Depression in reproductive-age women: assessment of infectious, endocrinological, and immunological correlates from an evolutionary perspective.

Amber N. Carrier
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DEPRESSION IN REPRODUCTIVE-AGE WOMEN:
ASSESSMENT OF INFECTIOUS, ENDOCRINOLOGICAL, AND
IMMUNOLOGICAL CORRELATES FROM AN EVOLUTIONARY PERSPECTIVE

by

Amber N. Carrier
B.S., University of Southern Indiana, 2005
M.S., University of Louisville, 2008

A Dissertation
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University of Louisville
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A Dissertation Approved on

April 2, 2012

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Cynthia Corbett

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Michael Perlin

______________________________
James Summersgill
For DJA, my beloved friend --

My words are the greatest gift I could ever give to you.
ACKNOWLEDGEMENTS

There are so many people that have given me help, advice, joy, laughter, and support on this journey of mine. I am most thankful for having had each one of you in my lives, and a nod in a simple acknowledgement section is not enough to repay you. For the time being, however, it will have to do.

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without your wit and constant assurance that I am awesome when I felt far less than so. Thank you for happily eating my stress cookies and for accompanying me on last-minute trips to awesome places. For an attorney, you make a great nurse. Becky Rankin Wagenaar, you are an amazing friend who has taken care of me when I needed you. Thank you for letting me crash at your place more often than I should have, and for giving me statistics advice when I woke up in the morning. Travis Gault, I essentially used you as a hotel. You have been incredible. Thank you for every bit of support you gave me in finishing this. Joshua Corum, your friendship has been so valuable to me, even more than the statistics advice and instruction on how to use SPSS. You have been a good friend and an even better partner in crime. Our mutual ability to distract each other from the schoolwork we should be doing is eclipsed only by our ability to hole up at Heine Brothers and get things done.

And finally, David Ahlgren, you refused to let me quit. Thank you for being that one person I could open up to about anything. Thank you for fielding all my text messages and for Dr. Who and Skyrim and BJ's Brewhouse and Gameworks and the mall and even for your constant teasing (occasionally it was a little much, but you know I secretly liked it). Thank you for introducing me to Explosions in the Sky, without whose music playing in the background I might never have finished this dissertation. Thank you for all the closeness and the laughter and for always believing in me. Thank you for making me promise that I would finish this PhD on multiple occasions. This one's for you.
ABSTRACT

DEPRESSION IN REPRODUCTIVE-AGE WOMEN: ASSESSMENT OF INFECTIOUS, ENDOCRINOLOGICAL, AND IMMUNOLOGICAL CORRELATES FROM AN EVOLUTIONARY PERSPECTIVE

Amber N. Carrier
May 11, 2012

Depression is one of the most frequent causes of disability worldwide. It can affect the psychological, social, and physical wellbeing of those that suffer from it, the majority of which are women. Depression has been linked to immune activation as well as serotonin depletion through a reduction in its precursor, tryptophan. *Chlamydia trachomatis* infection and estrogen can influence both the immune system and tryptophan levels, thereby biochemically inducing a depressive state. If this is the case, the use of exogenous estrogen through oral contraceptive pills (OCPs) could increase depressive symptoms, while the use of antibiotics to treat *C. trachomatis* infection could decrease depression.

This thesis examined whether depression status in subjects was correlated with infection and medication use. Women were screened for depression at their annual gynecological exam, during which time they would receive an STI screen as standard of care. The study was conducted at the University of Louisville GYN/OB Foundation clinic and Campus Health Services from 2009-2011. Subjects were given a Beck Depression Inventory (BDI) to assess depressive symptoms and asked to keep a calendar
of medication use. Subjects returned after one month to take a second BDI and their
scores and medication calendars were compared with their medical records, specifically
infection status and existing medical conditions.

BDI scores decreased significantly in the follow-up assessment relative to the
initial assessment. This decrease was correlated with the extent to which the subjects
used mood-altering medications. Birth control use was correlated with an increase in
depressive symptoms, but subjects who took mood-altering medication in addition to
birth control were not more depressed than those that did not take birth control. There
were insufficient data to correlate sexually transmitted infections, particularly C.
trachomatis, with depressive symptoms. These findings show that increased hormonal
birth control use is correlated with an increase in depression, but this depression is
ameliorated with the use of mood-altering medication. These results imply that anti-
depressive medication may be particularly effective in treating depression associated with
estrogen or oral contraceptive pills.
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Theodosius Dobzhansky once said, "Nothing in biology makes sense except in the light of evolution." [1] Evolution is a framework upon which all of biology rests; the concept of change over time in heritable traits has reverberations in each branch of biology, from anatomy to ecology to genetics. The works of scientists such as Charles Darwin, J.B.S. Haldane, Sewall Wright, and W. D. Hamilton have been accepted as fundamental to the modern understanding of the biological sciences. However, the field of medicine, which is founded on biological, chemical, and physical principles, has been slow to acknowledge how the core principles of evolution may influence the current and future practice of medicine.

One exception to this generalization is the field of population genetics, which describes evolutionary change in terms of changing gene frequencies, and has been integrated into medical sciences for over a half century [2]. Explanations of the disappearance of the ability to digest lactose as an adult, the lack of alcohol dehydrogenase in certain groups of people, and the phylogeny of pathogens in order to predict future epidemics all have origins in evolutionary biology [3].
Evolutionary medicine seeks to illustrate how and why the human body has innate vulnerabilities to disease, providing the clinician with new insight into how to respond to a set of symptoms because the clinician understands that the body works, as Nesse [3] puts it, as a "product of natural selection with traits more exquisite than any machine."

Though this level of understanding seems as if it would be intuitive to those who practice medicine, there has been some reluctance to directly apply evolutionary thinking to medical practice. This reluctance likely has its roots in our limited understanding of pathogens; we are still learning not only about the pathogenesis of infectious diseases but also how the body responds both acutely and chronically to these infections. Many of these pathogens, such as *Varicella zoster* in the case of shingles and *Helicobacter pylori* in the case of peptic ulcers and stomach cancer are cryptic in their presentation[4]. The pathology of other illnesses, such as multiple sclerosis and insulin-dependent diabetes mellitus, may be self-destructive immune responses to seemingly unrelated infections [5, 6]. Of particular concern are pathogens in which transmission is infrequent; these infections are likely to benefit from long dormant periods and mechanisms for the evasion of host defenses. A prime example of these types of pathogens is sexually transmitted infections [4, 7].

**STIs and Latent Infections**

Sexually transmitted infections are those that are commonly spread through vaginal, anal, or oral intercourse. These infectious may not be immediately symptomatic, and some, such as human immunodeficiency virus (HIV), can be present for many years.
before the patient begins to display overt signs of infection [4, 7]. Other STI patients, such as those that suffer from *Neisseria gonorrhoeae*, which causes gonorrhea, often display overt symptoms much earlier, though overt symptomatology is not always the case. One of the reasons for continued infection and transmission of STIs is that many sufferers, particularly women, do not display overt symptoms of disease. Because of this crypticity of infection, sexually transmitted pathogens can initiate long-term infections with less risk of eradication through medical treatment. Their prolonged infectiousness facilitates transmission over periods of time that span changes in sex partners [4].

Sexually transmitted infections are very common both in the United States and worldwide. Women bear a heavier STI burden than men. According to the Centers for Disease Control, there were over 590 cases of *Chlamydia trachomatis* infection and 105 cases of gonorrhea per 100,000 women in the United States, as opposed to 219 and 91 cases per 100,000 men for *Chlamydia trachomatis* and gonorrhea, respectively [8]. In the case of *Chlamydia trachomatis* infection, the burden on women is nearly three times higher than it is for men, and over 1.2 million cases of *Chlamydia trachomatis* were reported to the CDC in 2009, but many more infections may remain unreported [8]. Chlamydial infections in women are usually asymptomatic and if left untreated, lead to pelvic inflammatory disease, the number one cause of infertility related to obstructed fallopian tubes [8, 9].

One of the reasons that *Chlamydiae* are able to establish long-term infections is that they are obligate intracellular pathogens. Such pathogens are difficult for the immune system to effectively eradicate because they reside within host immune cells; an immune mechanism to battle such infections includes starving the pathogen by depleting
essential nutrients such as vitamins, iron, and amino acids [10, 11]. Over the course of its evolutionary history, however, genitally acquired strains of *C. trachomatis* have evolved the necessary genes to manufacture some of these nutrients, particularly tryptophan. Tryptophan is an essential amino acid that is restricted, or purposefully removed from circulation, by an enzyme called indoleamine 2,3-dioxygenase (IDO). The human body cannot manufacture tryptophan *de novo* and must obtain it from the diet, but *C. trachomatis* carries within its genome the genetic instructions for tryptophan synthase, an enzyme that manufactures tryptophan from other common molecules. In this way *C. trachomatis* infections may persist as the body's own pool of available tryptophan dwindles.

**Tryptophan and Serotonin**

Depletion of the body's tryptophan stores is ultimately harmful for synthesis pathways that depend upon availability of the amino acid. One of these synthesis pathways is that for serotonin, a monoamine neurotransmitter found in the GI tract and the central nervous system (CNS) [12]. Within the CNS, serotonin aids in regulation of emotional behavior, and as a result is often referred to as a "happiness hormone" despite the fact that it is not a hormone [13, 14]. Serotonin is also part of the synthesis pathway of melatonin, an important mood stabilizer and sleep regulator [12]. The most commonly prescribed class of antidepressants, selective serotonin reuptake inhibitors (SSRIs), act to increase the availability of serotonin in the synaptic cleft, and therefore increase the amount of serotonin available to bind to the postsynaptic receptor, by preventing the
reuptake of the monoamine [15]. This increase in serotonin availability helps the patient to stabilize mood and has been shown to reduce depressive symptoms a great deal in those with clinically significant depression [16]. The efficacy of SSRIs in those with milder depressive symptoms is often disputed because the effect of SSRIs for those with mild depression is small [17].

Reductions in serotonin availability due to decreased tryptophan may contribute to depressive symptoms because overall serotonin availability and tryptophan levels have been correlated [18-20]. Research has indicated that acute tryptophan depletion can induce depressive symptoms in individuals that had previously experienced depression [21]. It is thought that tryptophan depletion by the immune system may be the reason that proinflammatory cytokines induce both depression and "sickness behavior," which is physiologically almost indistinguishable from depression [22-26]. Such a process might be occurring in patients infected with *Chlamydia*, whose immune systems may restrict tryptophan in order to combat a chronic infection that has evolved a mechanism for evading tryptophan starvation by manufacturing its own.

*Chlamydia trachomatis* and Depression

If persistent *Chlamydia trachomatis* infection is depleting tryptophan stores, I hypothesize that women who are suffering from mild to moderate depression will have a higher frequency of undiagnosed *Chlamydia trachomatis* than women that show no depression (Chapter 2). If this is the case, upon diagnosis and treatment, not only should the infection be cleared, but also depressive symptoms should improve in the subject.
My thesis research attempted to test this idea. It was conducted at Campus Health Services, Belknap Campus and the University GYN/OB Foundation to examine if there is a correlation between undiagnosed *Chlamydia trachomatis* infection and depression, and if treatment of the infection ameliorates depressive symptoms in the subject (Chapters 3-4). Discussion and limitations of the study are included in Chapter 5.

Depression is a leading cause of disability [27, 28], and amelioration of some depression in the United States could save billions of dollars in health care costs and reduced productivity due to absenteeism [29]. There has been a great deal of research into the social circumstances and the biology of depression and its treatment, but little into the actual primary cause of depression [16, 30]. This dissertation seeks to explore a potential primary cause of this debilitating illness and provide an alternative treatment scenario for ameliorating depression that aims to eradicate the primary cause.
CHAPTER 2
DEPRESSION AND THE IMMUNE SYSTEM

Introduction

Depression is a debilitating illness affecting 15-35 million Americans [31]. Though most people typically experience a depressing situation at some point in their lives [32] major depressive disorder is defined as a prolonged and unusually severe period in which normal function is disturbed [33]. Depression has been ranked as the number one cause of disability worldwide [27-29] and the costs incurred through treatment and productivity loss are in the billions. Major depressive disorder has a distinct emotional, social, and physiological effect on the sufferers in all aspects of life [34]. Apart from the general malaise and the disability associated with low mood, depression takes a toll on the social and familial relationships in the sufferers' life.

There is no singular cause of major depressive disorder (MDD); rather, a number of social and biological factors have been identified as contributing to the development of disease. Factors such as childhood poverty and a history of abuse and neglect are widely known to be linked to depression in adulthood [35]. Major life changes and stressors, including death of a loved one, divorce, and job
pressure are known social triggers for the disorder [36]. Other factors including neurochemical imbalances, alcohol and drug abuse, and neuroanatomical differences also increase the predisposition for developing depression. It is likely that the development of depression is multi-factorial, with influences from each category of disease causation: genetic, non-infectious environmental, and infectious [37](Figure 1).

On the basis of high estimates of heritability and the physiological burden of the illness, some evolutionary psychologists believe that MDD may be an ancient behavioral adaptation that is somewhat maladaptive in modern environments [34]. Major depressive disorder usually begins during the first decade after reproductive maturity. Depression has been reported to result in late menarche, secondary amenorrhea, menstrual irregularity, and reduced live birth rate, decreasing overall female fertility [38]. Depression may persist evolutionarily because of compensatory benefits [34], such as socially appropriate behavior modification, improved social navigation, and avoidance of social risk [39-42]. Evolutionary psychologists also surmise that depression could be an adaptation that limits the transmission of causal infectious organisms[43] by encouraging the individual to rest and avoid others. Alternatively, it could be a side effect of the immune system's activation against the pathogen itself.
Figure 1. The triad of disease causation. Virtually every human illness is caused by at minimum genetic, infectious, or environmental, including social, factors; the majority of diseases are caused by a combination of the three.

Depression and the Immune System

There have been robust associations between chronic inflammation, stress, and depression. Major depressive disorder and other psychological disturbances are associated with increased host cell release of inflammatory markers and cytokines such as IFN-γ, IL-6, chemokines, and acute-phase proteins [44-49] (see Table 1 below). These markers are modified proteins that serve as signaling molecules in the immune system. An increase in concentrations of IL-6 and C-reactive protein has been observed in plasma of depressed patients. Elevated concentrations of IL-1β, TNF-α, and IL-6 have been found in the peripheral blood and cerebrospinal fluid of depressed and/or suicidal patients [47, 49-51]. Other chemokines and acute phase proteins elevated in patients with major
depression include α-1-acid glycoprotein, α-1-antichymotrypsin, haptoglobin[44, 52], human macrophage chemoattractant protein-1 (MCP-1), soluble intracellular adhesion molecule-1 (s-ICAM-1), and E-selectin. IL-2 production is reduced in depressed, dysthymic, and atypically depressed patients [50, 53, 54], but less so in the atypically depressed group that can still experience elevated mood in response to positive life events. These studies illustrate that there are differences in the presence of these immunological molecules in depressed vs. non-depressed patients, and that their concentrations may sometimes correlate with symptom severity [53, 54].

Table 1. Immune molecules associated with depression. This non-exhaustive list includes molecules linked to both depression and sickness behavior.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Type</th>
<th>Role in immune System</th>
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<tr>
<td>C-Reactive Protein</td>
<td>Acute phase protein</td>
<td>Binds to microbes and dead and dying cells to initiate complement binding during inflammation</td>
</tr>
<tr>
<td>Interferon-α</td>
<td>Type I Interferon</td>
<td>Produced by leukocytes, involved in innate immune response against viruses</td>
</tr>
<tr>
<td>Interferon-γ</td>
<td>Type II Interferon</td>
<td>Promotes NK cells, Th1 differentiation, leukocyte migration, antigen presentation on macrophages</td>
</tr>
<tr>
<td>Interleukin-1β</td>
<td>Cytokine</td>
<td>Produced by macrophages to mediate inflammatory response</td>
</tr>
<tr>
<td>Interleukin-2</td>
<td>Cytokine</td>
<td>Produced by Th1 cells; stimulates growth and differentiation of T-cells in response to antigen binding</td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>Cytokine</td>
<td>Secreted by T-cells and macrophages to stimulate immune response, particularly to infection</td>
</tr>
<tr>
<td>Tumor Necrosis Factor-α</td>
<td>Cytokine</td>
<td>Induces apoptosis, inflammation; inhibits tumorigenesis and viral replication</td>
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The question of exactly how cytokines may induce a depressive state remains in debate. Cytokines circulating in the bloodstream do not typically cross the blood brain barrier (BBB) but are transported into the brain via other means, such as circumventricular organs and carrier molecules [55]. Pro-inflammatory cytokines, particularly IL-1β, TNF-α, IFN-γ, and IL-6, partially mediate communication between the brain and the immune system through a saturable blood-to-brain transport system [55, 56]. It is possible that cytokines and the immune system may influence depression by shunting valuable precursor molecules away from pathways necessary for neurotransmitter production. Tryptophan (Trp), an essential amino acid, is the precursor for the production of the neurotransmitter serotonin (5-hydroxytryptamine or 5-HT) and its hormonal derivative, melatonin (N-acetyl-5-methoxytryptamine), both of which are involved in mood regulation [18, 19, 57-62]. Serotonin is largely responsible for mood regulation within the brain. The most commonly prescribed class of anti-depressants, SSRIs, act to inhibit the reuptake of the chemical in the presynaptic cleft within the brain, thereby increasing the amount available for action [15]. The immunomodulatory enzyme indoleamine 2,3-dioxygenase (IDO) is up-regulated by IFN-γ and other pro-inflammatory cytokines and functions to cleave tryptophan into kynurenine within extra-hepatic tissues [48, 61-69]. When IDO cleaves the indole ring of tryptophan [64], it not only depletes the host and parasite of an essential amino acid, but also produces proapoptotic precursors of the kynurenine (kyn) pathway and toxic oxygen radicals [61, 62, 66, 68, 70, 71]. Once this reaction takes place, there is less available tryptophan for serotonin production, and serotonin levels drop in the central nervous system, resulting in depressive symptoms [48]. Additionally, the intermediates of the kynurenine pathway,
collectively known as the kynurenines, have been shown in studies to be neurotoxic, although once transported away from the CNS they can be used to produce nicotinamide adenine dinucleotide (NAD), an important co-enzyme involved in energy metabolism [48] (Figure 2).

During serotonin production, tryptophan is hydroxylated by the enzyme tryptophan hydroxylase (TPH) into 5-hydroxy-L-tryptophan, which is cleaved by an aromatic amino acid decarboxylase into serotonin (Figure 2). Since its oxygenation by TPH is the rate-limiting step in the serotonin synthesis pathway, levels of tryptophan and serotonin have been correlated in cerebrospinal fluid (CSF) [18-20, 72, 73]. Additionally, the use of radiopharmaceutical tryptophan and PET scans have confirmed the correlation between trp levels and serotonin synthesis in the brain [72]. Polymorphisms in the TPH gene have been linked with suicidal behavior [18, 20] and variations in the levels of serotonin or its receptors and transporters have been associated with major depressive disorder. The addition of an acetyl group, followed by a methyl group, yields the neurohormone melatonin (Figure 2), produced by the pineal gland in the absence of light [74]. Melatonin disruption leads to seasonal affective disorder (SAD) through a mechanism that may involve stimulation of type 1 T-Helper (Th1) cells and other lymphocytes to produce cytokines such as IL-2, IL-6, and IFN-γ [74]. Exogenous tryptophan has been used as a treatment for both depression and sleep disorders, though the most common treatment for depression is currently the use of SSRIs [75].

When tryptophan is catabolized by IDO or tryptophan 2, 3-dioxygenase (TDO), a more specific enzyme that is known to exist only within the liver, the availability of the substrate for serotonin synthesis becomes limited. Depressive symptoms are found in
patients that have reduced serum tryptophan levels due to elevated IDO or TDO activity [19, 58, 60, 62]. Since interferons are also potent mediators of IDO activity [61, 64, 69, 74, 76, 77], elevated IDO activity and the subsequent drop in tryptophan levels due to inflammation could cause a decline in serotonin levels and a noticeable change in mood and demeanor.

Proponents of this "cytokine hypothesis of depression" are split on whether cytokines are the cause of depression or are the mechanism by which another "insult" leads to depression. Smith first suggested in 1991 that depression was associated with an increase in secretion of IL-1 by macrophages in his "macrophage theory of depression" [78]. Maes also believed that circulating monocytes and macrophages result in the increase of depression in patients [79]. Studies involving laboratory animals have shown that presence of inflammatory cytokines in the brain result in "sickness behaviors" that are indicative of depression, including anorexia, sleep disturbances, and the inability to feel pleasure. Inflammatory cytokines also have a stimulatory effect on the HPA axis, which modulates the stress response, depression, and immunity [44]. Cytokines disrupt proper serotonin, norepinephrine, and dopamine metabolism in neural centers that regulate emotional response. However, cytokines do not have the ability to cross the blood-brain barrier because of their size. Consequently, current research is focusing on exactly how these molecules can be found in the healthy brain. It is postulated that increased cytokine levels due to inflammation may degenerate the blood-brain barrier, thereby enabling cytokines and immune markers to enter the brain and influence mood [80].
Cytokines such as IL-6 and IL-2 are associated with high Hamilton Depression Scale (HAM-D) scores in patients with chronic illnesses such as decompensated heart failure, hepatitis C, certain cancers and multiple sclerosis [47, 62, 81, 82]. A study by Appels et al. in 2000 showed higher antibody titers in depressed and exhausted patients who were receiving treatment for a coronary lesion [83]. These patients also showed elevated IL-1β, IL-6, and TNF-α. Evidence that exogenous administration of cytokines (particularly IFN-α, TNF-α, and IFN-γ) results in an illness that is identical to major depression provides support to the argument that immune function, particularly inflammation, contributes to a depressive state [44, 84, 85]. This "sickness behavior" is often caused by up-regulation of IDO and tryptophan depletion, and it responds well to antidepressants [44, 86].

Medication administered to depressed patients has also been shown to have immunomodulatory effects. Antidepressants inhibit the production of proinflammatory cytokines and stimulate the release of anti-inflammatory cytokines; alternative antidepressant strategies such as electroshock therapy appear to attenuate the immune response, leading to decreased depressive symptoms [44]. Patients that have a history of impaired response to antidepressants typically display increased blood cytokine loads, particularly IL-6 [44, 52]. Anti-depressants treat immune-related depression but typically only address the mood-related symptoms rather than the outwardly physiologic symptoms such as lethargy, loss in appetite, and difficulty concentrating [78]. Additionally, cytokine antagonists such as IL-1 receptor antagonist appear to have anti-depressive effects [22, 78].
The impact of stress may also drive the development of immune-related
depression in an otherwise healthy patient. Literature has suggested that psychological
stress can stimulate the proinflammatory response [44]. Psychological stress is a causal
factor in major depression and stressful events often precede depressive episodes. Both
acute and chronic stresses are associated with increased production of proinflammatory
cytokines and a decrease in circulating anti-inflammatory cytokines.
Figure 2. Tryptophan and the IDO pathway. The amino acid tryptophan is converted by tryptophan hydroxylase ultimately into serotonin, a neuroamine responsible for the maintenance of mood in the brain. However, if tryptophan is cleaved by IDO it results in the production of the neurotoxic kynurenine. Kynurenine is cleared by conversion into other necessary macromolecules, notably niacin and NAD.
Depression and Co-morbidity

Depression is frequently co-morbid in illnesses in which immune dysfunction is apparent [78]. Many illnesses are known to involve or probably involve immune-related depression. Several of these diseases are considered below. I will first discuss immune-related depression in Polycystic Ovary Syndrome patients. Then I will discuss depression in atherosclerosis patients. Finally I will discuss the comorbidity of depression with infection.

Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is associated with alterations in mood. The characteristics associated with PCOS, including abdominal obesity, acne, and hirsuteness, can lead to a depressed mood [87]. Unfortunately, few studies have assessed the co-morbidity of PCOS and mental illnesses such as major depressive disorder. Twenty to sixty-five percent of PCOS patients are more depressed than controls [46, 80, 88-90]. Women with PCOS were far more likely to present with other mental illnesses, particularly somatic illnesses such as anorexia nervosa and sleep disorders [80]. BMI, age, education, marital status, and employment did not explain the association of depression with PCOS.

Though obesity is a known risk factor for depression, PCOS-related obesity does not account for the co-occurrence of major depressive disorder [80, 91]. In fact, obese women with PCOS have approximately six times the risk of developing major depressive disorder than obese women without PCOS, suggesting that a PCOS-related factor may be
influencing the development of depression [80, 92]. Obesity induces over-expression of such inflammatory markers as CRP and TNF-α, which is in turn associated with insulin resistance [46, 93]. Decreases in adiposity have been associated with decreases in freely circulating inflammatory markers [46, 99]. However, PCOS patients, including those with normal BMIs, experience chronic low-grade inflammation with elevated C-reactive protein, TNF-α, IL-6, neutrophils and total leukocytes compared with age and BMI-matched controls [94-99]. Another proinflammatory marker, macrophage migration inhibitory factor (MIF), is elevated in PCOS patients independently of weight [100]. The genes for cytokines involved in the PCOS-related chronic inflammatory response, including TNF-α and IL-6, have been investigated in order to deduce the nature of the association between inflammation and PCOS, but no PCOS-associated polymorphisms have as of yet been identified [98, 101].

Atherosclerosis

Depression is associated with atherosclerosis and may occur prior to diagnosis. Depressed patients with no existing knowledge of cardiovascular health status display increased arterial stiffness [102, 103]. Patients with diabetes mellitus (DM) who score higher than 10 on the Beck Depression Inventory are more likely to suffer from cardiovascular and dyslipidemic disorders [104]. This correlation is strong enough to suggest that physicians that notice depression in both DM and non-DM patients should consider a cardiovascular evaluation[104, 105]. Inflammation in the arterial wall is a key component of the cascade of atheroma formation. Leukocytes flood to the site of endothelial injury, and some of these, particularly macrophages, can sequester circulating
cholesterol and transform into foam cells within the lesion [106, 107]. Pro-inflammatory cytokines signal this inflammatory response, which can be detected both locally and systemically. In fact, circulating pro-inflammatory markers such as IL-6 and CRP can be used in a clinical setting to as a biomarker for atherosclerosis [106, 108]. Other immune markers correlated with depression are elevated in atherosclerotic patients; Elovainio et al. found that depression was only associated with atherosclerosis in women with high IDO activity [109].

Acute and Chronic Infections

A number of acute infectious illnesses such as influenza, gastroenteritis, and infectious mononucleosis (Epstein-Barr infection) are associated with both somatic and mood-related depression symptoms [55]. A combination of any or all of fatigue, decreased psychomotor skills, anorexia, somnolence, lethargy, aches, cognitive disturbance, depressed mood, a sense of guilt, and difficulty in decision making have been reported in those suffering from influenza and other viral infections. Viral infections stimulate the production of type I interferons and activate NK cells; once infection has been established CD8+ Tc cells and CD4+ Th1 cells mount a more precise antiviral response [110]. This response includes the production of IL-2, IFN-γ, and tumor necrosis factor by Th1 cells. Some viruses, particularly those that exist as chronic infections, can either evade host defenses or cause immunosuppression.

Extracellular bacterial pathogens incite an innate inflammatory response and cause to the release of inflammatory cytokines. However, innate immunity has little effect on intracellular bacteria, but these pathogens can induce a cell-mediated response
Cytokines secreted by CD4+ T cells, particularly IFN-γ, direct macrophages to assist in killing ingested pathogens. Many common bacterial infections in the United States, including *Campylobacter jejuni*, the *Salmonella* group, *Streptococcus pneumoniae*, and *Streptococcus pyogenes* can induce symptoms similar to depression, particularly malaise, muscle weakness, and fatigue [110-114]. These symptoms are due to the immune response to these infections. The sickness behavior associated with these infections is nearly synonymous with depression and depressive symptoms [22]. Patients that are receiving interferon treatment for various infections often develop depression as a side effect within as little as one month from the initiation of treatment.

Immune system-related depression is relatively common and sickness behavior is nearly identical to it. Because of this, it is logical to associate other illnesses in which an inflammatory response is generated with possible depression symptoms, particularly those with an infectious component. One bacterial infection that has not been examined in adequate detail with regard to its possible relationship to depression is *Chlamydia trachomatis*.

**Chlamydia and Depression**

*Chlamydia trachomatis* warrants particular scrutiny as a possible cause of depression due to some serovars' ability to produce tryptophan when IDO is elevated. Chlamydiae invade and live within host cells as obligate intracellular pathogens [64, 65, 115]. They exist within humans in two forms: the infectious yet metabolically inert
elementary body (EB), and the non-infectious, intracellular but metabolically-active reticulate body (RB) [65]. Conversion between the two forms takes place within the host cell. Three biovars of *C. trachomatis* have been linked with human disease: ocular trachoma (caused by serovars A, B, Ba, and C), genital trachoma (serovars D-K), and the severe and systemically invading lymphogranuloma venereum (LGV) (serovars L1, L2, and L3) [116]. There is very little cross-over between serovars and their infecting sites; the ocular serovars very rarely infect the genital tract, and the genital serovars generally only infect the eyes of infants when they pass through the birth canal [117]. The ocular and genital trachoma serovars are considered non-invasive, but the more rare LGV serovars proliferate within monocytes and lymphatic tissues and are highly invasive. The genital variants encode functional tryptophan synthase whereas the ocular variants do not [65, 116, 117]. A *trpRBA* operon codes for the tryptophan synthase alpha and beta subunits and the *trp* operon repressor. The deactivation of the repressor when tryptophan is scarce allows tryptophan synthase to produce tryptophan from indole, a readily available waste-product of many common vaginal flora [117]. This rescues *Chlamydia* from IFN-γ-induced tryptophan restriction in vitro.

As obligate parasites of human cells, chlamydiaceae can largely evade detection by the humoral immune system. Some cytokines, though, are effective against *C. trachomatis*. IFN-γ functions to clear *C. trachomatis* infection by up-regulating the production of IDO. When IDO cleaves the indole ring of tryptophan [64], it not only depletes the host and parasite of an essential amino acid, but also the by-product, kynurenine, is toxic to Chlamydiaceae [61, 62, 66, 68, 70, 71]. Tryptophan depletion starves Chlamydiaceae but often leads to the formation of a persistent, aberrant state of infection.
Under this state, infections can become chronic, particularly the genital strains that are able to synthesize tryptophan de novo and evade the immune system's IDO response.

Because of the ability of some persistent strains of *C. trachomatis* to synthesize tryptophan, a long-term infection is possible, even during immune activation and tryptophan restriction by IFN-γ and IDO. However, long-term immune activation and elevated IFN-γ levels may lead to other types of pathology such as the occurrence of depression due to a loss of serotonin. Because of the crypticity associated with *C. trachomatis* infections, particularly in the 70% of infected women that are asymptomatic, long-term *C. trachomatis* infection may lead to long-term symptoms of depression through biochemical means.

**Perimenstrual response to Infection and Depression**

Depressive symptoms in women are more frequent between puberty and menopause, and major depressive disorder (MDD) is twice as likely to occur in women as in men [66, 120-123]. Transitory depression is common and often occurs during major hormone fluctuation periods such as pregnancy, parturition, and menopause [120-122]. Estrogen fluctuation has been incriminated as a cause of this depression in part because of its effects on the IDO enzyme [123] and its temporal correlations with MDD [120]. Additionally, progesterone and its metabolites are correlated with perimenstrual symptoms such as fluid retention and emotional turmoil[124]. Both progesterone and estrogen dysregulate serotonin synthesis, and perimenstrual dysphoric disorder (PMDD)
and depression in women responds to treatment with SSRIs [125] and estrogen/progesterone containing low-dose birth control [123].

Estrogen and progesterone also modulate immune activity. Elevated progesterone during the luteal phase of the menstrual cycle is associated with a shift in immunity away from the $T_h1$ response, leading to a decrease in the production of IFN-$\gamma$ and other cytokines that are more effective in controlling intracellular bacteria[126]. Estrogen, which up-regulates IDO activity [126-128], is also elevated over the majority of the luteal phase. This appears to be a compensatory mechanism for the down-regulation of $T_h1$ activity during the luteal phase in order to provide some defense against intracellular bacteria by restricting tryptophan. This defense would be relatively ineffective against any genital serotypes of $C.\ trachomatis$ because they can synthesize their own tryptophan [126]. Accordingly, transitory depression and $C.\ trachomatis$ infection both covary within the menstrual cycle; both are exacerbated during the late luteal phase and the first few days of menstruation [124]. Depressive symptoms have been known to occur with sexually transmitted infections (STIs) [129] and $C.\ trachomatis$ in particular [28, 130].

In summary, depression is a debilitating illness with a distinct immunological component, and both hormones and infection might be key players in this interaction. The enzyme IDO functions to deplete tryptophan stores, and it is up-regulated by both estrogen and the immune system in response to intracellular infections such as $C.\ trachomatis$. If trp stores are depleted, there is less substrate with which to produce serotonin, and depression may result.
CHAPTER 3

MATERIALS AND METHODS

This clinical study (IRB 09.0226) of depression sought to determine whether antibiotic treatment of *Chlamydia trachomatis* infection was associated with a subsequent amelioration of depressive symptoms.

The goal was to enroll 100 patients from the University GYN/OB Foundation clinic that displayed evidence of depression during their annual gynecological check-up. This evidence included a physician's diagnosis of depression or the display of overt depressive symptoms. Subjects were pre-screened for eligibility by the physicians and medical assistants before their exams. Women between the ages of 18 and 40 who were non-menopausal (naturally or surgically), with relatively normal menstrual cycles and depressive symptomatology were invited to participate in the study. Each patient was compensated $10 at the initial visit, and $30 at a return visit one month later to assess depressive symptomology.

A medical assistant asked eligible patients if they would like to participate in a volunteer questionnaire study on mood in gynecology patients. Each interested subject was directed to the researcher who gave her a flyer and explained the details of participation, the need to keep records on her medication use, and to return for a follow-up visit one month after the initial visit.
The patient was then given time to consider participation away from the researcher. If she agreed to participate she returned to the researcher to begin the consent process.

The consent process consisted of the patient signing a Subject Informed Consent Document (SIC, Appendix A) and a HIPAA Research Authorization (HIPAA-RA, Appendix B) document. The researcher discussed each document with the subject, starting with the SIC so that the subject was aware of the scope and risks of the study before continuing. Each paragraph was explained orally and time was given for the subject to read it if she so chose. Once the subject had signed the informed consent document, the researcher discussed the HIPAA-RA with the subject in a similar manner and explained to the subject that in order to participate in the study the researcher would need access to some of the subject's medical records. The researcher then detailed the security procedures that protect the subject's Personal Health Information (PHI). Consenting subjects signed two copies of each document; one remained with the subject while the second remained with the researcher. After the subject's first visit, the researcher presented each SIC document to the three Principal Investigators of the study for signatures. The signed document's photocopy was mailed back to the subject.

Once the SIC and the HIPAA-RA were completed, the subject was asked to complete Research Study Questionnaire (RSQ) to confirm her eligibility for the study and a Beck Depression Inventory II (BDI). The RSQ (Appendix C) consisted of six open-ended questions intended to confirm the subject's age and health status, as well as to ensure accuracy when matching the patient's medical records to her BDI and calendar results. The RSQ also confirmed that the patients were pre-screened correctly. The BDI
(Appendix D), a widely respected standard in the psychological community for quantifying levels of depression, was used to quantify the degree of depression in the subject. Each question was read to the subject to reduce any effects of variation in literacy and language on the results of the questionnaire or BDI.

The BDI assesses two measures of depression -- the psychological/cognitive symptoms and the physiologic (somatic or non-cognitive) symptoms [131]. The cognitive symptoms, including thoughts of suicide, pessimism, and past failure, are covered in questions 2, 3, 5-9, and 14. Somatic/non-cognitive symptoms, including loss of energy and agitation, are illustrated by questions 1, 4, 10-13, and 15-21. Question 1, which addresses sadness, and question 10, which addresses crying behavior, have been associated with both cognitive and somatic symptoms, but are often classified as primarily somatic [131, 132].

After the patient completed these documents, the researcher scheduled each subject to return in approximately four weeks for her follow-up appointment. The researcher explained to each subject how to complete the calendar, document all medication use, and mark the day menstruation begins. The researcher requested that the subject note if there was a significant life event (described orally as a "car accident, family emergency, etc.") that might influence the results during the study period. The researcher also explained that this information on life events could be helpful but was not required and that such an event would not exclude her from the study. The researcher then filled out the receipt (Appendix E), noting the subject's address and phone number, and gave the subject the first portion of her compensation. The subject was sent home.
with a calendar (Appendix F) stapled to a purple file folder so that the calendar could be readily accessed.

Deviations from the above protocol were made only in the event that the subject had a question about any aspect of the study. Such deviations were noted in the research database.

A few days prior to each follow-up appointment (average 2.5 days), the subject was given a phone call to remind her of the study appointment and to confirm that the subject would be able to attend and return her calendar. If the subject needed to reschedule the appointment, this accommodation was made. At the follow-up appointment, the subject completed a second BDI and RSQ. She was compensated $30 at this visit and filled out another receipt. See Figure 3 below for more information about the timeline of subject interaction.

Figure 3. Timeline of Events from initial subject interaction to follow-up.
The GYN/OB Foundation Clinic tests for *C. trachomatis* as part of the procedure associated with pap smears. It was expected that approximately 25% of the subjects recruited would test positive for *C. trachomatis*. If a patient tested positive for *C. trachomatis* or any other STI, the standard follow-up of the GYN/OB Foundation involved patient notification and mailing the standard antibiotic treatment to the patient. In the case of *C. trachomatis*, treatment was typically one dose azithromycin, 1 g by mouth. Some subjects, particularly those with penicillin allergies, were treated instead with rocephin (ceftriaxone) plus erythromycin base, amoxicillin, erythromycin ethylsuccinate, or doxycycline (Table 2). Patients were instructed to record antibiotic usage on their calendars, as well as on the follow-up RSQ. These documents, when compared with the subject's medical records, allow identification of subjects who were *Chlamydia*-positive but did not complete the prescribed medication regimen. The RSQ and calendar also allowed detection of any other medication use that might influence the results of the BDI and sometimes provided indications of a subject's immune status. The second BDI was intended to allow assessment of the antibiotic's effects on depressive symptoms. Each BDI was scanned upon completion to detect abnormally high scores that might indicate a need for immediate care from a mental health professional. If no such problem was found, the initial and final BDI were scored after the subject completed the study to maintain confidentiality and impartiality.
Table 2. Medications prescribed for treatment of *Chlamydia* infections.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Class</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azithromycin</td>
<td>Azalide (macrolide)</td>
<td>1g by mouth</td>
</tr>
<tr>
<td>Rocephin (ceftriaxone)</td>
<td>Cephalosporin</td>
<td>250 mg intramuscular injection</td>
</tr>
<tr>
<td>Erythromycin base</td>
<td>Macrolide</td>
<td>500 mg by mouth for 1 day</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>β-lactam</td>
<td>500 mg by mouth 3x/day for 7 days</td>
</tr>
<tr>
<td>Erythromycin ethylsuccinate</td>
<td>Macrolide</td>
<td>800 mg by mouth 4x/day for 7 days or</td>
</tr>
<tr>
<td></td>
<td></td>
<td>400 mg by mouth 4x/day for 14 days</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>Tetracycline</td>
<td>100 mg by mouth 2x/day for 7 days</td>
</tr>
</tbody>
</table>

After each subject completed her initial and final visit, both of her BDIs were scored and her medical records examined to determine her *Chlamydia*-positive status at the time of the initial visit. Any other diagnoses at this visit were also noted as well as any medications prescribed at the initial visit or later. When the researcher was taking notes only a three-digit study code identifier was used rather than the subject's actual name, and any notes on paper were shredded in a diamond shredder after they were entered into the subject database.

All study data were entered into a password protected spreadsheet on an Apple Mac Book Pro running Mac OS X 10.7.2 (Lion). The spreadsheet was password-protected for both changing and viewing the document. No subject identifiers (such as name, birth date, address, etc.) were entered onto the spreadsheet; the date of the BDI administration was therefore the only possible identifier entered. The subject's paper
documents, including her SIC, HIPAA-RA, RSQ, BDI, and receipts, were placed in the file folder with her calendar, marked with her three digit ID code on the tab, and stored in a locked filing cabinet in the Life Sciences Building at the University of Louisville. The only two individuals in possession of a key to this cabinet were Amber Carrier and Paul Ewald. All data from paper documents, excluding any subject identifiers, were entered into the subject database. Further analysis was conducted from this de-identified data set.

Revised Protocol

It was very difficult to obtain the number of subjects as originally planned. Explanations for this difficulty are discussed in Chapter 5. The following changes in protocol were made in a second phase of the study to facilitate recruitment.

1. In November 2010, I spoke with Dr. Phillip Bressoud, Executive Director of Campus Health Services, and Dr. Kari Zahorik, Medical Director of Campus Health Services, about the possibility of working at Campus Health Services, Belknap Campus (215 Central Ave, Louisville, KY 40208) as a second subject recruitment site. The expected advantages of this site were higher availability of potential subjects, familiarity of potential subjects with simple research projects, and a high rate of STI transmission among college students. This addition required an amendment to the IRB protocol, approved January 27, 2011. I therefore began to recruit participants from this office in January 2011.
2. Campus Health Services does not automatically test for STIs unless the test is covered by the subject's health insurance, and one third of the student population does not have health coverage. An arrangement was negotiated with Louisville Metro Public Health and Wellness Department (LMPHW) to test for Chlamydia and Gonorrhea in uninsured subjects. I was provided with the test kits, and the staff at CHS utilized the LMPHW kits with uninsured subjects that agreed to participate in this study. I couriered the kits to LMPHW for testing and the results were faxed back to CHS.

3. Purple post-it notes identifying subjects that might be eligible for the study were attached to the medical charts of women scheduled for an annual exam each day that I was present. This served to remind the medical assistants to pre-screen potential subjects.

4. Several subjects at Campus Health Services had privacy concerns with mailing addresses and requested that I return their fully signed SIC at their next visit. I complied with these wishes, and gave future subjects this option without prompting.

5. An advertisement for the study was placed in the SGA Student News. Once students responded via email, they were sent identical emails explaining the study and encouraging them to make an appointment at Campus Health. This was done to encourage potential subjects to make a gynecologic appointment at Campus Health, and served the greater public good by encouraging women to obtain their annual exam. A copy of the ad and the email can be found in Appendix G.
6. Because potential subjects were not often reporting depressive symptoms even though their BDI scores indicated low levels of depression, the decision was made to recruit all gynecological subjects into the study, not just those that were displaying overt depressive symptoms during their exam.

7. Two research staff members, Laura Ovaitt (PhD student) and Samiyyah Sledge (undergraduate student) were added to the study, and recruited subjects under my supervision. Any subjects recruited by them are noted.

Table 3. Timeline of events pertaining to the Study

<table>
<thead>
<tr>
<th>Date</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/22/09</td>
<td>IRB Approval of project</td>
</tr>
<tr>
<td>10/1/09</td>
<td>Recruitment Begins in GYN/OB Foundation</td>
</tr>
<tr>
<td>1/27/11</td>
<td>CHS added as second recruitment site</td>
</tr>
<tr>
<td>3/14/11</td>
<td>Advertisement submitted to Student News</td>
</tr>
<tr>
<td>9/21/11</td>
<td>Samiyyah Sledge added as study personnel</td>
</tr>
<tr>
<td>10/5/11</td>
<td>Laura Ovaitt added as study personnel</td>
</tr>
<tr>
<td>12/1/11</td>
<td>Recruitment ends at OB/GYN</td>
</tr>
<tr>
<td>12/1/11</td>
<td>Recruitment ends at CHS</td>
</tr>
<tr>
<td>1/31/12</td>
<td>Data Analysis</td>
</tr>
</tbody>
</table>

Subjects might alter their tendency to report depressive symptoms to accommodate the researcher or because of a psychological effects of medical attention or participation in the study. In such situations, the BDI test scores could change even
when the underlying state of depression was not altered by antibiotic treatment. The questionnaire was administered to subjects who were not infected with *C. trachomatis* so that any reductions in depression associated with antibiotic treatment could be compared with changes among subjects who were untreated. If the results showed that antibiotic treatment of *C. trachomatis* infection was associated with amelioration of depression, this association could serve as a basis for a follow-up case-control study.

**Statistical Analysis**

The presence of a non-parametric sample was confirmed via a Shapiro-Wilk test, a test appropriate for determining the normality of small sample sizes [133]. Normality plots were also constructed to graphically assess normality of the data. When distributions were non-normal, Wilcoxon signed rank test were used to determine whether amelioration of depression was associated with the second administration of the BDI. This test was chosen because the initial and final BDI scores are related and the differences can be considered ordinal. A Mann-Whitney U test was conducted when groups of paired scores were compared with each other. BDI sub-scores were assessed using cognitive vs. somatic symptoms to determine if the somatic symptoms that are similar to sickness behavior decreased when the subject took medication. Primary statistical analyses were conducted using SPSS version 20 [134], on a MacBook Pro running Mac OS X 10.7.2 (Lion). Power analyses were conducted using the freeware G*Power 3.1.3 for Mac [135, 136].
CHAPTER 4

RESULTS

Subject Demographics

Twenty-four subjects completed the study. Nine additional subjects completed the first BDI but did not complete the study or were otherwise non-compliant (Table 4). The majority of subjects self-identified as Caucasian (16 total, two from GYN/OB and 14 from CHS) but other categories included Hispanic (two from OB/GYN), Black (four total, two each from both sites), Asian (one from CHS) and of mixed race (one from CHS).

Descriptive Statistics

BDI scores and measures of variation in these scores for the two groups of study subjects are presented in Table 5. To assess whether the data met the requirements for parametric statistical testing, variances were compared and normality was evaluated.
Table 4. Subjects who did not complete the study or were otherwise non-compliant.

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Recruitment Site</th>
<th>Reason for Exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>007</td>
<td>OB/GYN</td>
<td>No Follow-up</td>
</tr>
<tr>
<td>008</td>
<td>OB/GYN</td>
<td>No Follow-up</td>
</tr>
<tr>
<td>009</td>
<td>OB/GYN</td>
<td>No Follow-up</td>
</tr>
<tr>
<td>011</td>
<td>OB/GYN</td>
<td>No Calendar</td>
</tr>
<tr>
<td>013</td>
<td>CHS</td>
<td>No Follow-up</td>
</tr>
<tr>
<td>017</td>
<td>CHS</td>
<td>No Follow-up</td>
</tr>
<tr>
<td>019</td>
<td>CHS</td>
<td>No Follow-up</td>
</tr>
<tr>
<td>020</td>
<td>CHS</td>
<td>No Follow-up</td>
</tr>
<tr>
<td>033</td>
<td>CHS</td>
<td>No Follow-up</td>
</tr>
</tbody>
</table>

Table 5. Descriptive Statistics for subjects for whom BDI data is available.

<table>
<thead>
<tr>
<th></th>
<th>GYN/OB</th>
<th>CHS</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean Age</strong></td>
<td>31</td>
<td>24</td>
<td>26</td>
</tr>
<tr>
<td><strong>Number of Subjects</strong></td>
<td>8</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td><strong>Mean Initial BDI</strong></td>
<td>27.38</td>
<td>8.75</td>
<td>14.68</td>
</tr>
<tr>
<td><strong>Mean Final BDI</strong></td>
<td>17.00</td>
<td>7.41</td>
<td>10.48</td>
</tr>
<tr>
<td><strong>Mean Difference</strong></td>
<td>-10.38</td>
<td>-1.34</td>
<td>-4.20</td>
</tr>
<tr>
<td><strong>Variance of Difference</strong></td>
<td>152.26</td>
<td>37.97</td>
<td>88.42</td>
</tr>
<tr>
<td><strong>Std. Error Difference</strong></td>
<td>4.36</td>
<td>1.50</td>
<td>1.88</td>
</tr>
</tbody>
</table>
The variances of the changes in BDI scores in the OBGYN (N=8) and CHS (N=17) populations were significantly different (Table 5, row 6, columns 1 and 2, F=8.286, p=0.009, Levene’s test for unequal variances). The initial BDI scores deviated significantly from normality, as did difference in BDI scores (final score - initial score) (p=0.002 and 0.010 respectively, Shapiro-Wilk test, Figure 4). The final BDI scores did not (p=0.073, Figure 4). The threshold used for statistical significance was $\alpha=0.5$. By this criterion, the changes in BDI scores in two subject pools were not significantly different from each other (N=25, p=0.086). The lack of statistical significance does not, however, provide a justification for combining the two populations because the nearness to statistical significance suggests that such a grouping would risk incorporation of a Type II statistical error, particularly since power is low ($\beta=0.59$ for the difference). Considering this indication and the significant differences between the populations in normality and variance of BDI scores (see above) tests were run separately on each group whenever sufficient data were available within each group.
Figure 4. Normality plots with initial BDI score (upper left), final BDI score (upper right), and difference (final minus initial, lower left)). Means are given in Table 5 (rows 3, 4, and 5 of column 3).

The BDI scores were significantly lower in the follow-up assessment relative to the initial assessment for the OB/GYN sample (p=0.035, Figure 5), but not in the CHS sample (p=0.36, Figure 6). A Fisher's combined probability test of both Wilcoxon-Signed Rank scores indicated that overall the level of depression in the follow-up tests did not significantly decrease (p>0.30, $X^2 = 3.812$, df=4). Subject 011 was included in this analysis because BDI data was available for her.
Figure 5. Wilcoxon Signed Rank of initial and final BDI scores of the OB/GYN sample.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total N</strong></td>
<td>8</td>
</tr>
<tr>
<td><strong>Test Statistic</strong></td>
<td>3.000</td>
</tr>
<tr>
<td><strong>Standard Error</strong></td>
<td>7.133</td>
</tr>
<tr>
<td><strong>Standardized Test Statistic</strong></td>
<td>-2.103</td>
</tr>
<tr>
<td><strong>Asymptotic Sig. (2-sided test)</strong></td>
<td>.035</td>
</tr>
</tbody>
</table>
Because of the similarity between sickness behavior and the non-cognitive symptoms of depression, the initial and final BDI scores were subdivided according to cognitive (affective) and non-cognitive (somatic) sub-scores (Table 6). The OB/GYN and CHS populations differed significantly within these categories for the initial and final scores (Table 6, row 1 versus row 4 and row 2 versus row 5; Table 7, rows 1 and 2.)
Table 6. Affective and Somatic BDI scores for subjects. The affective BDI scores for all subjects ranged from 0-13 (out of a possible 24), and the rough somatic BDI scores ranged from 0-32 (out of a possible 39).

<table>
<thead>
<tr>
<th></th>
<th>Affective</th>
<th></th>
<th>Somatic</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.E.</td>
<td>σ²</td>
<td>Mean</td>
</tr>
<tr>
<td>CHS</td>
<td>Initial</td>
<td>2.82</td>
<td>0.74</td>
<td>9.28</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>2.29</td>
<td>0.67</td>
<td>7.60</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>-0.53</td>
<td>0.44</td>
<td>3.27</td>
</tr>
<tr>
<td>OBGYN</td>
<td>Initial</td>
<td>9.43</td>
<td>1.29</td>
<td>11.62</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>5.86</td>
<td>1.60</td>
<td>17.81</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>-3.57</td>
<td>1.56</td>
<td>16.95</td>
</tr>
</tbody>
</table>

Table 7. Comparison of BDI sub-scores in the CHS and OB/GYN populations. Entries are p values for the inter-site difference in the initial scores, the final scores and the change in the scores (final minus initial). Significant results are marked with an asterisk (Mann-Whitney U tests). Means for these categories are given in Table 6.

<table>
<thead>
<tr>
<th></th>
<th>Affective</th>
<th></th>
<th>Somatic</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>0.0005*</td>
<td></td>
<td>0.0002*</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>0.016*</td>
<td></td>
<td>0.006*</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>0.262</td>
<td></td>
<td>0.095</td>
</tr>
</tbody>
</table>

The somatic sub scores were significantly lower on the final visit relative to the initial visit for the OB/GYN population but not for the CHS population (Table 8, row 2). A combined probabilities test indicated that overall the results were not significant (p > 0.30, X² = 3.79, df=4). The affective sub scores were not significantly lower on the follow-up visit for either group (Table 8, row 1: p > 0.30, X² = 3.40, df=4, combined probabilities test).
Table 8. Comparison of change in BDI sub-scores in the final visit relative to the initial visit. Entries are p values for the differences between final and initial visits. Means for each category are given in Table 6. * denotes a statistically significant difference.

<table>
<thead>
<tr>
<th></th>
<th>OB/GYN</th>
<th>CHS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affective</td>
<td>0.089</td>
<td>0.223</td>
</tr>
<tr>
<td>Somatic</td>
<td>0.028*</td>
<td>0.452</td>
</tr>
</tbody>
</table>

Only one subject tested positive for *C. trachomatis*. I therefore could not assess whether pre-existing *C. trachomatis* infection was associated with higher BDI scores.

Five subjects were infected with a sexually transmitted infection of some kind -- one each with trichomonas, chlamydia, and HPV; and two with bacterial vaginosis. Mean BDI scores for each group are presented in Table 9. The presence of a sexually transmitted infection was not significantly associated with initial, final, or overall change in BDI scores (p > 0.1 for all comparisons, Mann-Whitney U test). Subject 011 was included in this analysis because the BDI data was available for her. Because of the limited number of subjects that were STI-positive, only the pooled (CHS + OB/GYN) sample was analyzed.

<table>
<thead>
<tr>
<th></th>
<th>Infected</th>
<th>Not Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial BDI</td>
<td>16.00</td>
<td>14.35</td>
</tr>
<tr>
<td>Final BDI</td>
<td>8.80</td>
<td>10.90</td>
</tr>
<tr>
<td>Difference</td>
<td>-7.20</td>
<td>-3.45</td>
</tr>
</tbody>
</table>

Table 9. Mean BDI scores of subjects based on infection status. There were no significant differences in scores between those that were infected with an STI vs. those who were not.
**Medication Analysis by class**

The large variety of medications used by the subjects (Table 10) and the small number of subjects prohibited statistical analysis on a medication-by-medication basis. Other than hormonal birth control, few medications were taken by more than one or two participants. Many of the medications, however, belonged to a smaller number of classes of drugs, which were prescribed for the similar reasons (Table 10). Medication categories were therefore used as variables in lieu of particular medications and all tests were run on all subjects from both study groups combined.

<table>
<thead>
<tr>
<th>Medication Class</th>
<th>Individual Medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>OTC Pain Relievers</td>
<td>acetaminophen, aspirin, ibuprofen</td>
</tr>
<tr>
<td>Mood Medications</td>
<td>amitriptyline, bupropion, duloxetine, fluoxetine, lamotrigine, sertraline, trazadone, zolpidem</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>amoxicillin, azithromycin, clindamycin, doxycycline, metronidazole</td>
</tr>
<tr>
<td>Other OTC Medications</td>
<td>azo yeast, melatonin, pepto bismol, prilosec, zantac</td>
</tr>
</tbody>
</table>

Mood-altering medication is expected to ameliorate depressive symptoms. P-values associated with mood-medication are therefore reported as one-tailed. The use of a mood-altering medication was significantly correlated with a decrease in BDI scores (one-tailed $p=0.024$, Table 11 row 2).

None of the other classes of medications used were significantly associated with a change in BDI score, although the increase in BDI scores associated with hormonal birth
control was sufficiently close to statistical significance to suggest that hormonal birth control might actually be associated with less improvement in BDI score ($p=0.078$, Table 11 row 4).

Table 11. Tests for associations between change in BDI scores and medication use (Mann-Whitney U Tests). *denotes statistically significant difference

<table>
<thead>
<tr>
<th>Medication Class</th>
<th>Taken</th>
<th>Not Taken</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
</tr>
<tr>
<td>OTC Pain Relief</td>
<td>16</td>
<td>-4.56</td>
</tr>
<tr>
<td>Mood Medication</td>
<td>6</td>
<td>-9.83</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>5</td>
<td>-8.40</td>
</tr>
<tr>
<td>Birth Control</td>
<td>12</td>
<td>-1.33</td>
</tr>
<tr>
<td>Other OTC</td>
<td>5</td>
<td>-2.60</td>
</tr>
</tbody>
</table>

**Birth Control Use**

Because associations with birth control bordered on statistical significance in several results, I analyzed the BDI results by grouping the subjects into those who used birth control versus who did not.

Birth control pills used by all participants in the study were combination estrogen/progesterone oral contraceptive pills (OCPs) (Table 12). The specific progesterone varied based on the subject's prescription, but all subjects' oral contraceptives were formulated with ethinyl estradiol (EE), a bioactive derivative of estradiol (E2). The majority of subjects taking OCPs were exposed to 0.030-0.035mg of EE with each pill.
Table 12. Subjects using oral contraceptive pills. Prescriptions varied with regard to dose and type of progestational compound. Subject 029's prescription contained a different formulation for each week of her cycle.

<table>
<thead>
<tr>
<th>Subject</th>
<th>OCP Brand</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>003</td>
<td>low ogestrel</td>
<td>0.03 mg EE and 0.3 mg norgestrel</td>
</tr>
<tr>
<td>014</td>
<td>levora 0.15mg</td>
<td>0.03 mg EE and 0.15 mg levonorgestrel</td>
</tr>
<tr>
<td>015</td>
<td>gianvi</td>
<td>0.02 mg EE and 3 mg drospirenone</td>
</tr>
<tr>
<td>016</td>
<td>ortho tri-cyclen</td>
<td>0.035 mg EE and 0.18 mg norgestimate</td>
</tr>
<tr>
<td>021</td>
<td>sprintec</td>
<td>0.035 mg EE and 0.250 mg norgestimate</td>
</tr>
<tr>
<td>022</td>
<td>ortho tri-cyclen</td>
<td>0.035 mg EE and 0.18 mg norgestimate</td>
</tr>
<tr>
<td>024</td>
<td>levora</td>
<td>0.03 mg EE and 0.15 mg levonorgestrel</td>
</tr>
<tr>
<td>025</td>
<td>ortho tri-cyclen</td>
<td>0.035 mg EE and 0.18 mg norgestimate</td>
</tr>
<tr>
<td>026</td>
<td>sprintec</td>
<td>0.035 mg EE and 0.250 mg norgestimate</td>
</tr>
<tr>
<td>027</td>
<td>levora</td>
<td>0.03 mg EE and 0.15 mg levonorgestrel</td>
</tr>
<tr>
<td>029</td>
<td>tri-sprintec</td>
<td>Week 1: 0.035 mg EE and 0.18 mg norgestimate; Week 2: 0.035 mg EE and 0.215 mg norgestimate; Week 3: 0.035 mg EE and 0.25 mg norgestimate</td>
</tr>
<tr>
<td>030</td>
<td>unspecified</td>
<td></td>
</tr>
</tbody>
</table>

Reports on the use of OCPs and their effects on depression vary; some studies have found that OCPs are correlated with a worsening of depressive symptoms in women with severe perimenstrual symptoms and/or a predisposition toward major depressive disorder [137, 138], while other studies have not confirmed this relationship. The use of OCPs for relief of severe perimenstrual syndrome (PMS) or perimenstrual dysphoric
disorder (PMDD) often treats only the pain and abnormal menstrual flow associated with these disorders and not the associated affective and somatic aspects of depression [139]. Since research indicates that estrogen influences the serotonergic pathway by up-regulating tryptophan restriction [123], exogenous administration of low-dose estrogen, such as that in an estrogen-containing OCP, may increase depressive symptoms in some women.

This study indicated that there is a trend toward the use of OCPs and the worsening of depressive symptoms. Subjects who took OCPs and mood medication had significantly improved BDI scores relative to those who used OCPs but did not use mood medication (p=0.031, Table 13, row 5). This improvement was significant for the affective sub scores (p=0.015, Table 13, row 3) but not in the somatic (p=0.089, Table 13, row 4). However, subjects who did not take OCPs did not have improved BDI scores when using mood-related medication (p=0.467, Table 14, row 5). This difference indicates that subjects who used birth control may have initially been more depressed than those that did not use birth control (Table 11, row 4), but the use of mood-altering medications ameliorated these depressive symptoms.

Table 13. Tests for an association of BDI scores with mood-altering medication, among subjects who used birth control (Mann-Whitney U tests). * denotes a statistically significant difference

<table>
<thead>
<tr>
<th></th>
<th>Mood Meds (n=2)</th>
<th>No Mood Meds (n=10)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Initial BDI Score</td>
<td>21.00</td>
<td>14.14</td>
<td>8.30</td>
</tr>
<tr>
<td>Final BDI Score</td>
<td>8.00</td>
<td>9.90</td>
<td>9.30</td>
</tr>
<tr>
<td>Change in Affective Sub score</td>
<td>-5.00</td>
<td>2.82</td>
<td>0.30</td>
</tr>
<tr>
<td>Change in Somatic Sub score</td>
<td>-8.50</td>
<td>0.71</td>
<td>-0.75</td>
</tr>
<tr>
<td>Change in Overall Score</td>
<td>-13.00</td>
<td>4.24</td>
<td>1.00</td>
</tr>
</tbody>
</table>
Table 14. Tests for an association of BDI scores with mood-altering medication, among subjects who did not use birth control (Mann-Whitney U tests). All p-values are one-tailed.

<table>
<thead>
<tr>
<th></th>
<th>Mood Meds n=4</th>
<th>No Mood Meds n=8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Initial BDI Score</td>
<td>23.00</td>
<td>14.14</td>
</tr>
<tr>
<td>Final BDI Score</td>
<td>14.75</td>
<td>9.90</td>
</tr>
<tr>
<td>Change in Affective Sub score</td>
<td>-2.75</td>
<td>2.82</td>
</tr>
<tr>
<td>Change in Somatic Sub score</td>
<td>-5.50</td>
<td>0.71</td>
</tr>
<tr>
<td>Change in Overall Score</td>
<td>-8.25</td>
<td>4.24</td>
</tr>
</tbody>
</table>
CHAPTER 5

DISCUSSION AND FUTURE CONSIDERATIONS

Summary of Findings

The original goal of the study was to determine if changes in depressive symptoms were correlated with infection status, and if treatment of the infection led to an amelioration of depressive symptoms. I had hoped to study the association of depression with *Chlamydia trachomatis* but difficulties in subject recruitment led to a broadening of scope.

The use of mood-altering medications such as fluoxetine (Prozac) and lamotrigine (Lamictal) was positively associated with the subject's change in BDI score. These medications are intended to alter mood symptoms and therefore this effect was not unexpected. Though these medications are classified in different drug classes (Appendix H), they are prescribed for the same effect -- to ameliorate depressive symptoms and mental illness in the patient.
The effect of birth control on increasing depressive symptoms approached significance in this study. The uncertainty associated with this finding reflects ambiguity about the roles of hormonal birth control in the literature. The effect of birth control, particularly OCPs, on depressive symptoms is controversial. The results of this study show, however, that depression in subjects taking hormonal birth control and mood-altering medications declined over the course of the study. In contrast, subjects who took mood medication but not hormonal birth control did not show such a decline.

The exogenous estrogen introduced by OCPs may be up-regulating IDO, causing increased tryptophan restriction and therefore less serotonin synthesis. If this is the case, medications that are prescribed to reduce the re-uptake of serotonin, and therefore increase its availability in the synapse, may counteract the effects of estrogen in reducing serotonin synthesis. Though a number of subjects took mood-altering medications, only three used an SSRI, making it difficult to statistically show an association.

The decline in BDI scores during follow-up could result from a "placebo effect" that arises from medical attention. Contact with a physician alone can be therapeutic, thereby improving the mood of subjects [140]. Since no physician contact occurred at the follow-up appointment, subjects that experience improved mood due to physician contact may have increased BDI scores, and therefore worse depression symptoms, without the physician's visit. Alternatively, anxiety about an upcoming doctor's visit and outright iatrophobia, or fear of doctors [141], are not uncommon phenomena and can result in some inflation of the initial BDI scores, since subjects were recruited at their annual exam. These subjects would have improved BDI scores at the follow-up since a doctor was not seen.
Limitations of the Study

A variety of real and potential confounding factors limit the conclusions that can be drawn. As the study was clinical in nature and relied heavily on self-reported data, it was subject to the limitations of subjective clinical research. These issues, discussed in detail below, included recruitment, compliance, the self-report nature of the BDI, and the truthfulness of the subject. Additionally, protocol changes complicate interpretations.

Recruitment

The most critical limitation on the study was subject recruitment. Despite the offer of compensation for their time and travel, it was difficult to identify women who were willing to participate. Several factors may have negatively affected recruitment. One was subject referral. The physicians and the medical assistants were responsible for the initial mention of the study to potential subjects. This was accomplished either by mentioning the study directly or arranging for me to speak with the potential subject before her exam. Often no mention of the study was made during the potential subject's appointment, in which case the opportunity to recruit was lost.

A second factor impeding subject recruitment was the subject's own hesitation to participate in the project. The most common reason, cited through personal communication with the subject at both CHS and the GYN/OB Foundation, was lack of time to stay for the initial visit. Subjects also mentioned that they were unable to return for the follow-up appointment. Since the initial visit took around 20-30 minutes total, subjects who had to return to work, go to class, or had other time constraints felt unable
to take the time both to consent to participate and fill out the Beck Depression Inventory. Several subjects approached at the GYN/OB Foundation did not directly decline participation but instead checked out and left immediately after the exam; staff speculated that these women held some distrust of the nature of the research due to not understanding the parameters. The medical assistants reported to me that these women seemed confused about the project and its scope despite my explanation to them.

A substantially larger subject population would have allowed testing of the original hypothesis that antibiotic treatment of *C. trachomatis* led to an improved mood. The general tendency for mood scores to improve on follow-up visits, however, indicates that detection of an effect of antibiotic treatment would have been more difficult than originally anticipated; the improvement in mood among subjects treated with antibiotics would have to be greater than the more general improvement that was documented by this study.

**Compliance**

Failure to return for the follow-up visit was a major factor in subject non-compliance. Eight subjects failed to return for their follow-up visits, despite repeated attempts to reschedule. An additional subject from the GYN/OB Foundation neglected to return her calendar but did complete a follow-up BDI. Without both the subject's final BDI scores and medication calendar, she was unable to be included in the final analysis.

A number of subjects failed to note all of the information requested on their calendars. Most included the names of the medications that they were taking, but a few named a medication class rather than the exact medication itself. The most common
piece of information omitted, however, was the start date of menstruation. Only 16 of the 24 subjects who completed the study included this information on their calendars. Only two subjects reported the use of depo provera, an injectable birth control that commonly alters menstruation frequency; these two subjects were also non-compliant subjects and were not included in the final analysis. It is unknown why these women did not complete this portion of the study.

**Self-report nature of the BDI**

As the BDI is a self-reported measure of depression, it is subject to the limitations of a subjective test [131, 142]. Although studies have indicated that it is a reliable tool in comparison to physician diagnosis [143], subjects may gauge their symptoms differently depending on any number of factors. The subjects may have exaggerated or minimized their symptoms based on the perceived desire of the researcher; for example, if the subject believed the researcher was hoping for a high score, she might be tempted to exaggerate her symptoms in an effort to please the researcher [142]. Subjects might be less likely to engage in such behaviors if the BDI was filled out in private or at home via postal mail, but these options would introduce other potential confounding factors. In an attempt to alleviate this risk, the researcher took precautions to not imply preferred results in either direction.

The subject's current health status and daily mood changes may have also influenced the study. If the subject was having a "bad day" on the date of either her initial or final visit, she might be feeling more emotional, and therefore exaggerate the results of the test. In this case, the test would be a measurement "in the moment" rather
than over the long term. The converse is true for a perceived "good day." Additionally, the stress of the physician's office visit, any negative news administered by the physician, or any current or ongoing health problems may influence how the subject responds on the BDI. A recent short-term illness such as a cold or the flu may also alter the subject's short-term mood and therefore alter her BDI score. This should be partially accommodated for by the subject's report of her medication use on her calendar.

Truthfulness

The study is also limited by the subject's truthfulness and accuracy in reporting all medication use on her calendar. If the subject did not know the name of a medication that was used, forgot to note a medication on her calendar, or labeled a medication incorrectly, the results of the study could be altered. Additionally, if a subject was using a medication incorrectly and was uncomfortable reporting this for fear of reprisal, her calendar results and potentially her BDI results would be inaccurate. It was stressed to the subjects to be as truthful, accurate, and honest as they could, and subjects were informed that the Health Insurance Portability and Accountability Act (HIPAA) required us to maintain confidentiality even in cases of illegal drug use. Each subject was reminded that the only situation where confidentiality would be breached is if the subject was an immediate danger to herself and/or others, and in this case we would only discuss it with her physician. However, some subjects may have omitted this information despite assurances of privacy.
Changes in Protocol

There were changes made to the study protocol that may have had a minor effect on the results. Subjects at GYN/OB Clinic were read their surveys, while those at CHS were allowed to read them themselves. The decision was made to read the subjects at GYN/OB Clinic their questions due to possible comprehension issues since we could not be assured of the education level of the participants. However, it may have made subjects feel less at-ease with the study personnel. At CHS, all of the subjects were in college or graduate school, so reading comprehension was less of a concern. Though this could have potentially influenced the results, any influence would likely be minor.

A Model for the Development of Depression

Because of the multifactorial nature of the development of mental illness, it has historically been difficult to model the development of major depressive disorder. Many of the variables that influence depression development also have a single or bi-directional causal relationship on each other. For example, Kendler et al. developed two separate models for men [144] and women [145] based on 18 risk factors, 17 of which were shared and the single discordant factor (low parental warmth for men vs. disturbances at home for women) was part of the same childhood risk factor category. Both models had a relatively good fit despite their complexity, but neither model addressed how inflammation and sex hormones might influence the incidence of an episode of major depression. A simpler model developed by Wichers et al [146] teased out how genetic
influences that alter vulnerability to depression can have both a direct effect on
depression development and an indirect effect by influencing negative life events, but this
model also neglected to consider an immune system component of depression or the role
of hormones. With the overall burden of depression higher for women, it is logical to
consider how physiological differences between men and women may influence the
development of depression. Because of the strong correlation of depression with sickness
behavior, a well-balanced model for the development of depression should include an
infectious/immune component as well. The development of a new model of depression
that addresses hormonal milieu and the immune/infectious component is not within the
scope of this study, but future work in this area may necessitate the construction of a new
model that addresses these factors.

Implications and Future Directions

The results of this study suggest that anti-depressive medications may be
particularly effective in ameliorating the depression associated with OCPs. This
possibility needs to be explored in detail. It is possible that subjects who experience
negative emotional side effects from birth control may benefit from use of an SSRI, or
tryptophan supplementation in their diet, because OCPs may deplete tryptophan levels.
Considering that many women who begin taking oral contraceptive pills discontinue
them within the first year of use because of PMS-like side effects [147], preemptively
addressing the depression-like side effects could lead to a reduction in OCP
discontinuation and ultimately fewer unplanned pregnancies. Since the use of hormonal
contraceptives is correlated with disease exacerbation, and estrogen up-regulates tryptophan restriction, it may be prudent to determine the depression and immune status of patients before hormonal contraception is prescribed, and to suggest non-hormonal or low-dose alternatives for patients who may be at risk for developing depression or other illnesses.

These results from this study must be considered preliminary because of small sample size and uncontrolled variables. With a larger sample size and evaluation of correlates of medication use, the validity of the hypotheses raised by this study could be better evaluated. Blood draws to determine serum cytokine, IDO, and antibody levels may shed more light on the relationship between depression and the immune system.
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SUBJECT INFORMED CONSENT DOCUMENT

EXPLORATORY STUDY ON MOOD AMONG GYNECOLOGY PATIENTS

IRB assigned number: 09.0226

Investigator(s) name & address:

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Amber N. Carrier (812) 454-4852

Site(s) where study is to be conducted:

University Gynecology and Obstetrics Foundation
550 South Jackson St.
Louisville, KY 40202

Campus Health Services
Cardinal Station Health Center, 215 Central Avenue, Suite 110
University of Louisville
Louisville, KY 40208

Phone number for subjects to call for questions:

Paul Ewald, PhD (502) 852-8816
Amber N. Carrier (812) 454-4852

Introduction and Background Information
You are invited to take part in a research study because you are a gynecology patient experiencing normal menstrual cycles between the ages of 18 and 40 who has experienced notable shifts in mood. The study is being conducted under the direction of Dr. Paul Ewald PhD, University of Louisville Department of Biology, Dr. Christine Cook MD, UofL Department of Obstetrics and Gynecology and Dr. Rich Lewine PhD, UofL Department of Psychology. Approximately 100 local subjects will be invited to participate. Your participation in this study will last for one (1) month.

Purpose

The purpose of this study is to examine changes in mood in relation to other medical factors such as illness or infection, use of medications and hormonal cycles.

Procedures

This first part of the study is predicted to last approximately twenty (20) minutes. During this visit, you will be asked to read and sign this informed consent form. You will be asked to fill out a questionnaire asking about your menstrual cycle, medical conditions and medication usage. The questionnaire will also be used to check and see if you are, in fact, eligible to take part in the study. If you are eligible, you will be asked to answer questions to assess your mood today. You may refuse to answer any questions that makes you uncomfortable or may make you prosecutable under the law. At home, you will be asked to fill out a calendar every day regarding what medications you take and when, as well as questions about your period. You will also be asked to return in four weeks to complete the same questionnaires and turn in your calendar. At the second visit, you will spend approximately five (5) minutes filling out the questionnaire and BDI. When you have filled out the documents on the first day you will receive $10 compensation for your time. Once you have filled out both documents the second time, you will be compensated an additional $30 for your time and travel.

Potential Risks

There are no known physical risks linked with filling out these questionnaires or calendar. The only known risk associated with this study is the psychological stress of answering questions about your mood. In addition, you may suffer harms that we have not seen before. You may suffer harms that are worse than we have seen.

Benefits

The possible benefits of this study include a more precisely directed treatment for your mood that may make your symptoms better. It is possible, however that the information may not benefit you directly but, the information learned in this study may be helpful to others.

Alternatives

Instead of taking part in this study, you could choose to not participate and can drop out at any time. If you decide not to participate in this study or stop participating at any time, you will not lose any benefits for which you may qualify.

Research Related Injury

As set forth in the Potential Risks section, there are no known risks or discomforts associated with the study.
competing the questionnaires or calendar in this research study. Neither the study site nor the study doctors have set aside money to pay for treatment of any injury. You and your insurance will be billed for the treatment of these injuries. Before you agree to take part in this research study you should find out whether your insurance will cover an injury in this kind of research. You should talk to the study doctor or staff about this. If you are injured, there is no money set aside for lost wages, discomfort, disability, etc. You do not give up your legal rights by signing this form. If you think you have a research related injury, please call Dr. Paul Ewald at (502) 852-8816.

Compensation

You will be compensated for your time, inconvenience, or expenses while you are in this study. The total compensation is forty dollars ($40). You will receive ten dollars ($10) at enrollment with the completion of the first set of questionnaires. You will receive thirty dollars ($30) more at your second visit in four (4) weeks at the completion of the second set of questionnaires and turning in your calendar.

Because you will be paid to be in this study the University of Louisville must collect your name, address, social security number, ask you to sign a W-9 form, and keep records of how much you are paid. You may or may not be sent a Form 1099 by the University. This will only happen if you are paid more than $600 in one year by the University. We are required by the Internal Revenue Service to collect this information and you may need to report the payment as income on your taxes.

This information will be protected and kept secure in the same way that we protect your other private information. If you do not agree to give us this information, we can't pay you for being in this study. You can still be in the study even if you don't want to be paid.

Costs

If you are injured by the research, there may be additional cost for participating in the research. Otherwise there will be no additional cost to you. However, you or your insurance company will be billed for all office visits and procedures that are part of routine medical care. It is your responsibility to find out what costs, if any, your insurance company will cover before taking part in the study.

HIPAA Research Authorization

The Health Insurance Portability and Accountability Act of 1996 (HIPAA) provides federal safeguards for protected health information (PHI). Examples of PHI are your name, address, and birth date. PHI may also include your medical history, results of health exams and lab tests, drugs taken and results of this research study. Your PHI may not be used or shared without your agreement, unless it meets one of the HIPAA exceptions. If you agree to take part in this research you may be required to sign a "Research Authorization" form. This allows the use and sharing of your PHI by those listed in the "Research Authorization."

Confidentiality

Total privacy cannot be guaranteed. We will protect your privacy to the extent permitted by law. If the results from this study are published, your name will not be made public.
The following may look at your research and medical records:

- The sponsor and others hired by the sponsor to oversee the research
- The University of Louisville Institutional Review Board, Human Subjects Protection Program Office, Privacy Office and others involved in research administration at the University
- People who are responsible for research and HIPAA oversight at the institutions where the research is conducted
- Government agencies, such as: (List all that apply)
  - Office for Human Research Protections,
  - Office of Civil Rights,
- People responsible for billing, sending and receiving payments related to your participation in the study.

Security
Your data will be kept private by being stored on a password-protected computer that is only accessible to the investigators and study staff. Completed questionnaires will be stored in a locked file cabinet located in the Biology Department and/or the researcher's residence.

Conflict of Interest
There are no conflicts of interest regarding your participation in this study.

Voluntary Participation
Taking part in this study is completely voluntary. You may choose not to take part at all. If you decide not to be in this study, you won't be penalized or lose any benefits for which you qualify. If you decide to be in this study, you may change your mind and stop taking part at any time. If you decide to stop taking part, you won't be penalized or lose any benefits for which you qualify.

You will be told about any new information learned during the study that could affect your decision to continue in the study.

Termination
Your study doctor has the right to stop this study at any point. Your study doctor may take you out of this study with or without your okay. Reasons why this may occur include: determining that you do not suffer from shifts in mood or that you were not seen for a routine gynecological exam.

Participation in Other Research Studies
You may take part in this study if you are currently in another research study. It is important to let your doctor know if you are in another research study.

Contact Persons
If you have any questions, concerns, or complaints about the research study, please contact Dr. Paul Ewald at (502) 852-8816 or Amber Carrier at (812) 454-4852.
IRB #09.0226: Exploratory Study on Mood Among Gynecology Patients

If you have any questions about your rights as a research subject, you may call the Human Subjects Protection Program Office at (502) 852-5188. You may discuss any questions about your rights as a research subject, in private, with a member of the Institutional Review Board (IRB). You may also call this number if you have other questions about the research, and you cannot reach the study doctor, or want to talk to someone else. The IRB is an independent committee made up of people from the University community, staff of the institutions, as well as people from the community not connected with these institutions. The IRB has reviewed this research study.

Concerns and Complaints

If you have concerns or complaints about the research or research staff and you do not wish to give your name, you may call the toll free number 1-877-852-1167. This is a 24 hour hot line answered by people who do not work at the University of Louisville.

Acknowledgment and Signatures

This informed consent document is not a contract. This document tells you what will happen during the study if you choose to take part. Your signature indicates that this study has been explained to you, that your questions have been answered, and that you agree to take part in the study. You are not giving up any legal rights by signing this informed consent document. You will be given a copy of this consent form to keep for your records.

Do you want your primary care physician notified that you are a subject in this study? □Yes □No

Printed Name of Subject/Legal Representative Signature of Subject/Legal Representative Date Signed

Printed Name of Person Explaining Consent Form Signature of Person Explaining Consent Form (if other than the Investigator) Date Signed

Printed Name of Investigator Signature of Investigator Date Signed

LIST OF INVESTIGATORS
Paul Ewald, PhD
Richard Lewine, PhD
Christine Cook, MD
Amber Carrier, PhC

PHONE NUMBERS
(502) 852-8816
(502) 852-3243
(502) 561-7441
(812) 454-4852

UNIVERSITY OF LOUISVILLE INSTITUTIONAL REVIEW BOARD
DATE APPROVED: 6/12/09 VALID THRU: 6/11/12

Consent version date: ________ Page 5 of 5

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APPENDIX B

AUTHORIZATION FOR USE AND DISCLOSURE OF YOUR HEALTH INFORMATION FOR RESEARCH

<table>
<thead>
<tr>
<th>IRB#</th>
<th>Study Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Exploratory study on mood among gynecology patients</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name (Last Name, First Name, MI)</th>
<th>Email Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ewald, Paul; Cook, Christine; Lewine, Richard</td>
<td><a href="mailto:Pw.ewald@louisville.edu">Pw.ewald@louisville.edu</a></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mailing Address – Include University Department (if applicable)</th>
<th>Telephone Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS 218, Dept of Biology, UofL, Lou., KY 40292</td>
<td>(502) 852-8816</td>
</tr>
</tbody>
</table>

Please read this form before you sign it.

In our research, we will look at and may share information about you and your health. Federal law requires that health providers and researchers protect this information and keep it private (confidential). "We" or "us" in this document refers to the following places (institutions, facilities, and practices) that are checked (✓).

**Affiliated Sites**

- University of Louisville (Do not remove this check.)
- Jewish Hospital & St. Mary's Healthcare
- Norton Healthcare, Inc., including Kosair Children's Hospital
- University of Louisville Hospital/J. Graham Brown Cancer Center

**Non-Affiliated Sites**

- Louisville Metro Public Health & Wellness
- KY Cabinet for Health & Family Services
- Seven Counties Services
- Other(s):

**University of Louisville Research Foundation (ULRF) Clinical Sites**

- Children & Youth Clinic
- Dentistry Clinics (Undergraduate DMD; Graduate, Perio, Endo and Ortho; Oral Surgery and OPR at ACB; Faculty Practice, Graduate Pedodontic Clinic)
- Family Medicine – (Newburg and Central Station; also Geriatrics and Sports Medicine at Central Station)
- Harambee Nursing Center
- Kidney Disease Program (Dialysis Unit and UL Renal Transport Lab)
- Neonatal Follow Up Program

**Faculty Practice Group Sites**

- University Anesthesiology Associates, PSC
- University Radiological Associates, PSC
- University Physicians Associates (UPA)/ UPG – Radiology, PSC
- University Emergency Medicine Associates, PSC
- University Family Practice Associates, PSC
- University Physicians Associates (UPA), PSC
- University Medical Associates, (UMA), PSC
- Associates In Dermatology, PLLC
- University Neurologists, PSC
- Neurosurgical Institute of Kentucky, PSC

Form: Research Authorization - FINAL (Rev 02-16-09)
The law allows us to look at and share your health information for research, if you agree to let us do this and if we protect it as required.

This form explains how we will look at and share your health information, as well as, who may see it and use your information. If you sign this form, it means you are letting us look at and share information for research.

1. **Health information about you from the items checked below may be looked at or given out to others.**

- Consultation reports
- Diaries and questionnaires
- Discharge summaries
- Records of your operation(s)
- Medical progress notes
- Photos, videotapes, or digital or other images
- Healthcare provider orders
- History and physical exams
- Records about the study device
- Records about the study drug and other drugs you may be taking
- Laboratory, x-ray, and other tests
- Other:

OR

WE WILL NOT BE LOOKING AT ANY OF THE ITEMS LISTED BELOW FOR THIS STUDY.

OR

THE INFORMATION WE MAY LOOK AT OR GATHER FOR THIS RESEARCH MAY INCLUDE:

- HIV / AIDS status
- Hepatitis infection
- Sexually transmitted diseases
- The diagnosis and treatment of a mental health condition
- Other reportable infectious diseases

2. **The following people or groups may share, receive and/or look at your information:**

- The people and organizations listed on this form to conduct, analyze, and understand this study;
- You or your personal representative;
- Others as allowed or required by law;
- Government entities that have the responsibility to oversee this research;
- The offices and departments responsible for oversight of research at the University of Louisville;
- Health care providers and others where you receive care during your participation in this study;
- Health care providers and others, as appropriate, for compliance oversight; and
- People responsible for sending and receiving payments related to your participation in the study.
In addition, the groups checked below may share, receive and/or look at your information:

- The sponsor of the study and the people that the sponsor may contract with for the study. The name of the sponsor is:
- Investigators and research staff at other places that are participating in the study;
- An outside institutional review board (human subjects review board)
- The Data Safety Monitoring Board
- Other:

If you have questions about who these people or organizations are, you may ask us.

3. While we are required to protect your health information, once any information leaves our institutions, we cannot promise that others will keep it private (confidential).

4. The information we look at or give to others as part of the research will be analyzed and further studied to answer the research questions and to make sure that the research was done correctly.

5. You have the following rights:

You do not have to sign this form. However, if you do not sign this form you will not be able to take part in this research. This will not change the health care or health care benefits you would otherwise receive.

You may cancel the permission you have given in this form at any time. This means you can tell us to stop using and sharing your information. If you cancel your permission:

- We will stop collecting information about you.
- You may not withdraw information that we had before you told us to stop.
  - We may already have used it or shared it.
  - We may need it to complete the research.
- Staff may follow-up with you if there is a medical reason to do so.

To cancel your permission, you should complete a written "Revocation of Research Authorization" form. Please send completed form to:

Institutional Review Board
MedCenter One, Suite 200
501 E. Broadway
Louisville, KY 40202

A revocation form may be obtained from your study doctor, designated personnel or from the Human Subjects Protections Program Office website (http://louisville.edu/research/humansubjects/subject-information). If you have any questions, call the Human Subjects Protections Program Office at (502) 852-5188.

6. The time period when information can be used or shared ends when all activities related to this study are completed.

7. Your access to your health information [✓] will [ ] will not be limited during this study.
If you do not know what something means, you may ask us. Before you sign this, you may talk it over with someone you trust. You will be given a copy of this form after you have signed it.

FOR ADULTS (OR MINORS) CAPABLE OF GIVING AUTHORIZATION:

<table>
<thead>
<tr>
<th>Subject's Signature</th>
<th>Date Signed</th>
<th>Printed Name</th>
</tr>
</thead>
</table>

FOR CHILDREN OR ADULTS NOT CAPABLE OF GIVING AUTHORIZATION:

<table>
<thead>
<tr>
<th>Signature of Parent/Surrogate/ Guardian/Health Care Agent for Subject</th>
<th>Date Signed</th>
<th>Printed Name</th>
</tr>
</thead>
</table>

Relationship of representative (Surrogate) to Subject:

NOTE: THE PRINCIPAL INVESTIGATOR MUST:
- PROVIDE A COPY OF THE SIGNED AUTHORIZATION TO THE SUBJECT
- RETAIN THE ORIGINAL SIGNED AUTHORIZATION IN THE RESEARCH RECORD
- PLACE A COPY OF THE SIGNED AUTHORIZATION IN THE SUBJECT'S MEDICAL RECORD
Exploratory Study on Mood among Gynecological Patients
18-40 Years-old

Name: __________________________ date of birth: ____________

Are you here for a routine exam? Yes / No

What was the first day of your last menstrual period: ________________

Did you have a pap smear and lab tests performed today? Yes / No

Are you on any medications or have you taken any medicine in the last thirty (30) days (including vitamins, herbal supplements, topical ointments, suppositories)? Yes / No
If yes, what ____________________________

______________________________

Do you have any medical conditions? Yes / No
If yes, what medical condition(s) do you have? ________________

_____________________________
APPENDIX D

BDI-II

Instructions: This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, and then pick out the one statement in each group that best describes the way you have been feeling during the past two weeks, including today. Circle the number beside the statement you have picked. If several statements in the group seem to apply equally well, circle the highest number for that group. Be sure that you do not choose more than one statement for any group, including Item 16 (Changes in Sleeping Pattern) or Item 18 (Changes in Appetite).

1. Sadness
   0 I do not feel sad.
   1 I feel sad much of the time.
   2 I am sad all the time.
   3 I am so sad or unhappy that I can't stand it.

2. Pessimism
   0 I am not discouraged about my future.
   1 I feel more discouraged about my future than I used to be.
   2 I do not expect things to work out for me.
   3 I feel my future is hopeless and will only get worse.

3. Past Failure
   0 I do not feel like a failure.
   1 I have failed more than I should have.
   2 As I look back, I see a lot of failures.
   3 I feel I am a total failure as a person.

4. Loss of Pleasure
   0 I get as much pleasure as I ever did from the things I enjoy.
   1 I don't enjoy things as much as I used to.
   2 I get very little pleasure from the things I used to enjoy.
   3 I can't get any pleasure from the things I used to enjoy.

5. Guilty Feelings
   0 I don't feel particularly guilty.
   1 I feel guilty over many things I have done or should have done.
   2 I feel quite guilty most of the time.
   3 I feel guilty all of the time.

6. Punishment Feelings
   0 I don't feel I am being punished.
   1 I feel I may be punished.
   2 I expect to be punished.
   3 I feel I am being punished.

7. Self-Dislike
   0 I don't feel particularly unattractive.
   1 I feel I look worse than I used to be.
   2 I am disappointed in myself.
   3 I dislike myself.

8. Self-Criticism
   0 I don't criticize or blame myself more than usual.
   1 I am more critical of myself than I used to be.
   2 I criticize myself for all of my faults.
   3 I blame myself for everything that happens.

9. Suicidal Thoughts or Wishes
   0 I don't have any thoughts of killing myself.
   1 I have thoughts of killing myself, but I would not carry them out.
   2 I would like to kill myself.
   3 I would kill myself if I had the chance.

10. Crying
    0 I don't cry anymore than I used to.
    1 I cry more than I used to.
    2 I cry over every little thing.
    3 I feel like crying, but I can't.

---

Subtotal Page 1

Continued on Back
11. Agitation
0  I am no more restless or wound up than usual.
1  I feel more restless or wound up than usual.
2  I am so restless or agitated that it's hard to stay still.
3  I am so restless or agitated that I have to keep moving or doing something.

12. Loss of Interest
0  I have not lost interest in other people or activities.
1  I am less interested in other people or things than before.
2  I have lost most of my interest in other people or things.
3  It's hard to get interested in anything.

13. Indecisiveness
0  I make decisions about as well as ever.
1  I find it more difficult to make decisions than usual.
2  I have much greater difficulty in making decisions than I used to.
3  I have trouble making any decisions.

14. Worthlessness
0  I do not feel I am worthless.
1  I don't consider myself as worthwhile and useful as I used to.
2  I feel more worthless as compared to other people.
3  I feel utterly worthless.

15. Loss of Energy
0  I have as much energy as ever.
1  I have less energy than I used to have.
2  I don't have enough energy to do very much.
3  I don't have enough energy to do anything.

16. Changes in Sleeping Pattern
0  I have not experienced any change in my sleeping pattern.
1a  I sleep somewhat more than usual.
1b  I sleep somewhat less than usual.
2a  I sleep a lot more than usual.
2b  I sleep a lot less than usual.
3a  I sleep most of the day.
3b  I wake up 1–2 hours early and can't get back to sleep.

17. Irritability
0  I am no more irritable than usual.
1  I am more irritable than usual.
2  I am much more irritable than usual.
3  I am irritable all the time.

18. Changes in Appetite
0  I have not experienced any change in my appetite.
1a  My appetite is somewhat less than usual.
1b  My appetite is somewhat greater than usual.
2a  My appetite is much less than before.
2b  My appetite is much greater than usual.
3a  I have no appetite at all.
3b  I crave food all the time.

19. Concentration Difficulty
0  I can concentrate as well as ever.
1  I can't concentrate as well as usual.
2  It's hard to keep my mind on anything for very long.
3  I find I can't concentrate on anything.

20. Tiredness or Fatigue
0  I am no more tired or fatigued than usual.
1  I get more tired or fatigued more easily than usual.
2  I am too tired or fatigued to do a lot of the things I used to do.
3  I am too tired or fatigued to do most of the things I used to do.

21. Loss of Interest in Sex
0  I have not noticed any recent change in my interest in sex.
1  I am less interested in sex than I used to be.
2  I am much less interested in sex now.
3  I have lost interest in sex completely.
APPENDIX E

University of Louisville
Human Subject Receipt for Compensation

(Please keep this portion of form in department with all study records)

<table>
<thead>
<tr>
<th>Participant Name</th>
<th></th>
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<tbody>
<tr>
<td>Participant Code</td>
<td></td>
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<tr>
<td>Study Name</td>
<td></td>
</tr>
<tr>
<td>Amount of Compensation</td>
<td></td>
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<tr>
<td>Date of Compensation</td>
<td></td>
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<tr>
<td>Signature of Participant</td>
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</tbody>
</table>

(Please return this portion of form to Controller's Office along with petty cash reconciliation or payment request.)

<table>
<thead>
<tr>
<th>Participant Code</th>
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<tbody>
<tr>
<td>Amount of Compensation</td>
<td></td>
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<tr>
<td>Date of Compensation</td>
<td></td>
</tr>
</tbody>
</table>
Please write down what medicines you take and when you take them. Also, mark when you start your period. Please fill this out everyday to make sure it is correct. Please note when you are scheduled to return to fill out the second pair of questionnaires and receive the rest of your compensation.

<table>
<thead>
<tr>
<th>Sun</th>
<th>Mon</th>
<th>Tue</th>
<th>Wed</th>
<th>Thu</th>
<th>Fri</th>
<th>Sat</th>
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<td>29</td>
<td>30</td>
<td>31</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For questions, please contact Carrie Doyle at 502-489-1904 or Amber Carrier at 812-454-4852
APPENDIX G

STUDY AD:

Female? Between 18 and 40? Is it time for a Check-up?

If you’re female and it’s time for your annual check-up, you may be eligible to participate in a simple research study on mood. Eligible patients will fill out two short questionnaires on their mood and take home a calendar to track medication use. They will then be asked to return the calendar in a month and repeat the questionnaires. If you qualify, you will be compensated $10 at the first visit and $30 at the second for your time and participation. For more information about the study, please contact Amber N. Carrier at amber.carrier@louisville.edu

STUDY EMAIL:

Thank you for your inquiry about the study on mood in gynecology patients.

To be eligible for the study, patients must be female, between the ages of 18 and 40, and present for an exam at Campus Health Services, Belknap Campus. You may be excluded from the study if you are not having certain tests done at your annual exam. Additionally, certain chronic diseases that affect the menstrual cycle may exclude you from the study (birth control is okay), but many other chronic conditions are okay. Your provider will determine your eligibility at your appointment.

Our goal is to correlate health status, the menstrual cycle, and medication use with changes in mood over the course of the month. Eligible patients are recruited at the time of their annual exam. They are asked to fill out a couple of short questionnaires concerning their mood. They then take home a calendar on which they mark the days that they take certain medicines and when they start their period. After about a month, the patient is asked to return to fill out the questionnaires again. Each visit is brief (approximately 20 minutes at the first one, and 5-10 minutes at the second one). To compensate each patient for her time and participation, she will receive $10 at the first visit and $30 at the follow-up a month later, for a total of $40 for completing the study.
Please note that we do not cover the medical costs associated with your exam and any follow-up treatment. Please contact your healthcare insurer to determine if your health care coverage includes the cost of an annual exam.

If you would like to schedule an appointment for your exam, please contact Campus Health Services, Belknap Campus at (502) 852-6479. At your exam, your provider will confirm if you are eligible to enroll in the study if you choose. The best times to schedule your appointment in order to ensure that I will be present and able to enroll you are on Monday, Wednesday, and Friday all day and Tuesday and Thursday afternoon. Other times I cannot guarantee that I will be available. Please contact me regarding Wednesday availability if that is when your exam is scheduled. If you cannot schedule an appointment at a time that I will be present, contact me and we can work out a time to enroll you in the study within a few days of your exam.

If you have any further questions please feel free to contact me. I appreciate your interest!

Sincerely,

Amber N. Carrier
## APPENDIX H

### MEDICATIONS USED IN THE FINAL ANALYSIS

Drug names were taken from the National Library of Medicine and from Chemfinder.com

<table>
<thead>
<tr>
<th>Medication</th>
<th>Ingredient of</th>
<th>Common Use</th>
<th>Drug Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>Tylenol, Lortab, Midol</td>
<td>relieves pain from headaches, menstrual periods, muscle aches, colds and sore throats, and to reduce fever</td>
<td>analgesic, antipyretic</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>Elavil, Endep</td>
<td>anti-depressant, migraine prevention</td>
<td>tricyclic antidepressant</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>Amoxil, Trimox, Augmentin, Amoclan</td>
<td>treats bacterial infections of respiratory tract, some STIs, urinary tract infections, and ear/nose/throat infections</td>
<td>penicillin-like antibiotic</td>
</tr>
</tbody>
</table>
Medication: Aspirin
IUPAC Name: acetylsalicylic acid
Ingredient of: Aspirin, Excedrin
Common Use: used to relieve swelling and pain, reduce fever, thin blood
Drug Class: salicylate

Medication: Azithromycin
IUPAC Name: (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-15-oxo-11-\{3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranoside\}-l-oxa-6-azacyclopentadec-13-yl 2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranoside
Ingredient of: Zithromax, Zmax
Common Use: treats bacterial infections of the lungs, reproductive tract, ears, skin, and throat
Drug Class: macrolide antibiotic

Medication: Bupropion
IUPAC Name: (±)-2-(tert-Butylamino)-1-(3-chlorophenyl)propan-1-one
Ingredient of: Wellbutrin, Zyban
Common Use: anti-depressant, SAD treatment, anti-smoking
Drug Class: atypical antidepressant

Medication: Clindamycin
IUPAC Name: methyl 7-chloro-6,7,8-trideoxy-6\{-[(4R)-1-methyl-4-propyl-L-prolyl]amino\}-1-thio-L-threo-α-D-galacto-octopyranoside
Ingredient of: Cleocin
Common Use: treats bacterial infections of the lungs, skin, blood, internal organs, and female reproductive tract
Drug Class: lincomycin antibiotic

Medication: Diphenhydramine
IUPAC Name: 2-(diphenylmethoxy)-N,N-dimethylethylamine
Ingredient of: Benadryl, Sominex, Theraflu, Triaminic, PM variations of OTC pain relievers such as Advil and Tylenol
Common Use: relieves eye irritation, sneezing, runny nose, and allergy side effects. Also used to treat motion sickness and insomnia
Drug Class: antihistamine
Medication: Doxycycline
IUPAC Name: (4S,4aR,5S,5aR,6R,12aS)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide
Ingredient of: Vibramycin, Periostat
Common Use: treats bacterial infections of respiratory tract, skin, genital, and urinary systems; can also be used to prevent malaria
Drug Class: tetracycline antibiotic

Medication: Duloxetine
IUPAC Name: (+)-(S)-N-Methyl-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl)propan-1-amine
Ingredient of: Cymbalta
Common Use: anti-depressant, GAD, pain due to diabetic neuropathy and fibromyalgia
Drug Class: SNRI

Medication: Ethinyl estradiol*
IUPAC Name: 19-Nor-17α-pregna-1,3,5(10)-triien-20-yne-3,17-diol
Ingredient of: Present in almost all oral contraceptives, see below
Common Use: Prevents ovulation
Drug Class: synthetic bioactive estradiol derivative

*ethinylestradiol is the estrogen component of most combination oral contraceptive pills (OCPs) on the market, and all of the ones taken by participants in this study.

Medication: Fluoxetine
IUPAC Name: N-methyl-3-phenyl-3[4-trifluoromethyl]phenoxy]propan-1-amine
Ingredient of: Prozac, Sarafem
Common Use: anti-depressant, anti-anxiety (as Prozac), PMDD (as Sarafem)
Drug Class: SSRI

Medication: Ibuprofen
IUPAC Name: iso-butyl-propanoic-phenolic acid
Ingredient of: Advil, Motrin, Midol
Common Use: pain relief, reduce swelling and tenderness, dysmenorrhea
Drug Class: NSAID

Medication: Lamotrigine
IUPAC Name: 6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine
Ingredient of: Lamictal
Common Use: anticonvulsant (for epilepsy), bipolar I disorder
Drug Class: anticonvulsant

Medication: Metronidazole
IUPAC Name: 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethanol
Ingredient of: Flagyl, Prostat
Common Use: eliminates infections of reproductive tract, gastrointestinal tract, and skin
Drug Class: antibiotic, amebicide, antiprotozoal

Medication: Sertraline
IUPAC Name: (1S,4S)-4-(3,4-dichlorophenyl)-N-methyl-1,2,3,4-tetrahydronaphthalen-1-amine
Ingredient of: Zoloft
Common Use: anti-depressant, panic attacks, PTSD, social anxiety, OCD, PMDD
Drug Class: SSRI

Medication: Trazadone
IUPAC Name: 2-{3-[4-(3-chlorophenyl)piperazin-1-yl]propyl}[1,2,4]triazolo[4,3-a]pyridin-3(2H)-one
Ingredient of: Desyrel, Oleptro
Common Use: anti-depressant
Drug Class: serotonin modulator/SARI

Medication: Zolpidem
IUPAC Name: N,N-dimethyl-2-(6-methyl-2-p-tolylimidazo[1,2-a]pyridin-3-yl)acetamide
Ingredient of: Ambien
Common Use: insomnia
Drug Class: sedative-hypnotic
CURRICULUM VITAE

Amber N. Carrier
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EDUCATION
• Doctor of Philosophy in Biology with a focus on Evolutionary Medicine, expected graduation May 2012, University of Louisville
• Master of Science in Biology, December 2008, University of Louisville (GPA 3.694)
• Bachelor of Science in Biophysics, May 2005, University of Southern Indiana (GPA 3.5)
• Associate of Science, August 2002, Henderson Community College (GPA 4.0)

PROFESSIONAL EXPERIENCE

Assistantship and Fellowship Awards
• Dissertation Completion Fellowship, University of Louisville (Spring 2012)
• Graduate Teaching Assistantship, University of Louisville (Fall 2006-Summer 2008, Spring 2009-Summer 2011)
• Graduate Service Fellowship, University of Louisville (Fall 2008)
• National Academies Christine Mirzayan Science and Technology Policy Graduate Fellowship (Fall 2008)

Students Trained/Supervised
• Samiyyah Sledge, Undergraduate
• Laura Ovaitt, Graduate

Grants
• Graduate Student Council Travel Grant, Fall 2011, $300
• Graduate Student Council Travel Grant, Spring 2011, $350
• Graduate Student Council Travel Grant, Fall 2009, $250
• University of Louisville COSW Travel Award, Summer 2008, $255
• Graduate Student Council Travel Grant, Summer 2008, $250
• University of Louisville Intramural Research Incentive Grant, Fall 2007, $4000
• Graduate Student Council Travel Grant, Fall 2007, $250

Posters
• Amber N. Carrier, Caroline M. Doyle, and Paul W. Ewald, Ph.D. “Interactions between perimenstrual immunosuppression, infection, and clinical depression” Posters at the Capitol, Frankfort, KY February 2009.
• Justin E. Farris, Jessica A. Perpich, Christopher K. Brown, Amber N. Carrier, Susanna K. Remold, Ph.D. “P. aeruginosa use of niches in the human household environment.” Kentucky Academy of Sciences Annual Meeting, November 2007
• Amber N. Carrier, Paul W. Ewald, Ph.D., and Susanna K. Remold, Ph.D. “Environmental niches and colonization cycling of bacteria in the lungs of cystic fibrosis patients” Research Louisville, Louisville KY, 2007

Talks and Seminars
• "Immunity, Infection, and Mood: How Treatment of Undiagnosed Chlamydia Infection May Improve Depression and Mood in Women of Reproductive Age." Graduate Research Symposium, April 2011
• "The Exploration of Women's Mental and Reproductive Health Using an Evolutionary Framework." University of Kentucky Graduate Interdisciplinary Conference, April 2010
• "Through Darwin's Eyes: The Exploration of Women's Mental and Reproductive Health Using an Evolutionary Framework." Graduate Research Symposium, March 2010
• “Depression and Infection.” Program on Disease Evolution Discussion Group, University of Louisville, February 2009
• “Polycystic Ovary Syndrome.” Program on Disease Evolution Discussion Group, University of Louisville, February 2007.

Work Experience
• National Academies, National Academy of Engineering, Mirzayan Policy Fellow, Fall 2008 (assisted the following boards and publications)
  o Committee on Women in Science, Engineering, and Medicine
  o National Academy of Sciences and the Committee on Women in Science, Engineering and Medicine and the Committee on National Statistics. Gender Differences at Critical Transitions in the Careers of Science, Engineering, and Mathematics Faculty. National Academies Press, 2009
  o Committee on Capitalizing on the Diversity of the Science and Engineering Workforce in Industry –
  o EngineerGirl! Website
• National Academy of Engineering
• Christine Mirzayan Science and Technology Policy Fellowship Program

Conferences Attended
- University of Louisville Graduate Research Symposium, Spring 2011
- University of Louisville Graduate Research Symposium, Spring 2010
- University of Kentucky Graduate Interdisciplinary Conference, Spring 2010
- Educational Credit Union Conference, Spring 2010
- Idea Festival 2009
- From Doctorate to Dean or Director: Sustaining Women through Critical Transition Points in Science, Engineering, and Medicine – Committee on Women in Science, Engineering, and Medicine, National Research Council, Fall 2008
- National Conference on College Women Student Leaders, Washington, D.C., Summer 2008
- National Conference on Graduate Student Leadership, Lexington KY, Fall 2007, Reporter
- Kentucky Academy of Sciences Annual Meeting, Louisville, KY, Fall 2007

Elected Positions
- Graduate Student Council President (May 2008-May 2010)
- Graduate Student Council Treasurer (May 2007-May 2008)
- Biology Graduate Student Association Vice-President (May 2007-May 2008)
- Student Government Association Senator (December 2007-May 2010)

Other Appointments
- Class Act Federal Credit Union Supervisory Committee (Fall 2010-May 2011)
- Class Act Federal Credit Union Quality Control Committee (Fall 2009-Fall 2010, Chair)
- Vice-President of Human Resources Search Committee (Summer 2009-Spring 2010)
- Student Government Association Development Board (Summer 2009-May 2010, Chair)
- Provost’s Ad-hoc Budget Advisory Committee (Spring 2009-present; Chair, subcommittee on New Ideas; member; subcommittee on Closing Programs and efficiencies)
- Graduate Council (Summer 2008-May 2010)
- Student Government Association Executive Board (Summer 2008-May 2010)
- University of Louisville Commission on the Status of Women (Winter 2008-Spring 2011)
- Biology Graduate Student Association Fundraising Committee (Fall 2006-Spring 2008)
- Graduate Student Council Events Committee (Spring 2007-Spring 2008)
- University parking and Appeals Committee (Fall 2007-Spring 2008)
- Student Organizations Board (Fall 2007-Spring 2008)
- University of Louisville Strategic Planning Committee (Fall 2007)

Courses Taught
• Human Anatomy and Physiology Laboratory, University of Louisville (Spring 2007, Spring 2009-Present)
• Principles of Biology Laboratory, U of L (Spring 2008)
• Medical Microbiology Laboratory, U of L (Summer and Fall 2007, Summer 2008, Summer 2009, Summer 2010)
• Introduction to Biology for Non-Majors, U of L (Fall 2006)

PROFESSIONAL AND SCHOLASTIC SOCIETIES
National Science Teachers Association, Golden Key International Honor Society, Omicron Delta Kappa Leadership Society, American Society of Microbiology, Sigma Zeta National Science and Mathematics Honor Society, American Association for the Advancement of Science, American Association of University Women, Association of Women in Science, Society for the Scientific Study of Sexuality

HONORS
• Office of LGBT Services Amber Carrier Outstanding Student Ally Award (Spring 2011; this was the inaugural year for the award and it was named in my honor)
• Student Government Association Senator of the Year (Spring 2010)
• Outstanding Graduate Student Award (Spring 2009)
• Christine Mirzayan Science and Technology Policy Graduate Fellow of the National Academies (2008)
• University Delegate to the Biennial National Conference on Graduate Student Leadership (2007)
• Biology Graduate Student Association Service Award (2007)