The role of folic acid in promoting reproductive health and pregnancy outcomes

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Thesis

THE ROLE OF FOLIC ACID IN PROMOTING REPRODUCTIVE HEALTH
AND PREGNANCY OUTCOMES

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ANKITA PRASAD

ABSTRACT

Throughout the past several years, there has been growing interest in folic acid as a crucial micronutrient that has several beneficial health outcomes. Given folic acid’s crucial role in DNA synthesis and methylation, it is involved in several processes, especially during development. Although folic acid has been studied previously with several disease processes such as neural tube defects, different types of cancer and even Alzheimer’s disease, specific attention has not been given to folic acid and reproductive health and other pregnancy outcomes such as implantation, embryogenesis and live birth. This thesis will examine the specific effects that folic acid could have in maintaining the reproductive health in women and men as well. The conclusion was reached that folic acid plays a critical role in several phases of reproductive health including maintenance of reproductive hormone levels in woman, protection against anovulation and spontaneous abortions as well improving sperm quality in males. Taken together, folic acid could be an inexpensive, convenient supplement for maintaining reproductive health as well as for couples who are attempting to begin and maintain a healthy pregnancy.
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LIST OF ABBREVIATIONS

B12    Vitamin B12
CAD    Coronary Artery Disease
CDC    Centers for Disease Control and Prevention
CH3    Methyl Group
CRC    Colorectal Cancer
DHFR   Dihydrofolate reductase
ICSI   Intra-cytoplasmic sperm injection
IVF    In Vitro Fertilization
LMP    Last Menstrual Period
PCOS   Polycystic Ovary Syndrome
PLCO   Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial
THF    Tetrahydrofate
INTRODUCTION

Reproductive Health and Diet

Diet has an important influence on a variety of health outcomes, especially reproductive health, and has been studied in detail over the years. A common theme has been that modification of lifestyle factors, such as dietary intake of fiber, can have a real effect on reproductive hormone concentration and particular outcomes in women’s health such as ovulation and eventual pregnancy (Mumford et al., 2011) Furthermore, specific diets such as a high protein diet, could moderately improve certain endocrine and metabolic outcomes in overweight women who are diagnosed with conditions such as polycystic ovary syndrome (PCOS)(Moran et al., 2003). The two previous studies are examples showing that diet can be a cause of changes in reproductive health as well as a treatment for those who have reproductive health syndromes such as PCOS.

Folate and Reproductive hormones

Folate has been studied with a variety of health outcomes but most importantly is its relationship to heart disease. One study found that a combination of folic acid and vitamin B12 supplementation did not lower the risk of heart disease (Albert et al., 2008). Another study similarly found that folic acid did not further reduce homocysteine levels, which is related to heart disease, in postmenopausal women (Ghezzi et al., 2004). In reproductive health, folate has been a particular interest during pregnancy because of its known effects in preventing birth defects (Case et al., 2014). The Centers for Disease Control (CDC) guidelines recommend that women take 400 micrograms every day to prevent birth defects as well as provide healthy cells in the body the necessary nutrients
to make new cells (Center for Disease Control, 2012). Given the relationship of folic acid with many crucial processes in the body, more research on the mechanism of action of folic acid is warranted.

Although there have been many studies on the beneficial effects of folic acid during pregnancy, there are few studies that examine the role of folate or folic acid in healthy, premenopausal women. One key study found that healthy, premenopausal women who had high source of synthetic fiber in their diet had higher progesterone levels and a decreased risk of anovulation (Gaskins et al., 2012). Understanding the pathways that folate affects in normal premenopausal women may give us insight into the variety of dietary factors that affect women’s health and may even promote a more healthy lifestyle. Furthermore, studying factors in diet that affect women’s health can help unravel the myriad of factors that help women maintain their health and well-being.
Specific Aims/Objectives

Many previous studies have demonstrated the effect of folic acid on women’s reproductive health, especially focusing on outcomes such as neural tube defects. Little is known about the effects of folate on a variety of markers of women’s health, such as reproductive hormone levels or ovulation, both of which contribute to a woman’s reproductive success. Furthermore, although the relationship between birth defects and folic acid supplements has been studied extensively, there has not particular focus on folic acid and live birth outcomes or other pregnancy outcomes such as folic acid improving chances of becoming pregnant. Lastly, folic acid has some positive effects in both men and women but it is unclear whether a couple’s therapy of folic acid could improve pregnancy outcomes.

The goal of the present study is to examine the relationship between folate and reproductive health, in both men and women, and pregnancy outcomes. Further, identifying areas of further research are another important component of this thesis. Specifically, this thesis will,

- Assess serum folate levels in a sample of healthy, premenopausal women who were not supplemented with folate and effects on a variety of reproductive hormones and ovulation
- Determine whether folic acid plays a role in other pregnancy outcomes, beside neural tube defects, which includes implantation, embryogenesis and maintenance of pregnancy
• Determine whether there is any precedence for couples therapy with folic acid supplementation in relationship to pregnancy

Further examination of folate levels and the relationship between diet and women’s health will help us understand the myriad of outcomes that diet can affect. Also, due to folate’s interactions with several processes in the body, having a greater understanding of folate’s mechanism of action will help determine what key factors are necessary for maintenance of reproductive health as well as what serum concentrations of micronutrients such as folic acid, are necessary for a successful pregnancy.
BACKGROUND

**Folate and Folic Acid**

Both folate and folic acid have been proven to have several beneficial health impacts in women. Although used interchangeably, folate refers to the naturally occurring compound that is found in several foods such as beans, brussels sprouts, spinach and some fruits (Konings et al., 2001). Folic acid, also known as synthetic folate, is the fully oxidized form that is often used in multivitamins as well as fortified foods such as cereal and other grains (Winkels et al., 2007). Both intake of folate and folic acid are known to increase serum folate levels, although according to some studies, naturally occurring folate found in food provides 78% of the bioavailability of synthetic folic acid (Winkels et al., 2007). Many studies investigate to roles of both folate and folic acid in relation to a variety of health outcomes. This paper will consider the roles of both compounds with a particular emphasis on folic acid supplementation and its effects in women.

**Folic Acid fortification**

Folic acid fortification is common in cereals and other grains within the U.S. and now around the world. One of the major drivers for requiring mandatory fortification of grains and flour in the US starting in 1998 was the prevention of neural tube defects in pregnant women (Solomons, 2007). The beneficial effects of folic acid in the preventing birth defects, specifically neural tube defects such as anencephaly, spina bifida and encephalocele has been in the literature since 1964 (Wald, 1991). In the 1980s, one of the first large randomized control trials was able to show a 72% protective effect of folic acid
preventing neural tube defects in a cohort of women who have had one or more neural tube defects in prior pregnancies when compared to a control population (Wald, 1991). After this study and a few other similar studies that showed the protective effect of folic acid (Laurence et al., 1981; Smithells et al., 1980).

Folic acid fortification was undertaken because minimal success was found by purely using supplementation in preventing birth defects. Although the proven efficacy of folic acid in preventing neural tube defects is undeniable, many pregnancies are unplanned and the window of action (before and up to 8 weeks of pregnancy) of folic acid passes if supplementation of folic acid is solely relied upon (Doležálková & Unzeitig, 2014; Bestwick et al., 2014). Many countries still rely upon supplementation as the main mechanism for achieving appropriate serum folate levels instead of fortification due to possible risks of increase in certain cancers with folate supplementation (see Figure 1 below) (Jägerstad, 2012). Furthermore, it was found that countries that do not have the mandatory fortification, such as all countries in the European Union, have had an increase in severe health inequalities due to younger women and non-Caucasian women having a decreased rate of folic acid supplementation adherence (Bestwick et al., 2014). The problem of supplementation is further compounded not only by low compliance of women but also with an inadequate amount of natural folate taken through dietary sources (Doležálková & Unzeitig, 2014). In lieu of these concerns, folic acid fortification was received as an option with greater success to solve an avoidable public health problem such as birth defects.
Figure 1. Map of world with wheat flour fortification status. Many countries have undertaken fortification due to some evidence showing prevention of neural tube defects and may also reduce cancer risks (taken from (Jägerstad, 2012)).
The success of folic acid fortification has been supported in the literature by stating that there is an increase in serum folate of women as well as a 19% decrease in neural tube defects and any harmful effects of folic acid have come from extra supplement use, not fortification (Berry et al., 2010; Quinlivan & Gregory, 2003). Although some studies have pointed out that the increase in folic acid fortification has increased blood serum levels of folate twice as much as Food and Drug Administration’s (FDA) predicted values (Choumenkovitch et al., 2002; Quinlivan & Gregory, 2003). Whether this increase over predicted values of serum folate has harmful effects is still being evaluated and is not conclusively determined, although detecting even low levels of unmetabolized folic acid in serum may be concerning (Quinlivan & Gregory, 2003). Although folic acid fortification’s main goal was eradication of neural tube defects, there are other residual effects that folate can effect that will be further discussed in the next section.

**Serum folate levels and various health effects**

The importance of a high serum folate level, whether through natural food or supplementation, is key in several disease processes due to folate’s involvement in the processes of DNA, RNA, and protein methylation, and even DNA synthesis and further DNA repair (Crider et al., 2011; Gong et al., 2014). The figure below demonstrates dietary folate’s action in the DNA Synthesis and methylation process (Figure 2). As Kim et al. describes, folate is considered a water soluble B vitamin whose major role is to help with the transfer of one-carbon moieties and further act as a cofactor for synthesis of purines and thymidylate, as depicted in the figure below (Kim, 2004).
Figure 2. Simplified scheme of folate metabolism involving DNA synthesis and methylation. B12, vitamin B12; DHFR, dihydrofolate reductase; CH3, methyl group; THF, tetrahydrafolate (Copied from (Kim, 2004)).
Due to folate’s critical role in essential genetic processes, folate deficiencies can promote several disease processes such as “intestinal cancers, vascular disease, cognitive decline and neural tube defects” (Stover, 2009). Several studies have examined these disease processes and a sample is provided below to highlight the importance a correct dosage of serum folate levels in a healthy lifestyle with a particular focus on women’s health.

**i. Cancer mechanisms and folic acid**

As mentioned previously, folate deficiencies can be a key factor in helping to develop certain disease processes. In the specific case of cancer, it is a multifactor disease that has a variety of causes from environmental and genetic to diet and exercise. There are a few theories on how folate deficiency particularly affects cancer processes as hypothesized by Anne Molloy and John Scott in a recent review article (Molloy & Scott, 2001). The first theory being that reduced folate status reduces the ability of cells undergoing replication to be able to provide sufficient thymidine for the cell which eventually causes incorrect base pairs to be incorporated in the DNA and can give rise to malignant cells (Molloy & Scott, 2001). This theory follows the logic presented earlier that folate is directly implicated with optimal DNA function as well as cancer’s development from abnormal gene repair that has been discussed in several papers (Molloy & Scott, 2001). Another complementary theory of folate deficiency affecting cancer development is the rise in homocysteine levels which can interfere with the process of DNA methylation and thus prevent certain genes from being down regulated and leading to cancer (Molloy & Scott, 2001). These two theories provide some basis for
the mechanism of folate relating specifically to cancer and further emphasize the need of having adequate amount of folate in our diet through foods and/or supplements.

On the other hand, there is some evidence that over supplementation of folic acid poses other risks specifically with relation to cancer. Ever since food fortification in the United States with folic acid, it has become easier for women to obtain an adequate serum folate levels. With a variety of folic acid supplements available in the market to further boost the folate status, there has been some concern over recent years that over supplementation can lead to risk of certain cancers such as breast and colon rectal cancer. Many of these supplements are unregulated which further causes concerns on what the health effects are. Described in the figure below is how both folate deficiency as well as folate supplementation can lead to a risk of cancer (Figure 3). As depicted in the figure, the unknown steps are demarcated with a question mark to represent that fact that although there are some postulated mechanisms, we are still unsure of the exact mechanism. This warrants further research on the development of cancer from folate deficiency and folate supplementation.

Given the wealth of research that has been done on breast and colorectal cancer with respect to folate status, this paper will briefly discuss them below.
Figure 3. Dual modulatory role of folate in carcinogenesis. Cancer develops over decades, if not a lifetime, through different stages of premalignant lesions (intraepithelial neoplasia, IEN) in the target organ. Folate deficiency in normal tissues predisposes them to neoplastic transformation and modest supplemental levels suppress, whereas supraphysiologic doses of supplementation enhances, the development of tumors in normal tissues. In contrast, folate deficiency has an inhibitory effect, whereas folate supplementation has a promoting effect, on the progression of established neoplasms. The effect of folate deficiency and supplementation on the progression of early precursor or preneoplastic IEN lesions to more advanced stages of IEN and to frank cancer is unknown at present. The mechanisms by which folate exerts dual modulatory effects on carcinogenesis depending on the timing and dose of folate intervention relate to its essential role in one-carbon transfer reactions involved in DNA synthesis and biological methylation reactions (Taken from(Kim, 2006)).
**ii. Breast cancer and folic acid**

One of the most studied cancers when it comes to women’s health is breast cancer and its relationship to high serum folate levels. Breast cancer, like other cancers, develops from a very complex pathway that includes environmental, genetic and social factors. Folate, through its interactions with the genome can mediate the development and outcome of breast cancer in women. Several studies have found mixed associations of folic acid supplementation and breast cancer development. In a recent study done by Babyshkina et al., the different effects of folate related SNPs on the development and progression of breast cancer were studied in premenopausal and postmenopausal women from the Western Siberian region of Russia (Babyshkina et al., 2013). This study found that certain folate related SNPs polymorphisms (MTHFR (677C>T), MTHFD1 (1958G>A)) were inversely associated with progression-free survival in premenopausal women while other polymorphisms (MTFD1 (1958AA)) were prognostic of progression-free survival in the same premenopausal women (Babyshkina et al., 2013). This study illustrates the genetic effects folate has on the development of breast cancer in women of various hormonal statuses (pre v. post menopausal).

Due to folate’s essential role in DNA/RNA and other genetic processes, folate deficiency has been shown to be related the progression of cancer. Several studies have examined the effects of folate intake on the risk of breast cancer in different populations. For example, among African American women, there is a great risk for developing more aggressive breast cancer and at a younger age when compared to European women (Gong et al., 2014). Although the exact reasons for the difference between the races is unclear, it
has been shown that among premenopausal African American women, a larger intake (quartiles 3, 4 in table 1 below) of natural folate from food reduces the risk of developing breast cancer (Gong et al., 2014). In European women, the highest quartiles of synthetic folate intake resulted in an increased risk of breast cancer (Gong et al., 2014).

An important point to remember is that cancer, like many other disease processes involves a series of complex process of which serum folate levels may be on contributing process. Other processes may also affect the development of cancer, such as age, race, hormonal status, family history etc. are critical to consider but will not be further discussed in this paper.

A recent review article further describes several epidemiologic studies, which concern folic acid and breast cancer. This article emphasize that due to the discrepancy in several studies over folic acids and its indirect relationship with breast cancer, supplementation with folic acid alone can not be strongly advised as a precaution to breast cancer (Kim, 2006). Even in studies that did find a significant decrease in folic acid supplementation and breast cancer, there is a risk of residual confounding which makes it difficult to conclude that folic acid is the main reason for protection against breast cancer (Kim, 2006).
### Table 1. Associations for risk of breast cancer by quartiles of folate (natural folate and synthetic folate) intake in all AA women and by menopausal status in the Women’s Circle of Health Study (adapted from (Gong et al., 2014)).

**Natural folate from food (mcg/day)**

<table>
<thead>
<tr>
<th>Quartile (mcg/day)</th>
<th>Q1 (≤159.6)</th>
<th>Q2 (159.7–230.6)</th>
<th>Q3 (230.7–315.1)</th>
<th>Q4 (&gt;315.1)</th>
<th>p for linear trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>236</td>
<td>250</td>
<td>111</td>
<td>123</td>
<td>125</td>
<td>127</td>
</tr>
<tr>
<td>189</td>
<td>205</td>
<td>0.88</td>
<td>0.65–1.18</td>
<td>0.44–1.02</td>
<td>92</td>
</tr>
<tr>
<td>153</td>
<td>189</td>
<td>0.74</td>
<td>0.53–1.03</td>
<td>0.32–0.84</td>
<td>86</td>
</tr>
<tr>
<td>163</td>
<td>183</td>
<td>0.74</td>
<td>0.50–1.09</td>
<td>0.33–1.00</td>
<td>68</td>
</tr>
</tbody>
</table>

**Synthetic folate from fortified food (mcg/day)**

<table>
<thead>
<tr>
<th>Quartile (mcg/day)</th>
<th>Q1 (≤78.1)</th>
<th>Q2 (78.2–129.8)</th>
<th>Q3 (129.9–188.6)</th>
<th>Q4 (&gt;188.6)</th>
<th>p for linear trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>214</td>
<td>228</td>
<td>97</td>
<td>112</td>
<td>118</td>
<td>116</td>
</tr>
<tr>
<td>180</td>
<td>208</td>
<td>0.92</td>
<td>0.69–1.25</td>
<td>0.57–1.42</td>
<td>96</td>
</tr>
<tr>
<td>159</td>
<td>199</td>
<td>0.93</td>
<td>0.67–1.30</td>
<td>0.53–1.42</td>
<td>85</td>
</tr>
<tr>
<td>188</td>
<td>192</td>
<td>1.16</td>
<td>0.80–1.67</td>
<td>0.85–2.55</td>
<td>72</td>
</tr>
</tbody>
</table>

p for linear trend

0.11                0.06                0.83
Lastly, there has recently been a clinical trial that showed a negative effect of folic acid supplementation on the development of breast cancer (Stolzenberg-Solomon et al., 2006). The novel finding from this cohort as a part of the “Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial” is that postmenopausal women who were taking \( \geq 400 \) mug/d of supplemental folic acid had a higher risk of developing breast cancer than those who did not take any supplemental folic acid (Stolzenberg-Solomon et al., 2006).

Furthermore, a few papers discuss the effects of folic acid in combination with alcohol consumption affecting the risk of breast cancer with conflicting results. Alcohol is an interesting confounder because it has been proven to be an inhibitor of folic acid metabolism (Hillman & Steinberg, 1982). This inhibition of folate metabolism could lead to a folate deficiency, which has been shown in several epidemiologic studies to increase the risk for developing breast cancer (Kim, 2006). Alcohol has also independently been cited as a major risk factor for breast cancer development (Kim, 2006). This relationship between alcohol directly increasing risk of breast cancer and especially in women with low folate intake has been supported by several epidemiological studies over the years (Baglietto et al., 2005; Stolzenberg-Solomon et al., 2006; Tjønneland et al., 2007).

Although previous research has supported the connection between folic acid and alcohol, more recent meta analysis of several studies that dietary folate either has no effect (Liu et al., 2014) or its effects are dependent on the dosage and timing of both folate ingestion and alcohol (Chen et al., 2014). These conflicting results about breast cancer and folic acid status reveal the wide scope of folic acid’s interaction with cancer machinery and
further studies must be done to determine other mechanism and actions of folate throughout the body.

**iii. Colorectal Cancer (CRC) and Folic Acid**

Folic acid is also implicated with a variety of other health factors such as colorectal cancer, heart disease and Alzheimer’s disease. For each of these conditions, folic acid has been implicated in both men and women. In the early 1990s, before fortification of flour with folic acid has taken hold, the rates of colorectal cancer were notably high. After fortification took hold in 1998, the incidence of colorectal cancer drastically decreased and some studies attribute the reduction to the increasing rates of serum folate levels due to fortification (Keum & Giovannucci, 2014). Although a few other studies have found that increasing serum levels of folate may promote instead of prevent colorectal cancer, there are several factors that must be considered when evaluating the risk of colorectal cancer (Jennings & Willis, 2014). Some of these factors mentioned include the (1) a long period of time that elapse between folate ingestion and possible protection from CRC; (2) dosage of allowed in supplement changing; (3) the fortification of folic acid in 1990s differentially impacted each race; and (4) improvement in cancer screening over time in the U.S. (Keum & Giovannucci, 2014). When these factors are considered together with folate acid fortification it seems that fortification has contributed to the decrease in CRC rates in both men and women. Further research is necessary to confirm this theory.
iv. Coronary Artery Disease (CAD) and Folic Acid

Folic acid has also been shown to be related to heart disease. When there is a deficiency of folic acid, rising levels of homocysteine can occur. Hyperhomocysteinemia has been directly implications for heart disease because it is a risk factor for coronary artery disease (CAD) and can help further the symptoms and development of CAD by destroying endothelial cells (Yi et al., 2014). A meta-analysis done by Yi et al., found that folic acid supplementation (400 µg/day) can lower plasma homocysteine levels in patients that are already diagnosed with CAD and a larger dosage of folic acid can further improve endothelial cell’s function and lower the chances of having a recurrent cardiovascular problem (Yi et al., 2014). This shows yet another disease process that serum folate levels is involved with.

Further concern with folic acid and homocysteine levels is their relationship with reproductive hormone levels, especially in women. It has been found by one study that plasma homocysteine levels are lower due to changing sex steroid hormone levels over the lives of women when compared to men especially during pregnancy and before the women start menopause (Hak et al., 2000). Specifically, postmenopausal women were found in this study of have higher homocysteine levels then premenopausal women as demonstrated by the figure below (Hak et al., 2000). The figure further describes that homocysteine concentrations continue to rise the longer that the woman is in menopause. This is another important example of folic acid affecting disease processes in relationship to reproductive hormone levels. Further research in studying the interaction between
reproductive hormone levels and the role of folic acid is necessary in order to fully understand the range of action of this very important micronutrient.

**Figure 4. Geometric mean values (95% confidence interval) of plasma homocysteine concentration (µmol/l).** The figure shows a dose dependent response when comparing homocysteine concentration to menopausal status (years since menopause started). Measurement were taken in 93 pre- and 93 postmenopausal women, categorized in three groups by time since menopause, and adjusted for age, creatinine, body mass index (BMI), smoking habit (yes, no) and alcohol intake (Taken from (Hak et al., 2000)).
SUMMARY OF BACKGROUND

In the previous section, several disease processes which may be related to folate levels have been discussed. Firstly, folic acid fortification was undertaken due to the evidence that supplementation prevents devastating neural tube defect in newborn babies. Given that folic acid plays such a crucial role in DNA synthesis and methylation, several studies started to examine other health effects that could occur as a result of folic acid fortification. One of the major areas of study has been cancer and folic acid where it has been found that both very low serum folate status (due to folate deficiency) and very high serum folate status (due to folate supplementation) could promote the development of cancer, especially breast cancer and colorectal cancer. Further studies are necessary in order to understand the exact mechanisms and the direction of the effect of folic acid when it comes to cancer. Folic acid deficiency has also been implicated with rising homocysteine levels, which have been known to contribute to heart disease. Homocysteine levels are also related to reproductive hormone levels.

The relationship of folic acid with several disease processes shows that it is a crucial micronutrient in the human body that can have various effects. The relationship between folic acid and pregnancy outcome (not related to neural tube defects) and/or other reproductive hormone levels is of need of further investigation. Since folic acid is crucially involved in so many disease processes, understanding its role in reproduction could help understand the critical processes that occur during development and help provide patients with proper advice when it comes to counseling them on which micronutrients are crucial before, during and even after pregnancy.
PUBLISHED STUDIES

Folic acid has been implicated in a variety of disease processes and many studies have aimed to understand the biochemical process that folic acid undergoes. One of the crucial aspects of understanding any disease process is realizing the different effects can occur and different stages of life. The rest of this thesis will be divided into examining the effects of folic acid in premenopausal women and throughout pregnancy. Given at each of these stages there are a variety of different hormone levels and other disease processes interacting with one another, it is crucial to examine the effects on each of these groups separately. Furthermore, another relatively new concept that has come up in the literature is a couples approach therapy with folic acid. Examining the effects of folic acid in women and in couple therapy could eventually improve live pregnancy rates and have other health benefits that have not been thoroughly examined. This section will examine folic acid in relation to these four different groups and determine which areas are in need of further research.

Premenopausal women and folic acid

Although there have been many studies looking at the effects of folic acid supplementation in terms of neural tube defects, cancer risk, Alzheimer’s disease and other disease processes, relatively few studies have critically examined the role that folic acid has in healthy premenopausal women, whether they are attempting pregnancy or not. This study is crucial because understanding the role in health women could provide further understanding of folate status in our body and the various health processes that it can affect. Furthermore, understanding what folate normally does can help physicians
and patients chose the correct treatment and dosage when processes do not occur as planned. Folic acid in healthy women is studied in several key processes: ovulation and the disease process of endometriosis.

One critical study that examines the role of folic acid in health premenopausal women is a study done by the Schisterman group at the National Institute of Child Health and Human Development called the BioCycle Study (Wactawski-Wende et al., 2009). This study has several strengths and weaknesses as outlined in the paper. The most critical strength of this study is that many papers in reproductive health examine women with certain diseases or conditions whereas the BioCycle study was one of the first studies that aimed to look at healthy, premenopausal women that are not attempting pregnancy nor are on any birth control or other prescribed medications due to known medical conditions (Wactawski-Wende et al., 2009). Given the strict restriction criteria, investigators were able to eliminate several possible confounders through study design alone. This allowed examination of several women’s health parameters including, but not limited to, folic acid (Gaskins et al., 2012).

Researchers became interested in the effects of folic acid in healthy premenopausal women due to the results in animal studies. Investigation in animals has been inconclusive: in pigs, folic acid supplementation has increased litter size due to increased embryo size and increased embryo weight during pregnancy (Guay et al., 2004). This is due to folate possibly increasing progesterone levels and promoting ovulation in the pigs (Guay et al., 2004). Furthermore in rats, it has been found that either an excess or a deficiency in folic acid partially inhibits ovulation (Willmott et al., 1968). This
evidence follows the previous discussion that an ideal concentration of folate in the blood is necessary for several key processes including, but not limited to DNA methylation and now ovulation. Lastly, in the rhesus monkey, a folate deficient diet caused a deficiency in follicular cells as well as a decrease in production of progesterone (Mohanty & Das, 1982). Both of these actions would cause a decrease in the probability of ovulation in the rhesus monkey due to folic acid. Although there are several animal studies that describe the effects of folic acid on ovulation and reproductive hormone levels, whether a similar effect would occur in humans is unclear. Gaskins et al. found that synthetic folate (otherwise known as folic acid supplements), was found to increase serum progesterone levels and decreased risk of anovulation (Gaskins et al., 2012). This was the first study of its kind to examine folic acid and its prepregnancy effect in premenopausal women with no known existing reproductive conditions (Gaskins et al., 2012). As shown in the table below, women who had the most synthetic folate intake (3rd tertile) had a 16% (95% CI, 1–34%) higher luteal progesterone level when compared with the women with the lowest synthetic folate intake (1st tertile) (Gaskins et al., 2012). As mentioned previously, synthetic folate is better absorbed by the body than natural folate in food which may contribute to this finding (Winkels et al., 2007).
Table 2. Association between dietary folate intake and estradiol and progesterone in the BioCycle Study. (Taken from (Gaskins et al., 2012)).

<table>
<thead>
<tr>
<th>Types of Folate (µg/day)</th>
<th>Tertile* (Median)</th>
<th>% Change</th>
<th>95% CI</th>
<th>P for trend</th>
<th>% Change</th>
<th>95% CI</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folate Equivalents</td>
<td>1 (325.0)</td>
<td>REF</td>
<td>REF</td>
<td>0.97</td>
<td>REF</td>
<td>REF</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>2 (444.9)</td>
<td>−2.68</td>
<td>(−12.98, 8.85)</td>
<td>6.38</td>
<td>(−8.54, 23.73)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 (682.0)</td>
<td>0.65</td>
<td>(−8.43, 10.63)</td>
<td>16.03</td>
<td>(−2.62, 38.25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synthetic Folate</td>
<td>1 (100.9)</td>
<td>REF</td>
<td>REF</td>
<td>0.56</td>
<td>REF</td>
<td>REF</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>2 (157.9)</td>
<td>−6.09</td>
<td>(−16.45, 5.55)</td>
<td>12.01</td>
<td>(−4.03, 30.74)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 (270.6)</td>
<td>2.33</td>
<td>(−7.04, 12.64)</td>
<td>15.70</td>
<td>(0.05, 33.80)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grain Folate</td>
<td>1 (83.2)</td>
<td>REF</td>
<td>REF</td>
<td>0.89</td>
<td>REF</td>
<td>REF</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>2 (138.7)</td>
<td>4.79</td>
<td>(−3.10, 13.31)</td>
<td>5.76</td>
<td>(−5.79, 18.73)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 (201.9)</td>
<td>3.04</td>
<td>(−8.08, 15.51)</td>
<td>4.66</td>
<td>(−7.00, 17.78)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cereal Folate</td>
<td>1 (14.0)</td>
<td>REF</td>
<td>REF</td>
<td>0.58</td>
<td>REF</td>
<td>REF</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>2 (53.7)</td>
<td>−1.58</td>
<td>(−11.05, 8.89)</td>
<td>1.69</td>
<td>(−8.91, 13.51)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 (192.4)</td>
<td>3.73</td>
<td>(−2.78, 10.68)</td>
<td>1.71</td>
<td>(−11.23, 16.53)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural Folate</td>
<td>1 (116.1)</td>
<td>REF</td>
<td>REF</td>
<td>0.24</td>
<td>REF</td>
<td>REF</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>2 (166.1)</td>
<td>−13.55</td>
<td>(−24.37, −1.18)</td>
<td>7.08</td>
<td>(−7.24, 23.61)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 (242.0)</td>
<td>−13.74</td>
<td>(−29.37, 5.34)</td>
<td>14.21</td>
<td>(−1.85, 32.88)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Tertiles of folate intake were compared with reproductive hormone levels. Synthetic folate had a significant difference in progesterone levels when comparing the highest tertile to the lowest tertile.  
1Results of weighted linear mixed models adjusted for age (continuous), race (White, Black, Other), BMI (continuous), mean total calorie (continuous), fiber intake (continuous), and other reproductive hormones (log-transformed, continuous) by inverse probability weights. Results are expressed as % change, (exp(β)−1)×100.
Furthermore, another key finding from this paper is that higher tertiles of intake of folic acid reduced the odds of anovulation (Gaskins et al., 2012). As shown in Table 3 below, synthetic folate as well as grain folate both decreased odds of anovulation. This follows the logic of folic acid increasing progesterone levels because an increase in progesterone levels will also simultaneously increase chances of ovulation, thereby decreasing chances of anovulation (Gaskins et al., 2012). Folic acid’s direct effect on anovulation further illustrates that its actions in women reproductive health is not limited to preventing neural tube defects during pregnancy but may have some effects even before conception. Folic acid’s effects preconception are a relatively new topic and require further research.

This finding of greater folic acid increasing progesterone levels is further supported by a study examining FertilityBlend, a nutritional supplement containing folic acid among other nutrients given to women who are attempting to become pregnant (Westphal et al., 2006). This study also found that an increase in mid-luteal progesterone rates and higher pregnancy rates in women who were taking the supplement when compared to a group of women not taking the supplement (Westphal et al., 2006). This provides supporting evidence that folic acid may produce changes in reproductive hormone levels specifically progesterone. One of the problems that is not resolved in this study is that since folate is one of the many ingredients in FertilityBlend, the beneficial effects of higher progesterone levels and increased pregnancy rates can not be solely attributed to folate alone and may also be due to other nutrients that are in the supplement.
Table 3. Association between dietary folate in tertiles and sporadic anovulation in the BioCycle study. (Taken from (Gaskins et al., 2012)).

<table>
<thead>
<tr>
<th>Types of Folate (µg/day)</th>
<th>Tertile (Median)</th>
<th>Adjusted Odds Ratio(^1)</th>
<th>95% CI</th>
<th>(P) for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folate Equivalents</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (325.0)</td>
<td>REF</td>
<td>REF</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>2 (444.9)</td>
<td>0.79</td>
<td>(0.34, 1.82)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (682.0)</td>
<td>0.49</td>
<td>(0.19, 1.23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synthetic Folate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (100.9)</td>
<td>REF</td>
<td>REF</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>2 (157.9)</td>
<td>0.68</td>
<td>(0.32, 1.45)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (270.6)</td>
<td>0.36</td>
<td>(0.14, 0.92)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grain Folate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (83.2)</td>
<td>REF</td>
<td>REF</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>2 (138.7)</td>
<td>0.72</td>
<td>(0.32, 1.63)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (201.9)</td>
<td>0.33</td>
<td>(0.14, 0.81)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cereal Folate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (14.0)</td>
<td>REF</td>
<td>REF</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>2 (53.7)</td>
<td>0.62</td>
<td>(0.30, 1.30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (192.4)</td>
<td>0.44</td>
<td>(0.20, 0.96)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural Folate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (116.1)</td>
<td>REF</td>
<td>REF</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>2 (166.1)</td>
<td>1.46</td>
<td>(0.58, 3.70)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (242.0)</td>
<td>1.40</td>
<td>(0.48, 4.08)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetable Folate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (28.4)</td>
<td>REF</td>
<td>REF</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>2 (56.0)</td>
<td>1.18</td>
<td>(0.50, 2.74)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (101.5)</td>
<td>1.03</td>
<td>(0.40, 2.64)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bean Folate(^2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (0.0)</td>
<td>REF</td>
<td>REF</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>2 (48.8)</td>
<td>0.11</td>
<td>(0.01, 0.85)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (160.4)</td>
<td>1.24</td>
<td>(0.44, 3.47)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Generalized linear mixed models adjusted for age (continuous), race (White, Black, Other), BMI (continuous), and mean total calorie and fiber intake (both continuous).

\(^2\) Due to the low intake of bean folate, intake was categorized into non-consumers and consumers above and below the median intake of consumers (81.4 mcg/day).

The paper by Gaskins et al. is one of the first papers to critically examine folic acid’s direct role in premenopausal women.
Furthermore, another recent review article described folic acid’s direct role in affecting the number of mature oocytes is through the homocysteine lowering action of folate which eventually protects oocytes from follicular growth due to the excess of oxidative stress that can be caused from higher than normal homocysteine levels (Papleo et al., 2011). This provides further evidence for a mechanism of folic acid affecting women’s health in more ways than only through neural tube defects and could have a direct effect on hormone levels, on ovulation and even pregnancy.

*Pregnancy and folic acid’s various roles*

Many studies have evaluated the effect of folic acid on neural tube defects post pregnancy, but the use of folic acid pre-pregnancy to promote chances of pregnancy and health of mother and child is still a debated topic. Interest in folic acid preconception has stemmed from animal studies, which have had conflicting results. Due to the convenience of mice as a model, several studies have used them to study the role of folic acid in conception. One study deprived mice of folate for five weeks and found that no significant difference in embryo implantation when compared to mice fed on a normal diet that included recommended levels of folate (Gao et al., 2012). As the paper mentions, folate is known to be involved in methylation reaction and five weeks deprivation of folate may have allowed the mice to use other stores for methylation, and a more chronic deprivation of folic acid could change the results (Gao et al., 2012). Furthermore, another study with mice found that folate levels can impact DNA methylation status of genes that regulate endometrial status (Ding et al., 2012). Although this study discusses genes that are critical in implantation of the embryo in mice, it is still unclear whether folate plays a
direct role with implantation or if its major role is after implantation has already occurred. Further research on this subtle distinction is necessary in order to develop recommendations and clinical trial in humans.

In animals, it has also been found that an excess of folic acid can also pose additional problems. In a recent study, mice who were fed a folic acid supplemented diet (with 20-fold higher folate content then recommended) when compared to a normal folic acid diet had significantly increased their risk of embryonic and further growth delays (Pickell et al., 2011). This underscores that although there is evidence that folic acid is beneficial during pregnancy, the correct dosage of folic acid supplementation required to produce beneficial effects still needs further research. This problem of the correct dosage for supplementation is also compounded by the fact that many foods in the US and other cities are fortified with folic acid. Although the mice in the above mentioned study were receiving much higher than the daily diet in most women, it is conceivable that women who are taking folic acid supplements could reach the same folate status (Pickell et al., 2011). Also, studying the various effects of folic acid during pregnancy in mice and other animals is useful in identifying possible mechanisms and performing randomized trials that would not be possible in humans. Many of these studies in animals examining folic acid and pregnancy have formed the scientific basis for several epidemiologic studies in humans looking at folic acid and implantation of embryo as well as other pregnancy outcomes such as live birth. These studies will be further discussed below.

A few studies have examined the relationship between folic acid status and live birth as an outcome. Just as has been found in the supporting animal studies, many
researchers question whether folic acid deficiency as well as over supplementation of folic acid can increase the risk of spontaneous abortions (George et al., 2002). Many conflicting results in previous studies have been due to differing sample sizes, not having matched cases and controls or differing methods of classifying folate status (Ray & Laskin, 1999). One study attempted to resolve some of these problems by conducting a matched case control study of women who had a spontaneous abortion and taking a blood sample at the time of the event (George et al., 2002). George et al. found that women who had low levels of plasma serum folate when compared to reference group were had a greater odds of having a spontaneous abortion and those women with higher plasma folate levels had no increase in risk of spontaneous abortion (See Table 4 below) (George et al., 2002). This study provides evidence that women who were in the average and above average ranges of serum folate levels may be protected from having a spontaneous abortion.

Another study the same year by Ronnenberg et al. also found similar results that women who were considered “folate deficient (less than 6.8 nmol/L)” more commonly had spontaneous abortions, as shown in Table 5 below (Ronnenberg et al., 2002). Ronnenberg also found that women who had both lower than normal folate (less than 40th percentile) and vitamin B6 (less than 80th percentile) serum concentrations had an increased risk of spontaneous abortions (adjusted OR 4.1, 95% CI 1.2, 14.4) when compared with women in the upper end of vitamin intake (Ronnenberg et al., 2002). Another more recent randomized trial further supports the idea that folic acid supplementation before and during first trimester of pregnancy, especially when
supplementation results in above the accepted daily range, is not associated with greater risk of miscarriage (Vila-Nova et al., 2013). Together these studies show that there is a connection between greater folate status during pregnancy and a reduced rate of spontaneous abortions. Given the results from recent studies, it does not seem likely that over supplementation with folic acid can lead to spontaneous abortions. Further, from these studies, it cannot be concluded that folic acid is the sole contributor to a spontaneous abortion, other factors must be considered as well that will not be discussed.

Table 4. Adjusted odds ratios for spontaneous abortion associated with folate levels stratified by gestational age and fetal karotype* (Taken from (George et al., 2002)).

<table>
<thead>
<tr>
<th></th>
<th>Case, No.†</th>
<th>Controls, No.†</th>
<th>Plasma Folate Level (ng/mL [nmol/L])</th>
<th>P Value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;2.19 (&lt;4.9) 2.20-3.95 (5.0-8.9) 3.96-6.16 (8.0-13.9) ≥6.17 (≥14.0)</td>
<td></td>
</tr>
<tr>
<td>Gestational age, wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-8</td>
<td>132</td>
<td>249</td>
<td>1.58 (0.75-3.26) Reference 0.46 (0.20-1.04) 0.49 (0.16-1.48) .06</td>
<td></td>
</tr>
<tr>
<td>9-10</td>
<td>181</td>
<td>352</td>
<td>1.58 (0.86-2.92) Reference 0.58 (0.31-1.08) 0.88 (0.44-1.75) .09</td>
<td></td>
</tr>
<tr>
<td>11-12</td>
<td>152</td>
<td>298</td>
<td>1.41 (0.70-2.65) Reference 1.30 (0.74-2.32) 0.64 (0.28-1.45) .33</td>
<td></td>
</tr>
<tr>
<td>Fetal karotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td>131</td>
<td>699</td>
<td>1.96 (1.09-3.46) Reference 0.90 (0.51-1.60) 1.05 (0.53-2.08) .11</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>83</td>
<td>899</td>
<td>1.11 (0.55-2.24) Reference 0.95 (0.44-1.84) 0.49 (0.18-1.36) .52</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>251</td>
<td>699</td>
<td>1.45 (0.90-2.33) Reference 0.93 (0.59-1.46) 0.77 (0.43-1.37) .27</td>
<td></td>
</tr>
</tbody>
</table>

* All values are odds ratio (95% confidence interval) unless other specified. Adjusted for maternal age, cigarette smoking, and caffeine consumption during pregnancy, education, parity, previous abortions, country of birth, body mass index, change of eating habits and pregnancy symptoms (i.e. nausea, vomiting, fatigue).
Table 5. Unadjusted and Adjusted Odds Ratios (95% Confidence Intervals) for Clinical Spontaneous Abortion* for different concentration of folic acid and vitamin B6 intake (Taken from (Ronnenberg et al., 2002)).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unadjusted</th>
<th>Age, BMI, and vitamin adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Folate (nmol/L)§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤6.60</td>
<td>1.9 (0.8, 4.7)</td>
<td>1.5 (0.6, 3.8)</td>
</tr>
<tr>
<td>6.61–8.40</td>
<td>1.4 (0.5, 3.6)</td>
<td>1.1 (0.4, 3.0)</td>
</tr>
<tr>
<td>8.41–10.10</td>
<td>0.8 (0.3, 2.2)</td>
<td>0.7 (0.2, 1.9)</td>
</tr>
<tr>
<td>10.11–12.60</td>
<td>0.8 (0.3, 2.2)</td>
<td>0.7 (0.3, 2.1)</td>
</tr>
<tr>
<td>≥12.61</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>P for trend</td>
<td>0.07</td>
<td>0.25</td>
</tr>
<tr>
<td>Low folate§</td>
<td>1.9 (1.1, 3.6)</td>
<td>1.7 (0.9, 3.1)</td>
</tr>
<tr>
<td>P</td>
<td>0.03</td>
<td>0.09</td>
</tr>
<tr>
<td>Vitamin B6 (nmol/L)§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤28.9</td>
<td>3.0 (1.0, 9.1)</td>
<td>2.5 (0.8, 7.8)</td>
</tr>
<tr>
<td>28.91–34.80</td>
<td>2.8 (0.9, 8.3)</td>
<td>2.9 (0.9, 8.9)</td>
</tr>
<tr>
<td>34.81–41.80</td>
<td>1.5 (0.4, 4.8)</td>
<td>1.4 (0.4, 4.6)</td>
</tr>
<tr>
<td>41.81–49.00</td>
<td>2.3 (0.8, 7.1)</td>
<td>2.1 (0.7, 6.4)</td>
</tr>
<tr>
<td>≥49.01</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>P for trend</td>
<td>0.06</td>
<td>0.13</td>
</tr>
<tr>
<td>Low vitamin B6§</td>
<td>2.4 (0.9, 6.2)</td>
<td>2.1 (0.8, 5.6)</td>
</tr>
<tr>
<td>P</td>
<td>0.07</td>
<td>0.13</td>
</tr>
<tr>
<td>Low folate and vitamin B6§¶</td>
<td>4.5 (1.3, 15.6)</td>
<td>4.1 (1.2, 14.4)</td>
</tr>
<tr>
<td>P</td>
<td>0.02</td>
<td>0.03</td>
</tr>
</tbody>
</table>

BMI = body mass index; other abbreviations as in Tables 2 and 3.
* Controls (no spontaneous abortion, n = 409) compared with patients (n = 49).
† Odds ratio adjusted for analytic batch only.
§ Adjusted for age, BMI, analytic batch, and either vitamin B6 or folate.
§§ Vitamin quintiles, with highest quintile used as referent.
¶ Vitamin concentration below the 40th percentile.
¶¶ Both folate concentration below the 40th percentile and vitamin B6 concentration below the 80th percentile. 27 patients and 205 controls.
* Vitamin concentration below the 80th percentile.
Another concern with folic acid supplementation and pregnancy outcomes is the timing of supplementation. Although most physicians recommend folic acid as a pre-pregnancy supplement in order to prevent neural tube defects, there is little evidence that folic acid itself can promote pregnancy if taken preconception. One very recent study by Gaskins et al. focused on a cohort from the Nurse’s Health Study II which only kept women in the study till their first pregnancy or miscarriage, whichever came first (Gaskins et al., 2014). This study is unique because using food frequency questionnaires, they were able to track the daily folate intake for several years pre-pregnancy and they found that risk of spontaneous abortion was 20% lower in women with highest quintile of folate intake (greater than 730 micrograms/d) when compared to the lowest quintile (0 micrograms/d) (Gaskins et al., 2014). It is important to review the timing of folic acid supplementation because many spontaneous abortions occur early on in pregnancy and studying a more overall view of the woman’s folate status may more clearly help researchers pinpoint the mechanisms that underlie the connection of folic acid status and spontaneous abortion. Furthermore, Gaskins et al. evaluated whether there was a dose dependent response to pre-pregnancy folic acid supplementation and risk of spontaneous abortion as portrayed in Figure 5 below (Gaskins et al., 2014). This figure shows that the trend for lower risk of spontaneous abortion for higher intake quintiles is significant whether the quintiles of folic acid or the categories of folic acid described in the paper are observed. A dose dependent response to folic acid is significant because it is useful information in determining what the ideal dosage is for supplementation and for fortification of folic acid.
Figure 5. Prepregnancy folate intake and adjusted absolute risks of spontaneous abortion. A. Total folate intake in quintiles. B. Supplemental folate intakes in categories. Adjusted risks are presented for the average age (35 years), total energy intake, (1700 kcal/d), year (1992), body mass index (18.5-24.9), smoking status (never smoker), physical activity (9.0-17.9 metabolic equivalent task-h/wk), history of infertility (none), martial status (married) and race (white) in our cohort (Taken from (Gaskins et al., 2014)).

The mechanism of how folate affects live births is still unclear. According to one review article, folate deficiency could possibly play a factor in spontaneous abortion by causing homocysteine levels in the body to rise which can cause damage to the
endothelial vasculature all over the body, including the placental vasculature (Ray & Laskin, 1999). Adequate blood flow is necessary to the placenta in order for crucial nutrients to be transferred to the fetus. Further developments on this theory include the study done by Ronnenberg et al. who did not find a significant relationship between increasing homocysteine levels and spontaneous abortions (Ronenberg et al., 2002). As cited in the study, the non significant results could be due to the small number of spontaneous abortions in this study or due to the fact that study examined first occurrence of spontaneous abortion, not recurrent episodes unlike previous studies (Ronenberg et al., 2002). Further research is needed in order to confirm the validity of this possible mechanism for folic acid action in the body.

Another possible mechanism for folate affecting spontaneous abortions could be that the placental tissue, which is known to be a major transporter and storage site for folate as well as vitamin B12 between the mother and the baby, could have some defect and not be able to properly transfer the folate to the fetus (Ray & Laskin, 1999). This could mean that regardless of the mother’s folate status, whether its sufficient or not, the fetus is still not receiving the proper nutrients necessary for its growth and development. As described earlier, folate plays a critical role in DNA synthesis and methylation, of which both processes are critical in a developing fetus and without adequate levels of folate in both the mother and the baby could severely affect survival and development of the baby. Both these postulated theories provide valid explanations of what the possible mechanism could be for folate’s actions during pregnancy but further studies are required
to determine which, if any, of these mechanisms can explain the effects of folic acid on spontaneous abortions.

**Couples approach to folic acid therapy**

In the paper thus far, the effect of folic acid throughout a women’s life have been discussed from ovulation to pre-pregnancy and during the pregnancy. A more recent theory in literature will now be discussed that focuses on couples therapy with folic acid to improve pregnancy outcomes. The scientific basis for folic acid being given to women has been previously discussed through various studies that have been presented in this paper including the promotion of serum progesterone levels, protection against spontaneous abortion and prevention of neural tube defects in the baby. Given national and international guidelines of folic acid fortification and supplementation, it is known that folic acid is overall beneficial in women who are attempting pregnancy. Whether similar effects are able to be seen in men is still debated and currently undergoing research. The following section will examine the role of folic acid in men’s reproductive health in relation to pregnancy and whether couples therapy is a viable option.

Men’s reproductive health has recently been an area of focus due to some studies finding a connection between men’s reproductive health, especially sperm and semen quality, affecting chances of pregnancy. Just as for women’s health, daily lifestyle and diet for men also came into question whether it would affect sperm and semen quality. As found in a study done by Schmid et al., men who consume more antioxidants (vitamin C, vitamin E and folate included) produced sperm that had less DNA damage (Schmid et al., 2012). The DNA damage in the sperm may pass on heritable genetic conditions to the
children. (Schmid et al., 2012). According to this current study, some of these heritable conditions in children could be prevented by having an adequate nutrition source of these antioxidants in both men and women’s daily diet at all times, but especially when attempting to become pregnant. Furthermore, another supporting study found that with increasing amounts of daily folate intake, there is a decrease in sperm aneuploidy which can cause several problems in children (see Figure 6 below) (Young et al., 2008). The figure below also portrays a dose relationship response whereas the increase in folate intake decreases the risk of having and aneuploidy within the sperm (Young et al., 2008). Specifically the study found that “722 micrograms or 1.8 times the recommended daily intake had between 20 and 30 percent lower frequencies of disomy X, disomy 21, sex nullisomy, and aggregate aneuplooidy compared with men with lower intake” (Young et al., 2008). Given the strong correlation between folic acid and aneuploidy in sperm, it is unlikely that this is due to any other confounding factor (Young et al., 2008). The main mechanism behind folate affecting the DNA in sperm is through similar mechanisms that have been previously described in this paper with folate affecting DNA methylation and synthesis, which would also affect the DNA in sperm. Both the Schmid et al. and Young et al. studies based their study on a previous study that was conducted in vitro and found that folate decreased physiologic concentrations of aneuploidy in lymphocytes (Beetstra et al., 2005; Wang et al., 2004).
Figure 6. Individual data points are shown along with adjusted negative binomial regression lines for total folate intake and aggregate aneuploidy ($\beta = -0.004, P = 0.01$). Covariates are held constant at their mean values. See Table III for covariates adjusted for in the final model. Aggregate aneuploidy is expressed as frequency per 10 000 sperm (Taken from (Young et al., 2008)).

Although there have been several studies that have examined the relationship between folic acid and prevention of DNA damage in sperm, not all studies have found a positive correlation. It is important to consider both sides of the issue of folic acid affecting semen quality before doctors and dieticians start recommending folic acid supplementation for men as well as women. Previous studies mentioned have only looked at large serum concentrations of folic acid due to supplementation and seen if it affects semen quality. In a study done by Eskenazi et al., they aimed to study whether daily
folic acid supplementation for men is necessary in order to see the effects on semen quality. Further research is necessary to see the true effects of folic acid in the diet affecting men’s sperm quality and if there are any negative effects of folic acid supplementation in men in general.

After examining the effects of folic acid with reproductive health and pregnancy outcomes in both men and women, many studies have turned to a couples approach to folic acid supplementation in order to increase chances of pregnancy and overall reproductive health. There has been an increased focus on the couples approach when discussing in vitro fertilization (IVF) and intra-cytoplasmic sperm injection (ICSI). This may be because when undergoing these expensive and time-consuming therapies, the quality of both the sperm and the egg must be considered. In a recent study evaluating pre-pregnancy diets in “subfertile couples” undergoing either IVF or ICSI, it was found that in women, a Mediterranean diet rich in folate had a 40% increased chance of pregnancy when undergoing IVF or ICSI when compared to a “health conscious-low processed” food diet that did not focus on a high folate intake (Vujkovic et al., 2010). Vujkovic et al. attributed the success of the Mediterranean diet to the folate intake because they observed a higher levels of folate in blood and follicular fluid of the women on the Mediterranean diet (Vujkovic et al., 2010). Just as previously shown in males, the strong correlation between dietary folate and increased pregnancy outcomes does not
appear to be due to other confounding factors. Furthermore, another review article outlined the importance of folate in all processes of reproductive health from pre-pregnancy to conception/implantation of embryo to embryogenesis and neural tube defects and other heritable defects in children (Cetin et al., 2010). Cetin et al. emphasized that although there are many animal studies that show the importance of folic acid during these critical processes of pregnancy, much more research is needed in humans in order to determine if the effects are the same as in other species (Cetin et al., 2010).

**DISCUSSION**

Folic acid is a critical micronutrient that plays a valuable role in various processes throughout the body and across a lifetime. As outlined throughout this paper, folic acid may play a role in certain types of cancer, Alzheimer’s disease, heart disease and other processes. Another highly researched and established role of folic acid is the prevention of neural tube defects in infants when taken throughout pregnancy. Although folic acid has been studied in relationship to various conditions, a particular focus towards folic acid, reproductive hormone levels and increasing the chances of pregnancy has not been given critical attention.

After researching several different papers, it has been found the folic acid plays a role at several stages throughout a woman’s reproductive lifetime. First, a diet rich in folate could support high progesterone levels and reduce the chances of anovulation as shown by the Gaskins et al. study (Gaskins et al., 2012). Furthermore, other studies also found evidence that folic acid when taken pre-pregnancy and during pregnancy can
produce a lower risk of spontaneous abortion. Lastly, other studies found that folic acid may be beneficial for men in protecting against DNA damage in sperm and semen. All of these studies taken together show that there may be benefit in providing a couple based therapy for folic acid. A couples based therapy could help promote pregnancy and provide a better understanding of what happens to women during pregnancy. This thesis critically examined the role of folic acid throughout a woman’s reproductive cycle and its beneficial effects during pregnancy. It also identified key areas of further research that can be done in order to further understand the mechanisms behind folic acid and its effects during pregnancy.

Diet plays a crucial role in reproductive health and especially during pregnancy. As shown in Figure 7 below, nutritional status of the woman, as well as the couple, is important to examine at all stages of pregnancy and even post pregnancy due to unforeseen health effects that can occur and are related to dietary intake (Cetin et al., 2010). Given the time frame and range of events that can happen at any point in time during the pregnancy, it is important to understand folic acid’s possible role in preventing negative outcomes such as infertility, miscarriage or preventable heritable genetic defects (Cetin et al., 2010). Since folic acid is involved with DNA synthesis and methylation, it is intimately involved in several cellular processes before and during the delicate development stages of pregnancy. Understanding a micronutrient, like folic acids, direct role in this process is crucial because it could provide a greater understanding of how adverse conditions develop during pregnancy. Although some of the studies presented earlier provide some correlation to folic acid and pregnancy outcome, more research is
necessary to understand the exact mechanism and any adverse outcome for folic acid supplementation.

Figure 7. Different pregnancy stages that represent a continuum, from the preconceptional to the post-partum period. Several and specific malformations and pregnancy-related disorders may originate during each phase. Preconception, conception, implantation, placentaion and embryo- or organogenesis are stages of the periconceptional period, which represents a critical step in determining fetus development. From the nutritional point of view, firstly preconceptional nutrition affects the overall health of childbearing-age women, thereby their reproductive potential. Subsequently, perconceptional maternal nutrition influences both the onset and the early stages of pregnancy, affecting both maternal health and conceptus development throughout the entire pregnancy. LMP: last menstrual period. (Taken from (Cetin et al., 2010)).
Furthermore, another reason further research is required with folic acid and pregnancy outcomes is that most papers provided different ranges of folic acid supplementation which they saw an effect. The dosage of folic acid supplementation that is required on top of fortification has been highly debated in order to find a balance between benefits for women and any other harm that could be caused by excess serum folic acid. In order to find a safe and effective dosage, more studies need to focus on which range of supplementation has beneficial effects above the amount of folate that is already provided by the fortification of grains of folic acid.

Although many studies as mentioned before have discussed the beneficial direct role that folic acid fortification and supplementation plays in women, few scientists are now turning their attention to the beneficial effects that folic acid may play in men’s reproductive health as well. Knowing that folic acid may protect the DNA in sperm from damage and aneuploidy and prevent heritable defects in children is a relatively new finding that has not been thoroughly tested and needs further randomized control trials in human subjects to see if it could directly impact pregnancy outcomes. Men’s reproductive health has not been a large focus in the field when compared to women’s reproductive health but given recent developments in research, there are crucial micronutrients, like folic acid, that are also essential to increasing a couple’s chances of becoming pregnant. Since folic acid deficiencies are relatively simple and inexpensive to reverse, greater attention to the deficiencies in both men and women are necessary and clinical trials to more clearly assess the positive and negative effects of folic acid supplementation in both men and women are crucial.
Given the necessity of folic acid for a healthy and fully functioning reproductive system in both men and women, a couples approach to folic acid supplementation would be a cost effective and beneficial preventative treatment especially with couples that are struggling to become pregnant. Some of the studies that have been presented in this thesis focused of IVF couples and folic acid supplementation levels in women alone. When the beneficial effects of supplementation on pregnancy rates are seen when women are the ones that are supplemented, it is unclear whether the beneficial effects of folic acid would increase when both women and men are supplemented preconception. Future studies should consider supplementation of both men and women and examining the outcome on reproductive hormones as well as pregnancy rates.

Due to the increasing exposure of IVF and ICSI procedures, several couples are seeking therapy sooner than clinically indicated, many women (48.1%) started using therapy early with less than twelve months of trying (Sanders et al., 2014). Due to the higher possibility of several side effects when using therapies of IVF and ICSI such as “low birth weight, increased likelihood of preterm delivery, an increased incidence in placental abnormalities and of pregnancy related hypertension,” giving couples other options such as folic acid supplementation or other dietary modifications could help them avoid these expensive treatments with possible devastating side effects (Sanders et al., 2014). In order for folic acid to be recommended as a therapy, more studies need to be done in order to determine the correct dosage and whether the combined folic acid supplementation in men and women affect pregnancy and live birth outcomes.
Also, more couples must be aware of other options such as diet modification and folic acid supplementation before being preemptively referred to other therapies such as IVF and ICSI. The focus on supplementation has been in IVF couples due to fertility issues that they may have had in the past, folic acid supplementation may be a viable option for couples not attempting IVF who have had problems becoming pregnant. Folic acid could be a cost effective preventative therapy for couples who are having reproductive health issues. Future studies, especially randomized control trials, are necessary to determine dosage of folic acid and the effectiveness of couples therapy, especially couples attempting to get pregnant.

CONCLUSION

Folic acid is a micronutrient that plays a critical role in several disease processes throughout the body as discussed throughout this thesis. Specifically, this thesis focused on folic acid’s role in affecting reproductive hormone levels and promotion of pregnancy. After reviewing several recent studies that examined folic acid’s relationship to reproductive hormone levels, it was found that folic acid supplementation could increase reproductive hormone levels (progesterone) and chances of ovulation. Folic acid also plays a direct role in pregnancy outcomes that are not related to neural tube defects. Women who had higher levels of serum folic acid levels had decreased risk of spontaneous abortion. There is also some evidence that folic acid may promote the chances of implantation of embryo. Lastly, studies have shown that sperm quality in men has improved with higher serum folate levels. Since folic acid affects both men and
women’s reproductive health, couples therapy may also be a viable alternative to expensive therapies such as ICSI or IVF.

These several factors taken together show that folic acid does play a critical role in improving reproductive hormone levels and pregnancy outcome. Although this thesis explored several beneficial effects of folic acid in relation to reproductive health, further research is necessary, especially for dosage and whether there are any negative effects, in order to definitively suggest folic acid as a supplement to improve overall reproductive health and pregnancy outcomes. Since folic acid is a relatively easy and cost effective compound to manufacture and administer, it is necessary to have further studies that can help establish sound recommendations for couples wanting to become pregnant.
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