REI Clinical Trial Workshop

OPTIMAL DIETARY STATUS IN COUPLES TRYING TO CONCEIVE: IS THERE A ROLE FOR ANTIOXIDANT VITAMINS?

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BACKGROUND

Human reproduction is inefficient with only one-fourth to one-third of fertilized human eggs likely to survive to produce a term delivery (Baird, 1986). The great majority of the failures occur in the early weeks after ovulation, with relatively little loss after clinical recognition of a pregnancy. Women who experience a delay before achieving a recognized conception also have elevated rates of early unrecognized pregnancy loss and clinical spontaneous abortion (Gray, 2000). Delayed time to pregnancy and early pregnancy loss may share common risk factors that affect implantation and embryogenesis. Although relatively little is known about factors affecting conception and early unrecognized pregnancy loss, a growing body of scientific literature suggests that environmental and lifestyle factors have important roles (Goldman, 2000). The evidence has led scientists to hypothesize that diet, particularly micronutrients such as antioxidants, and conditions leading to oxidative stress, may influence the timing and maintenance of a viable pregnancy (Ruder, 2008).

Promoting preconception health is a key public health strategy to increase fertility and decrease the morbidity and mortality associated with adverse maternal and fetal outcomes (CDC, 2006; CDC, 2007). Researchers have conducted clinical trials and observational studies that have shown that maternal periconceptional folic acid supplementation reduces the incidence of neural tube defects and other congenital malformations (Wilcox, 2007; Czeizel, 1992; Finnell, 2004). The 1991 Medical Research Council Vitamin Study, a multicenter, randomized, double-blind clinical trial in which half of the almost 2,000 women participants, all of whom had at least one child with a neural tube defect, took folic acid prior to conception and through the first trimester, showed that periconceptional folic acid supplementation reduced the recurrence of neural tube defects by 70% (MRC, 1991). Yet CDC (2007) reported that among Oklahoma women who had recently delivered, 21.5% were not aware of the benefits of folic acid before they became pregnant, 74% did not regularly consume a multivitamin before becoming pregnant, and 85% did not receive preconception counseling from a health care professional. Periconceptional multivitamin use was recently found to significantly increase birth weight in African American infants (Burris, 2010).

Reactive Oxygen Species and Oxidative Stress

Oxidative stress (OS) describes the damaging effects of free radicals -- oxygen atoms or oxygen containing molecules that are produced normally as intermediaries in cellular processes and respiration, and in the degradation of fatty acids. Evidence from laboratory, animal, and clinical studies suggests that reactive oxygen radical levels play a role in the etiology of adverse reproductive events in women (Agarwal, 2003; Ruder, 2008). In living cells, reactive oxygen species (ROS) are formed continuously as a consequence of both biochemical reactions, such as reactions of the mitochondrial respiratory chain, and external factors. There is a wealth of scientific data suggesting that high levels of ROS promote lipid peroxidation, structurally and functionally alter DNA, damage cells, and contribute to chronic diseases such as cancer and heart disease.

Antioxidants are compounds that bond with free radicals before they can cause damage. Oxidative stress occurs when the formation of ROS exceeds scavenging by antioxidants, either as a result of decreased intakes or increased antioxidant utilization. There are five classes of antioxidants: enzymes (catalases, peroxidases, and superoxide dismustase); peptides (glutathione); phenolic compounds (vitamin E and plant flavonoids); nitrogen compounds (various amino acids); and

carotenoids (beta-carotene). Other agents may have antioxidant effects through replenishing mechanisms. Vitamin C, for instance, helps to recycle Vitamin E, and N-acetyl cysteine provides an important component of glutathione. Antioxidants such as vitamins C and E and antioxidant cofactors, e.g., selenium, zinc, and copper, are capable of disposing, scavenging, or suppressing the formation of ROS.

Experimental and animal studies point to several possible avenues through which OS may affect fertility and early pregnancy loss. A recent review has addressed folate, zinc, and antioxidants in the pathogenesis of subfertility, but no study has directly addressed the effects of OS on fertility in women (Ebisch, 2007). Pregnancy itself is a state of OS as a result of increased metabolic activity. Increased plasma thiols in pregnant women and increased placental lipid peroxides and decreased expression of antioxidants have all been documented (Myatt, 2004).

When there is imbalance between OS and antioxidants, pregnancy complications may occur. In both the human and rat, granulosa and luteal cells respond negatively to ROS, adversely affecting meiosis II progression, leading to diminished gonadotropin and anti-steroidogenic actions, DNA damage, and inhibited protein ATP production. Glutathione, a non-protein sulphydryl tripeptide and key cellular antioxidant, has also been identified as critical for oocyte maturation, particularly in the cytoplasmic maturation required for pre-implantation development and formation of the male sperm pronucleus. In bovine models, beta-carotene has been recognized for its ability to enhance cytoplasmic maturation. The contrasting relationship of antioxidants, detrimental for the progression of meiosis I, but beneficial for meiosis II, suggests a complex role for antioxidants and ROS in the ovarian environment (Ruder, 2008).

Epidemiology of Dietary and Diet-related Exposures to Oxidation and Fertility

We hypothesize that oxidative stress may influence the timing and maintenance of a viable pregnancy and that OS is an important mediator of conception. There is indirect evidence of the importance of OS, and its control with antioxidant intake, from studies of preconceptional multivitamin supplementation and enhanced fertility, perhaps by increasing menstrual cycle regularity (Czeizel, 1994). Caffeinated beverages are also associated with decreased fertility, possibly mediated by tubal disease or endometriosis, which are characterized by ROS in the hydrosalpingeal fluid and peritoneal fluid, respectively (Agarwal, 2003). The effects of body weight and change in body weight on OS are intriguing because both inadequate and excessive energy intakes have been associated with reduced fertility among women. Research in this area has focused on the effects of energy intake on hormonal patterns and menstrual cycles. Irregular menstrual cycles, ovulatory dysfunction, and older age at menarche have been associated with both low and high body mass index (BMI, kilograms per square meter), energy intake, and high levels of physical activity. Physical activity, in turn, is associated with an increase in ROS, but appears to be damaging to tissues only when the exercise is exhaustive. Prospective studies on the influence of other factors thought to influence fertility (e.g., body fat distribution, recurrent weight fluctuations, delayed time-to-pregnancy, and early pregnancy loss) are needed.

Smoking and alcohol are both known to decrease fertility in women, likely through an increase in OS. Among individuals undergoing IVF, smokers were found to have diminished follicular fluid (FF) activity of the potent antioxidant glutathione peroxidase (GSHPx). The mean GSHPx activity in follicles yielding oocytes that were subsequently fertilized was greater than that of the follicles with non-fertilized oocytes. Overall, smokers have been reported to have higher ROS in FF and have a lower IVF success rate (Paszkowski, 2002). Lower levels of FF beta-carotene and decreased IVF success have been found in smokers compared to non-smokers (Tiboni, 2004). No smoking-related difference in the FF or plasma concentration of vitamin E or lycopene was detected, suggesting that follicular loss of beta-carotene occurs in response to OS brought on with smoking. Beta-carotene has previously been reported to be one of the micronutrients whose concentration is most strongly influenced by smoking. Bolumar (2000) reported associations between tobacco use,

BMI and time to pregnancy from the European Study of Infertility and Subfecundity. Using information collected at ≥ 20 weeks gestation on 4,035 women, they found that conception was delayed among female smokers with BMI > 30 (Odds Ratio (OR) = 11.5; 95% Confidence Interval (CI) 3.7-36.2) or BMI < 20 (OR = 1.7; 95% CI 1.1-2.8), compared to normal weight smokers (BMI = 20.0 – 24.9). Analysis of non-smokers in the cohort showed no association between BMI and time to pregnancy; however, women who did not become pregnant, regardless of smoking status, were not studied. In another study, female partners of couples planning a first pregnancy who consumed alcohol while they were trying to conceive had a significantly reduced odds of conceiving over a period of six menstrual cycles, compared to women who voluntarily abstained from alcohol (Jensen, 1998). After multivariate adjustment, compared to women who abstained from alcohol, women who consumed 1-5 drinks/week had an OR of becoming pregnant = 0.61 (95% CI 0.40-0.93), whereas women who consumed 6-10 drinks/week had an OR = 0.55 (95% CI 0.36-0.85), and women who reported 11-15 drinks/week had an OR = 0.34 (95% CI 0.22-0.52), (p for trend = 0.03). A positive dose response relationship between alcohol consumption and ovulatory factor infertility has also been reported in a case-control study (Grodstein, 1994). Compared with non-drinkers, the OR for ovulatory infertility was 1.3 (95% CI 1.0-1.7) for moderate drinkers (consuming < 100g alcohol or < ~ 7 drinks alcohol/week) and 1.6 (95% CI 1.1-2.3) for heavy drinkers (consuming >100g alcohol/week).

We examined vitamins C and E, beta-carotene, and lycopene levels, and fruit and vegetable intake in the first 250 couples undergoing infertility treatment as part of the Fast Track and Standard Treatment Trial (FASTT), collected using the 1998 Block Food Frequency Questionnaire (Goldman, 2005). Because these couples were undergoing care for infertility, they may be more health conscious than the population as a whole. For example, only 10% of participants were current smokers and over 40% of respondents reported regular vitamin supplement use. However, even in this population where participants may have made dietary changes, many individuals consumed far less than the recommended amounts for antioxidant nutrients and fruits and vegetables. More than one-third of the women and half of the men reported nutrient intakes limited in either vitamin C or E, when diet and dietary supplement use were considered. Sixty-eight per cent of the women and 74% of the men consumed fewer than the recommended five daily servings of fruits and vegetables.

STUDY OBJECTIVES

The working hypothesis of this study is that women with low antioxidant levels have a longer time-to-pregnancy compared to women with higher levels of antioxidants.

A prospective study with dietary assessment at baseline, prior to conception, is underway to assess the role of antioxidants and oxidative stress on time-to-pregnancy. A second objective is to determine what role antioxidants and oxidative stress have in early, unrecognized pregnancy loss.

Baseline assessments include dietary intake using diet recalls and a food frequency questionnaire, a personal history survey, a lipid profile, and blood and urine specimens from the women. Women also collect daily first morning urine samples from the time when they begin to attempt pregnancy until they become clinically pregnant, up to six menstrual cycles, for hCG analysis. Men complete diet recalls, a food frequency questionnaire, a personal history survey, and provide a blood specimen. Dietary recalls and food frequency questionnaires are repeated at three and six months for couples who have not conceived. Baseline blood specimens are being analyzed for the antioxidant vitamins C, E, and the carotinoids. Oxidative DNA damage is assessed in the women using two urinary tests, 8-hydroxy-2'-deoxyguanosine (8OH2'dG) and F2α-isoprostanes.

The next step will be to design and conduct clinical trials of optimal intervention strategies that consider the nature, dose, timing, and acceptability of dietary supplementation with antioxidant vitamins.

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POINTS TO CONSIDER WHEN DESIGNING A CLINICAL TRIAL

The following list includes some of the questions that should be considered in designing a clinical trial.

Research objectives:	What are the primary and secondary objectives of the trial? Have the questions been framed properly such that they can be answered by the trial you design?
Response variables:	What is (are) the primary response variable(s)? What specific endpoint will you use? Will you use direct or indirect measures, or both? What economic data should be collected for the cost-effectiveness analysis? Should the cost of complications as well as interventions be included?
Trial design:	Is a randomized controlled trial the proper design to answer the questions posed? What is equipoise? Single center or multicenter?
Masking:	Is masking feasible in this study? If so, should you use single, double, triple masking?
Sample size:	How many patients do you need to have adequate statistical power to detect important differences between groups?
Patient population:	Is there an adequate number of patients with the condition under study in your population? Will results found in your population be generalizable to the general population? Are there particular groups you want to target?
Recruitment:	How will you maximize recruitment? Do you need to advertise? Are monetary incentives necessary?
Screening criteria:	What are appropriate inclusion and exclusion criteria for potential participants in your study? Will these criteria limit recruitment?
Randomization:	What method of randomization will you use?
Safety parameters:	How will you monitor participants for the emergence of known (or unknown) adverse effects of the treatments? Is an interim analysis indicated? What are your “stopping rules?”
Data collection:	Who will develop data collection instruments? Hand-written or computerized? What approach to monitoring and quality control will be used (especially in multicenter trials)? Who will be responsible for collecting and analyzing data from multiple sites?
Informed consent:	Who will develop the informed consent document? What models are available? Who will obtain consent? How often does the IRB meet?
Baseline assessment:	What baseline history, physical and laboratory information do you want to collect prior to the intervention?

Compliance:	Will you need to educate participants to insure compliance with the treatment regimens?
Follow-up:	Is loss to follow up a potential problem? If so, how will you minimize loss to follow up? What mechanisms will be in place for short-term and long-term (if appropriate) follow up?
Data analysis:	Will you specify the type of analysis to be used at the outset of the trial? Will you use the Intention-to-Treat rule for inclusion of participants in the analysis? How will you deal with missing data?
Reporting results:	Has authorship been agreed upon in advance? Is there agreement about the journals and submission of manuscripts for publication?

CLINICAL TRIAL RESOURCES

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