# PERICONCEPTIONAL FOLIC ACID SUPPLEMENTATION AND THE INCIDENCE OF PREMATURE DELIVERY: A PROSPECTIVE COHORT

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CERTIFICATE OF APPROVAL

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This is to certify that the MPH thesis of

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# ABSTRACT


## PERICONCEPTIONAL FOLIC ACID SUPPLEMENTATION AND THE INCIDENCE OF PREMATURE DELIVERY: A PROSPECTIVE COHORT

*Background*

Prematurity is the leading cause of perinatal and infant mortality, among infants born without fatal congenital defects, in the United States. Diets deficient in folic acid have been linked to an increased incidence of congenital anomalies and poor birth outcomes, including prematurity. While the evidence supporting folic acid supplementation for the prevention of some congenital anomalies is generous, the connection between folate supplementation and the incidence of premature births is less well characterized. This study aims to further elucidate the impact of folic acid supplementation upon the incidence of premature delivery. We hypothesize that the earlier women take folic acid supplements, the lesser likelihood of delivering prematurely. Additionally, women who take folic acid supplements will be less likely to deliver prior to gestation maturity than women who do not take folate at any time during their pregnancy.

*Population*

A prospective cohort of 1,847 pregnant women gathered from obstetric practices in Oregon from 1996-2000 was characterized by timing of folate supplement initiation, i.e. within the periconceptional period, sometime after 6 weeks gestation, or no supplementation at any time during pregnancy.

*Methods*

General characteristics linked to patterns of folic acid supplement use in this sample were identified by ANOVA. Furthermore, multivariate logistic regression was used to compare patterns of supplementation with the incidence of premature delivery. Two models assess, (A) the timing of folate supplement initiation and (B) any folate use at any time during pregnancy, with the incidence of premature birth.

*Results*

Neither folate supplementation overall ($p$=.382), nor its timing of initiation ($p$=.516), significantly affected the incidence of prematurity in this cohort of women. Factors significantly associated with premature delivery included the number of prenatal care visits ($p$<.0005), prenatal care in the first 12 weeks of pregnancy ($p$<.0005), the interaction between diabetes in pregnancy and number of prenatal care visits ($p$=.013), water source ($p$=.041), and whether women were referred for a fetal echocardiogram($p$<.0005).

*Conclusions*

This study did not confirm previously demonstrated associations between folate and premature delivery. While folic acid supplementation remains an essential component of prenatal care for the reduction of congenital anomalies, the results of this investigation do not suggest that is important in reducing the incidence of prematurity.

# PERICONCEPTIONAL FOLIC ACID SUPPLEMENTATION AND THE INCIDENCE OF PREMATURE DELIVERY: A PROSPECTIVE COHORT

## INTRODUCTION

Prematurity is the leading cause of perinatal and infant mortality, among infants born without fatal congenital defects, in the United States. Diets deficient in folic acid have been linked to an increased incidence of congenital anomalies and poor birth outcomes, including prematurity. While the evidence supporting folic acid supplementation for the prevention of some congenital anomalies is generous, the connection between folate supplementation and the incidence of premature births is less well characterized.

Considering the substantial individual, societal, and economic impact prematurity exerts, reducing the proportion of such births would be of substantial benefit. As routine folate supplementation is generally unproblematic for women to follow, lacks significant side effects at conventional doses, and is relatively inexpensive, it is an almost ideal intervention. If folic acid supplementation were to decrease the likelihood of delivering an infant prior to gestational maturity, significant reductions in prematurity and its consequences could be of significant public health importance. Therefore, this study aims to elucidate the possible impact of folic acid supplementation upon the incidence of premature delivery.

## BACKGROUND AND SIGNIFICANCE

*Consequences of Prematurity*

The United States continues to experience one of the highest perinatal mortality rates among developed nations; this, despite spending $1.4 trillion dollars each year on health care, which amounts to 14.1% of the Gross Domestic Product (GDP) (CDC, Vital Health Statistics 1985-91). In 1998, the United States ranked 28th of 38 countries reporting infant morality rates to the World Health Organization (WHO), with an annual infant morality rate of 7.2 deaths in the first year of life per 1,000 live births, compared to the lowest rate of 3.2 in Hong Kong (WHO, Child and Adolescent Health, Multi-Country Evaluation, http://www.who.int/imci-mce/). Among the U.S. population, prematurity is the leading cause of perinatal and infant mortality among those infants born without fatal congenital defects, and it is the leading cause of mortality among non-Hispanic Black newborns (Scholl, 2000). Worldwide, 75% percent of perinatal deaths occur among infants born prematurely, more than two-thirds of these deaths occur in the 30-40% of preterm infants who are born at less than 32 weeks gestation (Slatterly, 2002). Moreover, in 2003, of the more than 4 million babies born in the United States, 12.3% were delivered prematurely, a proportion consistent with previous years' estimates (CDC, National Vital Statistics Report, 2002). In other words, 1 in 8 newborns are born prematurely in the United States.

In addition to a significantly increased risk of mortality, surviving premature infants and their families endure considerable physical, emotional, and economic burdens. Among other diseases and conditions, premature neonates are at an increased risk of respiratory distress syndrome (hyaline membrane disease), bronchopulmonary dysplasia, intraventricular hemorrhage, and necrotizing

enterocolitis (Moss, 2002). Together with long-term chronic conditions also associated with prematurity, learning disabilities, childhood psychological conditions, retinopathy of prematurity, and developmental delay continue to impact the health of infants who survive into childhood. The emotional hardships placed upon families caring for a premature infant, while difficult to quantify, are not difficult to imagine and must not be discounted.

The economic costs associated with preterm delivery are substantial. Each year more than 2 billion dollars is spent preventing or treating the medical complications of prematurity in the United States (Gilbert, 2003). A severely ill newborn may spend several weeks or months in a neonatal intensive care unit (NICU). A recent analysis of the costs associated with prematurity in a large California cohort demonstrated that while the weekly cost of care for a term infant may be as high as $1,100, the cost rises to $2,600 at 36 weeks and to $202,700 at 25 weeks gestation (Gilbert, 2003). Intuitively, infants born at earlier gestations suffer greater morbidities, undergo more procedures, and experience longer hospital stays, thereby incurring greater cost than infants born closer to maturity. Yet even infants born between 34 and 36 weeks gestation, thus still premature but born after the majority of neonatal morbidities are likely occur, incur an average of $1,500 to $6,100 more expense than infants born at term (Gilbert, 2003). Extrapolated to a population scale, these costs quickly amount to extraordinary sums of money.

Recognizing the United States' discrepancy between dollars spent and perinatal outcomes, Healthy People 2010 aims to reduce the incidence of preterm delivery to 7.6% of births to help reduce the infant mortality rate from 6.9% in 2000 to 4.5% in 2010 (March of Dimes, Perinatal Profiles 2003 Edition, Peristat).

*Epidemiology of Prematurity*

Reducing the incidence of prematurity, however, is complicated by a long list of risk factors whose relative contributions are difficult to quantify. Moreover, the statistical significance of many of these characteristics has varied greatly among studies, further obscuring interpretations of risk. In general, preterm births- infants born before 37 weeks 0 days gestation, may be divided into 2 groups – (1) those that are indicated by complications that threaten fetal or maternal health and (2) those that occur spontaneously. Understandably, the risk factors for these two groups differ (Goldenberg, 2002). However, attributes associated with *all* preterm deliveries, regardless of etiology, include: multiple gestation, pre-pregnancy weight less than 55kg, first pregnancy, black race, younger than 17 years or older than 35 years at conception, and bleeding in the first or second trimesters (Gabbe, 2002; Goldenberg, NEJM 1998; Goldenberg, 2002; and ACOG Practice Bulletin, No 31 Oct 2001).

Accounting for 20-30% of premature births, the risk factor profile for indicated deliveries is comprised of a relatively definitive list of conditions that pose such a significant threat to mother and/or fetus that early delivery is initiated. These conditions can include pregnancy-associated hypertension, preeclampsia, gestational diabetes mellitus (GDM), placenta previa, placental abruption, and fetal complications such as intra-uterine growth restriction (IUGR) and various congenital anomalies (Goldenberg, 2002). As the incidences of these conditions often exhibit direct relationships with maternal age, advancing age is an additional risk factor for deliveries that are deliberately initiated prior to gestational maturity (Goldenberg, 2002).

In contrast, while spontaneous preterm births, the second group, account for an estimated 75% of all preterm deliveries the associated risks are more diverse and less well understood. In addition to the major risk factors for all-cause preterm delivery listed above, a key risk factor for spontaneous premature birth is a history of preterm delivery: a woman who delivered prematurely during a previous pregnancy has a 3-4 times increased risk of delivering prematurely in a subsequent pregnancy (Goldenberg, 2002). Additional risks for spontaneous premature delivery include genitourinary and intrauterine infections, maternal age less than 18 years, compromised cervical length or strength, trauma, and a maternal history of smoking or substance abuse during pregnancy (Gabbe, 2002 and Goldenberg, NEJM 1998). Hypothetical and suspected risk factors that remain controversial include those related to an increased level of perceived emotional and/or social stress, such as a stressful living environment, violence, poverty, and lack of a beneficial social support network (Department of Health and Human Services 2003; Ruiz 2003). While conclusions drawn by experts in the field are not unanimous on the impact of these risk factors upon the incidence of prematurity, they remain compelling foci of research.

*Folic Acid*

Whereas attempts to lessen the impact of the risk factors described above have not significantly decreased the incidence of prematurity, several key interventions have reduced the incidence of poor pregnancy outcomes over the last century. Among such interventions is the consensus recommendation that women of reproductive age ensure adequate folate acid quantities in their diet by consuming folate-rich foods and taking a 400 microgram supplement beginning before

conception to reduce the development of fetal neural tube defects (NTDs). Over the past 10 years, research has demonstrated that up to 70% of NTDs can be prevented with folic acid supplementation; this is often cited as one of the ten major advances in public health of the 20th century (CDC, Folic Acid Information and Recommendations for Health Professionals; Morris, 2005). In fact, the evidence supporting the essential role of folic acid in reducing NTDs was so persuasive that in 1998 the FDA began requiring140 micrograms of folic acid per 100 grams of grain be added to cereals, breads, pastas, and all foods labeled "enriched" (www.cdc.gov/folicacidnow). The FDA reasoned that increasing the availability of folate in the average American woman's diet would further decrease the incidence of NTDs. Since 1998, the mean serum folate level in American women of reproductive age has indeed risen and the number of infants born with spinal defects has decreased (March of Dimes, Folic Acid Quick Reference and Fact sheet).

These guidelines and FDA action follow research demonstrating that folic acid is an essential nutrient during pregnancy; folic acid plays a critical role in cell division and nucleic acid synthesis, serving as a cofactor in the transport of one-carbon chains (Scholl, 2000). Interference with these processes contributes to abnormal cell division in rapidly dividing cells. Folate-deficient blood, therefore, impairs the exponential cellular growth that is necessary for the increased rate of erythropoeisis, enlargement of the mammary gland, and the growth of the uterus, placenta, and fetus during gestation (Scholl, 2000). Folate requirements in pregnancy are further amplified by an increase in folate turnover and maternal urinary excretion (Siega-Riz, 2004).

Besides decreasing serum and red blood cell folate levels, diets deficient in folic acid also cause a rise in homocysteine. High levels of homocysteine have been strongly associated with NTDs and also powerfully predict vascular disease. In pregnancy, hyperhomocysteinemia's positive correlation with vascular disease is hypothesized to disrupt the health of the placenta and thus explain its association with low birth weight, premature delivery, recurrent abortions, preeclampsia, and placental abruption (Vollset, 2000). While genetic mutations may also contribute to hyperhomocysteinemia, in the absence of these mutations increased levels of homocysteine may indicate inadequate folate ingestion.

Considering its crucial role in maternal, fetal, and placental health, it is not surprising that folate deficiency has been associated with numerous fetal anomalies. In addition to neural tube defects, diets deficient in folic acid have been linked to an increased incidence of craniofacial abnormalities, conotruncal heart defects, midline abdominal defects, urogenital malformations, low birth weight, IUGR, and preterm labor (Morris, 2005; Scholl, 2000). While current evidence supports folic acid supplementation for the prevention of NTDs, the connections between folate supplementation and these other abnormalities are just now becoming more clearly defined. This study's focus on the association between folate and prematurity follows decades of suggestive, yet conflicting, data describing the relationship.

Observational studies in the early 1970's suggested that preterm birth was not statistically related to levels of folate measured in red blood cells (Hibbard, 1975) and only weakly associated with whole blood folate levels (Daniel, 1971). Similarly, a randomized clinical trial of 5,502 women in 1994 did not find an association between folate taken in the first 12 weeks of pregnancy and the likelihood of preterm delivery

(Czeizel, 1994). However, in 1996 Scholl et al demonstrated that decreased folate levels later in pregnancy (28 weeks gestation) were strongly associated with an increased risk of prematurity. After adjusting for numerous maternal characteristics, energy intake, and other risk factors, successively lower levels of folate intake were associated with a 1.9 (95% CI 1.0-3.6) to 3.4 (95%CI 1.9-6.1) times greater risk of preterm delivery (Scholl, 1996). Likewise, in a secondary data analysis using a control series from a case-control study of orofacial clefts, Shaw et al revealed that women who had taken folate-containing vitamins from 1 month prior to conception to at least the end of the first trimester had a reduced risk (OR=0.38 [95% CI 0.16- 0.88]) of delivering an infant prematurely than women who initiated vitamin use in the second month (OR=0.60 [95% CI 0.31-1.2] or third month of pregnancy (OR=1.0 [95% CI 0.46-2.2]) (Shaw, 1997). In two studies, hyperhomocysteinemia was associated with preterm delivery, although in the second study serum folate concentration was not significantly different in women who delivered prematurely from those who did not (Vollset, 2000; Ronnenberg, 2002). A systematic review and meta-analysis of nutritional interventions during pregnancy and preterm delivery published before July of 2002 did not support an association with folic acid; however, timing of supplementation was not addressed (Villar, 2003).

Most recently, two studies have reinvigorated the interest in folate's potential to decrease the likelihood of premature birth. In an incident case-control study, after adjusting for numerous risk factors, folate deficient women in their third trimester were almost twice as likely to delivery prematurely (OR =1.97 [95% CI 1.06-3.68]) than women with below adequate serum folate levels for pregnant women as established by the WHO (Marti-Carvajal, 2004). Similarly, in a prospective cohort

study, Siega-Riz et al found that deficient folate levels in the second trimester were significantly associated with premature delivery. Controlling for energy intake, folate ingestion from both diet and supplementation equaling less than 500 micrograms per day was associated with an increased risk of 1.8 (95% CI 1.4-2.6), while low serum and red blood cell folate levels were associated with 1.8 times (95% CI 1.3-2.5) and 1.7 times (95%CI 1.1-2.6) the likelihood of prematurity, respectively (Siega-Riz, 2004).

While evidence exists to dispute a relationship between folic acid and prematurity, considering the number of investigations that *have* demonstrated a protective effect attributable to folate, this association remains a critical area of research and is the impetus for this analysis. In particular, previous data suggests that the timing of supplement initiation may affect the outcome. Therefore, this study will contribute additional information to the ongoing discussion of folate's association with the incidence of prematurity and, in particular, attempt to clarify the effect of timing of supplement initiation upon the likelihood of preterm birth.

## OBJECTIVES

The primary objective of this analysis is to assess the impact of folate supplementation upon the incidence of premature delivery. Specifically this prospective cohort, beginning early in the second trimester of pregnancy, addresses whether the timing of supplement initiation influences the likelihood of delivering a preterm infant as well as the influence of folate supplementation at any time in pregnancy versus none at all. Utilizing a cohort of 1,847 women seeking prenatal care in Oregon between 1996 and 2000, this secondary data analysis is designed to answer 2 main questions:

(1) Are women who take folic acid supplements in the periconceptional period (3 months prior to until 6 weeks after conception) less likely to experience pre-term delivery (<37 weeks gestation) than women who begin supplementation after 6 weeks gestation, or women who do not take folic acid supplements at any time during their pregnancy?

(2) Are women who take folic acid supplements immediately prior to, or at any time during, their pregnancy less likely to experience pre-term delivery than women who do not take any folic acid supplements at any time before or during pregnancy?

This investigation also characterizes the prevalence of folate supplementation and timing of initiation among a cohort of women seeking prenatal care in Oregon. Noted as a significant factor in reducing the burden of several congenital anomalies, folate supplementation was recommended nationally by the U.S. Public Health Service in 1992 and the Institute of Medicine in 1998. As this cohort includes pregnancies occurring between 1996 and 2000, the prevalence of women taking folate is of interest in measuring the breadth of dissemination of this public health prevention strategy.

## RESEARCH DESIGN and METHODS

This prospective cohort study examines factors influencing the delivery of a preterm infant among a cohort of Oregon women between 1996 and 2000. The data for this analysis were originally collected for a study investigating the relationship between low maternal intake of folate (dietary as well as supplemental) and abnormal folate metabolism, with the incidence of congenital heart defects (CHD), specifically conotruncal defects. The resultant paper entitled, "Folate Supplementation in Early Pregnancy Reduces the Risk of Conotruncal Heart Defects and Ventricular Septal Defects" identified a 54% reduction in the risk of a fetus with a conotruncal heart defect or ventricular septal defect (VSD) in women taking folate supplements in the first 6 weeks after conception, the period of cardiogenesis (Morris, 2005). Additionally, the risk of a pregnancy affected with CHD was significantly greater in the period prior to 1998 after which time the FDA required enriched foods to be supplemented with folic acid.

### Study Population

From 1996 to 2000 two cohorts, the Fetal Echo and Routine Prenatal Care Cohorts, were recruited in the early second trimester of pregnancy (Figure 1). Women in both cohorts were eligible for participation for only one pregnancy; information concerning subsequent pregnancies was not collected. Multiple gestation pregnancies were excluded from both cohorts for this analysis (n=74). Following recruitment, all study procedures for each cohort were identical; measures pertinent to this study are discussed below. No information regarding the hypotheses was related to the participants at any time during the study.

For the purposes of this investigation, these two recruitment groups were condensed into one group, as any potential differences between them are not believed to be pertinent to the research question. While the analysis is not stratified by initial cohort, the original group assignment remains an independent variable under analysis and will thus reveal any important differences should they exist.

*Fetal Echo Cohort*

Included in this group are women who were referred for a fetal echocardiogram because their fetuses had a higher risk of congenital cardiac malformations based on a clinical assessment of risk factors; for example, a family history of CHD, maternal use of certain medications, or particular maternal illnesses such as pre-existing diabetes. Women presenting for an *initial* fetal echocardiogram at Oregon Health & Science University (OHSU), Providence St. Vincent Medical Center in Portland, St. Charles Memorial Hospital in Bend, OR, or for a level II ultrasound with fetal echocardiography at Legacy Emanuel Hospital and Medical Center in Portland were asked to participate. If women underwent more than one fetal echocardiogram, participants were recruited only at the time of the initial study. Women who gave their informed consent were interviewed prior to the fetal echocardiogram or ultrasound.

Excluded from this cohort were women carrying a fetus with a chromosomal anomaly (except for chromosome 22q11 deletion) or if the current or a prior pregnancy was affected with a NTD.

*"Normal," Routine Prenatal Care Cohort*

Pregnant women between 16 and 26 weeks gestation receiving routine prenatal care through OHSU between 1996 and 2000 were also recruited and consented prior to the interview.

Excluded from this cohort were women for whom a referral for fetal echocardiogram was indicated: pregnancy affected by a diagnosed chromosomal anomaly or NTD; history of congenital heart disease in mother of the baby or first-degree relative; maternal use of lithium, phenytoin, trimethadione, amphetamines, cocaine, antihypertensive medication, valproic acid, or insulin in pregnancy; maternal history of diabetes, phenylketonuria, connective tissue disease, or epilepsy.

Three components of the primary study's research design are the foundation upon which this study builds its analysis- the initial patient interview, nutritional information, and pregnancy outcome data.

## Data Source and Data Management

*Initial interview.*

Subjects in the Morris Folate and Congenital Heart Disease Study were interviewed using a standardized instrument administered by a trained research nurse. The data instrument utilized for this initial interview assessed demographic information and socioeconomic status; lifestyle factors including smoking history, alcohol use prior to and in this pregnancy, and occupational exposures; history of the present and any prior pregnancy, date of the first prenatal visit, and gestational age at interview as determined by earliest ultrasound exam or the first day of the last menstrual period in the absence of ultrasound data; history of congenital heart disease or associated syndromes in first-degree relatives; maternal medical history;

maternal use of prescription and over-the-counter medication in pregnancy; maternal-fetal disorders such as intrauterine growth retardation, fetal cardiac arrhythmias, and extra-cardiac malformations.

*Nutritional Information*

The use of a multivitamin, prenatal vitamin, vitamin supplement, other herbal or health supplements, or nutritional beverages was determined using memory aids. Each woman was provided with an individualized calendar with the date of conception marked in addition to the three-month period prior to conception and the first six weeks after conception; holidays and community events were recorded on the calendar to enable the woman to triangulate dates. A book containing pictures of all common multivitamins and prenatal vitamins was available at the interview to provide a visual memory to enhance recall. Each woman was asked about her consumption of vitamins or supplements in the three months prior to and in the first six weeks of pregnancy. In addition to multivitamin combinations, each woman was asked about additional use of individual vitamins A, B6, C, and E, selenium, iron, folate, zinc, beta-carotene, calcium or other health supplements. A database containing the content of each supplement was established in order to determine the actual folic acid consumption in each period.

The Block food frequency questionnaire was used for assessment of usual dietary intake (Block, 1992). This questionnaire asks the subject to record the frequency of consumption and usual portion size of 100 major food items. Each subject was asked to use only the period of pregnancy as the reference, beginning at date of conception. This questionnaire was then analyzed for consumption of thirty major nutrients.

*Outcomes*

All pregnancies were followed to completion whereupon information regarding conditions diagnosed during pregnancy were collected, including new onset GDM, IUGR, nonimmune hydrops fetalis, polyhydramnios, pregnancy-associated hypertension/preeclampsia/Syndrome of Hemolysis, Elevated Liver Enzymes, and Low Platelets (HELLP Syndrome), placental abruption, placenta previa, and preterm, premature rupture of membranes (pPROM). Gestational age was determined by medical record review, determined by the earliest ultrasound or the first day of the last menstrual period when an ultrasound was not performed. Pregnancy outcome and gestational age at pregnancy completion were also recorded, noting live birth, fetal death, miscarriage, or induced abortion, as well as the presence of any cardiac or extra-cardiac anomalies present at delivery. Pregnancies delivered before 37 weeks 0 days gestation were counted as premature and those delivered on or after 37 weeks and 0 days were classified as mature.

A trained registered nurse reviewed the records of all participants to collect delivery data included gender, birth weight, length, head circumference, APGAR (at 1 and 5 minutes), type of labor, type of delivery, spontaneous labor, labor induction, or cesarean delivery. If the delivery was an elective induction or a planned cesarean section the reason these options were chosen was recorded- patient preference or medically indicated due to fetal distress, oligohydramnios, suspected IUGR, dystocia, cephalo-pelvic disproportion (CPD), failure to progress (FTP), pregnancy associated hypertension/preeclampsia/HELLP, post-term, placental abruption, placenta previa, pPROM, breech/abnormal presentation, or other non-listed reason. It was also noted whether the infant was admitted to the NICU, and if so, the number of days

the infant spent in the unit as well as the eventual disposition of infant (death or discharged to home). Finally, the health care providers of children born to mothers in both cohorts were contacted at 6 weeks and 1 year of age to identify the presence of a cardiac or other congenital defect.

*Data Management*

The initial study data were collected and entered into a relational database designed for this study with range and logic checks, utilizing double key entry for key variables. A statistician working on the original project converted the initial data from SAS to SPSS for the purposes of this investigation.

## STATISTICAL ANALYSIS

All statistical analyses, including the construction of both models, hereafter referred to as "Model A: Folate Initiation" (folate supplementation initiated in the periconceptional period, after 6 weeks gestation, or no folate taken at any time during pregnancy) and "Model B: Any Folate" (any folate supplement at any time during pregnancy versus none at all), were undertaken using SPSS Version 13.0.

Both univariable and multivariable logistic regression were used to analyze the relationships between independent and dependent variables, focusing particularly upon whether the timing of folate supplement initiation has any correlation with the timing of pregnancy delivery.

*Predictor Variables*

- Model A: Timing of folate supplementation initiation: periconceptional, after 6 weeks gestation, never
- Model B: Any folate supplementation: at any time during pregnancy, none at all

*Outcome Variable*

- Preterm delivery : premature (delivery @ < 37 weeks, 0 days GA) or not premature (delivery ≥ 37 weeks, 0 days GA)

*Potential Confounding Variables*

- Original study cohort assignment
- Paternal factors: age, race, major comorbidity
- Maternal factors: age, race, education level attained, marital status, employment status, health insurance, major comorbidity
- Lifestyle factors: smoking, alcohol or other substance use, nutrition, hazardous chemical exposure
- Pregnancy history: Gravidity, family history or previous pregnancy affected by chromosomal anomaly or NTD
- Prenatal Care: Pre-pregnancy BMI, total pregnancy weight gain, planned pregnancy, conception prior to 1998, GA first suspected pregnancy, GA at first prenatal appointment, number of prenatal visits, morning sickness in the first 6 weeks pregnancy
- Pregnancy diagnoses: GDM, IUGR, nonimmune hydrops fetalis, polyhydramnios, HELLP, placental abruption, placenta previa, pPROM

*Statistical Power and Sample Size*

The two hypotheses tested in Models A and B were analyzed by the construction of 4 independent sample comparisons. In this manner, the primary independent variable (time of initiation of folate supplementation) for each of the potential initiation times were analyzed compared to other cohort sub-groups as constructed below.

- Periconceptional folate supplementation vs. folate supplementation after 6 weeks gestation
- Periconceptional folate supplementation vs. no folate supplementation
- Folate supplementation after 6 weeks gestation vs. no folate supplementation
- Folate supplementation at any time prior to or during pregnancy vs. no folate supplementation

This method of comparison has the advantage of investigating a sequential relationship between the timing of initiation and pregnancy outcome. Setting the confidence level at $\alpha=0.05$ and adjusting for multiple hypotheses using the Bonferroni correction approach, each of the 4 independent sample comparisons was tested at an $\alpha=0.0125$ ($\alpha=0.05/4$). Using PASS software, considering the fixed sample size of each of the 4 sub-groups, statistical power (1-ß) is calculated from the proportional difference in incident premature births between the groups compared in each independent sample.

It was assumed that given the relatively large sample size of 1,847, that the standard deviation of the outcome variable will be small (<1%), and that the proportion of premature births among all live births in this study will closely follow the U.S. population estimate of prematurity of 12%. Thus, the degree to which each comparison group differs from the sample estimate of 12% will influence the

statistical power. Considering the limitations of a secondary data analysis, it will not be possible to adjust the sample size to achieve greater power. However, it is estimated that folic acid supplementation initiated prior or within the first trimester of pregnancy can reduce the likelihood of fetal neural tube defects by more than 50% (www.cdc.gov/folicacidnow). Considering the national rate of premature births is currently 12% a similar 50% reduction in prematurity to 6% would be a clinically significant result of folate supplementation. Therefore the effect size sought was 6% or 0.06.

Using a 50% reduction in premature births from 12% to 6%, the estimated power for each of the 4 independent comparisons is greater that 0.80 for all comparisons for a level of $\alpha$=0.05. Therefore, the proposed analysis is adequately powered at the .0125 level of significance appropriate to the Bonferroni correction for multiple comparisons.

*ANOVA Evaluation*

Prior to the construction of regression models, several demographic and pregnancy related factors of *a priori* interest (maternal age, maternal race, Hispanic ethnicity, maternal education, health insurance, marital status, planned pregnancy, gravidity, parity, gestational age at first prenatal visit, number of prenatal visits) as well as outcome measures (preterm [dichotomous] and gestational age at delivery [continous], LBW [dichotomous] and infant weight [continuous]) were individually compared within between the levels of folate use defined for Models A and B using ANOVA. In Model A, which compares three folate use groups, differences between means were also assessed for significance by the post-hoc analyses Tukey Honestly Significant Difference (HSD), Least Significant Difference (LSD), and Bonferroni

approaches. A $p<0.05$ was deemed significant while differences were qualified as marginally significant if $p\leq 0.1$.

*Model Building: Univariate and Multivariate Analyses*

The process of logistic regression model building was identical for both models. All variables contained in the data set (for a complete list and explanation of the variables consult the Glossary) were first assessed by univariate logistic regression models. In addition, non-continuous categorical variables were tested against the dichotomous outcome variable, preterm birth, using the contingency table method (Hosmer and Lemeshow, p. 92-95) and continuous variables were assessed using histogram, boxplot, and T-test analyses. Variables reaching a significance level of $p<0.250$ were carried over into multivariable analyses.

Multivariable analyses were undertaken in groups such that clinically correlated subsets of risk factors were analyzed together. Thus, factors significant at the $p<0.250$ level in univariate analyses were divided into groups including variables related to the study itself, demographics, maternal pre-pregnancy factors, maternal behavioral factors, maternal dietary factors, medical pregnancy factors, and fetal factors. Within each subset of factors, variables were tested through a backwards stepwise procedure whereby all variables of a subset were entered into a multivariable model and the least significant were removed sequentially until all factors in a subset achieved a significance of $p<0.250$. Then all previously removed variables were re-entered to assess for significance in the reduced model and re-admitted to the model if the p-value was $<0.250$.

After completing this process for all subsets, the remaining variables were added into one composite multivariable analysis. The least significant variables were

then removed sequentially until all variables attained a significance of $p<0.10$. Previously removed variables were again re-tested for significance and remained in the model if the p-value was $<0.10$. There were two exceptions to this algorithm-mother's age and race, and remained in all models as they are potentially clinically relevant factors in the evaluation of pregnancy outcome. Any model constructed to explain pregnancy outcomes without these demographic factors would be difficult to interpret clinically and would not be easily applicable. Additionally, independent variables for which any of the cells were "0," were excluded at this stage of the analysis. This occurred for only one variable, presence of a maternal connective tissue disease.

Once the preliminary main effects models were obtained, continuous variables were assessed for the assumption of linearity. All continuous variables in both models satisfied the assumptions for linearity. Interactions were then evaluated by testing each clinically significant interaction individually with the main effects in both models. Additionally, the folate variables in each model were tested for interactions against clinically relevant variables that were previously excluded from the preliminary main effects model. The additional interactions tested included those between folate use and BMI, binge drinking, smoking, education level, gestational age at 1$^{st}$ prenatal care visit, number of prenatal care visits, and planned pregnancy. All interactions significant at the $p<0.10$ level were retained in the model. All significant main effects and significant interactions from the previous model building were then entered into a large model and any variables that did not achieve significance of $p<0.10$ were removed, except for those main effects participant in an interaction. This process resulted in the final model.

*Testing of the Final Models*

After the final models were established each underwent an assessment of fit to evaluate the overall "fit" of the model to the data, as well as an examination of the individual components of the summary statistics. The assessment of fit includes the following Goodness-of-Fit Statistics: Pearson, Deviance with respect to the number of covariate patterns, Hosmer and Lemeshow, Classification Tables, Receiver Operating Characteristic Curves (ROC), and Cox & Snell and Nagelkerke $R^2$. The influence of individual data points were then assessed through diagnostic testing using DfBetas/ Standardized Betas, Unstandardized and Standardized/Pearson Residuals, Change in Deviance, Leverage, and Cook's Distance values in numerical as well as in graphic form. Neither model revealed significant or influential outliers, leverage points, or fit problems and no modifications were required.

## RESULTS

*Study Participants*

The demographic characteristics of all 1,847 participants are presented in Table 1. At the time of study enrollment, pregnant women in this cohort were, on average, 27.3 ± 6.3 years, 89.7% white, and 10.7% Hispanic; 57.6% were educated beyond the high school level, 37.3% were publicly insured, and 62.6% were married.

*Pregnancy Related Characteristics*

Pregnancy-related characteristics of the entire cohort of women are presented in Table 2. Less than half of participants (47.5%) were planning the current pregnancy. When asked about prior pregnancies, 26.0% of women reported a history of spontaneous abortion and 20.7% reported undergoing a therapeutic abortion. Just over fourteen percent of women (14.3%) carried a diagnosis of diabetes (including 4.0% with Type I, 5.2% with Type II, and 5.1% with gestational diabetes) during pregnancy. Over seven percent (7.6%) of women required insulin to manage diabetes in pregnancy, regardless of diabetes type. The mean gestational age at which women first suspected they were pregnant was 4.1 ± 2.5 weeks, the mean gestational age at the first prenatal visit was 9.7 ± 4.6 weeks, and the mean gestational age at the study interview was 22.3 ± 5.1 weeks. Finally, women reported an average of 12.3 ± 3.9 prenatal visits during their pregnancy.

*Folate Supplementation Characteristics*

Greater than 85% of women reported taking folic acid supplements at any time during pregnancy, while 68.7% of all pregnant women in the cohort began taking folate in the periconceptional period (Figure 2).

*Outcomes*

Within the entire cohort of women, 16.7% delivered infants before 37 weeks gestation and the mean gestational age at delivery was 37.9 ± 2.8 weeks. When analyzed by cohort, 22.5% of women in the Echo cohort experienced a preterm delivery, whereas 10.4% of women in the Routine Prenatal Care cohort delivered prematurely. This difference in proportion of preterm births between cohorts is statistically significant ($p<0.0005$). Regardless of mechanism, eighteen women (1%) experienced pregnancy termination before 24 weeks, the age of viability (Table 3).

*ANOVA*

Model A: Folate Initiation

Women who took folate in the periconceptional period, compared to women who did not take any folate in pregnancy, were statistically significantly ($p<0.05$) older, non-Hispanic and white, privately insured, more educated, married, had fewer pregnancies and fewer previous deliveries, were more likely to have planned the current pregnancy, had more and earlier prenatal visits, and greater infant birth weight (Table 4). Of particular interest to this study is that women who took folate in the periconceptional period were significantly more likely to deliver infants at an older gestational age when the outcome was measured as a continuous variable, and likewise, were less likely to deliver premature infants when measured as a dichotomous variable, than women who did not take any folate at any time during pregnancy.

Similarly, comparing women who took folate in the periconceptional period to women who took folate after the first 6 weeks of gestation, those who took folate earlier were statistically significantly older, non-Hispanic, white (marginal, Bonferroni

$p$=0.07), privately insured, more educated, married, more likely to have planned the current pregnancy, more and earlier prenatal visits, and greater infant birth weight.

Finally, women who started taking folate after 6 weeks gestational age were significantly whiter, married, had fewer pregnancies and fewer deliveries (gravidity marginal: Bonferroni $p$=.082) than women who never took folate at any time during pregnancy.

Model B: Any Folate

Women who took folate at any time during pregnancy were significantly older, non-Hispanic white, more educated, privately insured, married, fewer pregnancies and deliveries, more likely to have planned the current pregnancy, earlier prenatal care, more prenatal visits (marginal p=0.08), and greater infant birth weight than women who did not take any folate during pregnancy (Table 5). As in the first sub-group comparison in Model A, Model B demonstrates that women who took folate at any time during pregnancy were significantly more likely to deliver infants at an older gestational age when the outcome was measured as a continuous variable, and were less likely to deliver premature infants when measured as a dichotomous variable than women who did not take folate at any time during pregnancy.

*Univariate Analyses*

As described in the Methods section, prior to model construction variables were tested individually by logistic regression. This process yielded 49 variables significant at or below the $p<0.250$ level. Correlating coefficients, Wald Statistics, significance levels, and confidence intervals are listed in Tables 6 and 7. These 49 variables were then entered into multivariate models in clinically relevant groups as listed in Table 8.

When considered as univariate variables (see Table 6) the timing of folate initiation and any folate were suggestive of an associated with prematurity. As a single variable, folate initiation was marginally significant ($p=0.068$); women beginning supplementation in the periconceptional period were significantly less likely to deliver prematurely than women who never took folate (OR=0.68, [95% CI 0.49,0.94], $p=0.021$) and women initiating folate after 6 weeks gestation were insignificantly less likely than women who never took folate (OR=0.72, [95% CI 0.47,1.10], $p=0.125$) to deliver before gestational maturity. Any folate versus none at all was also significant as a univariate variable (OR=0.69, [95% CI 0.50,0.95], $p=0.022$).

*Multivariable Logistic Regression Models*

The process of model building and Goodness-of-Fit assessment outlined in the Methods section generated models for both Folate Timing and Any Folate that are similar, differing only slightly in magnitude of effect. Therefore, the models will be interpreted simultaneously in the discussion section with the odds ratios presented belonging to Model A (Figure 3). Please see tables 9 and 10 for differences in odds ratios, confidence intervals, and p-values.

Significant predictors of preterm birth in both models include the number of prenatal care visits, prenatal care in the first 12 weeks of pregnancy, diabetes in pregnancy, water source, and the initial group to which women were assigned in the primary study upon which this analysis is based. After adjusting for these relevant confounders, compared to women did not take folate at any time during pregnancy, women who initiated folate supplementation in the periconceptional period did not experience a significant reduction in risk (OR=0.88, [95% CI 0.60,1.29], $p$=0.125), and nor were women who began taking folate supplements after 6 weeks gestation (OR=0.76, [95% CI 0.47,1.22], $p$=0.250). Similarly, adjusted odds ratios did not demonstrate that women who took any folate in pregnancy were at a reduced risk of preterm delivery than women who did not take any folate during pregnancy (OR=0.85, [95% CI 0.59,1.23], $p$=0.382).

## DISCUSSION

This investigation suggests that neither prenatal folic acid supplementation, nor the timing of supplementation, affected the incidence of premature delivery in this cohort of women. While univariate analyses suggested a possible relationship between folic acid supplementation and prematurity, upon adjusting for confounding and effect modification by other variables, the multivariable logistic regression models did not find folate to be beneficial in reducing the incidence of preterm delivery. Factors that appeared to be associated with premature delivery included the number of prenatal care visits, prenatal care in the first 12 weeks of pregnancy, diabetes in pregnancy, water source, and the initial group to which women were assigned in the primary study upon which this analysis is based.

As this analysis was conducted under well-controlled circumstances with well-studied research instruments, and was adequately powered to demonstrate an effect if one truly existed, it is not likely that the lack of a demonstrated relationship between folate and preterm birth is due to errors in study design or management. One possible explanation for the absence of an effect is that about half of the cohort consists of women who may be at higher risk for premature delivery regardless of their folic acid supplementation history, i.e. the women referred for fetal echocardiography. Perhaps the innate risk these women carry cannot be reduced by folate supplementation and thus explains why folate use was not a significant predictor in the regression models. The final explanation for the lack in demonstrated effect could be that, in fact, prenatal folic acid supplementation may have no true effect on the incidence of premature delivery.

While the models constructed to evaluate the association of the timing of folate initiation and the incidence of prematurity do not find folate supplementation to be related to prematurity, the models do offer a few interesting points of analysis. First, in contrast to many previous studies, maternal age and race did not appear to influence the likelihood of premature delivery. This finding may suggest these maternal factors are not as strongly predictive of prematurity as previously thought. However, it is more likely that this cohort did not include enough women in the youngest and oldest age categories and was not ethnically diverse enough to demonstrate any potential differences that may in fact be present.

The final models do confirm other previously proposed risk factors for prematurity, including the number of prenatal visits and diabetes in pregnancy. First, as the number of prenatal visits increases from 0-9 visits to 10-16 visits the likelihood of premature delivery decreases by a factor of .11 (95% CI 0.08,0.16, $p<0.0005$). Similarly, women who attended more than 16 prenatal visits experienced a decrease in the odds of premature delivery by .15 when compared to women attending only 0-9 visits (95% CI 0.07,0.32, $p<0.0005$). In other words, greater than 9 prenatal care visits translated into a decreased likelihood of prematurity independent of other risk factors. One might conclude that the number of prenatal visits serves to quantify adequate prenatal care, wherein the greater number of visits increases adequacy and thus, decreases the likelihood of negative outcomes. Yet clinically, women attending more visits may require greater surveillance due to comorbidities that elevate the risk of a negative pregnancy outcome, including prematurity. Therefore, as half of this study's pregnancies may be deemed high risk by obtaining a fetal echocardiogram,

the number of prenatal visits and likelihood of delivery must be interpreted carefully and may not be generalizable to the general population of pregnant women.

Second, when considering the main effects without the interaction term in the model, women in this cohort who carried a diagnosis of diabetes in pregnancy were 4.8 times more likely to deliver prematurely than non-diabetic women (95% CI 3.25,7.04, $p<0.0005$). While in the final model diabetes in pregnancy is not a significant risk factor, this is due to its presence in the interaction with the number of prenatal visits. It is well described that compared to women without diabetes, women with poor glycemic control during pregnancy are more likely to deliver prior to 37 weeks gestation due to fetal macrosomia and placental health, among other maternal and fetal health issues. In general, the degree of glycemic control worsens with diabetes type; gestational diabetes poses the least risk, Type II greater risk, and Type I the greatest risk. Intuitively, women with diabetes of any type who require insulin to achieve adequate control, including by definition Type I, are at the greatest risk of negative birth outcomes.

Women with diabetes in pregnancy are likely to require more prenatal care visits to evaluate how adequately the diabetes is controlled and to closely monitor fetal development. Therefore, it is not surprising that there is a significant interaction between the number of prenatal care visits and diabetes in pregnancy. Intuitively, the more difficult a pregnant woman's diabetes is to control, the more visits she will likely attend. This analysis revealed that women who are diabetic and attend 10-16 prenatal visits are 6.7 times more likely to deliver prematurely than women with diabetes who attend 9 or fewer appointments (95% CI 4.94, 8.52 $p=0.006$). Curiously, women with diabetes who attend more than 16 prenatal care

visits are 3.2 times more likely to deliver prematurely than women with diabetes who attend less than 9 visits, however not at a statistically significant level (95% CI 1.38, 5.06 $p$=0.253). In other words, the risk of delivering prematurely is greater for women who attend less than 9 visits but less than women who attend 10-16 visits. While this final comparison cannot be deduced from this analysis, it may suggest that there is a continuum of both diabetes and the adequacy of prenatal care visits that are simultaneously exerting their effects on the outcome. When comparing the same interaction for women without diabetes, we see that there remains a protective effect of more prenatal care visits that is nearly equal for 10-16 visits (OR=0.1, 95%CI 0.08,0.16, p<0.0005) and greater than 16 visits (OR=0.2, 95%CI 0.07,0.32, p<0.0005) when both are compared to 0-9 visits. Diabetes and the efficacy of prenatal care are avid areas of research and future studies will contribute much to the interpretation of this interaction.

Prenatal care in the first 12 weeks of gestation is also significantly related to the outcome in this model, yet the direction of influence is contrary to conclusions drawn in previous studies. In this cohort women receiving prenatal care after the first trimester were less likely to deliver prematurely than women seeking care in the first 12 weeks of pregnancy by a factor of .35 (95% CI 0.24-0.52, $p$<0.0005). In other words, in this cohort later prenatal care was associated with a reduced likelihood of premature delivery.

The explanation for this relationship is unclear. In addition to the dichotomous variable in the present model, gestational age at the first prenatal visit was also analyzed as a continuous variable and as a categorical variable in which gestational age was divided into 1$^{st}$, 2$^{nd}$, and after the 2$^{nd}$ trimester. Each

orchestration of this risk factor was significant in the model, and each suggested that later prenatal care was associated with a decreased likelihood of prematurity. Considering the extent of the literature endorsing early prenatal care as a means of reducing pregnancy-related and infant morbidity, it is not likely that this model identifies information to the contrary. On the other hand, it is more probable that this cohort is at a higher risk for pregnancy-related morbidity and thus seeking early prenatal care represents a heightened concern for risk; women with medical problems, or a history of a complicated pregnancy, may be more likely to seek prenatal care earlier than women without such conditions. In this scenario, early prenatal care may be acting as a confounder between other risk factors and outcome.

Perhaps not surprisingly, the group into which women were initially recruited is associated with their likelihood of premature delivery. Women recruited into the Echo cohort but who delivered a normal infant were twice as likely to delivery prematurely than women in the Routine Prenatal care cohort (OR=2.0, [95% CI 1.45,2.76], $p<0.0005$). Similarly, women pregnant with a fetus with a conotruncal or VSD heart defect, or other congenital heart defect, were 3.2 (95% CI 1.78-5.77, $p<0.0005$) and 3.4 (95% CI 1.62-7.18, $p<0.0005$) times more likely to deliver prematurely than women in the Routine Prenatal Care cohort who delivered normal infants, respectively. Considering the potential anomalies associated with these defects, it is not surprising that women in the CHD groups are at an increased risk for premature delivery. Women in the Echo cohort, those who were recruited at the time of undergoing a fetal echocardiogram but delivered a baby without any identified heart defects, may also be at increased risk of premature delivery. For

example, the majority of women with pre-existing diabetes will obtain a fetal echo and many of these women deliver normal babies albeit prematurely.

Although this study did not identify a relationship between folate and prematurity, this model does suggest a novel risk factor related to the incidence of prematurity- water source. Considering the results of previous studies that have connected water contaminates to congenital anomalies, it does not seem likely that water source is a spurious finding in our model (Goldberg, 1990; Croen, 1997). In this cohort of women, those whose home water source was from a well were 1.58 times more likely to deliver prematurely than women whose homes received city water (95% CI 1.02-2.45, $p$=0.041). Whether this risk is associated with the water itself, or whether water source is a proxy for another risk factor such as rural versus urban residence, is unknown and cannot reliably be deciphered from this data set. However, as it remains a significant predictor after controlling for many known risk factors for prematurity, the influence of water source upon pregnancy outcome may be an important area of research to be investigated.

Finally, this study provides important descriptive information about a cohort of women receiving prenatal care in Oregon from 1996 to 2000 that is of public health importance. Our cohort reflected the mean age of pregnant women in Oregon and the United States, but contained a higher percentage of white women and fewer women of all other races and ethnicities (Oregon Vital Statistics, Annual Health Report 2003- Tables 2-7, 2-8; Hamilton, 2004). Study participants were more educated than the population of women giving birth in both Oregon and the U.S., and were more likely to be married than pregnant Oregon women and American

women (Oregon Vital Statistics, Annual Health Report 2003- Tables 2-7, 2-8; Hamilton, 2004).

Similar to state and national estimates, half of the pregnancies in this study were not planned (www.agi-usa.org). Interestingly, while 84.1% of American women receive prenatal care in the first trimester, 81.3% of Oregon women, and only 76.3% of this cohort of women received pregnancy-specific health care in the first 12 weeks of pregnancy (Oregon Vital Statistics, Annual Health Report 2003- Tables 2-7, 2-8; Hamilton, 2004). While the proportion of preterm births in the U.S. has remained relatively steady around 12.3% for several years, Oregon maintains a proportion of 9.7%. When considered in its entirety, however, our cohort had a preterm delivery proportion of 16.7%. Yet when divided into their original cohorts, 22.5% of women referred for an echocardiogram, and 10.4% of women receiving routine prenatal care, experienced a preterm birth. Thus, although women in the Echo cohort experienced a greater likelihood of preterm birth than the general population of Oregon and American women, women in the normal cohort were less likely than American women and slightly more likely than Oregon women to deliver before gestational maturity.

According to a 2004 survey by the March of Dimes, 40% of women 18-45 reported taking a multivitamin containing folic acid and 12% of women acknowledged that folic acid should be taken before conception to prevent birth defects (Carter, 2004). Yet this 2004 estimate follows nine years of steadily increasing knowledge and folic acid supplementation behavior. In 1996, when this study began, slightly less than 30% of reproductive-aged women reported taking a supplement containing folic acid and only 5% were knowledgeable of its importance

in preventing birth defects at the time of conception. These percentages increased to approximately 32% and 10%, respectively, in the year 2000 when this investigation concluded. Of our sample, 68.7% of women took folate during the periconceptional period, and 85.3% took folic acid some time during pregnancy. Thus, within this sample, female Oregonians are more aware of the importance of folic acid supplementation in pregnancy than national estimates. However, education efforts to increase knowledge regarding the importance of beginning supplementation prior to conception need to be strengthened.

*Limitations*

As this is a secondary data analysis, the limitations of this investigation may be divided into two separate categories- limitations inherently associated with a secondary analysis, as well as those that may be associated with the primary data. One of the major disadvantages of a secondary data analysis is that additional variables not pertinent to the primary hypothesis, but of interest to the secondary research question, cannot be collected. For example, a strong predictor of spontaneous preterm birth is a prior history of delivering an infant prematurely. Unfortunately, this data was not available from the original study data for the present analysis.

Similarly, one of the primary questions in this study was whether the timing of folate supplement initiation affects the incidence of prematurity. In constructing this hypothesis it was assumed that, like NTDs and CHD, folate deficiencies in the periconceptional period and first trimester would be associated with a greater risk of prematurity than later in pregnancy. However, as the recent investigations by Marti-Carvajal and Siega-Riz suggest, folate levels in the second and third trimesters may be

significantly related to prematurity. Whereas study interviews took place early in the second trimester, accurate assessment of folate supplementation in later pregnancy was not collected. It was assumed that once a woman began taking folate supplements she would continue taking them until the completion of her pregnancy. A brief subsequent analysis of this data revealed that approximately 20% of women who began taking folic acid supplements either before or during pregnancy stopped taking them at some point before the week they were interviewed. This proportion, however, is only an estimate and the true persistence of folate supplementing behavior is not known. As the true behavior is not known, there exist a few ways in which assuming persistence, if incorrect, could affect the interpretation of results. If women who do not take folate throughout pregnancy stop for reasons that are associated with their innate risk of prematurity, differential misclassification could result. For example, if women perceived themselves to be at a high risk for prematurity due to pregnancy complications, they may be less likely to stop all potentially beneficial interventions (such as folate) than women who believe themselves to be at very little risk of preterm birth. This type of association would bias the results away from the null hypothesis, incorrectly establishing correlations that do not truly exist. In contrast, if the reasons for stopping are not related to the known and unknown risks of prematurity, non-differential misclassification would result and thus bias the results away from the null-hypothesis, thereby diluting an effect if one were present.

An additional limitation of this analysis stems from the temporal setting in which the primary study was conducted. The period during which the data was collected, 1996 to 2000, includes two years before and after the FDA required grain

products to be fortified with folate. Therefore, serum levels of folic acid could conceivably be elevated in the second two years compared to the first, differentially affecting the incidence of prematurity between these two groups if an association exists. While Morris et al identified conception prior to 1998 as a significant variable in their model predicting congenital heart disease, it was not statistically significant in reducing prematurity. Although this analysis found that conception prior to 1998 is not a factor, this difference does exist and ideally this study would be conducted during a period of unchanging levels of folate in commercial food.

The limitations of this study attributable to the primary study include both random as well as systematic error that may affect the precision and accuracy of the study results, respectively. While the interview instrument was standardized, refined, and administered by trained interviewers, the random error introduced by a non-fully-automated instrument may reduce the precision of our measures. Similarly, the method by which gestational age was determined, ultrasound dating by fetal femur and crown-rump length in conjunction with time from last normal menstrual period, includes an element of error. Evaluating previous studies regarding the dating of fetuses without identified congenital anomalies, the standard deviation of the estimated gestational age via second trimester ultrasounds is approximately 3.3 days, or 0.47 weeks (Nackling and Backe, 2000). Therefore, we can assume that the measures of gestational age in this study are similarly characterized by this error. Finally, the diet data was assessed and analyzed by the Block Food Frequency Questionnaire and Analysis Program to evaluate the proportion of folate in each subjects' diet. Any inherent flaws or imprecision of this tool would also be transferred to the measurement of the independent variables. However, because all

of these above imprecisions are likely to affect all study subjects equally, they will not bias the results in the direction of either the null or alternative hypotheses.

Limitations of this study based upon systematic error may also affect the interpretation of the results. While the interviewers were trained to administer the questionnaire, it was not possible to blind interviewers to a woman's history of suspected potential fetal anomalies. Such knowledge may have affected the interviewer's approach to questioning, differentially affecting the interviewee's responses. Likewise, women who have recently been identified as carrying a fetus with a potential anomaly may respond to questions about their lifestyle and behavior during the period immediately prior and since conception differently than women who believe themselves to be carrying an unaffected fetus. The resulting potential recall bias could also reduce the accuracy of the interview and diet diary data. Unlike random errors, these systematic errors potentially bias the conclusions of the study, differentially directing the test statistics either toward or away from the rejection of the null hypotheses. Thus, the outcomes of this study must be interpreted in consideration of these potential inaccuracies.

Finally, the generalizability of this study may be constrained by the limited demographic diversity of the study population. The racial and ethnic constitution of the Oregon population, from which all members of the study were gathered, is less diverse than that of the entire U.S. population and, moreover, even less diverse than the population of pregnant Oregon women delivering during the same time period. Therefore, the conclusions drawn from this study sample may not reflect the effect of folate supplementation upon premature births for women of all races and ethnicities; hence, extrapolations must be generated cautiously. Furthermore, this

data was collected from women living in a developed country. While the plausible biological mechanisms underlying any potential result are likely to be among women of all nations, the impact of folate upon the incidence of premature births is likely to differ for each country reflecting the differential influence of numerous other factors. Thus, the conclusions drawn from this study may not be appropriately generalized to women living in other countries.

*Summary and Directions for Further Research*

While this study did not suggest that folic acid reduces the incidence of prematurity, the analysis did not focus on the latter months of pregnancy. Particularly following the 2004 papers of Siega-Riz and Marti-Carvajal, a prospective cohort monitoring pregnant women's persistence in folic acid supplementation from the period before conception until delivery would contribute much to this controversy surrounding this hypothesis. Furthermore, it would be of interest to prospectively follow not only reported dietary and supplement behaviors, but also to collect serum and erythrocyte folate levels, as well as genetic polymorphisms of folate metabolism. As technology advances, these indices will contribute additional strength to either support or refute a connection between folic acid supplementation and the incidence of prematurity.

In contrast to the significance of folic acid, this analysis identified well water as an independent risk factor for premature delivery when compared to city water. As previous studies have suggested a relationship between water contaminates and congenital anomalies, this association necessitates further investigation.

Finally, this study revealed that while a greater proportion of women in this cohort began taking folic acid before conception than national estimates, the

importance of taking folate supplements prior to conception needs to be encouraged and education efforts continued.

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**Figure 1. Study Population**

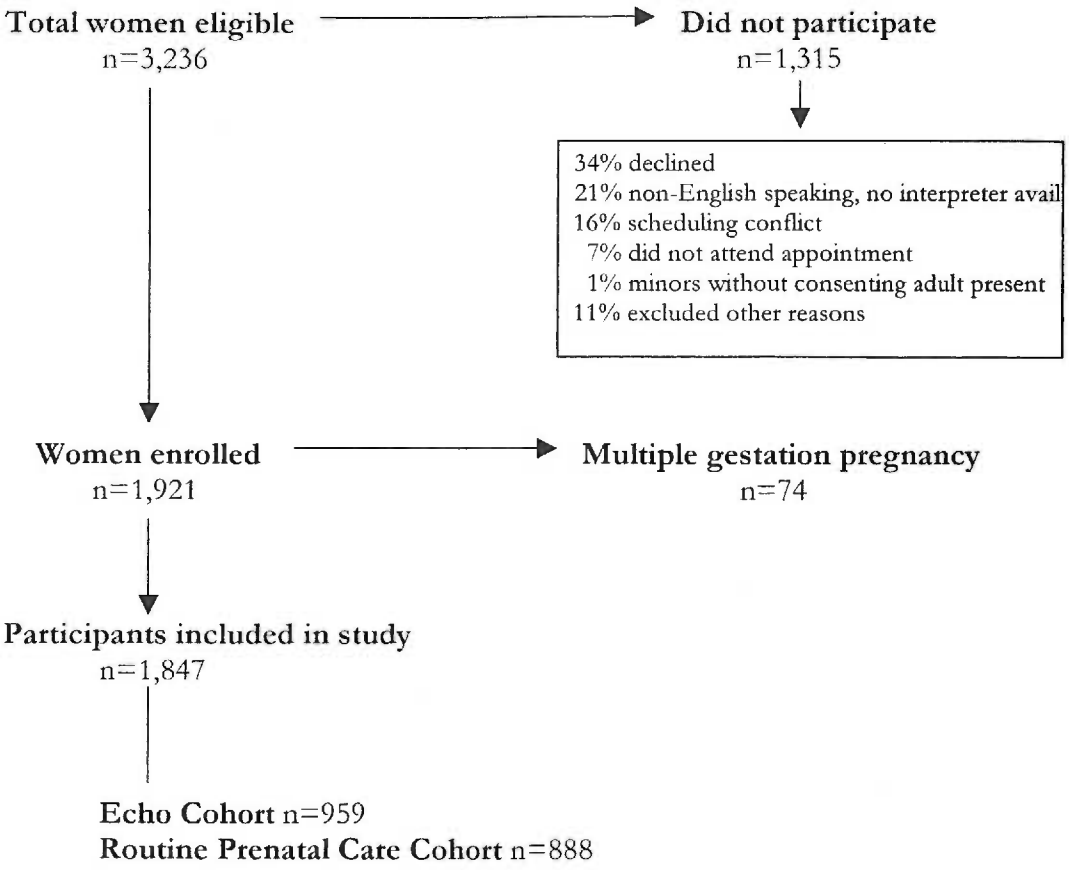


**Table 1. Demographic Characteristics**

| Characteristic | Value (n=1,847) |
|---|---|
| Maternal Age – years | |
| Mean | 27.3 |
| SD | 6.3 |
| Median | 27.0 |
| Range | 14-46 |
| Race – no. (%) | |
| White | 1657 (89.7) |
| Black | 93 (5.0) |
| American Indian/Alaskan Native | 27 (1.5) |
| Asian/Pacific Islander | 70 (3.8) |
| Hispanic – no. (%) | 198 (10.7) |
| Education – no. (%) [1] | |
| At least some: | |
| High School | 783 (42.4) |
| College | 752 (40.7) |
| Graduate School | 311 (16.9) |
| Health Insurance – no. (%) | |
| Public | 688 (37.3) |
| Private | 1028 (55.7) |
| Self | 131 (7.1) |
| Married – no. (%) | 1157 (62.6) |

[ ] number missing from estimate

**Table 2. Pregnancy Related Characteristics**

| Characteristic | Value |
|---|---|
| Planned Pregnancy - no. (%) | 878 (47.5) |
| Diabetes in Pregnancy - no. (%) | 264 (14.3) |
| Gestational diabetes - no. (%)[ξ] [6] | 94 (5.1%) |
| Type I Diabetes - no. (%) | 74 (4.0%) |
| Type 2 Diabetes - no. (%) | 99 (5.4%) |
| Insulin dependent - no. (%) | 141 (7.6%) |
| History of spontaneous abortion - no. (%) | 481 (26.0%) |
| History of therapeutic abortion - no. (%) | 372 (20.7%) |
| Gestational age first suspected pregnant [9] | |
| Mean | 4.09 |
| SD | 2.5 |
| Median | 4 |
| Min, Max, Range | -5, 22, 27 |
| Gestational age first Prenatal Visit [6] | |
| Mean | 9.73 |
| SD | 4.6 |
| Median | 9 |
| Min, Max, Range | 3, 35, 38 |
| Number of Prenatal Visits [35] | |
| Mean | 12.29 |
| SD | 3.9 |
| Median | 12 |
| Min, Max, Range | 0, 34, 34 |
| Gestational age at interview [3] | |
| Mean | 22.29 |
| SD | 5.1 |
| Median | 21.00 |
| Min, Max, Range | 12, 40, 28 |

[ ] number missing from estimate

*while gestational age was not available for 6 cases, the data point "preterm" was complete

ξAll women diagnosed with pre-existing diabetes (Type I or II) were from Echo cohort only; GDM includes 28 cases from Normal cohort. No women in Normal cohort required insulin for diabetes management.

## Figure 2. Timing of Folate Supplement Initiation

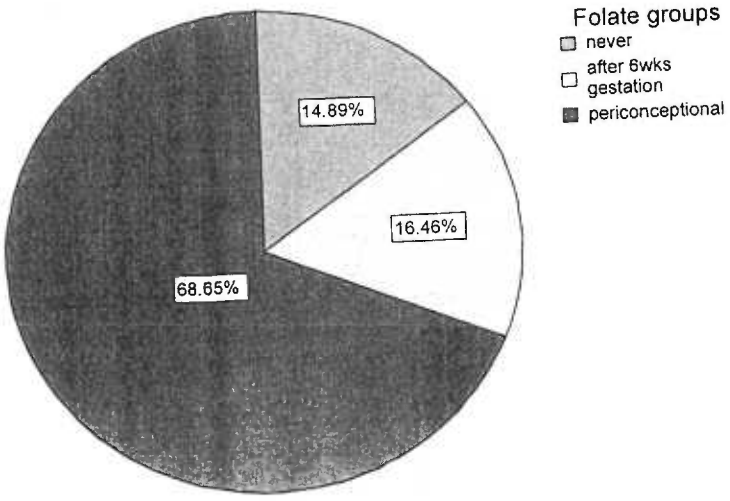


**Table 3. Pregnancy Outcomes by Cohort**

| Outcome | Entire Sample Value n=1,847 | Echo Cohort Value n=959 | Normal Cohort Value n=888 |
|---|---|---|---|
| Gestational age at delivery [6]* | | | |
| Mean | 37.93 | 37.37 | 38.53 |
| SD | 2.8 | 3.1 | 2.3 |
| Median | 38.0 | 38.0 | 39.0 |
| Min, Max | 18,44 | 18, 42 | 18, 44 |
| Pregnancy ending prior to 24wks [6] | 18 (1.0%) | 14 (1.4%) | 4 (0.4%) |
| Preterm – no. (%) | 308 (16.7) | 216 (22.5%) | 92 (10.4%) |

| | number missing from estimate

*while gestational age was not available for 6 cases, the data point "preterm" was complete

Table 4. ANOVA, Model A: Folate Initiation

| Variable | Group | Mean ± SD or Percent | p-value Periconcep vs. Never | p-value After 6wks vs. Never | p-value Periconcep vs. After 6wks |
|---|---|---|---|---|---|
| Maternal age | Periconcep | 28.2 ± 6.1 | <0.0005 | 0.10 | <0.0005 |
| | After 6wks | 25.0 ± 6.1 | | | |
| | Never | 25.8 ± 6.3 | | | |
| Maternal race (white) | Periconcep | 92.3% | <0.0005 | 0.01 | 0.07 |
| | After 6wks | 86.8% | | | |
| | Never | 81.1% | | | |
| Hispanic | Periconcep | 6.7% | <0.0005 | 0.85 | <0.0005 |
| | After 6wks | 19.7% | | | |
| | Never | 19.3% | | | |
| Level education (>1yr college) | Periconcep | 67.1% | <0.0005 | 0.93 | <0.0005 |
| | After 6wks | 37.8% | | | |
| | Never | 35.6% | | | |
| Public insurance | Periconcep | 29.5% | <.0005 | 0.93 | <0.0005 |
| | After 6wks | 53.9% | | | |
| | Never | 54.5% | | | |
| Married | Periconcep | 68.5% | <.0005 | 0.002 | <0.0005 |
| | After 6wks | 41.8% | | | |
| | Never | 54.2% | | | |
| Planned preg. | Periconcep | 55.0% | <0.0005 | 0.49 | <0.0005 |
| | After 6wks | 29.9% | | | |
| | Never | 32.7% | | | |
| Gest. age @ 1st prenatal visit | Periconcep | 9.1 ± 4.0 | <0.0005 | 0.34 | <0.0005 |
| | After 6wks | 11.4 ± 5.5 | | | |
| | Never | 11.0 ± 5.5 | | | |
| Number prenatal visits | Periconcep | 12.6 ± 3.8 | 0.04 | 0.20 | <0.0005 |
| | After 6wks | 11.5 ± 4.1 | | | |
| | Never | 11.9 ± 4.0 | | | |
| Gest. age @ delivery | Periconcep | 38.0 ± 2.7 | 0.02 | 0.11 | 0.75 |
| | After 6wks | 37.9 ± 2.9 | | | |
| | Never | 37.9 ± 2.3 | | | |
| Premature | Periconcep | 15.7% | 0.06 | 0.11 | 0.75 |
| | After 6wks | 16.4% | | | |
| | Never | 21.5% | | | |

Table 5. ANOVA: Model B, Any Folate

| Variable | Significance | Folate group is… |
|---|---|---|
| Maternal age | <.0005 | Older |
| Race | <.0005 | Whiter |
| Hispanic | <.0005 | Less hispanic |
| Level of education | <.0005 | More educated |
| Public insurance | <.0005 | Fewer publicly insured |
| Married | 0.002 | Married |
| Gravidity | 0.002 | Fewer deliveries |
| Parity | <.0005 | Fewer pregnancies |
| Planned pregnancy | <.0005 | More planned pregnancies |
| Gest. age @ 1st prenatal visit | <.0005 | Earlier |
| Number of prenatal visits | 0.08 | More |
| Gest. age @ delivery | 0.009 | Later |
| Premature | 0.021 | Fewer |
| Low birth weight | .609 | Same |
| Infant weight | .005 | Greater weight |

**Table 6. Univariate Analysis, Noncontinuous Variables**

| Variable | Categorical | Coefficient | Wald | Sig | Exp | 95% CI |
|---|---|---|---|---|---|---|
| Conception prior 1998 | | -0.136 | 1.342 | 0.247 | 0.873 | .694,1.098 |
| Defect type | | | 44.724 | <.0005 | | |
| | no defect | ref | ref | Ref | ref | ref |
| | conotruncal | -1.268 | 16.89 | <.0005 | 0.281 | .154,.515 |
| | incidental | -0.151 | 0.137 | 0.711 | 0.86 | .388,1.907 |
| | other | -0.322 | 0.59 | 0.442 | 0.724 | .318,1.649 |
| | VSD | -0.343 | 0.59 | 0.442 | 0.71 | .296,1.703 |
| Echo done | | -0.911 | 53.209 | <.0005 | 0.402 | .315,.514 |
| Hispanic | | 0.302 | 2.871 | 0.09 | 1.352 | .954,1.917 |
| Fetal arrhythmia | | -0.936 | 11.306 | 0.001 | 0.392 | .227,.677 |
| Maternal education | | | 15.281 | <.0005 | | |
| | high school | ref | ref | Ref | ref | ref |
| | college | -0.414 | 10.587 | 0.001 | 0.661 | .515,.848 |
| | graduate | -0.539 | 9.403 | 0.002 | 0.583 | .413,.823 |
| Married | | -0.225 | 3.582 | 0.058 | 0.799 | .633,1.008 |
| Work in pregnancy | | -0.296 | 6.439 | 0.011 | 0.744 | .592,.935 |
| Maternal congenital heart disease | | 0.35 | 2.157 | 0.142 | 1.419 | .890,2.264 |
| Smoked 3 mo prior | | 0.191 | 2.393 | 0.122 | 1.211 | .950,1.543 |
| Smoked 6wks after | | 0.155 | 1.489 | 0.222 | 1.168 | .910,1.499 |
| Drink 3mo prior | | -0.517 | 19.05 | <.0005 | 0.597 | .473,.752 |
| Drink 6 wks after | | -0.321 | 6.829 | 0.009 | 0.725 | .570,.923 |
| Amt 3 mo prior | | | 25.198 | <.0005 | | |
| | 0 | ref | ref | Ref | ref | ref |
| | 0.5 | -0.369 | 8.109 | 0.004 | 0.692 | .537,.891 |
| | 1.5 | -0.757 | 15.494 | <.0005 | 0.469 | .322,.684 |
| | 3.5 | -0.845 | 7.449 | 0.006 | 0.43 | .234,.788 |
| | 5.5 | -1.305 | 4.591 | 0.032 | 0.271 | .082,.895 |
| | 7 | -0.526 | 0.497 | 0.29 | 0.591 | .223,1.564 |
| Amt 6 wks | | | 9.938 | 0.077 | | |
| | 0 | ref | ref | Ref | ref | ref |
| | 0.5 | -0.222 | 2.807 | 0.094 | 0.801 | .618,1.038 |
| | 1.5 | -0.811 | 7.216 | 0.007 | 0.444 | .246,.803 |
| | 3.5 | -0.41 | 0.836 | 0.361 | 0.664 | .275,1.598 |
| | 5.5 | -19.878 | 0 | 0.999 | 0 | 0 |
| | 7 | 0.344 | 0.255 | 0.614 | 1.41 | .371,5.355 |
| Binge 3 mo | | -0.327 | 4.017 | 0.045 | 0.721 | .523,.993 |
| Water source | | 0.576 | 9.513 | 0.002 | 1.779 | 1.234,2.565 |
| Drink supp | | 0.368 | 3.569 | 0.059 | 1.444 | .986,2.115 |
| Any drink supp | | -0.199 | 1.705 | 0.192 | 0.82 | .608,1.105 |
| Fortified cereal | | | | | | |
| Morning sickness first 6 wks | | -0.242 | 4.205 | 0.04 | 0.785 | .623,.989 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Veggie diet | | -0.765 | 10.762 | 0.001 | 0.465 | .294,.735 |
| Diabetic diet | | 1.467 | 69.777 | <.0005 | 4.337 | 3.074,6.118 |
| Abnormal ultrasound | | 0.849 | 29.384 | <.0005 | 2.338 | 1.720,3.178 |
| Extra-cardiac anomaly | | 1.058 | 13.591 | <.0005 | 2.882 | 1.642,5.059 |
| Polyhydramnios | | 2.37 | 7.986 | 0.005 | 10.699 | 2.068,55.369 |
| Low birth weight | | 4.424 | 219.20 | <.0005 | 83.413 | 46.440,149.820 |
| Type I Diabetes | | 1.492 | 51.188 | <.0005 | 4.444 | 2.953,6.687 |
| Type 2 Diabetes | | 1.394 | 35.616 | <.0005 | 4.03 | 2.550,6.369 |
| Insulin dependent | | 1.44 | 65.256 | <.0005 | 4.22 | 2.976,5.985 |
| Any diabetes in pregnancy | | 1.281 | 75.011 | <.0005 | 3.600 | 2.694, 4.810 |
| Connective tissue dz | | 0.756 | 1.881 | 0.17 | 2.129 | .723,6.266 |
| Urinary tract infection | | 0.433 | 6.301 | 0.012 | 1.542 | 1.1,2.163 |
| Urinary tract inf 6wks | | 0.723 | 10.868 | 0.001 | 2.06 | 1.341,3.166 |
| Upper respiratory inf | | -0.163 | 1.799 | 0.18 | 0.849 | .669,1.078 |
| Group | | | 58.24 | <.0005 | | |
| | Normal cohort, normal baby | Ref | Ref | Ref | Ref | Ref |
| | Echo cohort, normal baby | .907 | 41.40 | <.0005 | 2.476 | 1.879, 3.264 |
| | Conotruncal, VSD | 1.456 | 29.54 | <.0005 | 4.289 | 2.537, 7.251 |
| | Other CHD | 1.381 | 16.50 | <.0005 | 3.978 | 2.043,7.746 |
| | | | 17.82 | .001 | | |
| Reference source | | | | | | |
| | OHSU | Ref | Ref | Ref | Ref | Ref |
| | Emanuel | .306 | 3.31 | .069 | 1.358 | .976, 1.888 |
| | Bend | -.152 | .059 | .808 | .859 | .253, 2.918 |
| | Kaiser | .722 | 10.81 | .001 | 2.059 | 1.339, 3.168 |
| | St. Vincent's | .804 | 6.98 | .008 | 2.235 | 1.231, 4.060 |
| | | | 9.784 | 0.02 | | |
| Prepregnancy Body Mass Index category | <19 | ref | Ref | Ref | ref | ref |
| | 19.0-24.9 | -0.492 | 5.728 | 0.017 | 0.611 | .408,.915 |
| | 25-29.9 | -0.275 | 1.473 | 0.225 | 0.76 | .487,1.184 |
| | >30 | -0.114 | 0.261 | 0.609 | 0.892 | .575,1.384 |
| | | | | <0.000 | | |
| | | | 127.18 | 5 | | |
| Number prenatal visits category | none | ref | Ref | Ref | ref | ref |
| | 1-5.0 | -20.41 | 0 | 1 | 0 | 0 |
| | 6-10.0 | -22.3 | 0 | 1 | 0 | 0 |
| | 11-15.0 | -23.247 | 0 | 1 | 0 | 0 |
| | 16-20 | -22.475 | 0 | 1 | 0 | 0 |
| | 21-25 | -21.823 | 0 | 1 | 0 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 26-30 | -21.805 | 0 | 1 | 0 | 0 |
| | >30 | -42.402 | 0 | 0.999 | 0 | 0 |
| Maternal age, 5 groups | | | 2.133 | 0.711 | | |
| | <20 | ref | Ref | Ref | ref | ref |
| | 21-25 | .118 | .376 | .540 | 1.126 | .771, 1.644 |
| | 26-30 | -.089 | .203 | .652 | .915 | .621, 1.347 |
| | 31-35 | -.115 | .307 | .579 | .892 | .594, 1.338 |
| | >36 | -.037 | .023 | .879 | .964 | .600, 1.547 |
| Maternal race | | | 5.6 | 0.133 | | |
| | white | ref | Ref | Ref | ref | ref |
| | black | 0.407 | 2.868 | 0.09 | 1.502 | .938,2.405 |
| | amer ind/Alaskan nat. | 0.6 | 1.982 | 0.159 | 1.822 | .790,4.198 |
| | asian/pacific islander | -0.281 | 0.712 | 0.399 | 0.755 | .394,1.450 |
| Folate group | | | 5.364 | 0.068 | | |
| | never | ref | ref | Ref | ref | ref |
| | >6wks gest | -0.328 | 2.357 | 0.125 | .721 | .474, 1.095 |
| | periconcep | -0.383 | 5.339 | 0.021 | .682 | .492, .943 |
| Any folate | | -0.372 | 5.265 | 0.022 | .689 | .501, 0.947 |

Table 7. Univariate Analysis, Continuous Variables

| Variable | Coefficient | Wald | Sig | Exp | 95% CI |
|---|---|---|---|---|---|
| Maternal Age | 0.001 | 0.02 | 0.891 | 1.001 | .983,1.020 |
| Prepregnancy weight | 0.001 | 1.05 | 0.305 | 1.001 | .999,1.004 |
| Prepregnancy Body Mass Index | 0.018 | 4.65 | 0.031 | 1.018 | 1.002,1.034 |
| Number prenatal visits | -0.08 | 23.77 | <.0005 | 0.923 | .894,.953 |
| Birth weight | -0.003 | 312.41 | <.0005 | 0.997 | .997,.998 |
| Gravidity | 0.042 | 1.86 | 0.173 | 1.043 | .982,1.109 |
| Parity | 0.047 | 0.99 | 0.319 | 1.048 | .956,1.149 |
| Daily folate 3mo prior | 0 | 2.48 | 0.116 | 1 | 1.000, 1.001 |
| Daily folate 6wks prior | 0 | 4.49 | 0.034 | 1 | 1.000, 1.000 |
| Daily B12 6wks after | -0.004 | 1.78 | 0.182 | 0.996 | .991,1.002 |
| Daily folate wk interview | 0 | 2.03 | 0.155 | 1 | 1.000,1.000 |
| Gest. Age suspected preg | -0.041 | 2.44 | 0.118 | 0.96 | .911,1.011 |
| Gest. Age at prenatal visit | -0.023 | 2.61 | 0.106 | 0.977 | .949,1.005 |

**Table 8. Variable subsets for multivariate analyses**

| Study Factors | Demographic Factors | Maternal Pre-pregnancy Factors | Maternal Behavioral Factors |
|---|---|---|---|
| Group | Race | Conceived prior 1998 | Smoked 3 mo prior |
| Reference source | Hispanic | Maternal heart disease | Smoked 6wks after |
| | Education level | Work in pregnancy | Drink 3mo prior |
| | Married | Pre-pregnancy BMI | Drink 6 wks after |
| | Maternal age | Numb prenatal visits | Amt 3 mo prior |
| | | Gravidity | Amt 6wks after |
| | | Gest age suspected pregnant | Binge drink 3 mo prior |
| | | Gest age 1st prenatal visit | |

| Nutrition Factors | Comorbidity Factors | Fetal Factors |
|---|---|---|
| Water source | Type I DM | Defect type |
| Drink supp | Type II DM | Diagnosis |
| Any drink supp | Insulin in preg | Echo done |
| Fortified cereal | Any diabetes in preg | Fetal arrhythmia |
| Veggie diet | Connective tissue dz | Extra cardiac anomaly |
| Diabetic diet | Urinary tract inf | Abnormal Ultrasound |
| Folate Initiation | Urinary tract inf 6 wks | Extra cardiac anomaly |
| Any folate | Upper respiratory inf | Polyhydramnios |
| Morning sickness 1st 6wks | | Nonimmune hydrops fetalis |

**Table 9. Final Multiple Logistic Regression Model- Model A: Folate Initiation**

| Variable | OR | 95% CI | P-Value |
|---|---|---|---|
| Maternal Age | | | .749 |
| <20* | - | - | - |
| 21-25 | 1.0 | .66, 1.55 | .954 |
| 26-30 | .9 | .55, 1.34 | .504 |
| 31-35 | .8 | .51. 1.30 | .390 |
| >36 | .8 | .45, 1.36 | .383 |
| Maternal race | | | .139 |
| White* | - | - | - |
| Black | 1.8 | 1.02, 3.19 | .044 |
| Amer. Ind/Alaskan Nat. | 1.6 | .60, 4.16 | .361 |
| Asian/Pacific Islander | .7 | .32, 1.65 | .439 |
| Prenatal care after 1st trimester | .4 | .24, .52 | <.0005 |
| Group | | | <.0005 |
| Normal | - | - | - |
| Echo | 2.0 | 1.45, 2.76 | <.0005 |
| Conotruncal or Ventricular Septal Defect | 3.2 | 1.78, 5.77 | <.0005 |
| Other Congenital Heart Defect | 3.4 | 1.62, 7.18 | .001 |
| Water source | 1.6 | 1.02, 2.45 | .041 |
| Number prenatal visits$^{\Psi}$ | | | <.0005 |
| 0-9* | - | - | - |
| 10-16 | .1 | .09, .18 | <.0005 |
| >16 | .1 | .07, .19 | <.0005 |
| Diabetes in pregnancy$^{\Psi}$ | 4.8 | 3.25, 7.04 | <.0005 |
| Numb prenatal visits x Diabetes in Pregnancy | | | .013 |
| 0-9 visits x Diabetes* | - | - | - |
| 10-16 visits x Diabetes | 6.7 | 4.94, 8.52 | .006 |
| >16 visits x Diabetes | 3.2 | 1.38, 5.06 | .253 |
| Numb prenatal visits x No Diabetes in Pregnancy | | | <.0005 |
| 0-9 visits x No Diabetes* | - | - | - |
| 10-16 visits x No Diabetes | .1 | .08, .16 | <.0005 |
| >16 visits x No Diabetes | .2 | .07, .32 | <.0005 |
| Folate Group | | | .516 |
| Never* | - | - | - |
| After 6 wks gestation | .8 | .47, 1.22 | .250 |
| Periconceptional | .9 | .60, 1.29 | .514 |

*Referent

$\Psi$ Values from main effects model without interaction

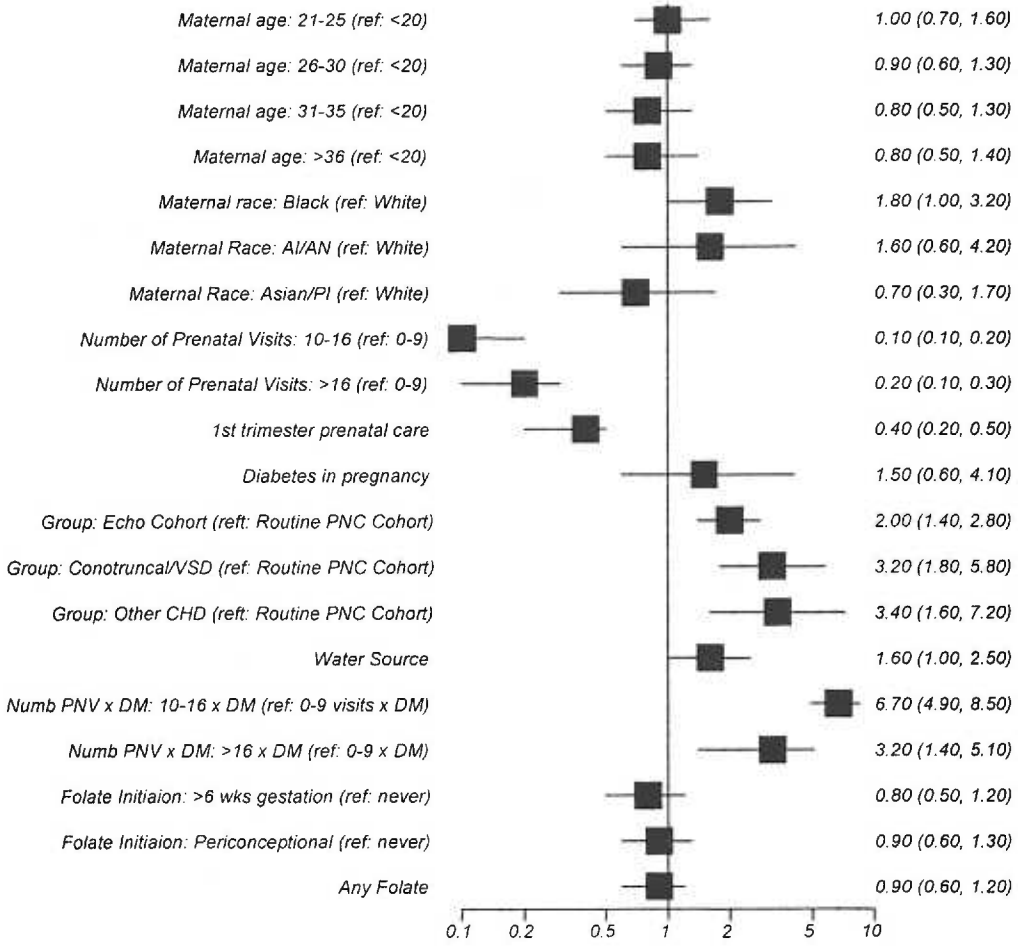
**Table 10. Final Multiple Logistic Regression Model- Model B: Any Folate**

| Variable | OR | 95% CI | P-value |
|---|---|---|---|
| Maternal age | | | .793 |
| <20* | - | - | - |
| 21-25 | 1.0 | .67, 1.58 | .886 |
| 26-30 | .9 | .57, 1.36 | .562 |
| 31-35 | .8 | .53, 1.33 | .451 |
| >36 | .8 | .47, 1.39 | .450 |
| Maternal race | | | .144 |
| White* | - | - | - |
| Black | 1.8 | 1.01, 3.16 | .047 |
| Amer. Ind/Alaskan Nat. | 1.6 | .59, 4.13 | .368 |
| Asian/Pacific Islander | .7 | .31, 1.63 | .425 |
| Prenatal care after 1st trimester | .3 | .23, .51 | <.0005 |
| Group | | | <.0005 |
| Normal | - | - | - |
| Echo | 2.0 | 1.45, 2.77 | <.0005 |
| Conotruncal or Ventricular Septal Defect | 3.2 | 1.78, 5.77 | <.0005 |
| Other Congenital Heart Defect | 3.4 | 1.64, 7.25 | .001 |
| Water source | 1.6 | 1.02, 2.46 | .041 |
| Number prenatal visits$^{\psi}$ | | | <.0005 |
| 0-9* | - | - | - |
| 10-16 | .1 | .10, .19 | <.0005 |
| >16 | .1 | .07, .20 | <.0005 |
| Diabetes in pregnancy$^{\psi}$ | 4.8 | 3.23, 7.00 | <.0005 |
| Numb prenatal visits x Diabetes in Pregnancy | | | .013 |
| 0-9 visits x Diabetes* | - | - | - |
| 10-16 visits x Diabetes | 6.7 | 4.95, 8.52 | .006 |
| >16 visits x Diabetes | 3.2 | 1.44, 4.94 | .244 |
| Numb prenatal visits x No Diabetes in Pregnancy | | | <.0005 |
| 0-9 visits x No Diabetes* | - | - | - |
| 10-16 visits x No Diabetes | .1 | .08, .16 | <.0005 |
| >16 visits x No Diabetes | .2 | .07, .32 | <.0005 |
| Any Folate | .9 | .59, 1.23 | .382 |

*Referent

$\psi$ Values from main effects model without interaction

## Figure 3. Final Multiple Logistic Regression Models



# GLOSSARY of VARIABLES and TERMS

- Abn u/s- history of abnormal ultrasound in current pregnancy
- Amt drink 3 mo prior- mean number drinks per occasion drinking 3 months prior conception
- Amt drink 6 wks- mean number drinks per occasion drinking 6 weeks after conception
- Any diabetes in pregnancy- diagnosis of GDM, Type I, or Type II in pregnancy
- Any drink supp- any drink supplements at any time during pregnancy
- Any folate 3 mo- any folate 3 months prior conception
- Any folate 6 wks- any folate 6 weeks after conception
- Any folate wk intvw- any folate week of interview
- Any folate day intvw- any folate day of interview
- Binge 3 mo prior- consume greater than 5 beverages in one period of alcohol consumption 3 months prior to conception
- Binge 6 wks after- consume greater than 5 beverages in one period of alcohol consumption 6 weeks after conception
- Birth weight
- Cohort- fetal echocardiogram or "normal," routine prenatal care cohort
- Conception prior 1998- current pregnancy conceived prior to January 1st, 1998
- CHD MOB- congenital heart defect in mother of baby
- CHD (congenital heart defect) type- no defect, conotruncal, incidental, ventricular septal defect, or other
- CT dz- connective tissue disease
- Diabetic diet- diabetic diet any time during pregnancy
- Diagnosis- specific congenital heart defect diagnosis in affected infants
- Drink 3mo prior- drink any alcohol 3 months prior to conception
- Drink 6 wks after- drink any alcohol 6 weeks after conception
- Drink supp- any drink supplements used at time of interview
- Echo done- fetal echocardiogram performed
- Extra cardiac anomaly- non-cardiac anomaly identified
- Fetal arrhythmia- fetal arrhythmia identified any time current pregnancy
- Folate time ab- took folate both 3 months prior to, and 6 weeks after conception
- Folate 6wks not 3 mo- took folate 6 weeks after but not 3 months prior to conception
- Folate 3mo not 6wk- took folate 3 months prior to conception but not 6 weeks after
- Folate group- folate in periconceptional period, sometime after 6 weeks gestation, none at all

- Folate dichotomous- folate taken at any time during pregnancy, none at all
- Fortified cereal- any fortified cereal ingestion at any time during pregnancy
- GA suspected pregnant- GA mother suspected pregnancy
- GA at prenatal visit- GA at 1st prenatal visit Gravidity
- Group- group assignment after data collection period closed: (1) conotruncal HD or VSD, (2) other CHD, (3) echo cohort, normal baby, (4) normal cohort, normal baby
- Hispanic- self-described Mexican American, Central American, South American, Puerto Rican, Cuban, Dominican, Unknown
- Insulin dependent- insulin dependent diabetes in current pregnancy regardless of diabetes type
- LBW (low birth weight)- infant delivered <2500g
- Marital Status- married, widowed, divorced, separated, never been married, unknown
- Maternal Age- calculated by DOB
- Maternal race- self-described white, black, american indian/alaskan native, asian/pacific islander, unknown
- Maternal education- high school, college, graduate
- Morning sickness 1st 6 wks- any morning sickness in first 6 weeks of pregnancy
- Often drink 3 mo prior- frequency of alcohol consumption 3 months prior to conception: everyday, 5-6 days/wk, 3-4 days/wk, 1-2 days/wk, never
- Often drink 6 wks- frequency of alcohol consumption 6 weeks after conception: everyday, 5-6 days/wk, 3-4 days/wk, 1-2 days/wk, never
- Never took folate- never took folate any time during pregnancy
- Nonimmune hf- non-immune hydrops fetalis
- Number prenatal visits
- Periconceptional period- period from 3 months prior to conception to 6 weeks after conception
- Parity
- Polyhydramnios- polyhydramnios identified any time current pregnancy
- Prepregnancy BMI- body mass index prior to pregnancy
- Prepregnancy weight
- Reference source- OHSU, Emanuel, Bend, Kaiser, St. Vincent's
- Smoke >100- maternal history smoking >100 cigarettes in lifetime
- Smoked 3 mo prior- maternal smoking 3 months prior to conception
- Smoked 6wks after- maternal smoking 6 weeks after conception
- Type I DM- maternal diagnosis Type I diabetes prior to conception
- Type 2 DM- maternal diagnosis Type II diabetes prior to conception
- URI (upper respiratory infection)- URI at any time during pregnancy
- UTI (urinary tract infection)- UTI at any time during pregnancy
- UTI (urinary tract infection) 6wks- UTI 6 weeks after conception
- Veggie diet- vegetarian diet any time during pregnancy

- Water source- home water source: city or well water
- Work in pregnancy- currently or previously working while pregnant