3.05 (0.94-9.97)
New York	3 (4.1%)	26 (8.8%)	0.48 (0.11-2.13)
North Carolina	2 (2.7%)	19 (6.4%)	[1]
Texas	1 (1.4%)	23 (7.8%)	[1]
Utah	5 (6.9%)	27 (9.2%)	0.77 (0.21-2.85)

* Percentages may not sum to 100 due to missing values

[1] OR not calculated for cell size ≤ 2

[2] Any reported use 1 month prior to pregnancy through three months after conception

[3] Any reported use 1 month prior to pregnancy through the date of infant's birth

[4] Any reported use 1 month prior to pregnancy through one month after conception

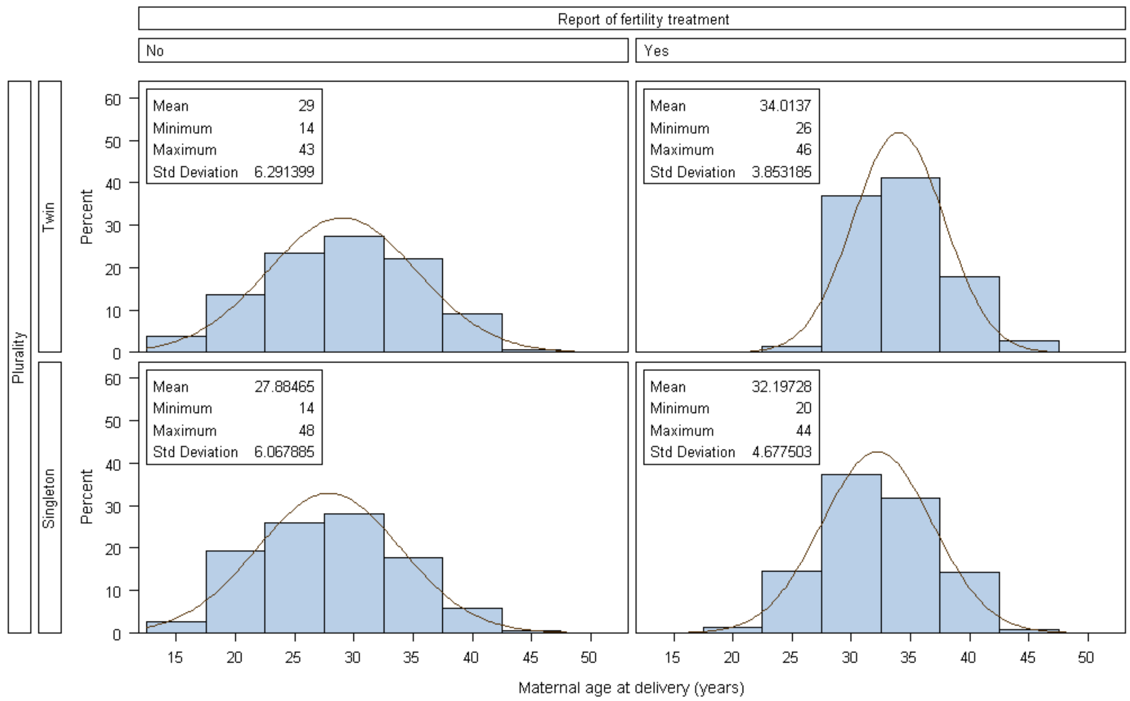

Figure 1: Distribution and descriptive statistics for maternal age by plurality and report of fertility treatment among mothers of liveborn infants with no major birth defects, National Birth Defects Prevention Study, 1997-2007

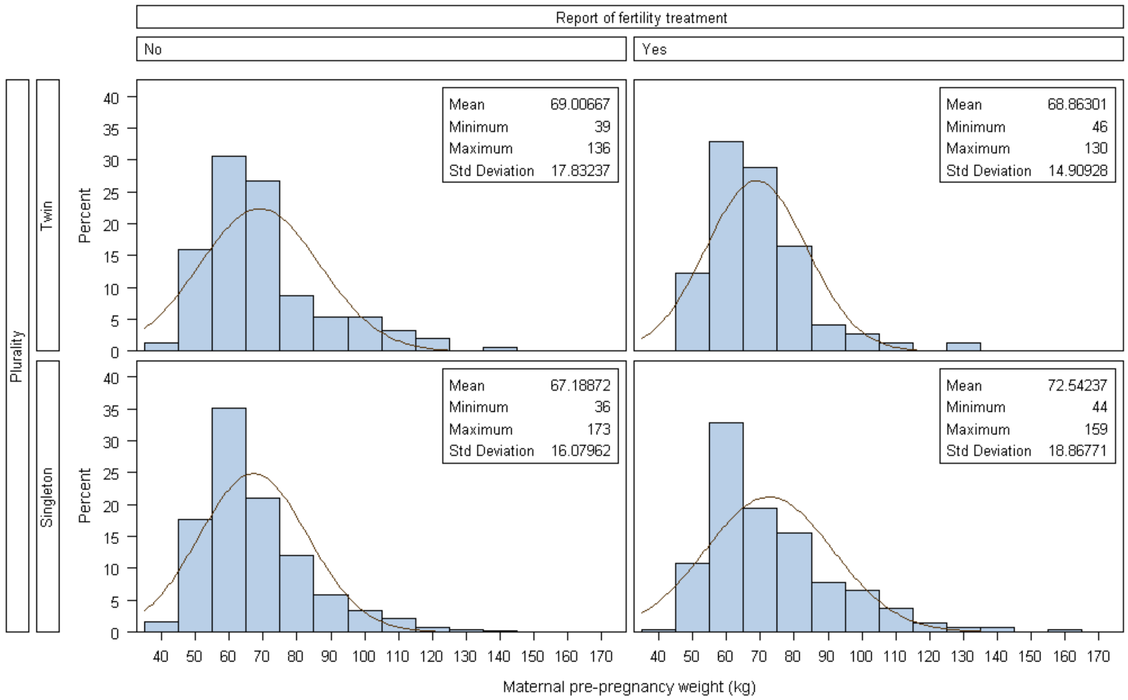

Figure 2: Distribution and descriptive statistics for maternal pre-pregnancy weight, in kilograms, by plurality and report of fertility treatment among mothers of liveborn infants with no major birth defects, National Birth Defects Prevention Study, 1997-2007

APPENDIX

We constructed directed acyclic graphs (DAGs) for each exposure of interest to use as tools for evaluating potential confounders and developing *a priori* multivariable logistic regression models (49). We based the variables eligible to be included in each DAG on a review of the literature. After carefully considering the possible relationships of each potential confounder with the exposure and outcome, we eliminated variables that did not act as confounders for the given association from the corresponding DAG (exceptions noted below). The DAGs that are described here are simplified: they do not include the relationships between potential confounders and are limited to data that could be obtained from the maternal interview. The arrows in the figures indicate the directed path that was assumed for each exposure-covariate, exposure-outcome, or covariate-outcome relationship. These paths do not necessarily indicate biologic causality, and do not include details of intermediate steps in these relationships (50). Although multiple DAGs are likely for any given scenario, only one DAG is described for each exposure.

Figure A presents the DAG we developed for the relationship between maternal age at delivery and twinning. Because fertility treatments are in the causal pathway between maternal age and twinning, controlling for them would lead to a biased estimate (51). However, we would also obtain a biased estimate for the independent association between maternal age and twinning if we did not control for fertility treatments (51). As this is true for any exposure in which fertility treatments are in the causal pathway, we elected to restrict all adjusted analyses to mothers that did not report any use of fertility treatments. Figure B describes the DAG for maternal age at delivery and twinning for the restricted population of mothers who did not use fertility treatments. All other figures describe models of exposure-twinning relationships among mothers that did not report any use of fertility treatments.

The DAG for maternal race/ethnicity and twinning is described in Figure C. This is the simplest DAG, with study site as the sole potential confounder. Variables such as maternal age and body mass index were considered as potential confounders, but these variables were determined to be intermediates in the causal path between race/ethnicity and twinning.

Figure D describes the relationship between maternal height and twinning. A secular trend in increasing adult average body height has been observed throughout the twentieth century, with speculation that this trend is due to advances in standard of living (52). Mother's year of birth was considered for this analysis rather than mother's age at delivery in order to better control for population differences in height over time; for the majority of mothers their age was unlikely to be related to their height. Socioeconomic status in childhood has been found to be a strong predictor of adult height, but could not be included in this analysis because the socioeconomic factors for which we had data (mother's highest level of completed education and annual household income) are measured in adulthood and may not accurately reflect the mother's childhood socioeconomic status (52).

Figures H and I describe the relationships between mother's highest level of completed education and annual household income, respectively, and twinning. Although maternal age at delivery could be considered an intermediate in the analysis of maternal education and twinning, as women who have college degrees are likely to delay childbearing, it could also be argued that maternal age is a potential confounder of this relationship, as older mothers have had more opportunity to complete higher levels of education (53). Therefore maternal age was included in this analysis in order to evaluate the direct effect of maternal education on twinning.

Similarly, in the analysis of mother's annual household income, maternal age at delivery was included as a potential confounder despite the fact that it could also be considered an intermediate in the income-twinning relationship. It has been observed that younger mothers are more likely to have come from a socioeconomically disadvantaged background, indicating that young maternal age is an intermediate effect (54). However, there is strong evidence that younger mothers, particularly teenage mothers, have lower incomes in later life than mothers who delay childbearing, indicating that maternal age may confound the relationship between income and twinning (55).

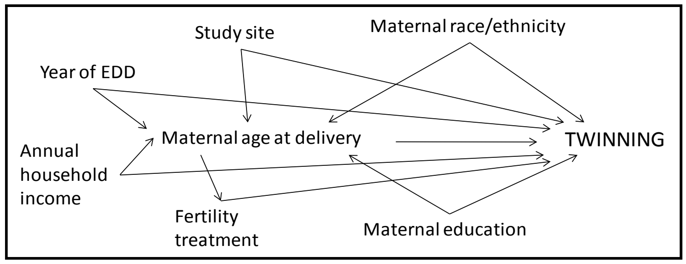

Figure A. Directed acyclic graph describing the relationships of maternal age at delivery and twinning with maternal race/ethnicity, study site, year of expected date of delivery (EDD), mother's annual household income, fertility treatments and maternal education as potential confounders.

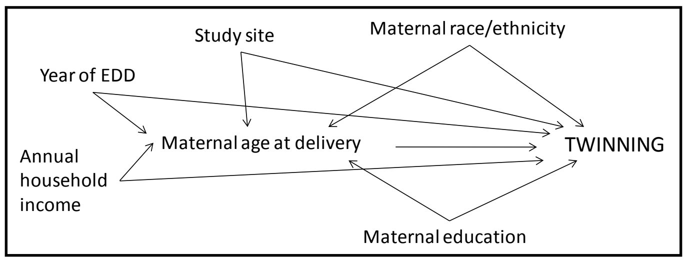

Figure B. After restricting analysis to women that did not undergo fertility treatments, this directed acyclic graph describes the relationships of maternal age at delivery and twinning with maternal race/ethnicity, study site, year of expected date of delivery (EDD), mother's annual household income and maternal education as potential confounders.

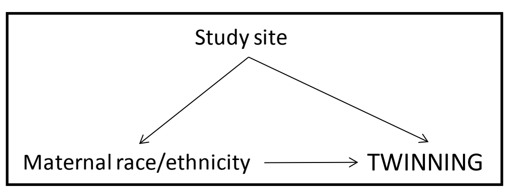

Figure C. Directed acyclic graph describing the relationship between maternal race/ethnicity and twinning, with study site as a potential confounder.

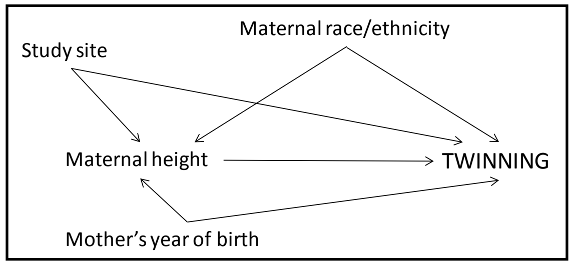

Figure D. Directed acyclic graph describing the relationship between maternal height and twinning, with maternal race/ethnicity, mother's year of birth, and study site as potential confounders.

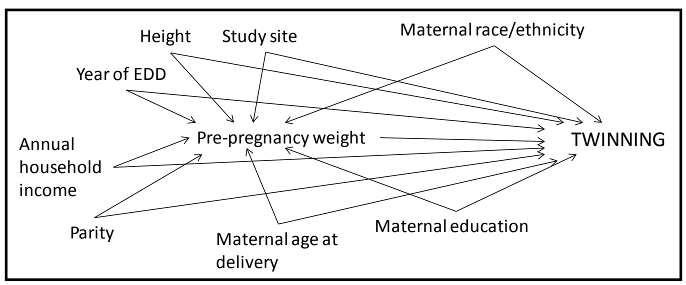

Figure E. Directed acyclic graph describing the relationship between pre-pregnancy weight, in kilograms, and twinning, with maternal race/ethnicity, age at delivery, education, annual household income, height, parity, study site, and year of expected date of delivery as potential confounders.

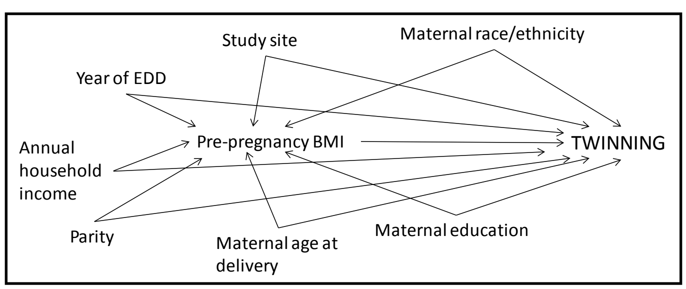

Figure F. Directed acyclic graph describing the relationship between pre-pregnancy body mass index and twinning, with maternal race/ethnicity, age at delivery, education, annual household income, parity, study site, and year of expected date of delivery as potential confounders.

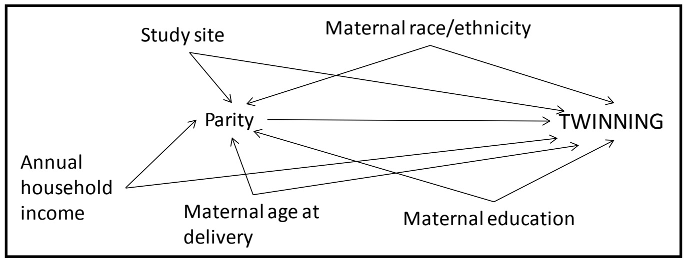

Figure G. Directed acyclic graph describing the relationship between parity and twinning, with maternal race/ethnicity, age at delivery, education, annual household income, and study site as potential confounders.

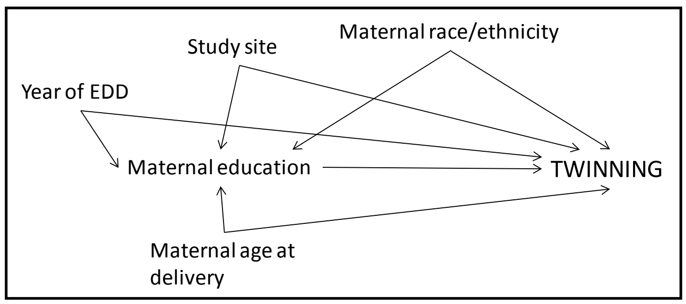

Figure H. Directed acyclic graph describing the relationship between the mother's highest level of completed education and twinning, with maternal age at delivery, race/ethnicity, study site and year of expected date of delivery as potential confounders.

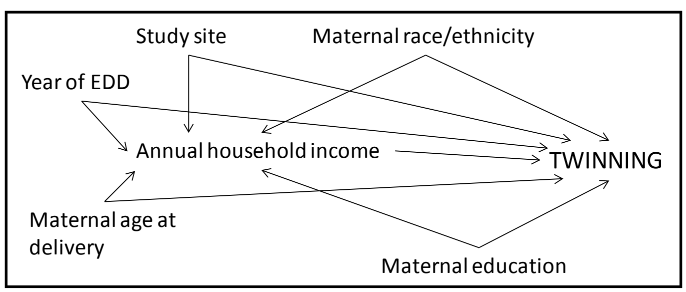

Figure I. Directed acyclic graph describing the relationship between annual household income and twinning, with maternal race/ethnicity, age at delivery, education, study site, and year of expected date of delivery as potential confounders.

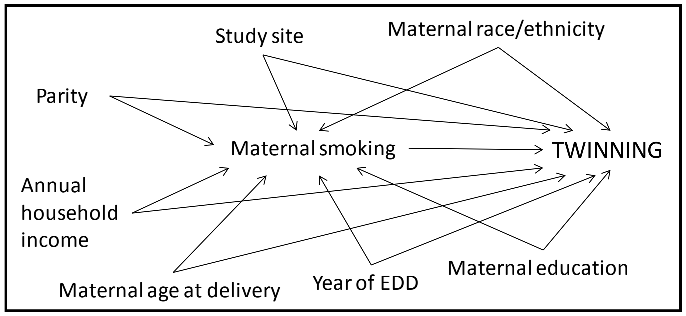

Figure J. Directed acyclic graph describing the relationship between maternal smoking during pregnancy and twinning, with maternal race/ethnicity, age at delivery, education, annual household income, parity, study site, and year of expected date of delivery as potential confounders.

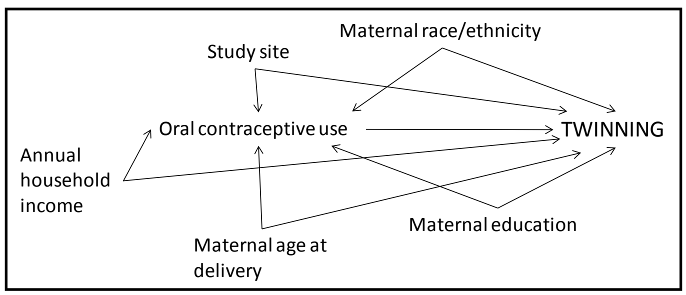

Figure K. Directed acyclic graph describing the relationship between periconceptional oral contraceptive use and twinning, with maternal race/ethnicity, age at delivery, education, annual household income, and study site as potential confounders.

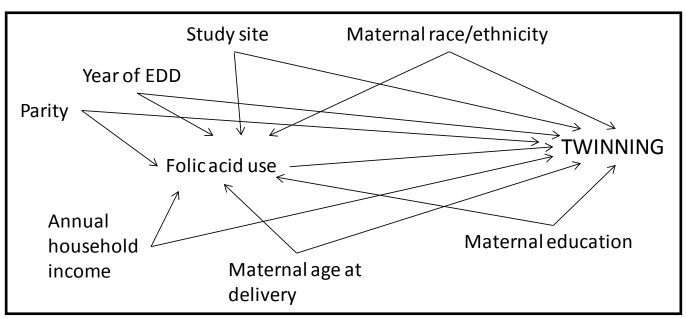

Figure L. Directed acyclic graph describing the relationship between periconceptional folic acid containing multivitamin use and twinning, with maternal race/ethnicity, age at delivery, education, annual household income, parity, study site, and year of expected date of delivery as potential confounders.