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Examination of Possible Protective Effect of Rhesus D Positive Blood Factor on Toxoplasma-related Depressive Symptoms in Pregnancy

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Examination of Possible Protective Effect of Rhesus D Positive Blood Factor on

Toxoplasma-related Depressive Symptoms in Pregnancy

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree
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DEDICATION

I would like to first dedicate this work to my committee. You're encouragement and guidance has allowed me to be in the position I am in today. This process although extreme, has been entirely worthwhile and I will forever be grateful for each of you and your contributions to my success.

I would also like to dedicate this work to my children, Emily and Brian. I have spent my life trying to be an example for you. I hope by completing this you will always know there is nothing you cannot achieve.

I would also like to dedicate this work to my precious supportive family and friends, your patience and understanding, has never weakened through this process. Your support has given me the strength to complete this venture.

Finally, to my parents, without them none of this would be possible. They have prayed every day for me and have always said there was nothing I could not do if only I put my mind to it. You have raised us to know that with the strength of God nothing is impossible. I am truly blessed.

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Abstract

Toxoplasma gondii infects approximately one third of the population worldwide. There is strong evidence that a relationship between *T. gondii* titer and depressive symptoms exists. There is also evidence suggesting a protective effect of RhD positive blood factor on toxoplasma-induced behavioral and personality changes. This protective effect may influence the relationship between *T. gondii* and prenatal depressive symptoms. The purpose of this secondary data analysis was to examine the possible protective effect of RhD positive blood factor on prenatal depressive symptoms in 56 pregnant women with *T. gondii* infection. The cross-sectional design was utilized to answer the question "Does positive RhD blood factor provide a protective effect on prenatal depressive symptoms of patients infected with *T. gondii* when controlling for ethnicity, race, income, marital status, age and stress?" The conceptual model hypothesized that there was a relationship between socio-demographic variables (age, income, marital status, race, and ethnicity), stress, positive *T. gondii* titers, RhD positive blood factor, and prenatal depressive symptoms. Pearson correlations and multiple regression were utilized to explore the aims of this study demonstrated in the four statistical models. Significant relationship between stress and positive *T.gondii* seropositivity on prenatal depressive symptoms was identified. There was no significant relationship identified between RhD positive blood factor on the pregnant women infected with *T. gondii* which could be attributed to the small sample size.

Chapter One

Introduction and Background

Toxoplasma gondii, an intracellular protozoan parasite transmitted through cat feces, infects approximately 30% of the population worldwide, with a higher prevalence in women than men; (23.7% versus 10.9%) (Flegr & Striz, 2011). Since the early 1920's *Toxoplasma gondii* (*T. gondii*) infection was known to be transmissible to the unborn fetus, resulting in severe brain damage and/or death (McAuliffe, 2012). The biology of the life cycle of *T. gondii* begins with only felids shedding the infected, environmentally resistant, oocytes of the parasite in the fecal material. The infection is then transmitted when the human inadvertently ingests the infective oocytes, such as while cleaning litter boxes, gardening where cats defecate, playing in sand boxes and/or ingesting uncooked meat or contaminated food or water. Once ingested, the oocyte converts to the tachyzoite form and spreads through the body which can cause sudden acute disease. After the primary infection, the parasites become confined to intracellular cysts in the form of bradyzoites, becoming dormant for months to years (Dyer & Stoltenow, 2001).

Acute toxoplasmosis, although responsible for devastating effects for the infant including hydrocephaly, intra-cerebral calcifications, microcephaly, and miscarriage in the immune compromised, is easily misdiagnosed as common bacterial or viral infections (Flegr & Striz, 2011). *Toxoplasma gondii* may become dormant for years after the acute phase within the human host resulting in specific behavioral and physiological effects (Flegr & Striz). The early studies of *T. gondii* infection reported that behavioral changes occurred in the intermediate host (Flegr, Zitkova, Kodym, & Frynta, 1996). Latent *T. gondii* infection is characterized by life-long cysts

within the brain and muscle. (Flegr & Havlicek, 1994). There is evidence supporting possible influences of latent *T. gondii* on rat and human behaviors and personality attributes (Flegr, Zitkova, Kodym, & Frynta, 1996). Latent Toxoplasma-induced personality changes are reported as behavioral changes, personality changes, gender specific changes, and possible links to psychiatric disorders (Lindova, Priplatova, & Flegr, 2011). There appears to be a positive correlation between length of latent toxoplasmosis infection and decreased intensity of superego strength, suggesting a toxoplasma-induced willingness to accept group moral standards (Lindova, Priplatova, & Flegr).

Flegr and Hrda (1994) found no significant influence of acute toxoplasmosis on human personality or behavior. Chronic modification of the immune system in response to the parasite in the brain may be responsible for effects noted in chronic, latent infection. Flegr and Havlicek (1999) conducted research on a group of 191 women in the Czech Republic, where *T. gondii* prevalence is above 50%. The toxoplasma-infected women were found to have "higher intelligence, lower guilt proneness, and possibly higher ergic tension" (p.22). Flegr, Kodym, and Tolarova (2000) followed-up with research that recognized increased toxoplasma-induced personality differences correlated with longer durations of infection.

Flegr (2010) discussed the evidence for a strong association between toxoplasmosis and schizophrenia. Flegr suggested that the long term effects of the *T. gondii* infection might trigger the psychiatric disorder. There is also evidence of an increased risk of suicide and risk of traffic accidents in latently infected individuals (Flegr, Novotna, Lindova & Havlicek, 2008; Novatna et al., 2008; Yereli et al., 2006). Flegr (2010) suggested a potentially protective effect of the Rh positive blood group in response to the deteriorating psychomotor and psychiatric toxoplasma-induced effects. Holub et al., (2011) provided evidence of the possible protective effect of Rh

factor in the toxoplasma positive schizophrenia patients. The Rh positive patients exhibited decreased schizophrenic symptoms. Ling, Lester, Mortensen, Langenberg, and Postalache, (2011) provided evidence of the association between patients seropositive *T. gondii* and increased risk for suicide. Girardi et al. (2012) provided evidence of the relationship of depression and increased suicide rates in women soon after delivery. There is well-known relationship between prenatal and postpartum depression (Whisman, Davila, & Goodman, 2011). Arling et al., (2009) also provides evidence of higher *T. gondii* titers in suicide attempters compared to controls. Groer et al., (2011) found an association between depressive symptoms and *T. gondii* titer in infected pregnant women.

Human blood is categorized within two main systems: the ABO system and the Rhesus (Rh) system. The RhD protein is the RhD gene product and a major component of the Rh blood group system and carries the D-antigen, the strongest blood group immunogen (Pilgrim, Lloyd-Jones, & Rees, 2009). When RhD negative mothers deliver a child that is RhD positive, the fetal cells left in the maternal circulation post delivery may cause the mother to develop anti-D antibodies and become RhD sensitized. Although the sensitization is not likely to affect the present fetus, the sensitization may result in future Rh positive pregnancies ending in hemolytic disease in the newborn. Providing prophylactic anti-D to RhD-negative mothers to prevent hemolytic disease of the fetus and newborn has been considered one of the most successful clinical applications of antibody-mediated immunosuppression (Kumpel, 2008,). The rates of RhD negative Caucasian, African, and Asian women are about 15%, 8%, and 1% respectively. (Flegr et al., 2009).

Flegr, Novotna, Lindova and Havlicek (2008) suggest "the RhD protein acts as an ion pump of uncertain specificity and unkown physiological role" (p.476). Novotna et al. (2008)

provide evidence of a protective effect of the RhD protein on toxoplasma-induced psychomotor performance impairment. The protective effect resulted in increased reaction times of the RhD positive participants. Kankova, Sulc and Flegr (2010) offer evidence supporting the protective effect of RhD positive blood factor against increased weight gain in pregnant women with latent toxoplasmosis. No research to date has examined the protective effect of RhD positive blood factor, *T. gondii* and prenatal depressive symptoms. The identification of the potential unique relationship between the protective effect of RhD positive blood factor, latent toxoplasmosis-induced effects and prenatal depression may have significant worldwide implications.

The World Health Organization predicts that depression will be the second leading cause of premature death and morbidity by 2020 and suicide is one of the most common causes of maternal death in the year following delivery in developed countries (World Health Organization, 2012). Wylie, Hollins Martin, Marland, Martin, and Rankin (2011) characterized depression as psychological behavioral changes such as a depressed mood, hopelessness, anxiety, excessive fatigue, psychomotor agitation, appetite and sleep disturbances, guilt and/or feelings of inadequacy. Prenatal depression occurs in up to 20% of pregnant women, but is not well understood and has also been associated with negative pregnancy and fetal outcomes and is suggested to be a predictor of postpartum depression (Stillwaggon, Carrier, Sautter, & McLeod, 2011).

Statement of Problem

Toxoplasma gondii, an intracellular protozoan parasite, infects over 30% of the population worldwide. Latent toxoplasma-induced behavioral and personality effects on the intermediate host are significant with evidence that latent *T. gondii* causes personality and behavioral changes, postpartum depression and anxiety. There is strong evidence that a

relationship between *T. gondii* titer and depressive symptoms exists. There is also evidence suggesting a protective effect of RhD positive blood factor on toxoplasma-induced behavioral and personality changes. This protective effect may influence the relationship between *T. gondii* and prenatal depressive symptoms. Research suggests that prenatal depression is a predictor of postpartum depression. If this unique relationship between the protective effect of RhD positive blood factor and toxoplasma-induced depression in pregnant women is present, this may increase the effectiveness of predicting postpartum depression, thus, reducing the second leading cause of suicide in post partem women with depression.

Statement of Purpose

The purpose of this exploratory investigation was to examine the protective effect of RhD positive blood factor on the relationship between *T. gondii* and prenatal depressive symptoms. The covariates, ethnicity, race, income, marital status, age, stress, and RhD positive blood factor were evaluated for their relationship to prenatal depressive symptoms of patients infected with *T. gondi*. Controlling for these covariates, the unique relationship of Rh positive blood factor on prenatal depressive symptoms of *T. gondii* infected patients was examined. The exploration of this potentially unique relationship may provide support for future research into the possible protective effect of Rh positive on post partum depression, the second leading cause of maternal death today.

Specific Aims

The proposed research question was "Does positive RhD blood factor provide a protective effect on prenatal depressive symptoms of patients infected with *T. gondii* when controlling for ethnicity, race, income, marital status, age, and stress?" The specific aims for this research are as follows:

1.) Examine the relationship of ethnicity, race, income, marital status, age, and stress on prenatal depressive symptoms in 56 positive *T. gondii* infected pregnant women who are between 16-25 weeks gestation.

2.) Examine the relationship of positive *T. gondii* status and prenatal depressive symptoms.

3.) Examine the relationship of Rh status and prenatal depressive symptoms.

4.) Examine the moderating effect of Rh status on the relationship between *T.gondii* and prenatal depressive symptoms.

Definitions of Relevant Terms

Ethnicity is defined as Hispanic or Latino and non-Hispanic or Latino. Race is defined as American Indian/Alaska Native, Asian, Native Hawaiian or other Pacific Islander, Black or African American, and White.

Wylie, Hollins Martin, Marland, Martin, and Rankin (2011) characterized depression as psychological behavioral changes manifesting in symptoms such as a depressed mood, hopelessness, anxiety, excessive fatigue, psychomotor agitation, appetite and sleep disturbances, guilt and/or feelings of inadequacy.

Assumptions

The assumption was made that the assays utilized to identify *T. gondii* positive infection are validated through manufacturer standards. The assumption was made that the instruments utilized would measure the covariates as defined. The self-report nature of the demographic and psychosocial data may affect reliability and validity of the scores.

Relevance and Significance

Pregnant women are currently provided with information warning against the risks of toxoplasmosis and ways to avoid the infection, but no formal screening is recommended. Although congenital toxoplasmosis is a preventable disease in pregnancy, estimated 500-5000 infants are born each year in the United States resulting in a significant emotional and economic burden from the sequelae of the disease for the parents and society (Montoya & Remington, 2008). Stillwaggon, Carrier, Sautter, and Mcleod (2011) estimate maternal serologic screening for *T. gondii* during pregnancy would provide a savings of $620/child screened, also reducing the number of congenital cases to 1/10,000.

Although there are many studies examining prenatal and postpartum depression, there is only one study was found examining the relationship of *T. gondii* and prenatal depressive symptoms. Additionally, no studies have been found on the relationship of the protective effect of RhD positive blood factor in pregnant patients with *T. gondii* and prenatal depressive symptoms. The ability to identify a protective effect of RhD positive blood factor on prenatal depressive symptoms of patients infected with *T. gondii* may allow for improved screening techniques and may provide improved surveillance for postpartum depression and suicide post-delivery to avoid or reduce the profound negative maternal and fetal outcomes. Including toxoplasma surveillance and subsequent identification of Rh negative women at risk for psychological behavior changes and postpartum depression may lead to a reduction of maternal suicide, the second most prevalent cause of maternal death in the United States.

Chapter Two

Review of the Literature

The research domains of *T. gondii*, Rhesus D blood factor, prenatal depression and postpartum depression were utilized to conduct a comprehensive literature review using Academic Search Premier, Cochrane Database of Systemic Reviews and the Cumulative Index to Nursing and Allied Health Literature (CINAHL), Cochrane Central Register of Controlled Trials, MEDLINE, PsycINFO, and PubMed databases first in isolation and then jointly using the Boolean operator *and*. The literature review was divided into areas of relevance for convenience of the review. The review was categorized by primary headings of *Toxoplasma gondii*, personality and behavioral changes, Rhesus D blood factor, preterm and postpartum depression. Within each area the content of research was presented chronologically.

State of the Science: *Toxoplasma gondii*

Since the early 1920's *T. gondii* infection was known to be transmissible to the unborn fetus, resulting in severe brain damage and/or death (McAuliffe, 2012). For the past forty years pregnant women have been warned regarding the dangers of exposure to cat feces and subsequent risk of exposure to *T. gondii*. The Centers for Disease Control and Prevention (CDC) noted that over a third of the world's human population was estimated to carry *T. gondii* infection with an 11% seroprevalence in women of childbearing age (CDC, 2009). The more recent statistics reveal that the *T. gondii* oocytes excreted by cats in their feces, infect approximately 30% of the population worldwide, with a prevalence rate significantly higher in females than in males (23.7% and 10.9%, respectively) (Flegr & Striz, 2011). Transmission of *T.*

gondii to humans occurs in various ways such as, consumption of raw or undercooked meat containing the tachyzoites in cat feces, or eating poorly washed fruits and vegetables contaminated with oocytes (Adesiyun et. al., 2011). The detection of *T. gondii* immunoglobulin (IgG) is used as evidence of past exposure/infection and detection of immunoglobulins M (IgM) for the evidence of acute/current infection (Adesiyun).

Although one third of the population of the United States is infected with the *T. gondii* parasite, the *T. gondii* infection rate, in other areas of the world, is estimated to be 10 to 90% (Stokkerman, Schwartzman, Keenan, Morrissette, Tilney, & Roos, 1996). Stokkerman et al. described the *T. gondii* parasite as an 'opportunistic pathogen' of the immunocompromised due to the reactivation of the cysts which lay dormant for the host's life. The rodent research conducted by Salibay and Claviera (2005) provided evidence identifying rodents as chronic carriers of the toxoplasma infection, which remains within the host rodents even though not immune compromised.

The prospective cross-sectional survey conducted by Kolbekova, Kourbatova, Novotna, Kodym and Flegr (2007) on 3290 military personnel in Prague, Czechoslovakia, identified risk factors for transmission of the *T. gondii* infection. The risk of infection increased significantly with age (25-34) when compared to ages younger than 25 years, childhood residence in towns with less than 10,000 residents being married, and being a professional member of the military. Toxoplasma infection risk was also significantly higher in subjects that ate raw meat, were cat, dog or rabbit owners, and had the blood groups A, B, or AB. The authors reported an overall seroprevalence of 23% with 26.3% in male population and 29 – 37% in pregnant women (Kolbekova, Kourbatova, Novotna, Kodym, & Flegr).

Acute *T. gondii* acquired during pregnancy with transmission to the fetus usually has devastating yet preventable effects on the fetus resulting in but not limited to visual and hearing loss, mental and psychomotor disabilities, seizures, blood disorders and possible death (Montoya & Remington, 2008). Most of the pregnant women who acquire the disease during pregnancy are asymptomatic. In the United States, serological screening on all pregnant women, although optimal for detecting the disease, is not mandatory. Unlike the United States, countries such as France and Austria require serological testing in pregnancy for early detection of the disease.

Serological testing is done to detect and quantify the *T. gondii* antibodies in the serum of pregnant women and to establish if the disease exposure is recent or in the distant past. The negative IgG and IgM would suggest no infection has occurred, while a positive IgG and negative IgM indicates the infection has occurred in the distant past. The diagnosis of the disease is facilitated not only by the serology but also by history and examination of the pregnant patient. The addition of the fetal ultrasound also provides valuable information related to the presence of fetal abnormalities including brain, spleen, and hepatic abnormalities, and ascites. Although the disease is treatable with antibiotic therapies, primary prevention utilizing educational material continues to be the predominant focus for prevention of *T. gondii* in pregnant women. In addition to this primary educational prevention, women who are found to be sero-positive for *T. gondii* must be advised of their options related to termination of their pregnancy, and antibiotic therapy in utero (Montoya & Remington, 2008).

Flegr, Hrda, and Kodym (2005) conducted an analysis of the clinical records of 758 women tested in their 16th week of pregnancy for toxoplasmosis. One hundred seventy-four women were found to be positive for *T. gondii* and significantly below normal body weight for their 16th week of gestation. Although when length of gestation and maternal age were also

examined there were no significant differences in patients who had positive and negative *T. gondii* titers. The lower body weight reported in this research supports the negative influence of *T. gondii* on pregnant women (Flegr, Hrda, & Kodym).

Kankova, Holan, Zajicova, Kodym, and Flegr (2010) suggested the modulation of the immunity in mice infected with latent toxoplasmosis. The mice demonstrated a decreased production of interleukin-2 and nitric oxide and synthesis of DNA. Knowing that approximately 30% of the world's population is infected with latent toxoplasmosis, it was suggested that the *T. gondii* associated immunosuppression might result in a serious public health concern (Kankova et. al.). Flegr and Striz's (2011) research on rodents provides evidence of the potential immunosuppressive effects of latent toxoplasmosis also suggesting subsequent serious public health concerns.

Personality and Behavioral Changes

Flegr, Zitkova, Kodym, and Frynta (1996) found a high incidence of latent *T. gondii* in London, New York, and Paris with an estimated 22%, 32%, and 84% of pregnant women, respectively, as an opportunity to investigate the influences of *T. gondii* on human behavior. The research data were collected over 26 months in 1992-1994. The Cattell's 16-factor questionnaire, (form A), measures personality characteristics. Two experimental sets consisted of the first set of 224 men and 170 women students from Charles University, Prague, and the second set of 190 men from various Prague hospitals, all infected with acute toxoplasmosis within the past 13 years. The relationships of toxoplasmosis, gender, and the toxoplasma-gender interactions on personality factors were evaluated utilizing a multivariate analysis of covariance (MANCOVA). Although the toxoplasmosis and gender factors on personality factors were not significantly different, the interaction of toxoplasmosis-gender on personality factors was highly significant.

Five personality factors were isolated as significantly influencing the difference in the toxoplasmosis-gender interaction. Flegr, Zitkova, Kodym, and Frynta's (1996) research suggested the existence of a correlation between human personality and the duration of the toxoplasmosis. The data revealed that there was more likelihood that the correlation suggested the duration of toxoplasmosis induced personality changes versus the personality changes being the cause of the probability of becoming infected with *T. gondii*.

Havlicek, Gasova, Smith, Zvara, and Flegr's (2000) research supports the supposition that after the initial acute infection, the infection moves into a dormant/latent phase in which the cysts form and survive in the tissues for the duration of the human hosts lifespan. The double blind study was conducted in the Institute of Haematology and Blood Transfusion, in Prague. The sample for the experimental set included 69 men and 47 women, and donors of thrombocytes, who participated in reaction time testing over three time frames of one, two and three minute intervals. Computerized psychomotor testing and enzyme-linked immunosorbent assay (ELISA) testing for IgG and IgM antibody titers for *T. gondii* were performed. Complement fixation tests (CFT) were conducted to differentiate between acute and latent toxoplasmosis. CFT titers between 1:8 and 1:128 were considered positive for latent toxoplasmosis. Analysis of covariance (ANCOVA), controlling for age, revealed a significantly longer reaction time for toxoplasmosis positive subjects versus the toxoplasmosis negative subjects. The correlation between duration of toxoplasmosis and slower reaction times became significantly longer with each time frame interval. These results demonstrated the deteriorative effects of latent toxoplasmosis on subject's concentration over the three time intervals (Havlicek, Gasova, Smith, Zvara, & Flegr).

Flegr et al (1996) rodent research data have suggested that although asymptomatic, rats with latent toxoplasmosis became immune-compromised, leading up to and including death. Although latent toxoplasmosis is known to be asymptomatic, studies have been conducted suggesting the manipulative effect of the dormant parasite on the human personality (Flegr et al., 1996; Flegr, Kodym & Tolavora, 2000; Flegr, Priess, Klose, Havlicek, Vitakova, & Kodym, 2003; Flegr & Havlicek, 1999). Flegr, Hrda, and Kodym (2005) tested 758 women in their 16th week of gestation for *T. gondii*. The women positive for *T. gondii* were found to have a significantly lower body weight at 16 weeks than women who were negative for *T. gondii* ($p =$ 0.02). Although there were unusual findings suggesting longer length of gestation for patients positive for *T. gondii*, the negative impact on general health of this population may be a more significant factor (Flegr, Hrdy & Kodym).

Simpore, et al. (2006) provided evidence of increasing seroprevalence of *T. gondii* in sub-Saharan Africa with an increase in the human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV). This may support the negative impact of latent *T. gondii* on general health of the immunocompromised. It is more likely that the immune compromise of these diseases leads to increased susceptibility for primary infection and increased reactivation of latent infections. Of the 336 pregnant women in an antenatal clinical at Saint Camille Medical Centre in Ouagadougou, Burkina Faso, 207 were HIV positive, 129 were HIV negative, and 25.3% (85/336) were found to have positive *T. gondii* IgG antibodies (Simpore et al).

Skallova et al. (2005) reported the prevalence of *T. gondii* infection was 34.1% in 205 men and 27.1% of the women. The research suggests that the participants with latent toxoplasmosis were at higher risk for schizophrenia. The missing link between toxoplasmosis and schizophrenia was speculated to be inflammation-induced increases in dopamine. Also the

decreased novelty seeking behaviors of the toxoplasma infected blood donors was also linked to this increase in dopamine (Skallova et al.). Skallova, Kodym, Frynta, and Flegr (2006) provided evidence of a probable change in the dopaminergic system induced by *T. gondii* infection. Of the mice utilized in this research, the *T. gondii* positive females were found to be more explorative of the environment in which they were placed and there was a slight weight loss coinciding with the immune system compromise. Although they were unable to provide a definitive link between possible *T. gondii*-induced behavioral changes, future research combining neurochemical and ethological studies may facilitate a better understanding of the physiological mechanism of *T. gondii*-induced behavioral changes (Skallova, Kodym, Frynta, & Flegr).

Manipulation theory is characterized by the ability of the parasite to modify the behavior of the host through an evolutionary process to increase the efficiency of the parasitic transmission to the uninfected host (Kankova et al., 2007; Flegr, 2007; Voozen, 2007). The manipulation of behavior found in *T. gondii* positive animals impairs motor performance (Hutchinson, Aitken, & Wells, 1980), produces learning deficits (Witting, 1979), increases trapability (Webster, 1994), and causes loss of cat urine odor avoidance (Berdoy, Webster, & MacDonald, 2000). Based on the manipulation theory, Kankova, Kodym, Frynta, Varinova, Kubena, and Flegr (2007) investigated the influence of latent toxoplasmosis on the secondary sex ratio in mice suggesting that a deviated sex ratio was a product of the manipulative activity of the parasitic organism, *T. gondii*. In conjunction with the manipulation theory, evidence has reported that pregnant women with latent *T. gondii* have a higher incidence of delivering male offspring rather than female offspring, at a rate of 250 males for every 100 females (Kankova, Sulc, Nouzova, Fajfrlik, Frynta, & Flegr, 2007).

Rhesus D (RhD) Blood Factor

Gunel, Kalelioglu, Gedikbasi, Ermis and Aydinli (2011), reviewed case studies reviewed, finding that 75% of the participants had the RhD-positive blood factor, 17.5% RhD-negative blood factor, and 7.5% discordant tests. These data are supported by previous research reporting approximately 15% of the world's population is RhD negative (Branger & Winer, 2006). The utilization of the anti-D prophylaxis has significantly reduced the incidence of hemolytic disease in fetuses and newborns (Kumpel, 2008; Pilgrim, Lloyd-Jones, & Rees, 2009).

Latent toxoplasmosis is reported to have 20 to 60% prevalence in the world's population (Flegr, Klose, Novotna, Berenreitterova, & Havlicek, 2009). Flegr et al., (2009) provided evidence of an increased incidence of traffic accidents in toxoplasma-infected military drivers when compared to toxoplasma free military drivers. In toxoplasma infected military drivers, the RhD positive blood factor had a protective effect. The 709 toxoplasma infected RhD positive drivers were found to have significantly fewer traffic accidents when compared to the 181 toxoplasma infected RhD negative drivers (p=0.028). The RhD positive blood factor was found to have a protective effect on the toxoplasma-induced impaired reaction times (Flegr et al.).

In 2010, Flegr, Novotna, Fialova, Kolbekova, and Gasova reported a significant difference in toxoplasma-associated personality changes between 302 RhD positive and RhD negative *T. gondii* infected blood donors in Prague. The Cattell's 16PF and Cloninger's Temperament and Character Inventory (TCI) personality instruments were utilized to identify differences when comparing personality of RhD positive and RhD negative T gondii infected patients. The Cattell's 16PF is known to have opposite results for males and females with latent toxoplasmosis, thus analysis was repeated separately for males and females to accommodate for the known differences. The results from this research support the suggestion of a protective

effect of RhD positive blood factor on latent toxoplasmosis personality changes (Flegr et al., 2009).

Prenatal Depression

Maloni, Park, Anthony and Musil (2005) conducted a study designed to replicate three previous studies measuring depressive symptoms of 89 hospitalized high-risk antenatal women between 20-33 weeks gestation on bed rest across time periods (Heaman, 1992; Maloni et. al., 1993; Mercer & Ferketich, 1988). The pregnant women's depressive symptoms were measured utilizing three standardized instruments; Multiple Affect Adjective Checklist-Revised (MAACL-R), Profile of Mood States (POMS), and Center for Epidemiologic Studies of Depression Scale (CES-D). The study purpose was to measure the depressive symptoms to provide evidence of validity and reliability of the scores utilizing the three instruments. The women within the study were diagnosed as high-risk antenatal patients due to the increased concern for their medical outcomes to either themselves or their fetuses. The results of the study provided evidence of validity and reliability of the scores utilizing the MAACL-R and the POMS to detect change over time. The measurement instruments were given upon admission, at 2 weeks and at 4 weeks. Convergent validity was recognized as moderately high for all time frames. Test-retest reliability correlations between the admission and 2 week measurements ($n = 89$) for the three measurements MAACL-R, POMS, and CES-D were .57, .61, and .63, respectively, and from admission through 2 and 4 weeks ($n=37$). The MAACL-R and POMS remained significantly correlated .61, .43, .42, .66, .71, and .50, respectively, across time. This research supports the development of interventions based on accurate assessment of the depressive symptoms, utilizing either of the MAACL-R and the POMS standardized instruments (Maloni et al.).

Lancaster, Gold, Flynn, Yoo, Marcus, and Davis (2010) provide a systematic review of risk factors for depressive symptoms during pregnancy. Approximately 12.7% of pregnant women will experience a major episode of depression during pregnancy and within a year of childbirth. The American College of Obstetrics and Gynecologists recognize the importance of depression during pregnancy enough to warrant recommending that all pregnant women be screened for depression during each trimester (American College of Obstetricians and Gynecologists, 2006). Limitations of the systematic review were recognized; the cross-sectional designs limited the ability to draw causality conclusions and the screening tools used were not diagnostic of depression and utilized various cutoffs, therefore the predictability of the risk factors was limited. Although limitations were recognized the significance of recognizing depressive symptoms and predictive risk may facilitate more effective interventions for prenatal/postnatal depression (Lancaster et al.).

To date, only one study of prenatal depression and anxiety in women positive for *T. gondii* was found (Groer et al., 2011). Of the 414 pregnant women measured at between 16-25 weeks gestation, the 44 *T. gondii* positive women had a correlation of higher *T. gondii* immunoglobulin titer related to anxiety and depression during pregnancy. The Profile of Mood States- depression/dejection score (POMS-D) was utilized to measure the depressive symptoms of the subjects (Groer et al.). Bansil et al.'s(2010) research suggested that depression was associated with adverse maternal and fetal outcomes. Bansil reported the estimated prevalence of depression during pregnancy ranged from 7.4% to 15%. Depression has negatively influenced pregnancy outcomes through the correlation of depression to harmful health behaviors such as drug abuse, alcohol abuse, self-medication, and cigarette use, poor nutrition with inadequate weight gain, which leads to inadequate placental function leading to adverse fetal outcomes

(Bansil et. al.). These significant negative outcomes support the need for screening during pregnancy for *T. gondii* (Groer et al.).

Post-natal Depression

The World Health Organization estimates that depression will be the second leading cause of premature death and morbidity by 2020 and suicide is one of the most common causes of maternal death in the year following delivery in developed countries (World Health Organization, 2012). A critical analysis of the literature on post-natal depression (PND) was conducted by Wylie, Hoolins-Martin, Marland, Martin and Rankin (2011). Girardi et al. (2012) found higher risk for depression and suicide in the post-partum period. Post-natal depression is characterized by "depressed mood, hopelessness, anxiety, excessive fatigue, psychomotor agitation, appetite and sleep disturbance, guilt and/or feelings of inadequacy" (Wylie et al., p. 49.). Beck (2001) identified 13 predictors of post-natal depression which included prenatal depression. Also the National Institute for Health and Clinical Excellence (NICE) (2007) incorporated the concept of predicting and detecting mental health problems related to recognizing mental health problems during pregnancy to include prenatal depression as a predictor of post-natal depression.

British National Institute for Health and Care Excellence (NICE) clinical guidelines recommends that early detection of the predictive signs of post-natal depression may afford more effective evidenced-based interventions for decreasing the incidence of post-natal depression and the adverse emotional effects on women and children (NICE). NICE guidelines recognize post-natal depression as part of a continuum and has provided recommended questions to facilitate a more accurate diagnosis of post-natal depression through the utilization this continuum and the predictive value of prenatal depression (NICE). High quality interventions developed as a result

of this early recognition and more consistent use of the predictor, prenatal depression, may lead to greater prevention of the second leading cause of maternal death in the first year post birth (Wylie et al., 2011).

Research conducted in China and Jordan provide supportive evidence of an increased risk of postpartum depression when prenatal depressive symptoms were present resulting in an increased risk for suicide (Wan, Moyer, Harlow, Fan, Jie, & Yang, 2009; Mohammed, MMid, Gamble, & Creedy, 2011). The supporting evidence provided by the Ling et al. research of the associated increased risk of seropositive *T. gondii* women and suicide rates in women suggest improved focused interventions for this population (2011).

Gaps in Research

Evidence was reviewed of the negative behavioral and personality changes related to the long-term influence of the parasite, *Toxoplasma gondii* and the negative *T. gondii* influence on prenatal depressive symptoms. Evidence was reviewed discussing the protective effect of RhD positive blood factor on the latent *T. gondii* infected subjects. Research conducted in 20 European countries resulted in an association of seropositive *T. gondii* women and suicide rates (Ling, Lester, Mortensen, Langenberg, & Postolache, 2011). However there is no research to date examining the potentially protective effect of RhD positive blood factor on *T. gondii* infected pregnant patients with depressive symptoms. Identifying this unique relationship between latent toxoplasmosis, prenatal depressive symptoms, and the protective effect of RhD positive blood factor may support the need for routine prenatal screening for toxoplasmosis and allow for improved interventions in postnatal depression prediction resulting in fewer suicides, the second leading cause of maternal death during the first year post delivery.

Chapter Three

Method

The purpose of the research was to explore the possible protective effect of RhD positive blood factor on prenatal depressive symptoms in *T. gondii* positive pregnant patients. Identifying the possible protective effect of RhD positive blood factor may provide information to improve interventions for the treatment of post natal depression. Chapter three describes the methodology utilized within the research. The chapter begins with an introductory section followed by the identification of the design with rationale, a description of the setting, population, and sample including inclusion and exclusion criteria. Instrumentation is described and validity and reliability of the scores were reviewed. Procedures are described in detail to include Institutional Review Board submission and approvals, recruitment, informed consent, and data collection procedures. The data analysis plan and rationale of analysis are included.

Design

The research is a secondary data analysis of the parent study on the relationship between *T. gondii* and prenatal depression (Groer et. al., 2011). The design of this research was a cross-sectional design. Exploratory research seeks to investigate the phenomenon and the manner in which it is manifested and other factors to which it is related. The researcher sought to explore the possible protective effect of Rhesus D positive blood factor on Toxoplasma-related depressive symptoms in pregnancy. Figure 1 represents the Logic model for the research.
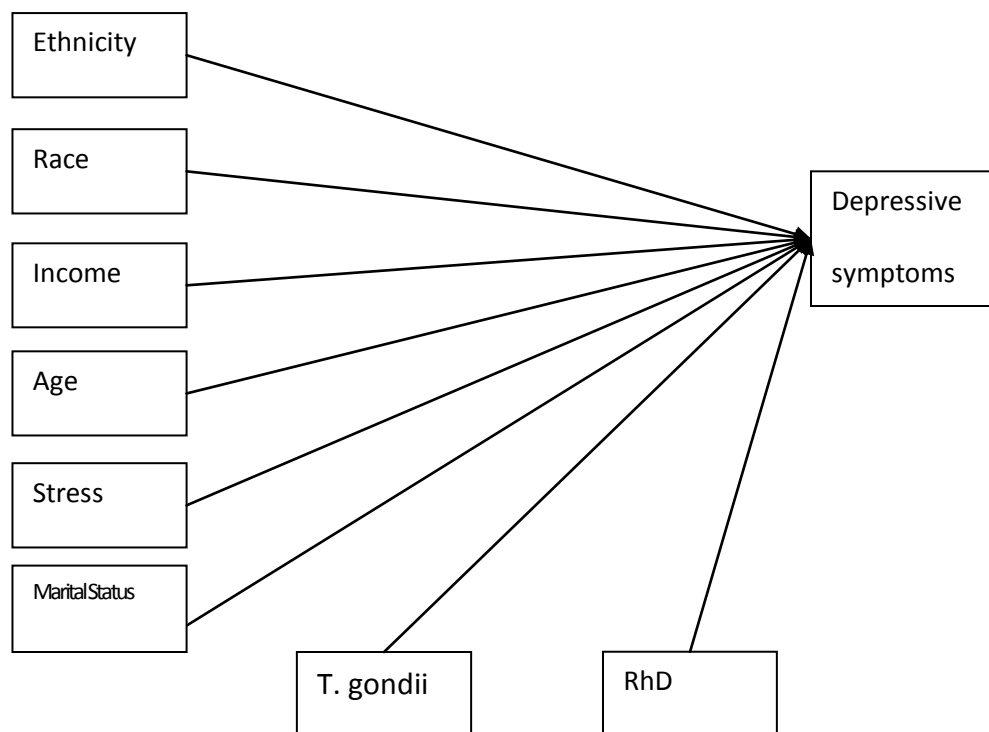
Figure 1 - Logic Model

Setting

The original data were obtained at two large obstetrical practices which were under the auspices of the University of South Florida Department of Obstetrics and Gynecology in Tampa, Florida. These practices supervised over 2500 births per year therefore 600 women between 16-25 weeks gestation were not difficult to recruit. The setting for the secondary data analyses was the lab at the University of South Florida, Tampa, Florida. The RhD status of the participants was already gathered by Groer's research team at Tampa General Hospital from medical records and recorded on Excel spreadsheets and subsequently added to the parent research. The

researcher was granted access to the 217 de-identified participants' blood samples which were not yet tested for *T. gondii* when the initial study was done and these samples were analyzed for *T. gondii* in the lab at the College of Nursing on the University of South Florida, Tampa, Florida.

Population and Sample

The target population was healthy pregnant women 16-25 weeks gestation (*n*=631) recruited by two large obstetrical practices by trained recruiters from 2006-2009. Participants were given information about the research and provided informed consent at a prenatal visit and were enrolled at a subsequent visit. Of those eligible, 44% declined to participate. Exclusion criteria included immune diseases, immune altering medications, in vitro fertilization, human immunodeficiency virus disease, illegal drug abuse, extreme thinness, and age <18 and >45 years of age. All participants were evaluated for depressive symptoms.

The University of South Florida is located within an ethnically diverse city. The population of Tampa, Florida, at the time of the parent research was 317,647. With an ethnicity breakdown of 64.2% Caucasian, 26.1% Black or African Americans, 0.4% American Indian or Alaskan Native, 2.2% Asian. 0.1% Native Hawaiian or other Pacific Islander, and 19.3% Hispanic or Latino (http://quickfacts.census.gov). The sample (*n*=631) was representative of the ethnically diverse population.

Measures

All participants completed a demographic questionnaire which solicited information regarding age, race, income, education, family history of thyroid disease, height, weight, exercise, problems during labor, birth history, mode of delivery, infant gender, postpartum complications, yearly income, family size, the presence of any chronic or acute health problems, smoking, current medications, birth history, work status, exercise, and breastfeeding or bottle-

feeding history. For the purposes of the secondary data analyses, the author examined ethnicity, race, income, marital status, age, stress, level of depressive symptoms, RhD blood factor status and *T. gondii* titer.

Profile of Mood States

The Profile of Mood States (POMS) (McNair et al, 1992) is a 65-item instrument designed to elicit feelings over the past week, including the day of measurement. There are six subscales: tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue-inertia, and confusion-bewilderment. Experience of mood is reported on a 5-point summated rating scale, Likert-type, with responses ranging from 0 (not at all) to 4 (extremely). The scores of the POMS have been found highly reliable and valid in a number of studies of adults in a variety of life circumstances. The internal consistency ranges from .87 to .92 and test-retest reliably from .68 to .74 (McNair et al.). The POMS was considered ideal for assessing the whole range of depressive symptoms in postpartum.

Profile of Mood States (POMS-D)

The POMS-depression/dejection score (POMS-D) demonstrated a high correlation with the Beck's Depression Index-II ($r = 0.81$) (Malouff, Schutte, & Ramerth, 1985). In the parent research the POMS was selected because it is known as an excellent screening instrument for dysphoric disorders. The aims of the parent research were to examine general negative moods during pregnancy and through postpartum period. The depression subscale (POMS-D) includes 15 items that may be used as a stand-alone measure. The response metric: (0) Not at All (1) A Little (2) Moderately (3) Quite a Bit (4) Extremely, quantifies the frequency of experiencing a feeling over the duration of a day and over the past 7 days. Higher scores on the POMS indicate higher levels of dysphoric moods. Reliability of the POMS scores as indicated by alpha

coefficients of .95 for depression and .92 for anxiety is favorable (Conn, Taylor, & Abele, 1991; Boyle, 1987).

Perceived Stress Scale

The Perceived Stress Scale (PSS) was utilized to measure the "degree to which situations in one's life are appraised as stressful" (Cohen, Karmarck & Mermelstein, 1983, p.385). In the parent study the 14-item, likert-type scale, was utilized to measure the participants' level of perceived stress. The scale ranges from 0 (never), 1 (almost never), 2 (sometimes), 3 (fairly often), and 4 (very often). Internal consistency was demonstrated at .84, .85, and .86, respectively, in three samples of young adults. Convergent and criterion validity were found to be excellent, the predictive validity declined over time (Cohen, Karmarck, & Mermelstein).

RhD Blood Factor

For the purposes of this secondary data analysis, the RhD blood factor in the parent data base was recoded to either negative or positive, RhD negative (0) and RhD positive (1). The researcher received permission from the graduate student, who collected the RhD blood factors, to utilize the RhD blood factors collected from her research.

T. gondii

In the parent research 414 participants' stored blood samples (-80°C) from 2006-2009 were assessed for quantitative IgG antibodies to whole Toxoplasma organisms. The results were defined as positive or negative based on comparison with the reactivity of specific antibody standards assayed with the plasma samples. A positive *T. gondii* infection was determined at a level greater than 18 International Units (IU).

For the purposes of the secondary analysis Dr. Groer's laboratory conducted the assay of the remaining 217 samples utilizing the VIR-ELISA assay and Dr. Groer reran all of the previously positive titers (*N*=44) with the same assay kit so that results were comparable.

Procedures

Dr. Maureen Groer, the principal investigator of the parent study granted permission for the researcher to utilize existing data.

Institutional Review Board (IRB) Submission

Institutional Review Board approval was required for the modification of the parent IRB for the secondary data analyses. Permission for the researcher to be added to the original approval was submitted by the principal investigator, Dr Maureen W. Groer. The researcher requested an expedited IRB to be allowed access to the data from the parent research. All of the data and specimens utilized within the research remained de-identified to the researcher.

Contact/Recruitment

Official participant recruitment was conducted in the parent research. No additional recruitment was required for these research questions. Inclusion and exclusion criteria for the secondary analysis remained the same. Exclusion criteria are described earlier in sample section.

Informed Consent

Informed consent was conducted in the parent research per procedure. There was no additional informed consent required for the secondary data analyses.

Data Analysis

Data analysis was performed to answer each research question. Descriptive statistics were utilized in the parent research to describe the study variables. Data were examined for normality and log transformed. As in the parent research, a Pearson correlation was performed between the *T. gondii* IgG titers and the POMS-D scores.

Analysis is described through the corresponding aims:

Aim #1: Examine the relationship of ethnicity, race, income, marital status, age, stress,

and RhD blood factor on prenatal depressive symptoms in 56 *T. gondii* infected pregnant women 16-25 weeks gestation. Aim #1 was addressed utilizing multiple-linear regression. In Model 1, the POMS-D, was regressed onto the set of demographic variables; ethnicity, income, marital status, age, and stress. Significance tests on the regression weights for each predictor were used to determine importance. An R^2= .20 was utilized to define level of fit, *df*=6.

Aim #2: Examine the relationship of *T. gondii* titer and prenatal depressive symptoms. *T. gondii* titer was added to the Model 1, resulting in *df*=7.

Aim #3: Examine the relationship of Rh status and prenatal depressive symptoms. The Rh status was added to Model 1 resulting in remaining *df*=8.

Aim #4: Examine the moderating effect between Rh status and *T. gondii* on prenatal depressive symptoms. A product term was created to identify the test for the moderating effect. The *T gondii* titer, Rh status and product term will be added to Model 1 to test for the moderating effect (interaction) effect of Rh resulting in *df*=9.

In the parent research data, there was a positive correlation of *T. gondii* IgG titer with the POMS-D (r = .37; $p < .01$) with the relationship remaining significant when controlling for the effects of race, number of pregnancies, cigarette smoking, and age. Scatter plots from parent research reflect the POMS depression scores increased as the *T. gondii* titers increased. The researcher expected that when adding the additional participants' *T. gondii* results the relationships would remain significant. There has been research data supporting the protective effect of positive RhD blood factor (Flegr et. al., 2009, 2010), therefore, the researcher expected to find the effect of positive *T. gondii* on depressive symptoms to be moderated by the positive RhD blood factor. Due to the multiple influences of the covariates on prenatal depressive

symptoms, the RhD positive test of interaction was conducted while controlling for the covariates, ethnicity, race, income, marital status, age, and stress, known to contribute to depression.

A fundamental component of research is the critical appraisal of the statistical analysis of the data, and thus the magnitude of the influence of the independent or predictor variables on the dependent variable, the effect size (Polit & Sherman, 1990). In the parent research there was a significantly positive correlation of the *T. gondii* IgG titer with the POMS-D depression subscale ($r = .37$, $p < .01$), with the relationships remaining significant when controlling for the covariates. The standardized differences between the means of the groups were divided by the pooled standard deviations to elicit a Cohen's *d,* medium effect size, 0.5. To detect a medium effect size with .05 criterion of statistical significance level, and resulting power of .80, the sample size of 54 was required. In the parent data 10.5% of the original 414 participants tested positive for *T. gondii*, so it was expected that approximately 23 of the additional 217 participants would test positive for *T. gondii*.

Chapter Four

Results

Preliminary analyses

Missing data and normality. Of the 631 participant's blood samples in the parent

research, 414 participants were tested for *T. gondii* resulting in 44 participants positive for the

infection. The analysis of the remaining 217 participants' blood samples resulted in an additional

12 participant's positive for the *T. gondii* infection. To achieve a more comparable sample the

previous 44 positive blood samples were rerun by Dr. Groer with the same assay as the 12 new

T. gondii positive blood samples. Three samples of the 44 previously reported as positive had a

result of less than 18 IU/ml, a negative result for *T. gondii* in the rerun. The combined samples

resulted in a total sample of 56 participants positive for *T. gondii* infection.

Data were examined utilizing SPSS FREQUENCIES, DISTRIBUTIONS and exploration

of means to identify skewness, outliers and missing values. Data were transformed using

Logarithm (10) to reduce skewness, the number of outliers and improve the normality of the data

(Tabachnick & Fidell. 2007).

Pearson correlation was utilized for the correlation of the interval level data. Both listwise

and pairwise Pearson correlations were conducted. A listwise Pearson correlation was conducted

to identify the number of variables that were identified for both variables, *T. gondii* (Toxoigmg)

and RhD positive blood factor (RHpn). Utilizing pairwise Pearson correlation the researcher was

able to see how many variables were present independently and thus the researcher was able to

identify the degree of correlation and sample size in order to determine if the utilization of the

variable resulting in a decrease of sample size was reasonable. Upon examination of the variables, the addition of the variables, income and marital status, would reduce the already small sample size to less than 22. Also there was no significant relationship between these variables and the dependent variable, therefore income and marital status were eliminated from the models. The Rhesus positive blood factor variable was also found to be missing in 11 participant's reducing the sample size to 45.

Description of the sample. Participants ranged in age range of 19-44 years of age (*M*=29.44; *SD*=6.61). A majority of the sample self reported race as Hispanic origin (*n*=25; 55.6%), while 12 participants self-reported as Caucasian (26.7%), six participants self-reported as African American (13.3%), and two self-reported as Asian/Pacific Islander (3.6%). The ethnicity of the population was primarily Hispanic (*n*=25; 55.6%). The racial distribution of the sample of positive *T. gondii* participants is described in Table 1.

Table 1.

Racial distribution of sample of positive T. gondii participants

Race	N	%
Hispanic Origin	25	55.6%
Caucasian	12	26.7%
African American	6	13.3%
Asian/Pacific	2	3.6%

Additional demographic information analyzed in the study included income and marital status. Six participants reported income less than $14,999 (13.3%), three participants reported income less than $24,999 (6.7%), seven reported income less than $39,999 (15.6%), seven

reported income greater than 40,000 (13.3%) with twenty-three (51.1%) declining to report level of income. Marital status was reported as thirteen (28.9%) single, fifteen (33.3%) married, one (2.2%) divorced, and sixteen (35.6%) declined to answer.

Psychological variables. The mean score of the total POMS-depression/dejection score (POMS-D) score was 6.93 (*N*=45; *SD*=7.42) with range from 00 to 38. Higher scores indicate more depressive symptoms. The mean score for the Perceived Stress Scale Total score, (PSSTOT), was 21.8 (*N*=45; *SD*=6.79) with a range from 6 to 37. High scores also represent a higher degree of perceived level of stress (Cohen et al., 1983).

Physiological variables. The overall mean for the 45 participants positive for *T. gondii* was 85.08 IU (*SD*=44.72) with range from 18 to 200. The cut point for determining positive *T. gondii* infection was greater than 18 International units (IU).

The RhD blood factor was reported as negative (0) or positive (1). There were forty participants with the positive RhD blood factor (88.9%) and five participants with a negative RhD blood factor (11.19%). These percentages are the normal percentage distribution related to positive and negative RhD blood factors in the overall population.

Pearson correlations with respective means and standard deviations are represented in Table 2. As previously demonstrated in prior research (Groer et al., 2011), positive *T. gondii* and stress were significantly correlated to prenatal depressive symptoms. The secondary analyses also resulted in a significant correlation between positive *T. gondii* and stress to prenatal depressive symptoms; positive *T. gondii r*=.50, *p* < .01 and stress *r*=.34, *p* < .05. These correlations support evidence from the parent research. There was no correlation of RhD positive blood factor with the dependent variable, prenatal depressive symptoms. The interaction of *T. gondii* and RhD (TxR) was significantly correlated, when adding the T. gondii to the product

term, with prenatal depressive symptoms. The correlations between the variables utilizing

pairwise deletion; POMS-D total score (Tpomsd), age, Hispanic ethnicity (Hisp), Perceived

Stress Scale total score (PSSTOT), *Toxoplasma gondii* (Toxoigmg), RhD positive blood factor

(RHpn) and the interaction product term of T. gondii and RhD Positive blood factor (TxR) are

represented in Table 2.

Table 2.

Correlations prenatal depressive symptoms, psychosocial and physiological variables

	Total POMS-D	Age	Hisp	PSSTOT	*T. gondii*	RHpn	TxR
Total POMS-D	1.000	-.100	.221	.340*	.497**	.143	.344*
Age		1.000	.212	-.094	-.112	-.257	-.118
Hisp			1.000	.009	.200	.063	.314
PSSTOT				1.000	.234	-.063	.203
T. gondii					1.000	-.060	.336*
RHpn						1.000	.315*
TxR							1.000
Mean	.700	29.44	.489	21.80	85.09	.889	-.839
SD	.454	6.61	.506	6.79	44.72	.318	9.29
N	45	45	45	45	45	45	45

p = .05 * *p* = .01** *T. gondii* X Rhpn = (TxR)

Analyses of the Study Aims

Sequential regression was utilized to determine if the addition of the variables, *T. gondii*

and the RhD positive blood factor, and the product variable, while controlling for the

demographic variables would suggest these variables were contributing factors towards lower

prenatal depressive symptoms. Analysis was performed using the SPSS REGRESSION. Table 2

as described earlier displays the correlations between the variables utilizing pairwise deletion.

Aim #1: Examine the relationship of ethnicity, race, income, marital status, age, stress, and RhD blood factor on prenatal depressive symptoms in 45 positive *T. gondii* infected pregnant women 16-25 weeks gestation. Aim #1 was analyzed utilizing multiple linear regression. In Model 1, the POMS-D, depressive symptoms, were regressed onto the set of demographic variables; ethnicity, race, income, marital status, age, and stress. Significance tests on the regression weights for each predictor were used to determine importance. An R^2= .20 was utilized to define level of fit, *degrees of freedom* (*df*) =6. The variable race was found to not be a dichotomous variable therefore was removed from the regression. As previously described in this chapter the pairwise correlation demonstrated the rationale for reducing the number of variables utilized in the regression model to ethnicity (Hisp), age, and stress, reducing the *df*=3 for Model 1. Model 1 equation, $F(3, 41) = 2.941$, $p < .05$, $R=.421$ and $R^2=.18$. The adjusted R^2 value of .12 indicates that more than 12% of the variability in prenatal depressive symptoms is predicted by ones' age, being Hispanic, and level of stress.

Aim #2: Examine the relationship of *T. gondii* status and prenatal depressive symptoms. *T. gondii* titer was added to the Model 1, resulting in *df*=4.Table 3 illustrates the change from Model 1 to Model 2 with the addition of the positive *T. gondii* variable to the Model 2. In this equation, $F(4, 40) = 4.737$, $p < .01$, $R=.567$. The addition of *T. gondii* added to the prediction of prenatal depressive symptoms, $R^2=.32$. The F test for the change in R^2 when comparing Model 1 to Model 2 was significant at 8.50[**].

Table 3

Change from Model 1 to Model 2

| | | Prenatal Depressive Symptoms | |
| | | Model 2 | |
Variable	Model 1 *B*	*B*	95% CI
Constant	.363	-.070	-.656 - .797
Age	-.008	-.004	-023 - .014
Hispanic	.219	.136	-.112 - .385
PSSTOT	.022	.016	.002 - .034
T. gondii		.004	.001 - .007
R^2	.177	.321	
F	2.941*	4.737**	
ΔR^2		.144	
ΔF		8.508**	

Note. Dependent variable = POMS-D total score
N = 45. CI = Confidence interval.
*$p < .05$. **$p < .01$.

Aim #3: Examine the relationship of Rh status and prenatal depressive symptoms. The

RhD positive blood factor status was added to Model 1 resulting in remaining

df=5. The representation of the regression equation comparing Model 2 with Model 3

with the addition of the RH positive blood factor (RHpn) is represented in Table 4. The

comparison displays $F (5, 39) = 4.169$, $p < .01$, $R=.590$ and $R^2=.348$. The adjusted R^2 of

.27 indicates that the addition of the RhD positive blood factor, when added into the

model, minimally increased the variability predicted by the previous model. The F test

for the change in R^2 when comparing Model 2 to Model 3 was not significant at .212. The

comparison of the models is displayed in Table 4.

Table 4

Change from Model 2 to Model 3

		Prenatal Depressive Symptoms	
		Model 3	
Variable	Model 2 B	B	95% CI
Constant	-.070	-.279	-1.190 - .632
Age	-.004	-.001	-.020 - .019
Hispanic	.136	.113	-.137 - .363
PSSTOT	.016	.017	.001 - .035
T. gondii	.004	.004	.001 - .007
RHpn		.247	-.147 - .640
R^2	.321	.348	
F	4.737**	4.169**	
ΔR^2		.027	
ΔF		1.61	

Note. Dependent variable = POMS-D
*$p < .05$. **$p < .01$.

Aim #4: Examine the moderating effect between Rh status and *T. gondii* on prenatal

depressive symptoms.

A product term was created to identify the test for the moderating effect of *T*

gondii status and Rh status (TxRh). The interaction was added to Model 1 to test for the

moderating effect (interaction) effect of Rh resulting in *df*=6. The regression of the moderating

factor onto the dependent variable is represented in Table 5 with the comparison of Model 3 to

Model 4. When adding all of the independent variables including the interaction TxRh, F (6, 38)

$= 3.455$, $p < .01$, $R = .594$ and $R^2 = .353$. The interaction was not statistically significant ($p = .605$) representing no moderating factor. The F test for the change in R^2, when comparing Model 3 to Model 4, was not significant at .272. The comparison of the models is displayed in Table 5.

Table 5.

Change from Model 3 to Model 4

| | | Prenatal Depressive Symptoms | |
| | | Model 4 | |
Variable	Model 3 *B*	*B*	95% CI
Constant	-.279	-.205	-1.169 - .759
Age	-.001	-.001	-020 - .019
Hisp	.095	.095	-.167 - .357
PSSTOT	.016	.016	.003 - .034
T. gondii	.004	.004	.001 - .007
RHpn	.210	.210	.212- .632
TxRh		.004	-.011 - .019
R^2	.348	.353	
F	4.169**	3.455**	
ΔR^2		.005	
ΔF		.272	

Note. Dependent variable = POMS-D *$p < .05$. **$p < .01$.

The independent variables of stress and *T. gondii* were consistently found to be significant predictors of prenatal depression, where the addition of the RhD positive blood factor did not significantly contribute when regressed onto prenatal depressive symptoms. Model 1, with the log of age, ethnicity and stress in the equation, had a $R^2 = .177$, (adjusted $R^2 = .12$), F_{inc} (3, 41) = 2.941, $p < .05$. In Model 2,

after the square root of *T.gondii* was added to the prediction of prenatal depressive symptoms by the age, ethnicity and stress, resulted in R^2=.321, (adjusted R^2=.25), F_{inc} (4, 40) = .006, $p < .01$. The addition of square root of *T.gondii* to the equation with age, ethnicity, and stress resulted in a significant increment of R^2. In Model 3, with RhD positive blood factor added to the prediction of prenatal depressive symptoms by *T. gondii* and square root of age, ethnicity and stress, the result was R^2=.348, (adjusted R^2 = .27), F_{inc} (5, 39) = .212. The addition of the RhD positive blood factor did not reliably improve R^2. In Model 4, the addition of the product variable TxRh to the prediction of prenatal depressive symptoms by *T. gondii* and square root of age, ethnicity, stress, *T. gondii*, and RhD positive blood factor, the result was R^2=.353, (adjusted R^2 = .25), F_{inc} (1, 37) = .605. The addition of the product variable did not reliably improve R^2. The addition of neither the positive RhD blood factor nor the product variable significantly improve the reliability of R^2 in predicting prenatal depressive symptoms.

Chapter Five

DISCUSSION

The purpose of this study was to examine the possible protective effect of RhD positive blood factor on prenatal depressive symptoms in pregnant women with *T. gondii* infection. The cross-sectional design was utilized to answer the question "Does positive RhD blood factor provide a protective effect on prenatal depressive symptoms of patients infected with *T. gondii* when controlling for ethnicity, race, income, marital status, age and stress?" The conceptual model hypothesized that there was a relationship between socio-demographic variables (age, income, marital status, race, and ethnicity) and prenatal depressive symptoms. After preliminary analyses utilizing Pearson correlation, the variables income and marital status were eliminated from the analyses due to the significant reduction in sample size if utilized. Due to previous research evidence, a relationship between *T. gondii* and stress on prenatal depressive symptoms was also proposed. Although prior research (Flegr et al, 2005; Flegr et al, 2008; Flegr et al, 2009, & Flegr et al, 2010) provided evidence of the protective effect of RhD positive blood factor on the variable outcomes in *T. gondii* infected individuals, there was no research to date exploring a protective effect on pregnant women infected with *T. gondii.* Therefore, this study proposed a possible protective effect of positive RhD blood factor in*T. gondii* infected pregnant women on prenatal depressive symptoms. Finally, a moderating effect of RhD positive blood factor on the relationship between *T. gondii* and prenatal depressive symptoms was proposed. Pearson correlations and multiple regression were utilized to explore the aims of this study.

Sample size may account for our lack of findings of 45 pregnant *T. gondii* positive women. The percentage of women with RhD positive blood factor versus negative was 88.9% and 11.2%, respectively. These percentages are comparable to the research evidence provided by Branger and Wise (2006). The power analysis of the secondary analyses suggested 54 participants would result in a medium effect. Missing RhD blood factor data resulted in a reduction of the original sample of 56 pregnant participants infected with *T. gondii* to an N=45. The reduction of sample size may have attributed to the nonsignificant findings. The parent study did, however, find a significant relationship between stress and positive *T.gondii* seropositivity on prenatal depressive symptoms, which was replicated in this research.

Toxoplasma gondii infects approximately 30% of the population worldwide, with a higher prevalence in women than men, (23.7% versus 10.9%), (Flegr & Striz, 2011). The CDC estimates that 22.5% of the population 12 years and older in the United States is infected with *T. gondii* (CDC, 2013). Although Kolbekova, Kourabatova, Kodym, and Flegr (2007) reported an overall seroprevalence of 29-37% in pregnant women world-wide, the percentage of pregnant participants with positive *T. gondii* infection in the parent research was 9% of the 631 sample. The CDC (2013) named toxoplasmosis as one of the five leading neglected parasites in the United States, calling it the leading cause of death which is attributed to food borne illness. Acute toxoplasmosis continues to be responsible for devastating effects for the newborn infant including hydrocephaly, intra-cerebral calcifications, microcephaly, and miscarriage in the immune compromised (Montoya & Remington, 2008). To date, although literature regarding toxoplasmosis is distributed to pregnant women and they are encouraged them to avoid cat litter, wash produce well before consumption, and eat well cooked meat products, there is no recommendation for routine screening for *T. gondii* in all pregnant women.

There was a significant relationship between the level of stress and *T. gondii* infection on prenatal depressive symptoms. Higher levels of stress were associated with higher prenatal depressive symptoms reported by the *T.gondii* infected pregnant women. Both *T.gondii* infection and stress were associated with depressive symptoms. Which of the variables contributed more is unable to be ascertained. Although no significant relationship was demonstrated between T. gondii and stress, one might speculate that the increased stress may have reduced the body's ability to fight the *T. gondii* infection. Although there is evidence supporting the increase in depressive symptoms with *T.gondii* infection, this research did not examine the length of time the participants had the *T. gondii* infection. Research evidence suggests that as the length of the *T. gondii* infection or latency increases, so do the psychiatric disorders and personality changes which could deteriorate immune response to disease (Flegr & Hrda, 1994; Flegr & Havilcek, 1999; Flegr, Kodym, & Tolarova, 2000; Havlicek et al., 2001; Flegr, 2007; & Flegr, 2010). In addition, possible reactivation of the dormant *T. gondii* tachyzoites may have contributed to the inability of the body to fight the infection (Stokkeman et al., 1996). The ability to determine the latency and or reactivation state of the *T. gondii* infection would strengthen the results of this research.

Also, 51% of the sample self-reported Hispanic ethnicity. Research is suggestive of a higher *T. gondii* seroprevalence in women of Hispanic ethnicity which was supported by a significant relationship between *T. gondii* infection and Hispanic ethnicity in this research (Jones, Kruszon-Moran & Wilson, 2003; Groer et al, 2011). However, there was not a significant relationship between Hispanic ethnicity and prenatal depressive symptoms.

Although Flegr et al. (2009) found a significant protective effect of the positive RhD blood factor in *T. gondii* infected patients; this study did not find evidence of a significant

protective effect. When examining the relationship of the RhD positive blood factor and depressive symptoms, the small sample size cannot be ignored. Thus, the conceptual model of this research was not supported by the evidence. Although previous research evidence (Flegr et. al, 2005; Flegr et. al, 2008; Flegr et. al, 2009; Flegr et. al., 2010) supported the protective effect of RhD positive blood factor on *T. gondii* infected patients, no studies had been conducted on pregnant women infected with *T. gondii*. Because of the significant relationship between *T. gondii* infection and prenatal depressive symptoms and the subsequent predictive nature of prenatal depressive on post partum depression (NICE, 2007; Beck, 2001; Stillwaggon et al., 2011; Whisman et al., 2011; Girardi et al., 2012) further research on this relationship is warranted. This suggested research could be accomplished if pregnant women were screened for the presence of *T. gondii* infection routinely upon diagnosis of pregnancy. Improved screening for *T. gondii* may lead to improved surveillance of post partum depression and suicide post delivery to avoid or reduce the negative maternal and fetal outcomes.

Limitations

The sample size in this study is a limitation. Although the power analysis to elicit a medium effect suggested a sample size of at least 54 participants, due to missing data on the 57 positive participants infected with *T. gondii*, the sample size was reduced to a total of N=46. The small sample increases the risk of Type II error. A larger sample size of pregnant women, positive for the *T. gondii* infection, would allow for further exploration of the possible protective effect of positive RhD blood factor related to prenatal depressive symptoms. To date there has been only one study to identify the relationship between *T. gondii* infection in pregnant women and depressive symptoms. Therefore, further research is needed to examine this suggested relationship.

The cross-sectional, single group, design represents a collection of data from participants at a specific point in time. A cross-sectional design, although easy to do and economical, has no ability to identify which of the variables had the most influence overtime in this study. Also, the cross-sectional design limits the ability to draw causality conclusions. With the strong evidence of hosts' response to the *T. gondii* infection over time, a longitudinal study may provide a more in depth look at the data over time. This type of study may allow for a more in depth examination of the effects of individual variables over time.

Self-reporting was a limitation of the data collection of the psychological variables. Self-reported data, although versatile and directly to the point, is a weakness (Polit & Beck, 2004). How does the researcher know if the participant really feels the way they say they do or if they are merely reporting what they believe the researcher wants to hear? Most participants want to be seen as normal or less ill. The researcher must assume the self reported feelings are real.

Finally, there was some limitation to the *T. gondii* titers obtained in this study. Although the researcher attempted to stabilize the results of the titers by rerunning the titers performed on the parent study with the same manufacturers' analysis kit as this study, there is still literature (CDC, 2009) suggesting that the sensitivity and specificity of the ELISA kits vary among manufacturers. Presently there is no gold standard for which assay kit is preferred.

Implications

Strong evidence has been presented regarding the ability of the *T. gondii* infection to lay dormant for years after an acute episode within its human host resulting in specific behavioral and physiological effects (Flegr et al., 2000; Flegr et al., 2005; Flegr & Striz, 2011). Latent Toxoplasma-induced personality changes have been reported with possible links to psychiatric disorders (Lindova, Priplatova, & Flegr, (2011). The World Health Organization (2012) reported

that suicide is one of the most common causes of maternal death in the first year after delivery. Having provided evidence of the relationship between positive *T. gondii* and increased depressive symptoms, the ability to study *T. gondii* as it relates to prenatal and post partum depression more thoroughly has the potential to reduce this significant negative outcome. The prior research evidence suggests being of Hispanic ethnicity has a strong correlation to positive *T. gondii* infection. This patient population would need to be included in any future research.

Finally, although this study did not provide significant evidence of the protective effect of positive RhD blood factor on *T. gondii*-related depressive symptoms, there is strong evidence of a protective effect of RhD positive blood factor on the psychiatric symptoms of the *T. gondii* infection (Flegr et al., 2008; Flegr et al., 2009; Flegr et al., 2010). Further research is warranted to include pregnant women positive with the *T. gondii* infection and their related depressive symptoms. This research must include screening for the *T. gondii* infection among pregnant women. This screening may be the catalyst to the ability to predict prenatal and post partum depression among this vulnerable population.

Conclusion

The evidence of the relationship between *T. gondii* infected pregnant women and depressive symptoms was significant. Although not the focus of this research, there is also strong research evidence of devastating outcomes related to prenatal and post partum depression. The ability to screen pregnant women for *T. gondii* is available and should be considered as the next additional routine testing to be performed on all pregnant women. The identification of *T. gondii*, whether acute or dormant, may lead to a decline in the second leading cause of death in pregnant women in their first year post delivery. Educating pregnant women about the risks of *Toxoplasmosis,* when they become pregnant is not enough. The ability to identify a unique

relationship between latent *T. gondii* infection, prenatal depressive symptoms, and RhD positive blood factor may lead to the support needed to provide routine screening. The ability to improve interventions in predicting post partum depression may result in reducing the second leading cause of maternal death in the first year after delivery.

References

Adesiyun, A., Campbell, M., Rahaman, S., Bissessar, S., Stewart-Johnson, A., Dookereman, S., & Gittens-St Hilaire, M. (2011). Frequency of detection of immunoglobulins of *Toxoplasma gondii*, Leptospira spp., and Brucella abortus in livestock/farm and abattoir workers in Trinidad. *Journal Agromedicine, 16*(3), doi: 10.1080/1059924X.2011.581541.

American College of Obstetricians and Gynecologists. (2006). Committee opinion no.343: Psychosocial risk factors: Prenatal screening and intervention. *Journal of Obstetrics and Gynecologists, 189*, 267-273.

Arling, T. A., Yolken, R. H., Lapidus, M., Langenberg, P., Dickerson, F. B., Zimmerman, S. A.,…Postolache, T. T. (2009). *Toxoplasma gondii* antibody titers and history of suicide attempts in patients with recurrent mood disorders. *The Journal of Nervous and Mental Disease, 197*(12), 905-908.

Bansil, P., Kuklina, E. V., Meikle, S. F., Posner, S. F., Kourtis, A. P., Ellington, S. R., & Jamieson, D. J. (2010). Maternal and fetal outcomes among women with depression. *Journal of Women's Health, 19*(2), 329-334.

Beck, C. T. (2001). Predictors of postpartum depression: An update. *Nursing Research, 50*, 155-164.

Berdoy, M., Webster, J. P., & MacDonald, D. W. (2000). Fatal attraction in rats infected with *Toxoplasma gondii*. *Proceedings of the Royal Society of London, B267*, 1591-1594.

Branger, B. & Winer, N. (2006). Epidemiology of anti-D allo-immunization during pregnancy. *Journal of Gynecology, Obstetrics and Biological Reproduction, 35*(1), 1S87-1S92.

Centers for Disease Control and Prevention. (2009). *Toxoplasmosis.* Retrieved July 12, 2014,

from http://dpd.cdc.gov/dpdx/html/Toxoplasmosis.htm.

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A global perceived stress. *Journal of Health

and Social Behavior, 14(4)*, 385-396.

Conn V., Taylor, S., & Abele, P. (1991). Myocardial infarction survivors: Age and gender

differences in physical health, psychosocial state, and regimen adherence. *Journal of

Advanced Nursing, 16,* 1026-1034.

Dyer, N. W., & Stoltenow, C. L. (2001). Toxoplasmosis caused by the protozoan parasite

Toxoplasma gondii. Public Health Watch focus on Agriculture, V-1221. Retrieved July

12, 2014, from http://www.ag.ndsu.edu/ndsuag/gsearch?q=toxoplasma%20gondii .

Flegr, J. (2007). Effects of Toxoplasma on human behavior. *Schizophrenia Bulletin, 33*(3), 757-

760.

Flegr, J. (2010). Influence of latent toxoplasmosis on the phenotype of intermediate hosts. *Folia

Parasitologica, 57*(2), 81-87.

Flegr, J., & Havlicek, J. (1999). Changes in the personality profile of young women with latent

toxoplasmosis. *Folia Parasitologica, 46,* 22-28.

Flegr, J., & Hrda, I. (1994). Influence of chronic toxoplasmosis on some human personality

factors. *Folia Parasitologica, 41,* 122-126.

Flegr, J., Hrda, S., & Kodym, P. (2005). Influence of latent 'asymptomatic' toxoplamosis on

body weight of pregnant women. *Folia Parasitologica, 52*(3), 199-204.

Flegr, J., Klose, J., Novotna, M., Berenreitterova, M., & Havlicek, J. (2009). Increased incidence of traffic accidents in *Toxoplasma*-infected military drivers and protective effect RhD molecule revealed by a large-scale prospective cohort study. *BMC Infectious Diseases, 9*(72), 1-7.

Flegr, J., Kodym, P., & Tolarova, V. (2000). Correlation of duration of latent *Toxoplasma gondii* infection with personality changes in women. *Biological Psychology, 53,* 57-68.

Flegr, J., Novotna, M., Fialova, A., Kolbekova, P., & Gasova, Z. (2010). The influence of RhD phenotype on toxoplasmosis- and age-associated changes in personality pofile of blood donors. *Folia Parasitologica, 57*(2), 143-150.

Flegr, J., Priess, M., Klose, J, Havlicek, J., Vitakova, M., & Kodym, P. (2003). Decreased level of psychobiological factor novelty seeking and lower intelligence in men latently infected with the protozoan parasite *Toxoplasma gondii*. Dopamine, a missing link between schizophrenia and toxoplasmosis? *Biological Psychology, 63*, 253-268.

Flegr, J., Novotna, M., Lindova, J., & Havlicek, J. (2008). Neurophysiological effect of the Rh factor. Protective role of the RhD molecule against *Toxoplasma*-induced impairment of reaction times in women. *Neuroendocrinology Letters, 29*(4), 475-481.

Flegr, J., & Striz, I. (2011). Potential immunomodularity effects of latent toxoplasmosis in humans. *BMC Infectious Diseases, 11,* 274-280.

Flegr, J., Zitkova, S., Kodym, P., & Frynta, D. (1996). Induction of changes in human behavior by the parasitic protozoan *Toxoplasma gondii*. *Parasitology, 113,* 49-54.

Girardi, P., Pompili, M., Innamorati, M., Serafini, G., Berrettoni, C., Koukopoulos, A.,…Primiero, F. M. (2012). Temperament, post-partum depression, hopelessness, and suicide risk among women soon after delivery. *Women & Health, 51*, 511-524.

Groer, M. & Vaughan, J. H. (2013). Positive thyroid peroxidase antibody titer is associated with dysphoric moods during pregnancy and postpartum. *Journal of Obstetric, Gynecologic & Neonatal Nursing, 42(1)*, E26-E32.

Groer, M. W., Yolken, R. H., Xiao, J. C., Beckstead, J. W., Fuchs, D., Mohapatra, S. S.,...Postolache, T. T. (2011). Prenatal depression and anxiety in *Toxoplasma gondii*-positive women. *American Journal of Obstetrics and Gynecology*. doi:10.1016/j.ajog.2011.01.004

Gunel, T., Kalelioglu, I., Gedikbasi, A., Ermis, H., & Aydinli, K. (2011). Detection of fetal RHD pseudogene (RHDΨ) and hybrid RHD-CE-D form RHD-negative pregnant women with a free DNA fetal kit. *Genetics and Molecular Research, 10*(4), 2653-2657.

Hall, S. R. & Smith, A. P. (1996). Behavioral effects of infectious mononucleosis. *Neuropsychobiology, 33*, 202-209.

Havlicek, J., Gasova, Z, Smith, A. P., Zvara, K., & Flegr, J. (2001). Decrease of psychomotor performance in subjects with latent 'asymptomatic' toxoplasmosis. *Parisitology, 122*, 515-520.

Heaman, M. (1992). Stressful life events, social support, and mood disturbance in hospitalized and non-hospitalized women with pregnancy-induced hypertension. *Canada Journal of Nursing Research, 24*, 23-37.

Holub, D., Motlova, L. B., Dragomirecka, E., Rodriguez, M., Preiss, M., Novak, T.,...Flegr, J. (2011). Possible protective function of Rh factor in schizophrenia. *Psychiatrie, Supplemental 2,* 37-42.

Hutchison, W. M., Aitken, P. P., & Wells, B. W. P. (1980). Chronic *Toxoplasma* infection and

    motor performance in the mouse. *Annals of Tropical Medicine and Parasitology*, 74,

    507-510.

Jones, J. L., Kruszon-Moran, D., & Wilson, M. (2007). *Toxoplasma gondii* prevalence United

    States, Retrieved http://wwwnc.cdc.gov/eid/article/13/4/06-1355_article.

Kankova, S., Holan, V., Zajicova, A., Kodym, P., & Flegr, J. (2010). Modulation of immunity in

    mice with latent toxoplasmosis – the experimental support for the immunosuppression

    hypothesis of *Toxoplasma*-induced changes in reproduction of mice and humans.

    Parasitology Research, 107, 1421-1427.

Kankova, S., Kodym, P., Frynta, D., Vavrinova, R., Kubena, A., & Flegr, J. (2007). Influence of

    latent toxoplasmosis on the secondary sex ratio in mice. *Parasitology, 134,* 1709-1717.

Kankova, S., Sulc, J., Nouzova, K., Fajfrlik, K., Frynta, D., & Flegr, J. (2007). Women infected

    with parasite Toxoplasma have more sons. *Naturwissenschaften, 94*, 122-127.

Kankova, S., Sulc, J., & Flegr, J. (2010). Increased pregnancy weight gain in women with latent

    toxoplasmosis and RhD-positivity protection against this effect. *Parasitology, 137,* 1773-

    1779.

Kolbekova, P., Kourbatova, E., Novotna, M., Kodym, P., & Flegr, J. (2007). New and old risk-

    factors for *Toxoplasma gondii* infection: Prospective cross-sectional study among

    military personnel in Czech Republic. *European Society of Clinical Microbiology and*

    Infectious Diseases, 13, 1012-1017.

Kumpel, B. M. (2008). Lessons learnt from many years of experience using anti-D in humans for

    prevention of RhD immunization and haemolytic disease of the fetus and newborn.

    Clinical and Experimental Immunology, 154, 1-5.

Lancaster, C. A., Gold, K. J., Flynn, H. A., Yoo, H., Marcus, S. M., & Davis, M. M. (2010). Risk factors for depressive symptoms during pregnancy: A systematic review. *American Journal of Obstetrics and Gynecology, 202*, 5-14.

Lindova, J., Priplatova, L., & Flegr, J. (2011). Higher extraversion and lower conscientiousness in humans infected with toxoplasma. *European Journal of Personality*. doi:10.1002/per.838

Ling, V. J., Lester, D., Mortensen, P. B., Langenberg, P. W., & Postolache, T. T. (2011). *Toxoplasma gondii* seropositivity and suicide rates in women. *The Journal of Nervous and Mental Disease, 199*(7), 440-444.

Maloni, J. A., Park, S., Anthony, M. K., & Musil, C. M. (2005). Measurement of antepartum depressive symptoms during high-risk pregnancy. *Research in Nursing & Health, 28*, 16-26.

Maloni, J. A., Chance, B., Zhang, C., Cohen, A. W., Betts, D., & Gange, S. J. (1993). Physical and psychosocial side effects of antepartum hospital bedrest. *Nursing Research, 42*(4), 197-203.

Malouff, J. M., Schutte, N. S., & Ramerth, W. (1985). Evaluation of a short form of the POMS-depression scale. *Journal of Clinical Psychology, 41*, 389-391.

McAuliffe, K. (2012). How your cat is making you crazy. *The Atlantic Monthly, 1,* 36-44.

McNair, D. M., Lorr, M., & Droppleman, L. F. (1992). Profile of mood states (POMS) manual. San Diego, CA: Educational and Industrial Testing Services.

Mercer, R. T. & Ferketich, S. L. (1988). Stress and social support as predictors of anxiety and depression during pregnancy. *Advances in Nursing Science, 10*(2), 83-95.

Mohammad, K. I, MMid, B. N., Gamble, J., & Creedy, D. K. (2011). Prevalence and factors associated with the development of antenatal and postnatal depression among Jordanian women. *Midwifery, 27*, e238-e245.

Montoya, J. G., & Remington, J. S. (2008). Management of *Toxoplasma gondii* infection during pregnancy. *Clinical Practice, 47,* 554-566.

National Institute for health and Clinical Excellence (NICE). (2007). *Antenatal and Postnatal Mental Health*. NICE, London.

Novotna, M., Havlicek, J., Smith, A. P., Kolbekova, P., Skallova, A., Klose, J.,...Flegr, J. (2008). *Toxoplasma* and reaction time: role of toxoplasmosis in the origin, preservation and geographical distribution of Rh blood group polymorphism. *Parasitology, 135,* 1253-1261.

Pilgrim, H., Lloyd-Jones, M., & Rees, A. (2009). Routine antenatal anti-D prophylaxis for RhD-negative women: A systematic review and economic evaluation. *Health Technology Assessment, 13*(10), doi:10.3310/htal3100

Polit D. F., & Sherman, R. E. (1990). Statistical power in nursing research. *Nursing Research, 39,* 365-369.

Salibay, C. C., & Claveria, F. G. (2005). Serologic detection of *Toxoplasma gondii* infection in *Rattus* spp collected from three different sites in Dasmarinas, Cavite, Philippines. *Southeast Asian Journal of Tropical Medicine Public Health, 36*(suppl 4), 46-49.

Simpore, J. Savadogo, A., Ilboudo, D., Nadambega, M. C., Esposito, M., Yara, J., …Musumeci, S. (2006). *Toxoplasma gondii*, HCV, and HBV seroprevalence and co-infection among HIV-positive and negative pregnant women in Burkina Faso. *Journal of Medical Virology, 78*, 730-733.

Skallova, A., Kodym, P., Frynta, D., & Flegr, J. (2006). The role of dopamine in *Toxoplasma*-induced behavioral alternations in mice: An ethological and ethopharmcological study. *Parasitology,* 1-11.

Skallova, A., Novotva, M., Kolbekova, P., Gasova, Z., Vesely, V., Sechovska, M., & Flegr, J. (2005). Decreased level of novelty seeking in blood donors infected with *Toxoplasma*. *Neuroendocrinology Letters, 26*, 1-7.

Smith, A. P., Tyrrell, D. A.. J., Al-Nakib, W., Barrow, P. G., Leekam, S., & Trickett, S. (1989). Effects and after-effects of the common cold and influenza on human performance. *Neuropsychobiology*, 21, 90-93.

Stillwaggon, E., Carrier, C. S., Sautter, M., & McLeod, R. (2011). Maternal serologic screening to prevent congenital toxoplasmosis: a decision-analytic economic model. *PLOS Neglected Tropical Disease, 5*(9), e1333.

Stokkerman, T. J. W., Schwartzman, J. D., Keenan, K., Morrissette, N. S., Tilney, L. G., & Roos, D. S. (1996). Inhibition of *Toxoplasma gondii* replication by dinitroaniline herbicides. *Experimental Parasitology, 84*, 355-370.

Tabachnick, B. G., & Fidell, L. S. (2007). *Using Multivariate Statistics* (5th Ed). Boston: Pearson Education, Inc.

Voosen, P. (2007). Jaraslav Flegr: A manipulation hypothesis brain parasite influences human

    behavior, Czech scientist says. *The Prague Post*. Retrieved January 10, 2012, from

    http://www.praguepost.com/archivescontent/3144-jaraslav-flegr-a-manipulation-

    hyposthesis.

Wan, E. Y., Moyer, C. A., Harlow, S. D., Fan, Z., Jie, Y., & Yang, H. (2009). Postpartum

    depression and traditional postpartum car in China: Role of Zuoyuezi. *International*

    Journal of Gynecology and Obstetrics, 104, 209-213.

Webster, J. P. (1994). The effect of *Toxoplasma gondii* and other parasites on activity levels in

    wild and hybrid Rattus norvegicus. *Parasitology, 109*, 583-589.

Witting, P. A. (1979). Learning capacity and memory of normal and *Toxoplasma*-infected

    laboratory rats and mice. *Zeitschrift fur Parasitenkunde 61*, 29-51.

Whisman, M. A., Davila, J. & Goodman, S. H. (2011). Relationship adjustment, depression, and

    anxiety during pregnancy and the postpartum period. *Journal of Family Psychology*

    25(3), 375-383.

World Health Organization. (2012, January). *Maternal mental health and child health and*

    development. Retrieved January 10, 2012, from

    http://www.who.int/mental_health/prevention/suicide/MaternalMH/en/index.html

Wylie, L., Hollins Martin, C. J., Marland, G., Martin, C. R., & Rankin, J. (2011). The enigma of

    post-natal depression: An update. *Journal of Psychiatric and Mental Health Nursing, 18,*

    48-58.

Yereli, K., Balcioglu, I. C., & Ozbilgin, A. (2006). Is *Toxoplasma gondii* a potential risk for

    traffic accidents in Turkey? *Forensic Science International, 163,* 34-37.