CORRELATION OF POSTPARTUM MOOD DYSPHORIAS WITH VARIOUS PLASMA HORMONE LEVELS

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CHAPTER ONE

INTRODUCTION

Mood Disorders Associated with Pregnancy

Postpartum mood dysphoria in some form or other is exceedingly common and reported by the majority of mothers. Estimates of its frequency vary, but they range from 50 to 85 percent of postpartum women (Gundersen, 1996; Grush and Cohen, 1998; *Oklahoma DO*, 1996; Susman, 1996). It is neither a recent phenomenon, nor one peculiar to western culture, as Hippocrates described postpartum depression in ancient Greece (Grinspoon, 1997). Researchers have found similar rates of the disorder in Australia, Italy, the Netherlands, Greece, England, Nigeria, and Uganda (Grinspoon, 1997). However, Gundersen (1996) suggests it is more common in western countries than elsewhere.

Three types of mood dysphorias after delivery have been described: postpartum (or baby) blues is the mildest, postpartum depression is more severe, and postpartum psychosis is the most serious. It may be useful, however, to conceptualize these disorders as existing along a continuum, because there is sometimes overlap between the categories (Nonacs and Cohen, 1998). Further, some researchers still question the existence of a mood disorder unique to the postpartal period, as a few population-based studies have found similar rates of minor and major depression in puerperal and nonpuerperal women (Nonacs and Cohen, 1998).

Postpartum Blues

Postpartum blues may affect up to 85% of new mothers. It is characterized by transient mood swings that develop within the first two weeks after birth, often peaking between the third and seventh day after delivery. Symptoms include lability of mood characterized by either sadness or elation, irritability, crying, anxiety, insomnia, headache, appetite changes, fatigue, and feeling overwhelmed or oversensitive (Gundersen, 1996; Susman, 1996; Sutter et al., 1997). The blues typically lasts only a few days and resolves on its own without treatment (Gundersen, 1996).

Sutter et al. (1997) recently suggested that postpartum blues be subdivided into two categories, a so-called "transient light symptomology" and a "heavy postpartum blues" (HPPB), which is slightly more severe as measured by the 28-item Kennerly and Gath Blues Scale. Sutter suspects the differentiation may be helpful, because he speculates that those with HPPB are more likely than their less-affected counterparts to go on to develop true postpartum depression. However, for the purposes of this research, only the general term "postpartum blues" will be used.

Postpartum Depression

Postpartum depression (PPD) affects roughly 10-20% of new mothers. Although the *Diagnostic and Statistical Manual of Mental Disorders-Text Revision,* 4th edition (*DSM-IV-TR*) applies the term "postpartum onset" to depression that begins within four weeks of delivery. However, most epidemiologic studies consider postpartum depression to be depression that presents within six months of delivery (Hendrick et al, 1998). Onset of symptoms in patients with PPD is usually a few days or weeks after delivery at most (Cox et al, 1993; Kumar, 1994), and for the majority of affected women, the episode

lasts for three months or less (Cooper et al, 1988). McCoy (2001) reported the average duration of PPD as 6-9 months. Longitudinal studies indicate that a quarter of affected mothers are still depressed at 12 months (Gregoire et al, 1996), and up to 14 months (Susman, 1996). These findings in women with PPD contrast with the incidence of major depressive disorder in the general population of two cases per 100 person-years in women (Beckham and Leber, 1995).

Susman (1996) reported that the *DSM-IV-TR* criteria for major depressive episode are used to diagnose PPD. At least five of the symptoms listed must be present for most of the day, and nearly daily for at least two weeks. Symptoms must involve either depressed mood or markedly diminished interest or pleasure in all or almost all activities most of the day. Additional symptoms include significant weight loss/gain or decrease or increase in appetite, insomnia/hypersomnia, psychomotor agitation/retardation, fatigue or loss of energy, feelings of worthlessness or excessive, inappropriate guilt, impaired concentration or indecisiveness, and recurrent thoughts of death, suicidal ideation with or without a specific plan or suicide attempt (*DSM-IV-TR*, 2000).

Postnatal depression is not only distressing and unpleasant for the mother, it can have severe consequences for her offspring as well. Problems for the child may include alienation, indifference, detachment with lack of love, resentment, hate, and hostility. Such early failure to properly bond may have the potential to put the parent-child relationship at a more-or-less permanent functional disadvantage long after the mother's initial depression has resolved. Clearly, PPD needs to be addressed as a public health issue with significant societal ramifications (Kumar, 1997).

Postpartum Psychosis

Postpartum psychosis is rather rare, occurring only about 1-4 times per 1000 deliveries. It can begin within 1-3 months after delivery, but most data collected show a peak incidence 1-2 weeks postpartum. The condition may begin with insomnia and progress to confusion, memory impairment, irritability, anxiety, and intrusive thoughts (Gundersen, 1996). Further, it is characterized by severely depressed mood, hallucinations, or delusions (Grinspoon, 1997; *Oklahoma DO,* 1996) and may progress to symptoms similar to those associated with schizophrenia or a thought disorder (Susman, 1996). Hospitalization is generally required, because there is a substantial risk for infanticide and/or suicide. However, 95% of patients are better within 3 months (Susman, 1996).

CHAPTER TWO

REVIEW OF THE LITERATURE

The Etiology of Postpartum Mood Disorders

Perinatal Hormonal Events

As pregnancy progresses, the concentration of serum estriol, the main estrogen manufactured in the placenta from fetal androgens, steadily rises until its levels are 1000 fold greater than in the nongravid state. Estradiol is the chief estrogen produced by the ovaries in nongravid women of reproductive age. It is made in the placenta as well, but in smaller quantities than estriol. During pregnancy, serum estradiol concentrations increase 100 fold. In addition, serum progesterone, made at first by the corpus luteum of the ovary and later by the placenta, rises markedly as well. When the placenta is delivered at the birth of the child, levels of all three female sex steroids drop sharply. Plasma concentrations of both estradiol and progesterone reach pregravid levels five days later, while levels of estriol fall until they are virtually undetectable. In addition, beta-endorphin, human chorionic gonadotropin, corticotropin releasing hormone, and cortisol rise during pregnancy, peak around the time of delivery, and decline thereafter (Hendrick, 1998).

Rising levels of both estrogens stimulate production of thyroid binding globulin, which increases levels of bound triiodothyronine (T3) and thyroxine (T4). The subsequent decline in free T3 and T4 stimulates the release of thyrotropin releasing hormone (TRH) from the hypothalamus and, in turn, thyroid stimulating hormone (TSH) from the pituitary to compensate, thereby returning free T3 and T4 to normal levels. At delivery thyroid-binding globulin levels decline with the drop in estrogen, while negative

feedback on pituitary production of TSH allows free T3 and T4 to remain relatively stable. Prolactin rises during pregnancy and continues to rise for about two months in lactating women, although it declines rapidly and returns to pregravid levels within three weeks in non-lactating women (Hendrick, 1998). Oxytocin from the posterior pituitary helps maintain high prolactin levels during breastfeeding, and it also stimulates milk letdown (Nicholas et al, 1998; Hendrick, 1998).

Possible Biological/Endocrinological Causes

Role of Progesterone

Correlations among plasma levels of a variety of hormones and incidence of PPD have been conflicting and inconclusive. History of premenstrual syndrome (PMS) has been shown to correlate with incidence of PPD (Gundersen, 1996), and just before menses is the time during the menstrual cycle when progesterone levels fall.

English obstetrician Katharina Dalton published four studies over a 15-year period in which she tested prophylactic natural progesterone as a treatment for postpartum depression. After the first apparently successful experiment in 1971 (Dalton, 1971), she wrote a letter to the editor of *British Medical Journal* recommending the following standardized prophylactic treatment: For the first seven days after delivery, 100 milligrams of per day of intramuscular, injected natural progesterone was to be administered. Following the first week, 400 milligrams of natural progesterone suppositories twice daily were used for about two months or until menstruation resumed (Dalton, 1982).

Later, Dalton (1985) again attempted to show that prophylactic progesterone was successful in preventing recurrence of postpartum depression in women who had

experienced it with a previous birth. The research design's control, however, was patient information from previous studies. Since the women from earlier studies were under an entirely different set of circumstances, the results lack the scientific rigor expected of reliable research. Further, the experimental design failed to account for a possible placebo effect, and might therefore have overestimated the efficacy of the treatment (Filer, 1992). Dalton did claim, however, that progesterone prophylaxis decreased recurrence of PPD from the expected 68% to 10%. The dosage given was the same as she had recommended in 1982. It is important to note that the treatment was only effective as a prophylaxis; once symptoms of depression appeared, progesterone was not helpful in alleviating them.

In 1989 Dalton published again—this time reporting on 242 women who received prophylactic natural progesterone because they were at risk of recurring idiopathic PPD. Results showed that only 7% again experienced PPD after their next baby was born, compared to a 67% recurrence rate for the control group who did not receive progesterone (Dalton, 1989). Finally, in 1995, Dalton reported that prophylactic progesterone managed to prevent PPD recurrence in 92% of another group of 255 women (Dalton, 1995).

Another paper whose results tended to support Dalton's findings reported that the greater the fall in plasma progesterone after delivery, the higher the risk of depressive symptoms (Nott et al, 1976). However, some recent data have shown progestogens to exacerbate, rather than allay postpartum depression. Researchers in South Africa published data in 2000 that indicated that volunteers given depot norethisterone enanthate (a synthetic progestogen) within 48 hours of delivery and lasting 8-12 weeks

had significantly higher PPD scores than controls. The authors, including contributor K. Dalton, concluded, "there is no place for synthetic progestogens in the prevention or treatment of postnatal depression" (Lawrie et al, 2001). However, it may be worth noting that it was reported last year that teenagers who were given the synthetic progestin Norplant for contraception within two months after giving birth had similar incidence of depression as mothers who used other methods of contraception or who had Norplant inserted several months after delivery. This result surprised the authors, because they expected the synthetic progestin to cause a higher rate of depression in the teenagers. It has been reported that teens already have a higher incidence of postpartum depression than adults (Stevens-Simon et al, 2000). Controversy over the role of synthetic progestins in postpartum depression shows the need for additional research in this area.

It is likely impossible to make an objective comparison between natural progesterone, tested by Dalton, and the synthetic progestins in oral contraceptives. In fact, the so-called "19-nors" that are often used in oral contraceptives for their potent progestin activity are actually much like androgens by biochemical definition. Specifically, although they typically contain a total of 20-23 carbon atoms, they lack the carbon atoms found in the numbered positions 19, 20, and 21 in progesterone on the steroid nucleus and resemble testosterone around the molecule's D ring. In addition to their progestin-like qualities, 19-nor compounds are known to have a variety of androgenic activities that may contribute to side effects associated with 19-C progestin treatment (Williams and Stancel, 1996). Therefore, findings that synthetic progestins do not help postpartum depression do not necessarily negate Dalton's studies that showed that natural progesterone was an effective prophylaxis. In fact, Lawrie et al (2001) readily admitted

that natural progesterone's role in the prevention and treatment of PPD has not yet been properly evaluated in a randomized placebo-controlled trial. Dalton herself believed that one of the relevant differences between natural progesterone and synthetic progestogens lay in the body's ability to synthesize corticosteroids from progesterone, but not from synthetic progestins (Dalton, 1971).

It has been known for several decades that progesterone and some of its metabolites, such as allopregnanolone, as well as other steroids like deoxycorticosterone, can affect the CNS. One mechanism that does not involve the classical direct genomic pathway is the ability of progesterone, allopregnanolone, and deoxycorticosterone to exert an effect through the gamma aminobutyric acid (GABA) receptor complex. GABA is a neurotransmitter involved in inhibitory pathways in the CNS. Progesterone and its metabolites can help to stimulate the GABA receptors by facilitating GABA's binding to these same receptors via allosteric modulation. In fact, neuroactive steroids may function as endogenous anxiolytics that are produced in response to stress (Rodgers and Johnson, 1998). Some of the anxiety associated with postpartum depression might be attributable to the abrupt loss of high levels of progesterone.

Progesterone may oppose some of the antidepressant actions of estrogens, since progestins in general have antiestrogenic qualities. In 1996, Arpels reported that progesterone opposes estrogen's ability to decrease the activity of monoamine oxidase (MAO), the enzyme that breaks down epinephrine, norepinephrine and dopamine. Essentially, then, progesterone increases MAO activity. Given that the current concept of depression is modeled on a seeming functional deficiency in brain neurotransmitters, it is

also credible that progesterone could depress mood by reducing the quantity of biogenic amines.

Role of Estradiol/Estriol

Other predictors of postpartum blues are levels of free and total estriol (O'Hara et al, 1991). It is well-established that there are estrogen and progesterone receptors in the brain (Grinspoon, 1997). Estrogen has antidopaminergic properties (Gundersen, 1996), because it decreases D2 receptors and probably other dopaminergic receptors as well (Halbreich, 1997). Perhaps in some women, the sudden withdrawal of estrogens and their antidopaminergic activity after delivery might result in the psychotic symptoms associated with too much dopamine.

Estrogen increases serotonergic responsivity in postsynaptic neurons by upregulating the number of serotonin receptors present and, hence, the amount of serotonin they take up. Further, estrogens act as cholinergic agonists by increasing the activity of acetylcholine transferase in the preoptic area, amygdala, horizontal diagonal nucleus, frontal cortex and area CA1 of the hippocampus (Halbreich, 1997).

Estrogens have mixed effects on norepinephrine activity in the brain (Halbreich, 1997), but it is known that in some brain areas estrogens help to decrease the activity of monoamine oxidase, an enzyme that breaks down catecholamines (Arpels, 1996). In addition, estrogens act as a gamma-aminobutyric acid adjunct agonist by increasing binding of GABA agonists and their up-regulation of GABA receptors. These actions of estradiol suggest that estrogens may function overall as antidepressants (Halbreich, 1997).

Many of the signs and symptoms associated with postpartum blues and depression, including sleep disturbance, irritability, anxiety and panic, memory and cognitive dysfunction, and a decreased sense of well-being, are also experienced by women suffering from premenstrual dysphoric disorder (PMDD), the perimenopausal transition, and menopause. A unifying hypothesis is starting to emerge that views the entire spectrum of these female disorders as one of relative brain hypoestrogenism. The female brain may have a set point for an estrogen minimum, so that whenever estrogen levels drop below the set point, dysfunctions in mood, memory and cognition occur (Arpels, 1996).

Bolstering the above data are studies that indicate that transdermal estrogen is an effective treatment for severe PPD (Sichel et al., 1995; Gregoire et al, 1996; Grinspoon, 1997). In 1995 Sichel et al. studied four women with history of PPD and seven women with history of postpartum psychosis. Each of the women in the study had previously developed postpartum affective disorder within two weeks of giving birth, so the researchers hypothesized that the cause may have been a so-called "estrogen withdrawal state" that somehow modulated brain dopamine and serotonin. Each was given high dose oral estrogen immediately following childbirth. Even though the relapse rate was expected to be 35-60% without prophylaxis, only one of the eleven participants again developed postpartum affective disorder. The estrogen doses started out approximating levels during pregnancy then tapered gradually to follicular phase estradiol levels over four weeks (Sichel et al., 1995).

In 1996 Gregoire and colleagues reported that transdermal estrogen was effective in improving the symptoms of PPD. To reduce the risk of endometrial hyperplasia, after

three months of unopposed treatment, patients in the active group were given 10 mg dydrogesterone, a synthetic progestin, for 12 days per month (Gregoire et al, 1996). This study supports the notion that low levels of estrogen may be a contributing factor to PPD.

More recently, estradiol was administered sublingually to two patients with PPD, and, in a separate study by the same principal author, to two patients with puerperal psychosis. During the treatments, estradiol levels in serum rose coincidentally with decline of depressive or psychotic symptoms as measured by the Montogomery-Asberg Depression Rating Scale and the Brief Psychiatric Rating Scale. The authors of the studies concluded that estradiol may have a causal relation to both PPD and postpartum psychosis, and they considered estrogen to be a significant treatment for the condition (Ahokas et al, 1999; Ahokas and Aito, 1999).

Even research with animals is supporting the hypothesis that estrogen withdrawal after giving birth can put females at risk for depressive symptomology. Recently, female Long-Evans rats were given daily injections of estradiol and progesterone for 23 days to simulate pregnancy. At the end of the 23 days, one group continued to receive estradiol but not progesterone, while the other received no exogenous hormone at all. The group not receiving estradiol exhibited behaviors consistent with "depressive-like" symptoms, as evidenced by the Forced Swim Test, while the group that continued to receive estradiol did not. Both were compared to controls that had not been given hormone injections. The authors concluded that high levels of estradiol in rats attenuate "depressive-like" symptoms in simulated postpartum pregnancy (Galea, et al, 2001).

Another study supporting the hypothesis that a hypoestrogenic state after birth can contribute to postpartum depression was done by giving non-pregnant, healthy euthymic

women estradiol and progesterone in amounts that simulated levels occurring in late pregnancy. After eight weeks the steroids were abruptly withdrawn. Women who had a history of PPD were significantly more likely to develop symptoms of depression after the withdrawal of sex steroids than women who had not experienced PPD. The researchers concluded that the data provided direct evidence for the involvement of estrogens and/or progesterone in the etiology of PPD. Further, they suggested that women with a history of PPD may be sensitive to mood dysphorias associated with gonadal steroids (Bloch et al, 2000).

Most research with estrogens has focused primarily on pre- and postnatal levels of estradiol, rather than the more abundant but less potent placental estriol However, O'Hara et al reported in 1991 that levels of both free and total estriol were higher at 34, 36, and 38 weeks gestation in women who had the greatest tendency to develop postpartum blues during the first eight days after giving birth. They also found that women who met the criteria of their measuring instruments for postpartum blues were more likely to experience true postpartum depression by postpartum week nine as measured by the Beck Depression Inventory (O'Hara et al, 1991). It may be inferred, then, that their results support a hypothesis that hypoestrogenism is a possible cause of postpartum depression. Unfortunately, little, if any, follow-up research on estriol has been done to date.

Role of Cortisol

Additionally, a relationship between postpartum depression and a relatively high serum cortisol before delivery has been noted (Kendell, 1985; Okano and Nomura, 1992; Pedersen et al, 1993). A Japanese paper reporting on a study of 47 women showed that

those who developed the blues had significantly higher serum concentrations of cortisol than the non-blues group. They concluded that hyperadrenocorticism is important in the genesis of PPD, because there was no other consistent obstetric factor which predisposed women to develop the blues (Okano and Nomura, 1992). High cortisol levels are associated with psychiatric depression (Schimmer and Parker, 1996), although well-researched theories about the etiology of this phenomenon are scant. In general, hypotheses regarding the association of depression with diseases that are characterized by hypercortisolemia, such as Cushing's Syndrome, center around glucocorticoid receptors in the brain whose stimulation affects the production and release of various neurotransmitters (Schimmer and Parker, 1996).

In pregnancy cortisol levels rise, as well as their plasma binding proteins (CBGs) due to the effect of estrogens on plasma levels of CBG, TBG and SSBG (Williams and Stancel, 1996). Fetal cortisol production rises sharply during the last few weeks of gestation. This late gestational increase in cortisol secretion is important in preparing the fetus for the abrupt transition to extrauterine life. Cortisol influences lung maturation, increases stores of liver glycogen, induces intestinal transport systems and digestive enzymes, and promotes closure of the ductus arteriosus (Berne et al, 1993).

Role of Corticotropin Releasing Hormone (CRH)

In human pregnancy, from the onset of the second trimester, the placental cytotrophoblast and syncytiotrophoblast are major sources of CRH secretion. Fetal membranes, such as the amnion and chorion, also produce CRH (Chrousos, 1999). Plasma levels of CRH in the pregnant woman reach 100 times their pregravid titers during the last six to eight weeks of pregnancy (Majzoub and Karalis, 1999).

Paradoxically, cortisol increases CRH production by the placenta, rather than inhibiting CRH, as cortisol does in the hypothalamic-pituitary-adrenal axis. This increase in CRH has been corroborated by research in which women at risk for preterm delivery were given betamethasone, a synthetic steroid similar to cortisol, to stimulate fetal lung maturity. (Marinoni et al, 1998). Placental CRH may be an important component of a system that controls the normal maturation of the fetus and signals the initiation of labor (Majzoub et al, 1999). It is probably also responsible for vasodilation of the fetoplacental circulation by activating nitric oxide synthase, and it may cause the secretion of prostaglandins F2α and E2, which aid in the onset of labor (Chrousos, 1999).

Much of the circulating CRH during pregnancy is bound to CRH binding protein produced by the liver, and it is therefore unable to exert any physiological effect. However, during the last month or six weeks of gestation, the concentrations of this liver protein fall to about 40% of their previous level, and free CRH is thus more than doubled (Chrousos, 1999).

It is known that the postpartum period is generally characterized by a transient suppression of hypothalamic CRH, and therefore, a general decrease in cortisol production by the adrenals as well (Chrousos, 1999). Some researchers are postulating that this suppression in cortisol secretion may be partially responsible for the development of blues and/or depression. They hypothesize that estrogen ameliorates PPD, because it is known to serve as a direct stimulant for the hypothalamic release of CRH (Chrousos, 1999). Indeed, in 1996, a study showed that women who developed postpartum blues or depression had a significantly more severe and longer-lasting inhibition of the HPA axis function than women who did not develop blues or

depression. Those researchers postulated that suppressed ACTH might serve as a biochemical marker for PPD or blues (Magiokou et al, 1996).

Conversely, mounting evidence suggests that chronic overproduction of CRH by the hypothalamus may be a causative factor in depression as well. Many researchers have become persuaded that CRH-producing neurons bear most of the responsibility for the emergence of depressive symptoms in both men and women who have major depression (Nemeroff, 1998). Some scientists now recognize two different types of depression that are linked to hypo- or hypersecretion of CRH and to a state of dysregulation of the HPA axis. So-called "melancholic depression", characterized by hypophagia and hyposomnia, seems to be linked to hypothalamic overproduction of CRH. The resultant hypercortisolemia is also a feature of exercise-induced amenorrhea and anorexia nervosa, conditions typically associated with stress, depression, and overproduction of CRH (Young and Korszun, 1998). "Atypical depression", characterized by irritability, hyperphagia, and hypersomnia, are apparently linked to the underproduction of hypothalamic CRH as described previously. Other associates of atypical depression, besides the postpartum period, include seasonal affective disorder and chronic fatigue/fibromyalgia syndrome (Cizza et al, 1997). In any case, both melancholic and atypical dysphorias underscore the key role that many believe CRH now plays in mood regulation.

Role of Tryptophan

There is also a possible relationship between a failure of the normal rate of rise in serum tryptophan after delivery and blues or PPD (Handley, 1980; Kendell, 1985). Tryptophan (Trp or W), is an aromatic amino acid that is essential in the production of

serotonin, an important neurotransmitter in the brain (Gundersen, 1996). Several amino acids are reduced in the first trimester of pregnancy, perhaps due to hormonal changes. In the later months of gestation, high amino acid demand by the fetus is another likely cause of maternal serum amino acid depletion (Handley, 1980). L-tryptophan loading does not seem to relieve the symptoms of PPD in women with low tryptophan levels. However, it is thought that supplementing these women's diets with pyridoxine (Vitamin B6), a cofactor required for the neuronal utilization of tryptophan, may be an effective therapy (Gundersen, 1996).

Additional evidence now links serum tryptophan levels to both serotonin production and prolactin levels in the brain. In 1987 Cowen and Charig reported that intravenous administration of tryptophan to depressed patients who had lost less than 10 pounds of weight caused a significant blunting in the expected rise of prolactin and growth hormone. In contrast, depressed patients who had lost more than 10 pounds of weight exhibited a significant rise in plasma prolactin over controls when they were challenged with intravenous tryptophan. Cowen and Charig interpreted these results to indicate an abnormal serotonin-mediated neuroendocrine pathway in depressed patients and claimed it correlated with cortisol hypersecretion (Cowen and Charig, 1987).

Role of Prolactin/Lactation

In women who choose to breast-feed, levels of prolactin and oxytocin are higher than in those who do not nurse. In addition to its role in facilitating breast development and milk production, prolactin suppresses ovarian steroids to some degree, and often prevents ovulation. It is secreted in bursts during suckling and the older terms of "lactational

psychosis" and "milk fever" have long implied a mysterious link between breast feeding and mental illness (Stein, 1982).

In order to appreciate the significance of studies of prolactin in depression, it is critical to understand the psychobiology of the regulation of its release. Aside from studies of lactation, where prolactin levels are treated as correlates of breastfeeding or merely as incidental, research on men and non-lactating women have shown that dysregulation in prolactin secretion is a neuroendocrine abnormality that is often seen in patients with major depression. Although prolactin secretion is complex and has not yet been completely elucidated, it is clear that some neurotransmitters that have been implicated in the pathophysiology of depression are involved in regulating its release. Probably the best known is dopamine, also sometimes called "prolactin inhibitory factor" (PIH). Hypothalamic dopamine release prevents the anterior pituitary from producing and secreting prolactin in men and non-lactating women (Guyton and Hall, 2000). PIH is discussed in a subsequent section.

Less well-known than PIH's connection to prolactin is the role of the monoamine serotonin. Researchers are now calling prolactin a "window to the brain" regarding discovery of the biochemical basis of depressive illness (Nicholas et al, 1998).

Evidence shows that serotonin plays a stimulatory role in prolactin release. In 1984 research with fenfluramine, an anoretic drug and serotonin reuptake inhibitor, showed that prolactin response to administration of this drug was less in depressed patients than in controls. The authors concluded that the serotonergic system is less responsive in depressed patients than controls (Siever et al, 1984). This report is admittedly somewhat tangential to the subject of postpartum depression, nor is it evidence that serotonin

stimulates prolactin release. However, perhaps an important conclusion that may be drawn from Siever's study is that prolactin somehow plays an integral role in mood, since it seems to be tied so directly to serotonin release.

Certain serotonin agonists like buspirone and gepirone increase prolactin secretion, although that is not true across the board for all serotonin agonists. Further, extracts from the hypothalamus, such as thyrotropin-releasing hormone, vasopressin, vasoactive intestinal peptide, oxytocin, peptide-histidine-isoleucine, and an unidentified factor from the intermediate lobe apparently have prolactin-releasing activity. A host of other potential candidates have yet to be verified. The point is that links between prolactin secretion or its lack thereof and levels of certain mood-altering neurotransmitters and/or hormones may play a significant role in mood state. If researchers can assert with confidence that abnormal prolactin levels correlate with mood dysphorias, it seems likely that the prolactin titer changes seen during pregnancy and the postpartum period might well be associated with mood dysphorias during this stage (Nicholas et al, 1998).

Studies in the past, which have attempted to link high prolactin levels with depression or the prevention of it, have met with mixed, confusing and disappointing results. In 1983 Alder and Cox reported that mothers who totally breast-fed their babies for at least 12 weeks or who were taking oral contraceptives, had a higher incidence of postnatal depression than those who were not on the pill or who partially breast-fed. Among the 62 women who attempted breast-feeding, those most likely to have normal levels of endogenous hormones were those least likely to have depressive symptoms. The authors carried out their research by questioning a sample of women in Edinburgh, Scotland, 1-2 years after they had given birth. However, since the reports were retrospective, plasma

hormone levels were not tested (Alder and Cox, 1983). In 1986, the same primary author found that changes in sexuality or mood in postpartum women were not related to levels of prolactin or estrogen or to the return of follicular activity, which was delayed in persistent breast feeders (Alder et al, 1986).

A later paper (Auerbach and Jacobi, 1990) criticized the results of Alder and Cox (1983), asserting that postpartum depression in that research was poorly defined. Further, the authors (Auerbach and Jacobi, 1990) stated that the literature on PPD and the literature on lactation intersected so infrequently and so poorly that it was impossible at that time to identify clearly the relationship between the two elements and/or which influences the other. They pointed out that in order to conclude that breastfeeding mothers are at greater risk for PPD because their hormone levels are abnormally high in some cases and abnormally low in others would require acceptance of a faulty assumption: that lactation is an abnormal state. Lactation is the normal postpartum state for which every woman's body is prepared to function after the birth of the baby (Auerbach and Jacobi, 1990). Further, results of Alder and Cox (1983) seem to conflict with the generally established notion that the release of oxytocin during suckling is associated with feelings of well-being and relaxation in many women (Pryor and Pryor, 1991).

Susman and Katz (1988) reviewed anecdotal evidence from four of their own patients, three of whom had to be hospitalized for severe mood dysphoria, that cessation of breastfeeding in some way related to the onset of severe depression. In each case depression did not begin until weaning was attempted. The authors underscore and reiterate earlier work that speculates that absolute plasma levels of certain hormones may

not be as important as relative and changing amounts and the ratios of the various subtypes of estrogen. Further, these researchers postulate that in most cases postpartum depression is the result of biologic and psychodynamic factors combined, as though a genetic predisposition of some sort makes a woman more vulnerable to depression following certain psychological stressors.

Stowe and Nemeroff (1995) suggested that failure to nurse might possibly be a risk factor for postpartum depression. Although "failure to nurse" was not specifically defined, the article implied that it was the inability of the mother-baby couple to achieve a successful feeding. There was no differentiation, however, between inability of the infant to suckle and inability of the mother to produce or let down milk. Stowe and Nemeroff (1995) concluded that absence of breastfeeding in and of itself was not a significant risk factor for postpartum depression, but the results were less than conclusive..

More recently, Dr. Shaila Misri, a psychiatrist with 20 years of clinical experience with obstetrical patients, observed an association between postpartum depression and the cessation of nursing. However, his retrospective study of 51 women who experienced true postpartum depression revealed that 83% claimed the depression began before the cessation of breast-feeding. One interesting finding of Misri's (1997) research was that the majority of women in the study insisted on continuing nursing even though their depression was severe. Since very few mothers were willing to give up breast-feeding regardless of their mood, appropriate pharmacotherapy became a dilemma, because antidepressants are secreted in maternal milk (Misri et al., 1997).

Fortunately, thus far, infant plasma concentrations of tricyclic antidepressants and SSRIs have usually been below the detection limit of commerical laboratories (Epperson, 1999). Effects of antidepressants secreted in breast milk on the neural development of the infant are not yet known (Misri et al., 1997), but evidence seems to suggest that infants can tolerate the exposure without difficulty (Epperson, 1999). A conservative approach for a breast-feeding woman taking an antidepressant is to nurse the baby immediately prior to taking the medication or to pump milk when drug levels are likely lowest, then use that milk to bottle-feed when drug levels are expected to be higher (McCoy, 2001).

Role of Dopamine (PIH)

The major prolactin inhibitory factor (PIH) from the hypothalamus has been identified as dopamine. Dopamine acts via D2 receptors located on the lactotroph cells of the anterior pituitary gland. Dopamine inhibits prolactin secretion in several ways. First, dopamine inhibits the exocytosis of prolactin-containing granules in lactotroph cells via a G-protein-linked second messenger system that stops the production of cAMP. Without cAMP, calcium-mediated action potentials across the cell membrane cannot occur, and these are required for exocytosis of the prolactin-filled vesicles. Further, dopamine inhibits lactotroph cell division, and it also stimulates the self-digestion of prolactin-containing granules, a process known as crinophagy (Nicholas et al, 1998). Keeping in mind preceding information linking prolactin to mood state, dopamine's effect on prolactin secretion may tie it indirectly to depressive illness.

Dopamine (PIH) has also previously been reported to inhibit TSH release from the anterior pituitary (Hedge et al, 1987). Insufficient TSH eventually may lead to low

thyroid hormone output. Depression is a well-established consequence of hypothyroidism (Ascoli and Segaloff, 1996).

Role of Triiodothyronine/Thyroxine

There may be a relationship between thyroid dysfunction (lower free T3 and T4 levels) and PPD (Pedersen, 1993). Depression is often associated with hypothyroidism (Ascoli and Segaloff, 1996). In fact, a correlation between myxedema and depression following childbirth was recognized and successfully treated with animal thyroid extract as far back as 1888 (Cox and Holden, 1994). Alternatively, depression may also be a symptom of hyperthyroidism, although it is not nearly as common. The syndrome that can accompany excess serum levels of thyroid hormone includes apathy, lethargy, and depression, along with weight loss and tachycardia. Traditional therapy with antidepressants is not generally effective. Depression with hyperthyroidism is thought to be caused by depletion of catecholamines resulting from continual sympathetic nervous system stimulation (Leigh and Kramer, 1984).

During the postpartum period there is a gradual decrease in thyroid gland volume but a more rapid reversion of thyroxine-binding globulins to normal (Harris, 1993). It follows that T3 and T4 are slightly elevated for a time after pregnancy. However, a short time later, peaking at four to six months postpartum, transient hypothyroidism is found in 4% to 7% of postpartum women (Susman, 1996). It is known that approximately 5% of women develop thyroiditis postpartum (Kendell, 1985; McPherren et al, 1995). Postpartum thyroiditis is believed to be an autoimmune disorder, and antimicrosomal thyroid antibodies are typically present. This disorder tends to progress from early

hyperthyroidism one to three months postpartum, through a euthyroid phase, to a hypothyroid phase three to eight months postpartum (McPherren et al, 1995).

The enzyme that converts thyroxine (T4) into the more potent T3 is Type I 5-deiodinase. It is inhibited by glucocorticoids (Farwell and Braverman, 1996), which may be elevated during the perinatal period (Kendell, 1985; Okano and Nomura, 1992; Pedersen et al, 1993). It seems plausible that the lower levels of T3 present postpartum, perhaps in combination with low postpartum levels of sex steroids, could predispose postpartum women to PPD.

Most of the reported cases of both thyrotoxicosis and postpartum hypothyroidism during the postnatal period present with anxiety and depression (Stein, 1982). However, thyrotoxic patients can even present with symptoms that would suggest panic disorder (Susman, 1996). Interestingly, depression and anxiety are often associated with both hypo- and hyperthyroidism. Anxiety, however, is understandably more common in hyperthyroidism than in hypothyroidism, because many of its symptoms tend to be associated with hyperactivation of the nervous system. Cardinal features of anxiety include, among others, motor tension, inability to relax, and gastrointestinal complaints, all common complaints of postpartum depression (Leigh and Kramer, 1984).

It is also possible for women to have depressed mood with so-called "subclinical hypothyroidism." Subclinical hypothyroidism can be defined as the presence of normal thyroxine with elevated TSH. In fact, some 40% of subclinically hypothyroid patients—mostly women—have significant depressive symptoms (Pies, 1997). Custro et al. (1994) suggested that subclinical hypothyroidism might lower the threshold for the occurrence of depression or exacerbate depressive symptoms. When this variable is added to the

stresses and physical and mental adjustments of early motherhood, postpartum depression could well result.

Role of Antithyroid Antibodies

Autoimmune thyroiditis has two forms: a goitrous form and an atrophic form. In both cases thyroid autoantibodies can be found in serum. Apparently, activated T cells stimulate B cells to produce antibodies against thyroglobulin, thyroid microsomal enzyme (or peroxidase), and/or the TSH receptor. For testing purposes, only antimicrosomal antibodies are usually measured by enzyme-linked immunoassay (ELISA) or radioimmunoassay (RIA), in order to establish the presence of chronic autoimmune thyroiditis (Pies, 1997; Dayan and Daniels, 1996).

Autoimmune thyroiditis is strongly familial, but it can also be brought on by environmental factors such as excess iodine intake. A subclinical hypothyroid state characterized by serum thyroid antibodies and sometimes also elevated thyrotropin can persist for years before progressing to include low thyroxine. Mild, silent, transient postpartum thyroiditis is often a manifestation of chronic autoimmune thyroiditis (Dayan and Daniels, 1996). Elevated levels of antithyroid antibodies postpartum are associated with depression (Pies, 1997). No association was found between the presence of auto-antibodies and diagnosed depressed mood by Harris et al (1989), but an excess of depressive symptoms (though not major depression) was reported (Harris et al, 1992) in women who were positive for thyroid antibodies in the first eight months after delivery. That was true even when conventional tests for thyroid dysfunction did not show abnormalities. Results also showed that antibody-positive women had higher mean scores for depression on several scales regardless of whether they had become

hypothyroid. Harris concluded by speculating that cytokines may be released as thyroid antibody concentrations rise after delivery, and that these substances have an effect on the brain to cause many of the behaviors associated with postpartum depression.

Custro et al. (1994) showed that of nine patients recruited into their study who were suffering from major depression, five were subclinically hypothyroid. All five of these women tested positive for antibodies against thyroglobulin and/or thyroid peroxidase, revealing a symptomless autoimmune thyroiditis. In contrast, none of four euthyroid severely-depressed and none of 66 of the euthyroid minor-depressed subjects were seropositive for thyroid autoantibodies. Custro et al (1994) suggested that major depression is accompanied by unrecognized hypothyroidism in a significant proportion of women. Therefore, the possibility of autoimmune disease should be considered whenever depressed women display biochemical thyroid dysfunction.

Researchers estimate that about three in 100 postpartum women will experience depression that is related to positive thyroid antibody status and presence of normal blood levels of total and free T3/T4. Possible reasons for these findings include poor methodology and a general malaise due to the thyroiditis that is unrelated to actual thyroid hormone levels. Support for the latter hypothesis was found when T4 was administered prophylactically to thyroid-antibody positive women in the postpartum period. These women were found to develop depression just as often as their antibody-positive counterparts who did not receive T4. The authors of that study concluded that depression occurring in thyroid antibody positive women was likely associated with malaise that followed the thyroid antibody positive state rather than thyroid dysfunction (Harris, 1999).

Another hypothesis for the etiology of depression mediated by thyroid antibodies maintains that activated leukocytes produce cytokines which cross the blood brain barrier, attach to specific receptors, and mediate neurotransmission, thus possibly affecting mood (Leonard and Song, 1999). Indeed, cytokines have been implicated in the pathophysiology of the depression that is often associated with both infectious and non-infectious diseases, such as autoimmune conditions. Immune dysregulation has been found to precede the development of depression in some cases, which suggests that depression is not a psychological reaction to the illness, but rather causally related to immune activity. Further evidence for the role of cytokines in depressive symptoms can be found in studies in which cytokines are administered exogenously to volunteers. In one study, almost immediately upon receiving alpha interferon, interleukin-2, or tumor necrosis factor-alpha (TNF-α), subjects experienced the following symptoms: depressed mood, dysphorias, anhedonia, helplessness, mild to severe fatigue, anorexia and weight loss, hypersomnia, psychomotor retardation, decreased concentration, and confusion, all of which are associated with clinical depression (Yirmiya, 1998).

Psychosocial and Circumstantial Risk Factors

In addition to the key role physiology and endocrinology seem to play in mood state, certain psychosocial risk factors have been shown to correlate with the incidence of PPD, including primiparous pregnancy (Gundersen, 1996; Kendell, 1985; Nott et al, 1976), and personal history of depression or first-degree relative with depression (Gundersen, 1996; Kendell, 1985). Conversely, multiparity was cited as a risk factor for PPD (Righetti-Veltema et al., 1998). In another study, prenatal depression and lack of postpartal closeness to the husband correlated with postpartal depression, and together the two

accounted for nearly 40% of its variance (Logsdon et al, 1995). Other predisposing factors may include a problematic marital relationship (Cox et al., 1982; O'Hara, 1986; Susman, 1996; Epperson, 1999), depressive symptoms during pregnancy (Righetti-Veltema et al., 1998; Yonkers and Chantilis, 1995), high life stress, and obstetric complications at the time of delivery (O'Hara, 1986; Yonkers and Chantilis, 1995). Poverty is also a clear risk factor, as it has been found to be associated with more than twice the accepted rate of occurrence of PPD (Hobfoll et al, 1995). Coping difficulties may also be a factor. Righetti-Veltema et al. (1998) reported the incidence of separation from families countries, emotional upsets, and loss of job during pregnancy was greater in depressed than non-depressed mothers.

In a review of the medical records of 35 psychotic pregnant women hospitalized between 1979 and 1984 for nonorganic, psychotic symptoms, Rudolph and colleagues reported that the majority of the women with psychosis during pregnancy had repeated hospitalizations, lacked social and economic supports, had repeated pregnancies without benefit of contraceptive planning, and lacked proactive planning for child care arrangements during and after hospitalization (Auerbach and Jacobi, 1990). However, there is much firmer evidence for a consistent incidence of postpartum psychosis across cultural and ethnic divides than there is for blues or PPD; this observation, together with clinical data and historical evidence of an unchanging incidence rate of postpartum psychosis during the past 150 years, points to a primarily endogenous etiology for the psychoses, which may be triggered by the physiology of childbirth (Kumar, 1994).

A study of 327 Jewish Jerusalem women in 2000 showed an inverse relationship between orthodox affiliation and postpartum depressive symptomology. Scores on the

Edinburgh Postnatal Depression Scale tended to be higher in women with secular, rather than religious, orientation. Further, the researchers found psychiatric history, immigrant status, and poor support with newborn care to be other important predictors of depressive symptoms (Dankner, 2000).

A recent study in Turkey reported that high parity, defined in the study as having an average of 4.6 children, long marriage period, and low education level were all significantly associated with low mood in the first week after birth. In particular, grandmultiparity, short inter-pregnancy interval, and low educational level were found to have the most important effects on postpartum blues and/or depression. The researchers concluded that pregnant women with these risk factors should be screened early in the antenatal period and given education and social support to help them cope (Gurel and Gurel, 2000.)

Past History as a Predictor for At-risk Women

Women with a history of mood dysphorias are at increased risk of postpartum mood disturbances. A new mother who has experienced major depression at sometime in the past has a 30% risk of relapsing during the postpartum period (Nonacs, 1998). Mothers who previously have had PPD have a 50% risk for developing it again with subsequent children. Those who have had postpartum psychosis run a 70% risk of recurrence with future births (Nonacs and Cohen, 1998).

Purpose of Study and Hypothesis

The purpose of this study was to make a careful, but broad search for a particular physiological correlation for postpartum depression. The researchers hypothesized that specific, identifiable fluctuations in the level of one or more of the hormones to be tested

would be associated, at least in part, with depressive mood disorders commonly seen in women after childbirth. In particular, it was anticipated that exceptionally low progesterone and/or estradiol/estriol levels postpartum might correlate well with depressive symptomology. High postnatal cortisol was also expected to be a correlate of depression. Low postnatal prolactin associated with failure to breastfeed was another suspected correlate of low mood, as well as low postnatal T4 and presence of thyroid autoantibodies.

This research design contrasted with what had been done in the past in that more hormones were simultaneously tested and data analysis was specifically focused on searching for a *combination* of plasma hormone levels that may be related to mood dysphoria.

CHAPTER THREE

METHODOLOGY

Subjects

Subjects were patients from the OSUCHS clinic or the OSU Physicians clinic in Tulsa, Oklahoma. Each was over 18 years of age, in her third trimester of pregnancy, not expecting twins, and negative for drugs detected by a urine drug screen except, in some cases, tetrahydrocannabinol (THC). Further, the recruits said they had no reason to suspect that they might deliver by Cesarean section, and each scored below the threshold of depression on the Edinburgh Postnatal Depression Scale.

Rating Scales

Edinburgh Postnatal Depression Scale

The Edinburgh Postnatal Depression Scale (EPDS) was developed in Scotland in 1987. It is a 10-item self-report scale that has been used for many studies on postpartum depression and is easy to score. The authors reported that the sensitivity of this test is 86% and the specificity is 78%. A threshold score of 12/13 is used to screen for possible depression (Cox et al, 1987). One drawback to having a subject repeatedly take a self-scoring exam is the possibility for a drop in scores over time as a result of a retest effect. This problem is potentially serious in research in which comparison of symptom levels across time is critical (O'Hara et al, 1984). The original design for this study called for six postnatal mood assessments in the first 12 weeks after birth, but to minimize the retest effect and to increase compliance, the number was reduced to four.

Although the designers of the EPDS suggest that 12/13 be the cutoff scores for depression screens in research, they recommend a threshold of 10 if the exam is intended

as a screen to be used by paramedical personnel (Cox et al, 1987). One study in Geneva using the French translation of the EPDS "privileged the specificity of the diagnosis" and used 11/12 instead of 12/13 when women who scored a 12 gave "answers evocative of depressive symptoms" (Righetti-Veltema et al, 1998). This decision was made in light of a French paper that used the EPDS and recommended a score of 12 be used for research purposes and 11 for clinical ones. An earlier Australian study validated the EPDS cut-off score of 12.5 as being 100% sensitive for detecting major depression in nine postpartum women out of a group of 103. The authors also reported a specificity of 95.7% and a predictive value of 69.2% (Boyce et al., 1993).

Research using data from the Olmsted Medical Center and the Mayo Clinic in Rochester, Minnesota, revealed that routine screening for PPD with the EPDS was associated with more than doubling the rate of diagnosis of PPD in that population (from 3.7% to 10.7%). The authors of that study concluded that a high EPDS score was reliably predictive of a diagnosis of PPD, and they highly recommended the routine use of the EPDS at six weeks postpartum (Georgiopoulos et al, 2001). In light of the increasing popularity of the EPDS, it was chosen for use in this study (Appendix A).

It must be emphasized that the EPDS is a screen for depression, not a test that definitively diagnoses the condition. The accepted standard for diagnosing depression with certainty is the *Diagnostic and Statistical Manual of Mental Disorders-Text Revision,* 4th edition *(DSM-IV-TR)*. As mentioned previously, that text lists nine symptoms that are characteristic of a major depressive episode. At least five of those criteria must be present most of the day, nearly daily for at least two weeks in order to determine whether an individual is clinically depressed (Susman, 1996). These criteria

are an echo of the landmark "Research Diagnostic Criteria" publication by Spitzer et al, 1978. That paper listed eight symptoms characteristic of major depressive disorder and recommended that certain diagnosis be made on the basis of the presence of at least five of the eight symptoms for a period of at least two weeks. Probable diagnosis should be made if four symptoms are present for a period of at least one to two weeks (Spitzer et al, 1978). Certain diagnosis goes beyond the scope of the present study, so in light of the high sensitivity and specificity of the EPDS, as well as the ease of administering it, its results were allowed to stand alone. Volunteers who scored higher than 12 were immediately referred to their physicians for evaluation and/or treatment.

Originally, as its name suggests, the EPDS was devised to test women after they gave birth. Since that time, it has been validated for use during pregnancy, and the authors have suggested that its name be changed to "Edinburgh Perinatal Depression Scale" or "Edinburgh Depression Scale." It may even eventually be used to screen for clinical depression at times other than the perinatal period or to identify depressed fathers (Cox and Holden, 1994).

Visual Analog Scales

Although the EDPS is a useful tool for screening for depression, it is not designed to detect or quantify baby blues. Therefore, another self-scoring exam based on the work of Kendell et al (1981) was used to detect mood dysphoria that is not severe enough to be characterized as depression. The Visual Analog Scales consist of six individual scales on which subjects rate various mood states (Appendix C). One of the scales is positive and five are negative. Each test consists of a 10-cm line which has been labeled "not at all" on one end and "worse (better) than I have ever felt before" on the other. The examinee

rates her mood for the past 24 hours by placing a pencil dot somewhere along the continuum as a representation of her subjective rating of her feelings (O'Hara et al, 1990). Distance from the left end of the line can then be measured by the examiner and treated as the "score" the subject received on that particular question. A graph can be generated in which the patient's score is represented on the "y" axis and time in days is represented on the "x" axis. Visual analog scales have been used in previous studies of postpartum blues and depression (Cox et al, 1983; Kendell et al, 1981). Kendell (1981) suggested a separate graph for each question and generated them as described above.

Volunteers were given the VAS at about 36 weeks gestation when they came to the clinic for a regular prenatal appointment. Researchers, their assistants, or clinic staff explained the VAS, told the women to fill one out daily at the same time for 21 days, and reminded the women that they should pack the scales to take to the hospital, so that even the first few days after birth would be properly filled out. Volunteers were also told to return the VAS to the clinic when they came for their four-week postpartum follow-up appointment.

Procedure

Approximately 200 women total were recruited from the OSUCOM and the OSU Physicians clinics in Tulsa, Oklahoma. On days that the clinic saw obstetrics patients, we went through the roster of appointments and selected women to approach about the study that were over age 18 and already in their third trimester. If the patient was interested in being in the study, her urine was tested by employees of the in-house lab for evidence of amphetamines, barbiturates, benzodiazepines, cocaine, opiates, phencyclidine metabolites (PCP), tetrahydrocannabinol (THC), and tricyclic

antidepressants. If the drug screen was negative, the volunteer was given the EPDS. Several women were eliminated due to presence of THC in their urine, but entry criteria were relaxed as the study progressed, and eventually, four women who were positive for only this drug were allowed to remain in the study.

If the woman's initial EPDS score was below 13, she was enrolled. A lab technician then drew her blood if she was past 34 weeks gestation. Otherwise, the draw was postponed until further into the pregnancy. Of the approximately 200 women who were screened, about 120 were admitted into the study. After attrition due to various causes, 75 subjects finished the project. Each of these subjects met the major inclusion criteria of testing below 13 on the EPDS during her third trimester, a probable indication that she was not depressed (Cox and Holden, 1994).

The other inclusion criteria were established based on previous studies from other laboratories to facilitate comparison of results. Qualified subjects were at least 18 years of age, in agreement with several of the papers referenced for this research (Cowen and Charig, 1987; Gurel and Gurel, 1999; O'Hara et al, 1984; O'Hara, 1986; O'Hara et al, 1990; O'Hara et al, 1991). Further, the volunteers were not currently depressed nor taking prescription or illegal medications, except THC. Volunteers also had to be carrying only one baby and have reason to believe they would deliver vaginally. They were questioned directly to obtain this information.

Once before the birth of her child at about 36 weeks gestation, each woman's blood was drawn at the clinic where she was receiving her prenatal care. This was considered Time A. The blood was immediately spun and frozen and later taken to OSU College of Osteopathic Medicine to be stored in a laboratory freezer at –80 degrees Celsius until

testing. Assays to determine plasma concentrations of the various substances were performed at Regional Medical Laboratory of St. John's Hospital in Tulsa, Oklahoma. Levels of TSH, free T4, estriol, progesterone, cortisol, prolactin, and antibodies against thyroid peroxidase and thyroglobulin were all reported. If possible, a blood sample was also taken within 24 hours (Time B) of the volunteer's arrival at Tulsa Regional Hospital to give birth. After the birth the woman was again tested at 1, 4, 8 and 12 weeks postpartum for plasma levels of the same substances, except that estradiol was substituted for estriol. These blood draws were designated Time C, Time D, Time E, and Time F, respectively. The total duration of participation for an individual, then, was approximately 16 weeks. The clinic where she had received her prenatal care did each of the postpartum blood draws, as they had done the prenatal one. Except for the time of birth, each time the blood was taken, the subject again took the self-scoring EPDS exam. Note was made of whether she was breastfeeding exclusively, partially, or not at all.

Compensation

To encourage compliance, cover transportation costs, and compensate for time and other expense incurred, the women were paid $120 in a lump sum upon completion. Complete disclosure regarding payment was made at the time of enrollment. For each missed blood draw, $20 was deducted from the total. This approach was similar to that of previous researchers whose objectives most closely matched those of this study (O'Hara et al, 1984). If a volunteer's participation was terminated before the end of the study due to her likely development of depression as evidenced by EPDS score of >12, she was paid the same as if she had completed the entire protocol. However, no one in the study was told ahead of time that she would be dropped and paid in full if she

developed depression and elected on the advice of a physician to take a prescription medication.

Confidentiality and Informed Consent

Patient confidentiality was closely guarded. Individuals' identities were kept private by assigning random numbers for data analysis at the hospital laboratory. Questionnaires, plasma hormone level data, and any other pertinent information was kept in a locked file at the OSU Physicians' Medical Clinic. Once recorded, data were reported in the completed study as mean scores of all participants. The protocol and informed consent were reviewed and approved by the Oklahoma State University Center for Health Sciences Institutional Review Board (Appendices E and F).

Instruments

The prolactin and TSH tests were immunoassays that used direct chemiluminescence in a two-site sandwich immunoassay. With this method there is a direct relationship between the amount of prolactin in a sample tested and the amount of relative light units detected by the system, a Chiron Diagnostics ACS:Centaur.

The cortisol and free T4 tests were competitive immunoassays that used direct chemiluminescence. The hormone to be tested competed with acridinium ester-labeled cortisol or free T4 for binding to polyclonal rabbit antibody against the hormone of choice. With this method an inverse relationship exists between the amount of hormone in a sample tested and the amount of relative light units detected by the system, a Chiron Diagnostics ACS:Centaur. The instrument was carefully calibrated before each batch of tests was run, by testing control samples sent from the manufacturer.

The estradiol and progesterone tests were performed on an Immulite Analyzer. Both used direct chemiluminescence. The test for estradiol was a competitive immunoassay, while the test for progesterone was a sequential immunometric assay. An inverse relationship exists between the amount of estradiol in a sample tested and the amount of relative light units detected by the Immulite. With this method there is a direct relationship between the amount of progesterone in a sample tested and the amount of relative light units detected by the Immulite. Before each batch of tests was run, this instrument was carefully calibrated by testing control samples sent by the manufacturer.

The tests for both thyroglobulin antibody and peroxidase antibody were performed using a Biochem Personal Lab machine with kits and reagent from Wampole Laboratories. These kits employ the enzyme immunoassay (EIA) method. The machine was carefully calibrated before each batch was run, by testing control samples sent from the manufacturer.

The estriol test was not performed in-house at Regional Medical Laboratory of St. John's Hospital. Instead, it was sent to Quest Diagnostics, Incorporated, in San Juan Capistrano, California. There, extraction radioimmunoassay was performed to determine estriol concentrations. Information on the type of machine used was not available to Regional Medical Laboratory.

Statistical Analysis

Various portions of the results of the data collected were analyzed by multiple stepwise regression, simple linear regression, simple linear regression of differences, two-tailed t-test, and Chi Square. In the multiple backward stepwise regression, the scores on the EPDS were treated as the response or dependent variable, and hormonal

data as independent variables. The Backward Stepwise Technique was used to fit full model "all predictors". Insignificant predictors were removed one at a time until all predictors present were statistically significant. The hypothesis was that the final model should be able to predict an EPDS score based on independent variables and, thus, predict whether or not the subject is likely to be depressed.

Simple linear regressions were performed for each of independent variables separately to determine the effects these variables have on depression scores. Regressions were run each time data were collected. Contingency tables, expressing the relationship of the categorized depression (yes/no) to lactation (yes/no), were created for each time period. Chi square tests were performed to assess whether or not lactation occurred with more frequency with depression as determined by the EPDS scores. Chi square tests were also performed to determine whether there was a difference in the answers to questions 4 and 5 on the EPDS, dealing specifically with anxiety, between women who had a higher than normal prenatal progesterone level and those whose progesterone levels were not above the accepted ranges for normal. Two-tailed t-tests were performed by dividing the women into "depressed" and "not-depressed" at each of the postnatal time points and comparing hormone averages between the two groups.

CHAPTER FOUR

RESULTS

Demographics

Sixty-one of the 75 women who finished the study reported their age. The mean age of this subset of participants was 25 years old, and the range was 18-49. Sixty-six of the 75 volunteers reported marital status. Of those, 32 were married, three were divorced, five were separated, 26 were single, and none was widowed.

Table I summarizes the average EPDS scores obtained at 36 weeks gestation, as well as at 1, 4, 8, 12 weeks postpartum. The number of women reporting varied and was never as high as the total who participated, 75. Although all of the women admitted into the study were properly screened for depression prenatally, on some occasions the actual, numerical, prenatal EPDS scores were not recorded (Table I).

Table II provides information concerning the percentage of women whose EPDS scores fell within the suggested limitations for determining "not depressed", a score below 13. Percentage of women scoring within the range for probable clinical depression was somewhat arbitrarily broken down further into two ranges, 13-20 and 21-30, in order to provide a look at the differing numbers of those with severe depressive symptomology and those with probable depression of a less severe nature.

Table III provides a comparison of hormone ranges and means at each of the time points of the study with published normal range and average hormone values. Standard deviations for each of the study averages are also included. Units used throughout the results section to measure serum hormone concentrations are those used by clinicians and hospital laboratories for reference (Bakerman, 1994).

TABLE I

MEAN EPDS SCORES ACROSS TIME

	N	Mean EPDS*	Range of EPDS**
Prenatal EPDS	56	6.0	0-12
One Week EPDS	28	7.4	1-22
Four Week EPDS	56	6.5	0-23
Eight Week EPDS	38	5.6	0-25
Twelve Week EPDS	34	5.3	0-19

*A summary of the average Edinburgh Postnatal Depression Scale (EPDS) scores of volunteers for the time periods at which this test was administered are given.
**Range is the lowest to the highest reported score for that time period. (Lowest possible score is 0, and highest possible score is 30.)

TABLE II

EDINBURGH POSTNATAL DEPRESSION SCALE SCORES
BROKEN DOWN BY CATEGORY—SHOWN BY PERCENT*

	N	% Scoring 0-12	% Scoring 13-20	% Scoring 21-30
Prenatal	56	100	0	0
One week	28	78	18	4
Four weeks	56	82	16	2
Eight weeks	38	89	8	3
Twelve weeks	34	91	9	0

*Percentage of study participants whose scores on the Edinburgh Postnatal Depression Scale (EPDS) fell below 13, between 13 and 20, and greater than 21. Statistics are shown for each of the time periods surveyed.

TABLE III

COMPARISON OF NORMAL HORMONE RANGES
WITH STUDY RESULTS

36 Weeks Gestation

	Normal Range*	Study Range	Mean	S.D.**
TSH	0.47-6.8 mIU/mL	0.441-4.952	1.797	0.91
Free T4	0.65-1.5 ng/dL	0.570-1.14	0.809	0.11
PRL	9.7-208.5 ng/mL	25.600-369.000	161.061	66.0
Cortisol	2-25 mcg/dL	10.700-34.100	20.137	5.39
PGN	8,000-20,000 ng/dL	1,698-26,139	15,185	4,591
Estriol	7.2-28.0 ng/mL	2.050-28.150	10.216	4.79

One Week Postpartum

	Normal Range*	Study Range	Mean	S.D.**
TSH	0.47-6.8 mIU/mL	0.342-2.907	1.252	0.66
Free T4	0.65-1.5 ng/dL	0.81-1.33	1.061	0.14
PRL	2.8-208.5 ng/mL	9.4-330.3	94.773	75.5
Cortisol	2-25 mcg/dL	5.4-34.5	15.232	6.0
PGN	<150->2,000 ng/dL	0.043-369	55.274	63.3
Estradiol	30-400 pg/mL	7.157-261	30.23	42.4

Four Weeks Postpartum

	Normal Range*	Study Range	Mean	S.D.**
TSH	0.47-6.8mIU/mL	0.113-10.121	1.419	1.3
Free T4	0.65-1.5 ng/dL	0.0940-1.360	0.989	0.18
PRL	2.8-208.5 ng/mL	3.300-226.900	36.563	44.5
Cortisol	2-25 mcg/dL	3.700-24.600	9.450	3.75
PGN	<150->2,000 ng/dL	0-1,392	78.92	1,078
Estradiol	30-400 pg/mL	2.364-1,772	73.5	1,204

Eight Weeks Postpartum

	Normal Range*	Study Range	Mean	S.D.**
TSH	0.47-6.8 mIU/mL	0.493-10.176	1.568	1.45
Free T4	0.65-1.5 ng/dL	0.700-1.310	1.020	0.13
PRL	2.8-208.5 ng/mL	3.200-286.200	35.432	48.6
Cortisol	2-25 mcg/dL	4.000-26.500	10.998	4.78
PGN	<150->2,000 ng/dL	0.048-1,036	83.0978	203.5
Estradiol	30-400 pg/mL	5.206-276.000	35.920	52.2

Twelve Weeks Postpartum

	Normal Range*	Study Range	Mean	S.D.**
TSH	0.47-6.8 mIU/mL	0.5-34.7	2.278	5.2
Free T4	0.65-1.5 ng/dL	0.52-1.34	1.018	0.15
PRL	2.8-208.5 ng/mL	3.2-237.4	32.43	44.6
Cortisol	2-25 mcg/dL	3.0-21.9	10.71	4.7
PGN	<150->2,000 ng/dL	0.0-1356	109.64	282
Estradiol	30-400 pg/mL	0.0-222	36.30	43.5

*From laboratory kit package inserts and Bakerman, 1994.
**S.D.=Standard Deviation

Multiple Backward Stepwise Regression

The purpose of running any regression is to determine if there is a correlation between two variables such that the value of one can be used to predict the value of the other. For each variable, a so-called "F-statistic" is calculated, which is determined by dividing the estimate of the variability (square of the standard deviation) that the variable in question explains by the estimate of the remaining unexplained variability for all variables in the model. In a multiple backward stepwise regression, only variables whose F-statistics are considered significant remain in the model. Since the independent variables, hormone levels in this case, are sometimes themselves highly correlated with each other, the multiple regression is an appropriate test to run. This test allows for holding constant all significant independent variables except the one being considered, while examining the relationship between one hormone and its potential correlate or dependent variable, EPDS score (Steel, Torrie and Dickey, 1997).

Serum substances tested included prenatal estriol, postnatal estradiol, cortisol, progesterone, prolactin, TSH, free T4, thyroglobulin antibodies, and thyroid peroxidase antibodies. The manufacturer of the estradiol test stated that prenatal estriol would cross-react with the test for estradiol, thus artificially elevating estradiol levels. Therefore, only prenatal estriol was reported. In addition, subjects were asked if they were breastfeeding at 1, 4, 8 and 12 weeks. Data from the blood draw taken at birth were not used, because compliance was too low for meaningful analysis.

Significance for P values was set at < 0.15. Tests utilizing the rigorous standard of significance, $P < 0.05$, that are performed on small sample sizes can sometimes fail to detect significance that actually exists in the general population. Further, in the

backwards stepwise regression, $\alpha = 0.15$ is not unusual in that "PROC STEPWISE" in SAS, the command for running a multiple backward stepwise regression in a popular computer program for analyzing statistical data, uses $\alpha = 0.15$ as a default value. A type I error in statistics, defined as rejection of a true null hypothesis (Steel, Torrie, and Dickey, 1997), is not a dangerous thing in research of this nature, because of minimal risk. Further, it may lead to discovery of weak correlations between variables that would otherwise be missed.

Another adjustment made in the data analysis was the decision to use "maximum postnatal EPDS score". The original intent had been to examine hormone levels at each time period versus their corresponding EPDS scores at that same time, and those results are presented for one and four weeks postpartum—the times at which the most women were above the EPDS minimum score for depression. However, it was determined that if some of the correlations were actually the results of a delayed cause and effect of hormone on mood, the EPDS scores that reflected their effects might occur at some later time point than the one in which the hormone level was obtained. Further, cause and effect aside, certain interesting correlations might exist in which high postnatal EPDS scores preceded the hormone levels that were significantly associated with them. Therefore, pre- and postnatal hormone values were run against the maximum EPDS score obtained at any time point in the data set after a woman gave birth, an examination of potential postnatal correlations, so to speak. If compliance was minimal, that might translate to only one EPDS score available. It was unfortunate there was not a complete data set on each patient, and in some cases EPDS scores that might have even been

higher were missed, because a volunteer did not come in to be tested. However, this particular method of analysis proved workable given the situation.

Prenatal Blood Test (Time A)

Increases in prenatal cortisol correlated with significant increases in maximum depression scores (P = 0.0727) (Table IV). Increases in prenatal progesterone resulted in significant increases in maximum depression scores (P = 0.0360) (Table IV). Decreases in prenatal estriol correlated with significant increases in maximum depression scores (P = 0.0516) (Table IV).

The correlation coefficient (r) is 0.353. Another statistical calculation that may be useful is the coefficient of determination. This value, which is the square of the correlation coefficient and is designated "r^2", is an estimate for the percent of variability of y that the statistical model, or all of the variables in it, explains. In the multiple backward stepwise regression of data collected at 36 weeks gestation, then, there is only one coefficient of determination, even though there are three independent variables for which were obtained significant probability values. The result is: $r^2 = 0.1245$ (Table IV). In other words, about 12 ½% of the variability of EPDS scores can be explained by the variability in prenatal levels of serum estriol, cortisol, and progesterone. No increases or decreases of any other serum prenatal hormone or antibody levels correlated with increases in maximum depression scores (Table V).

TABLE IV

A SUMMARY OF SIGNIFICANT RESULTS (P < 0.15)
OF CORRELATION BETWEEN VARIOUS SERUM
HORMONE LEVELS AND MAXIMUM POSTNATAL MOOD TEST* SCORES
USING MULTIPLE BACKWARD STEPWISE REGRESSION

	Significant Variable	N	Parameter Estimate**	Std. Error	P value	r^2***
Prenatal	Cortisol	58	0.28984	0.15839	0.0727	0.1245
	E3****	58	-0.34402	0.17290	0.0516	
	PGN	58	0.00041303	0.00019219	0.0360	
Four Weeks	TSH	46	3.18554	1.11824	0.0067	0.2885
	Free T4	46	14.56071	6.00727	0.0196	
	Thyroid Ab	46	7.54419	2.21706	0.0015	
Eight Weeks	TSH	29	2.88991	1.59268	0.0807	0.1711
	Thyroid Ab	29	6.22138	3.48043	0.0851	

*Mood test administered was the Edinburgh Postnatal Depression Scale (EPDS).

**Parameter Estimate is sometimes referred to as "partial slope". It is the change in the dependent variable (y) divided by the change in the independent variable (x) or "rise over run", but with the stipulation that all other variables in the model are held constant. Parameter Estimate is included here, because it conveys the relationship of y to one particular x. However, since this is a multiple backward stepwise regression, the x in question could be related to the other x's in the model. Therefore, this value may not be shown as the steepness of a line on a graph. That is why the simple regressions were also performed.

***r^2 is the coefficient of determination, or percent of variability in EPDS scores that can be explained by the statistical model. It is reported for the entire test, rather than for individual variables, and is the square of the correlation coefficient.

****E3=serum estriol; PGN=serum progesterone; PRL=serum prolactin; TSH=serum thyroid stimulating hormone; Free T4=serum free thyroxine; Thyroid Ab=presence of serum antibodies against either thyroid peroxidase or thyroglobulin.

TABLE V

A SUMMARY OF NON-SIGNIFICANT RESULTS (P > 0.15)
OF CORRELATION BETWEEN VARIOUS SERUM HORMONE LEVELS
AND MAXIMUM POSTNATAL MOOD TEST* SCORES
USING MULTIPLE BACKWARD STEPWISE REGRESSION

	Variable	N	P value
Prenatal	Thyroid Ab**	58	0.9494
	TSH	58	0.4803
	Free T4	58	0.3929
	PRL	58	0.4418
One Week	Thyroid Ab	17	NA***
	Free T4	17	0.8276
	PRL	17	0.4886
	E2	17	0.7992
	Cortisol	17	0.5036
	PGN	17	0.4659
	Lact	17	0.5940
	TSH	17	0.4217
Four Weeks	Lact	46	0.7554
	PGN	46	0.8600
	Cortisol	46	0.9293
	E2	46	0.5545
	PRL	46	0.3415
Eight Weeks	PGN	29	0.8436
	Cortisol	29	0.8058
	Free T4	29	0.7096
	Lact	29	0.4107
	E2	29	0.3638
	PRL	29	0.2145
Twelve Weeks	Thyroid Ab	30	0.9544
	E2	30	0.4286
	PRL	30	0.4195
	PGN	30	0.3944
	Cortisol	30	0.2522
	Lact	30	0.5969
	TSH	30	0.4409
	Free T4	30	0.2795

*Mood test administered was the Edinburgh Postnatal Depression Scale (EPDS).

**Thyroid Ab=presence of serum antibodies against either thyroid peroxidase or thyroglobulin; TSH=serum thyroid stimulating hormone; Free T4=serum free thyroxine;. PRL=serum prolactin; PGN=serum progesterone; Lact=lactation.

***The SAS computer program for statistics eliminated thyroid antibodies from the beginning, because there were too many variables, not enough data, and the variation of thyroid was confounded with other variables. Elimination was also likely due to the fact that thyroid antibodies was a yes/no variable.

One Week Postpartum (Time C)

No increases of serum hormone levels at one week postpartum correlated with increases in depression scores taken at that same time point. Patient compliance was low at this time point, as only 18 subjects participated (Table VI).

No increases of serum hormone levels at one week postpartum correlated with increases in maximum postpartum depression scores (Table V). Patient compliance was low at this time point, as only 17 subjects participated.

TABLE VI
A SUMMARY OF NON-SIGNIFICANT RESULTS (P > 0.15)
OF CORRELATION BETWEEN VARIOUS SERUM
HORMONE LEVELS AND MOOD TEST* SCORES TAKEN AT
THAT SAME TIME POINT USING MULTIPLE BACKWARD
STEPWISE REGRESSION

	Variable	N	P value
One Week	Thyroid Ab**	18	NA***
	E2	18	NA***
	Free T4	18	0.7036
	PRL	18	0.7683
	Cortisol	18	0.4502
	PGN	18	0.3436
	TSH	18	0.6913
Four Weeks	E2	45	NA***
	PRL	45	0.6671
	Cortisol	45	0.5953
	PGN	45	0.3792
	Free T4	45	0.2091

*Mood test administered was the Edinburgh Postnatal Depression Scale (EPDS).

**Thyroid Ab=presence of serum antibodies against either thyroid peroxidase or thyroglobulin. E2=estradiol; Free T4=serum free thyroxine; PRL=serum prolactin; PGN=serum progesterone; TSH=serum thyroid stimulating hormone;

***The SAS computer program for statistics eliminated thyroid antibodies from the beginning, because there were too many variables, not enough data, and the variation of thyroid was confounded with other variables. Elimination was also likely due to the fact that thyroid antibodies was a yes/no variable.

Four Weeks Postpartum (Time D)

Increases in serum TSH at four weeks postpartum, when thyroid antibody was held constant as per the design of a multiple backward stepwise regression, correlated with highly significant increases in depression scores taken at that same time point (P = 0.0125) (Table VII). Positive thyroid antibody test (TPO and/or thyroglobulin) at four weeks postpartum, when TSH was held constant, correlated with *very highly* significant increases in depression scores taken at that same time point (P = 0.0066) (Table VII). No increases or decreases of any other serum prenatal hormone or antibody levels correlated with increases in depression scores taken at that same time point (Table VI).

Increases in serum TSH at four weeks postpartum, when free T4 and thyroid antibody were held constant as per the design of a multiple backward stepwise regression, correlated with highly significant increases in maximum depression scores (P = 0.0067) (Table IV). Increases in serum free T4 at four weeks postpartum, when TSH and thyroid antibody were held constant, correlated with highly significant increases in maximum depression scores (P = 0.0196) (Table IV). Positive thyroid antibody test (TPO and/or thyroglobulin) at four weeks postpartum, when TSH and free T4 were held constant, correlated with *very highly* significant increases in maximum depression scores (P = 0.0015) (Table IV).

The coefficient of determination, r^2, for this model is 0.2885 (Table IV). In other words, about 29% of the variability of maximum EPDS scores can be explained by the variability in levels of serum TSH, free T4, and presence or absence of thyroid antibodies. No increases or decreases of any other serum prenatal hormone or antibody levels correlated with increases in maximum depression scores (Table V).

TABLE VII

A SUMMARY OF SIGNIFICANT RESULTS ($P < 0.15$)
OF CORRELATION BETWEEN CONCENTRATIONS OF TWO SERUM
SUBSTANCES AND POSTNATAL MOOD TEST* SCORES
TAKEN AT THAT SAME TIME POINT USING MULTIPLE
BACKWARD STEPWISE REGRESSION

	Significant Variable	N	Parameter Estimate**	Std. Error	P value	r^2***
Four Weeks	TSH****	45	3.03481	1.16366	0.0125	0.2206
	Thyroid Ab	45	6.57342	2.30229	0.0066	

*Mood test administered was the Edinburgh Postnatal Depression Scale (EPDS).

**Parameter Estimate is sometimes referred to as "partial slope". It is the change in the dependent variable (y) divided by the change in the independent variable (x) or "rise over run", but with the stipulation that all other variables in the model are held constant. Parameter Estimate is included here, because it conveys the relationship of y to one particular x. However, since this is a multiple backward stepwise regression, the x in question could be related to the other x's in the model. Therefore, this value may not be shown as the steepness of a line on a graph. That is why the simple regressions were also performed.

***r^2 is the coefficient of determination, or percent of variability in EPDS scores that can be explained by the statistical model. It is reported for the entire test, rather than for individual variables, and is the square of the correlation coefficient.

****TSH=thyroid stimulating hormone; Thyroid Ab=presence of serum antibodies against either thyroid peroxidase or thyroglobulin.

Eight Weeks Postpartum (Time E)

Increases in serum TSH at eight weeks postpartum, when thyroid antibody results were held constant, correlated with significant increases in maximum depression scores ($P = 0.0807$) (Table IV). Positive thyroid antibody test (TPO and/or thyroglobulin) at eight weeks postpartum, when TSH levels were held constant, correlated with significant increases in maximum depression scores ($P = 0.0851$) (Table IV).

The coefficient of determination, r^2, for this model is 0.1711 (Table IV). In other words, about 17% of the variability of EPDS scores can be explained by the variability in levels of serum TSH, and presence or absence of thyroid antibodies when measured at eight weeks postpartum. No increases or decreases of any other serum prenatal hormone or antibody levels correlated with increases in maximum depression scores (Table V).

Twelve Weeks Postpartum (Time F)

No increases of serum hormone levels at twelve weeks postpartum correlated with increases in maximum depression scores (Table V). A possible reason for this is that the majority of women who were positive for thyroid antibodies had already been removed from the study since they became depressed.

Simple Linear Regression

Simple linear regressions are performed to determine if a correlation exists between the response variable and the individual explanatory variables. The effects of the many explanatory variables are investigated independently, however, and the co-linearity of these variables is not considered. Because of this, it is possible for certain correlations to appear significant in a multiple regression that were not significant in the simple linear regression.

Further, as the name implies, a simple linear regression assumes that any significant relationship would have the form of a straight line. Although perhaps presumptuous, because of computational ease, the straight line is sometimes selected as an approximation when it fits fairly well over the range of the independent variable involved. This is the case on occasion even when the true form is known to be non-linear. For each independent variable, a so-called "F-statistic" is calculated, which is determined by dividing the estimate of the variability (square of the standard deviation) that the variable in question explains by the estimate of the remaining unexplained variability. (Steel, Torrie and Dickey, 1997).

Serum substances tested included prenatal estriol, postnatal estradiol, cortisol, prolactin, progesterone, TSH, free T4, thyroglobulin antibodies, and thyroid peroxidase antibodies. In addition subjects were asked if they were breastfeeding at 1, 4, 8, and 12 weeks postpartum.

Prenatal Blood Draw (Time A)

No increases of serum hormone levels at 36 weeks gestation correlated with increases in maximum depression scores (Table VIII).

TABLE VIII

A SUMMARY OF NON-SIGNIFICANT RESULTS (P > 0.15) OF CORRELATION BETWEEN VARIOUS SERUM HORMONE LEVELS AND MAXIMUM POSTPARTUM MOOD TEST* SCORES USING SIMPLE LINEAR REGRESSION

	Variable	N	P value
Prenatal	Thyroid Ab**	58	0.9184
	TSH	58	0.4776
	Free T4	58	0.1554
	PRL	58	0.5368
	Cortisol	58	0.2144
	PGN	58	0.2500
	E3	58	0.2743
One Week	Thyroid Ab	29	0.8254
	TSH	30	0.8458
	Free T4	30	0.8590
	PRL	30	0.4107
	E2	27	0.8010
	Cortisol	30	0.7373
	PGN	26	0.4307
	Lact	24	0.9768
Four Weeks	TSH	57	0.3387
	Free T4	57	0.2895
	PRL	57	0.8566
	E2	56	0.4166
	Cortisol	57	0.2180
	PGN	55	0.4965
	Lact	53	0.4617
Eight Weeks	Free T4	36	0.4551
	E2	36	0.4249
	Cortisol	36	0.2832
	PGN	36	0.7298
	Lact	37	0.5992
Twelve Weeks	Thyroid Ab	35	0.6365
	TSH	35	0.4449
	Free T4	35	0.2620
	E2	34	0.5314
	PRL	35	0.8003
	Cortisol	35	0.2473
	PGN	35	0.7853
	Lact	33	0.6318

*Mood test administered was the Edinburgh Postnatal Depression Scale (EPDS).

**Thyroid Ab=presence of serum antibodies against either thyroid peroxidase or thyroglobulin; TSH=serum thyroid stimulating hormone; Free T4=serum free thyroxine; PRL=serum prolactin; PGN=serum progesterone; E3=serum estriol; Lact=lactation

*Mood test administered was the Edinburgh Postnatal Depression Scale (EPDS).

One Week Postpartum (Time C)

No increases of serum hormone levels at one week postpartum correlated with increases in depression scores taken at that same time point (Table IX). No increases of serum hormone levels at one week postpartum correlated with increases in maximum postpartum depression scores (Table VIII). Patient compliance was low at this time point.

TABLE IX

A SUMMARY OF NON-SIGNIFICANT RESULTS (P > 0.15) OF CORRELATION BETWEEN VARIOUS SERUM HORMONE LEVELS AND MOOD TEST* SCORES TAKEN AT THAT SAME TIME POINT USING SIMPLE LINEAR REGRESSION

	Variable	N	P value
One Week	TSH**	22	0.5406
	Free T4	22	0.3322
	PRL	22	0.3920
	Cortisol	22	0.4040
	PGN	21	0.6930
	E2	23	0.4124
Four Weeks	Free T4	50	0.6671
	PRL	50	0.5634
	Cortisol	50	0.3351
	PGN	48	0.2649
	E2	49	0.2918

*Mood test administered was the Edinburgh Postnatal Depression Scale (EPDS).
**TSH=serum thyroid stimulating hormone; Free T4=serum free thyroxine; PRL=serum prolactin; PGN=serum progesterone; E2=serum estradiol

Four Weeks Postpartum (Time D)

Increases in serum TSH at four weeks postpartum correlated with significant increases in depression scores taken at that same time point ($P = 0.0416$) (Figure 1). The correlation coefficient (r) is 0.286. The coefficient of determination, r^2, is 0.0820. In other words, about 8% of the variability of EPDS scores can be explained by the variability in serum thyroid antibodies measured at four weeks postpartum.

Figure 1. Simple linear regression of serum TSH at four weeks postpartum versus score obtained at that same time point on the Edinburgh Postnatal Depression Scale (EPDS). TSH is measured in uIU/mL. N = 50. P = 0.0416. Slope of the line is 2.5.

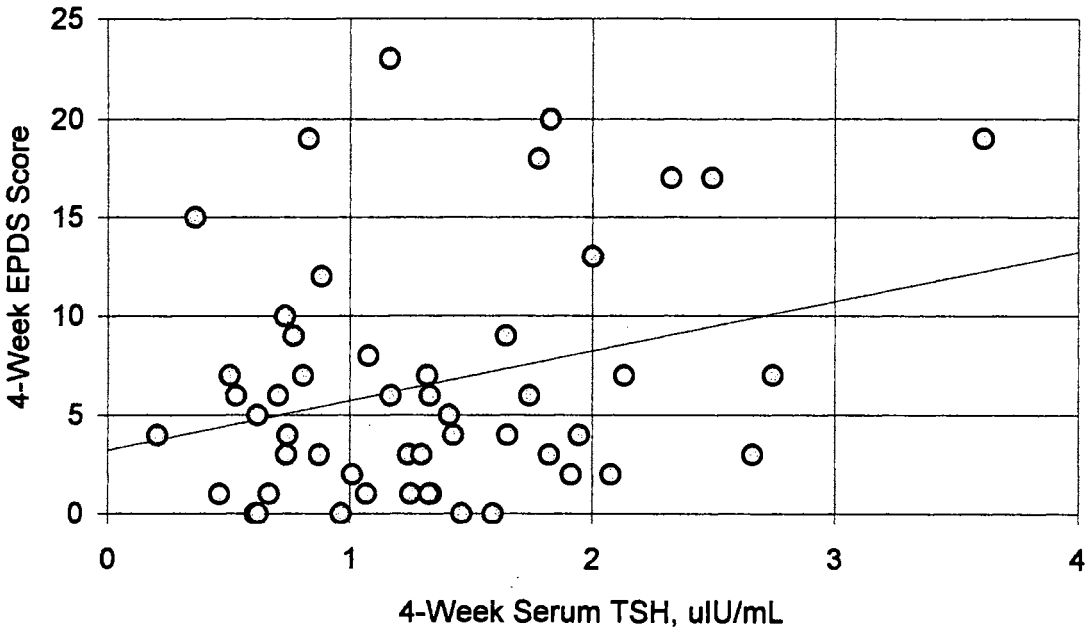

FIGURE 1

Serum TSH at Four Weeks Postpartum Versus Score Obtained at that Same Time Point on the Edinburgh Postnatal Depression Scale (EPDS)

Positive thyroid antibody tests (TPO and/or thyroglobulin) at four weeks postpartum correlated with significant increases in depression scores taken at that same time point ($P = 0.0428$) (Figure 2). The correlation coefficient (r) is 0285. The coefficient of determination, r^2, is 0.0811. In other words, about 8% of the variability of EPDS scores taken at four weeks postpartum can be explained by the variability in serum thyroid antibodies measured at four weeks postpartum. Patients with positive thyroid antibody tests had, on average, maximum EPDS scores that were almost 5 points higher than patients who did not have positive thyroid antibody tests. No other increases of serum hormone levels at four weeks postpartum correlated with increases in depression scores taken at that same time point (Table IX).

Positive thyroid antibody tests (TPO and/or thyroglobulin) at four weeks postpartum correlated with significant increases in maximum depression scores ($P = 0.0615$) (Figure 3). The correlation coefficient (r) is 0.249. The coefficient of determination, r^2, is 0.0621. In other words, about 6% of the variability of EPDS scores can be explained by the variability in serum thyroid antibodies measured at four weeks postpartum. Patients with positive thyroid antibody tests had, on average, maximum EPDS scores that were almost 5 points higher than patients who did not have positive thyroid antibody tests. No other increases of serum hormone levels at four weeks postpartum correlated with increases in maximum depression scores (Table VIII).

Figure 2. Serum thyroid antibody status (positive or negative) at four weeks postpartum versus score obtained on the Edinburgh Postnatal Depression Scale (EPDS) at four weeks postpartum. N=50. P = 0.0428. Positive thyroid antibody status is indicative of postpartum autoimmune thyroiditis (Pies, 1997).

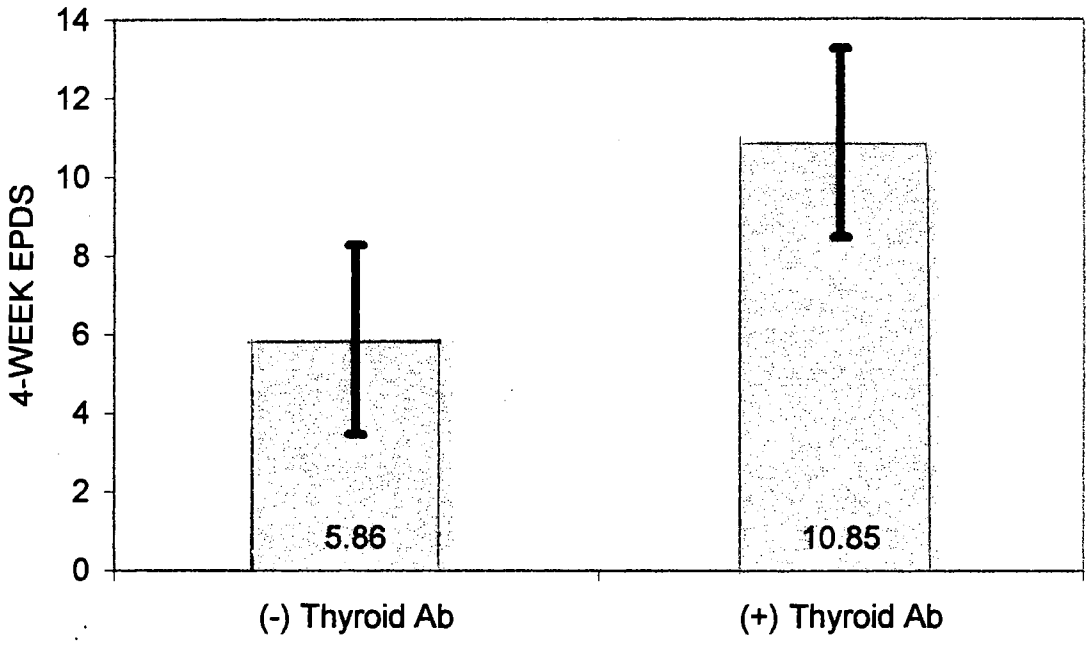

FIGURE 2

Serum Thyroid Antibody Status (Positive or Negative) at Four Weeks Postpartum Versus Score Obtained on the Edinburgh Postnatal Depression Scale (EPDS) at Four Weeks Postpartum

Figure 3. Serum thyroid antibody status (positive or negative) versus maximum score obtained on the Edinburgh Postnatal Depression Scale (EPDS). EPDS score could be obtained at any measured time point after birth, 1, 4, 8, or 12 weeks postpartum. N=56. P = 0.0615. Positive thyroid antibody status is indicative of postpartum autoimmune thyroiditis (Pies, 1997).

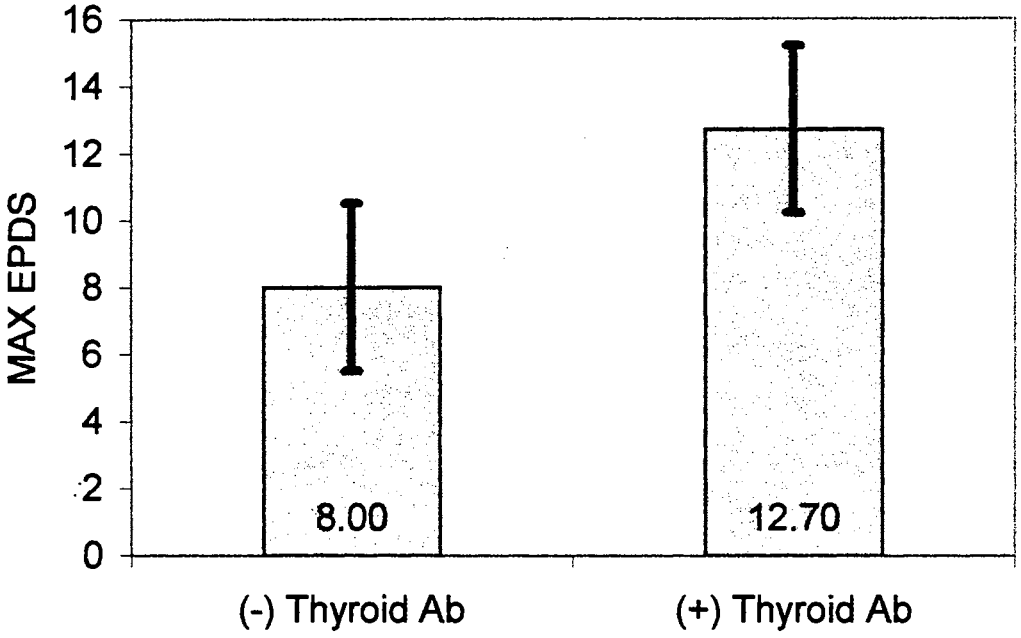

FIGURE 3

Serum Thyroid Antibody Status (Positive or Negative) at Four Weeks Postpartum Versus Maximum Score Obtained on the Edinburgh Postnatal Depression Scale (EPDS) over an 11-Week Period from One Week Postpartum through 12 Weeks Postpartum

Eight Weeks Postpartum (Time E)

Positive thyroid antibody test (TPO and/or thyroglobulin) at eight weeks postpartum correlated with significant increases in maximum depression scores ($P = 0.1015$). The correlation coefficient (r) is 0.281. The coefficient of determination, r^2 is 0.0792. In other words, about 8% of the variability of EPDS scores can be explained by the variability in serum thyroid antibodies measured at eight weeks postpartum. Patients with positive thyroid antibody tests had, on average, maximum postpartum EPDS scores that were just over 5½ points higher than patients who did not have positive thyroid antibody tests (Figure 4).

Figure 4. Serum thyroid antibody status (positive or negative) at eight weeks postpartum versus maximum score obtained on the Edinburgh Postnatal Depression Scale (EPDS). EPDS score could be obtained at any measured postpartum time point, 1, 4, 8, or 12 weeks. N=34. P = 0.10151. Positive thyroid antibody status is indicative of postpartum autoimmune thyroiditis (Pies, 1997).

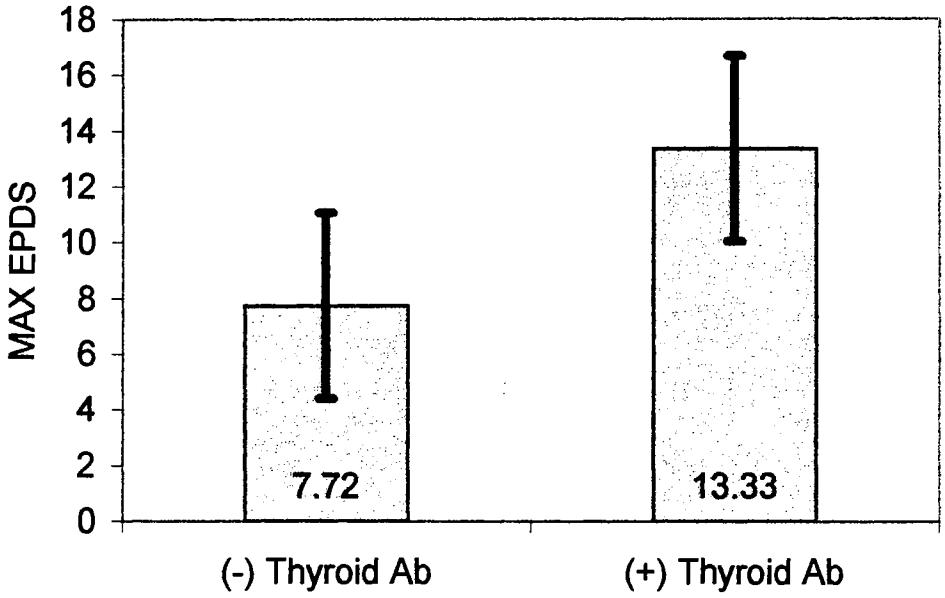

FIGURE 4

Serum Thyroid Antibody Status (Positive or Negative) at Eight Weeks Postpartum Versus Maximum Score Obtained on the Edinburgh Postnatal Depression Scale (EPDS) over an 11-Week Period from One Week Postpartum through 12 Weeks Postpartum

Increases in serum TSH at eight weeks postpartum correlated with significant increases in maximum depression scores (P = 0.0999) (Figure 5). The correlation coefficient (r) is 0.275. The coefficient of determination, r^2 is 0.0755. In other words, about 7½% of the variability of EPDS scores can be explained by the variability in serum prolactin measured in serum TSH at eight weeks postpartum.

Information from the simple linear regression can also be used to make a prediction about the maximum EPDS score in the twelve weeks postpartum using the value of serum TSH at eight weeks postpartum. The equation for the line describing this relationship is: max EPDS = 4.67 + 2.45(TSH). Therefore, if a woman's postpartum max EPDS was 13, her TSH value at 12 weeks should be 3.4 mU/mL. The upper limit of the normal range is 4.6 mU/mL (Bakerman, 1994). The lowest EPDS score that would predict a TSH value above the normal range is 17.

Figure 5. Simple regression of serum TSH at eight weeks postpartum versus maximum score obtained on the Edinburgh Postnatal Depression Scale (EPDS). EPDS score could be obtained at any measured postpartum time point, 1, 4, 8 or 12 weeks postpartum. TSH is measured in Mu/mL. N = 36. P = 0.0999. Slope of the line is 2.45.

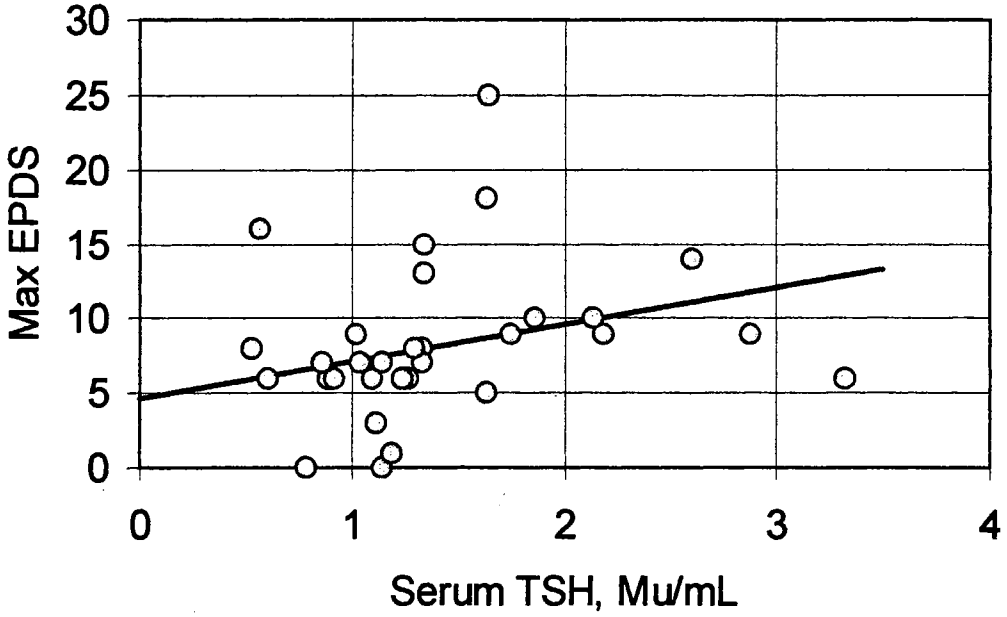

FIGURE 5

Serum TSH at Eight Weeks Postpartum Versus Maximum Score Obtained on the Edinburgh Postnatal Depression Scale (EPDS) over an 11-Week Period from One Week Postpartum Through 12 Weeks Postpartum.

Increases in serum prolactin at eight weeks postpartum correlated with significant increases in maximum depression scores ($P = 0.1092$) (Figure 6). The correlation coefficient (r) is 0.268. The coefficient of determination, r^2 is 0.0717. In other words, about 7% of the variability of EPDS scores can be explained by the variability in serum prolactin measured at eight weeks postpartum. No other increases of serum hormone levels at eight weeks postpartum correlated with increases in maximum depression scores (Table VIII).

Information from the simple linear regression can also be used to make a prediction about the maximum EPDS score in the twelve weeks postpartum using the value of serum prolactin at eight weeks postpartum. The equation for the line describing this relationship is: max EPDS = 6.50 + 0.05(PRL). Therefore, if a woman's postpartum max EPDS was 13, her prolactin value at 12 weeks should be 130 ng/mL, well above the upper limit of the normal range for a non-lactating woman, 18.5 ng/mL (Bakerman, 1994).

Figure 6. Simple regression of serum prolactin at eight weeks postpartum versus maximum score obtained on the Edinburgh Postnatal Depression Scale (EPDS). EPDS score could be obtained at any measured postpartum time point, 1, 4, 8 or 12 weeks postpartum. Prolactin is measured in ng/mL. N = 36. P = 0.1092. Slope of the line is 0.0515.

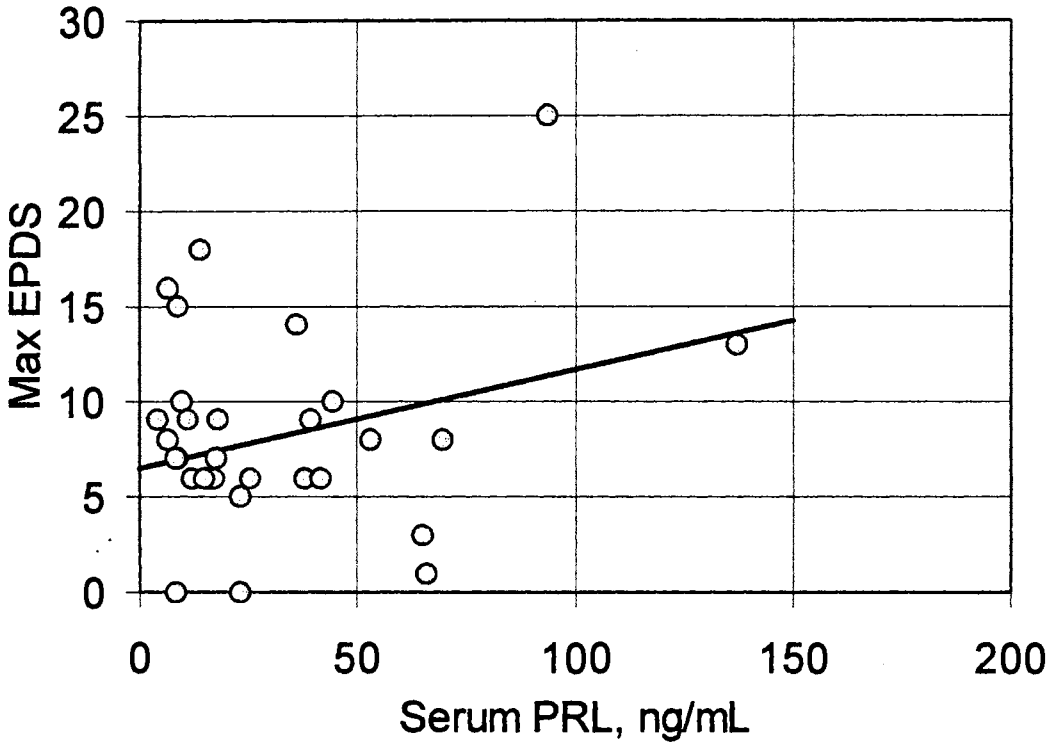

FIGURE 6

Serum Prolactin at Eight Weeks Postpartum Versus Maximum Score Obtained on the Edinburgh Postnatal Depression Scale (EPDS) over an 11-Week Period from One Week Postpartum Through 12 Weeks Postpartum

Twelve Weeks Postpartum (Time F)

No increases of serum hormone levels at twelve weeks postpartum correlated with increases in maximum depression scores (Table VIII).

Simple Linear Regression of Differences

As stated previously, the purpose of running any simple linear regression is to determine if there is a correlation between two variables such that the value of one can be used to predict the value of the other. Here, as in the other simple linear regression, the variables are independent of each other—one hormone and its possible correlation with mood score is examined at a time. In this analysis, however, the independent variables are the largest differences between two values of the same hormone at different times, and they are independent of each other. In other words, the value of a given hormone at Time A is subtracted from the postpartum value that is farthest from it. This absolute number serves as the independent variable.

Serum substances tested included prenatal estriol, postnatal estradiol, cortisol, progesterone, TSH, prolactin, and free T4. Increases in changes in serum cortisol values correlated with significant increases in maximum depression scores ($P = 0.0622$) (Figure 7). The correlation coefficient (r) is 0.233. The coefficient of determination, r^2, is 0.0541. That is to say, over 5% of the variability of EPDS scores can be explained by the variability in levels of serum change in cortisol from the prenatal draw to the lowest detected value among the postnatal draws. No other increases in changes in serum hormone values correlated with significant increases in maximum depression scores (Table X).

Figure 7. Simple regression of change in cortisol (from prenatal or postnatal maximum to lowest measured postnatal value) versus maximum score obtained on the Edinburgh Postnatal Depression Scale (EPDS). EPDS score could be obtained at any measured postpartum time point, 1, 4, 8, or 12 weeks postpartum. Cortisol is measured in mcg/dl. N = 64. P = 0.0622. Slope of the line is 0.2207.

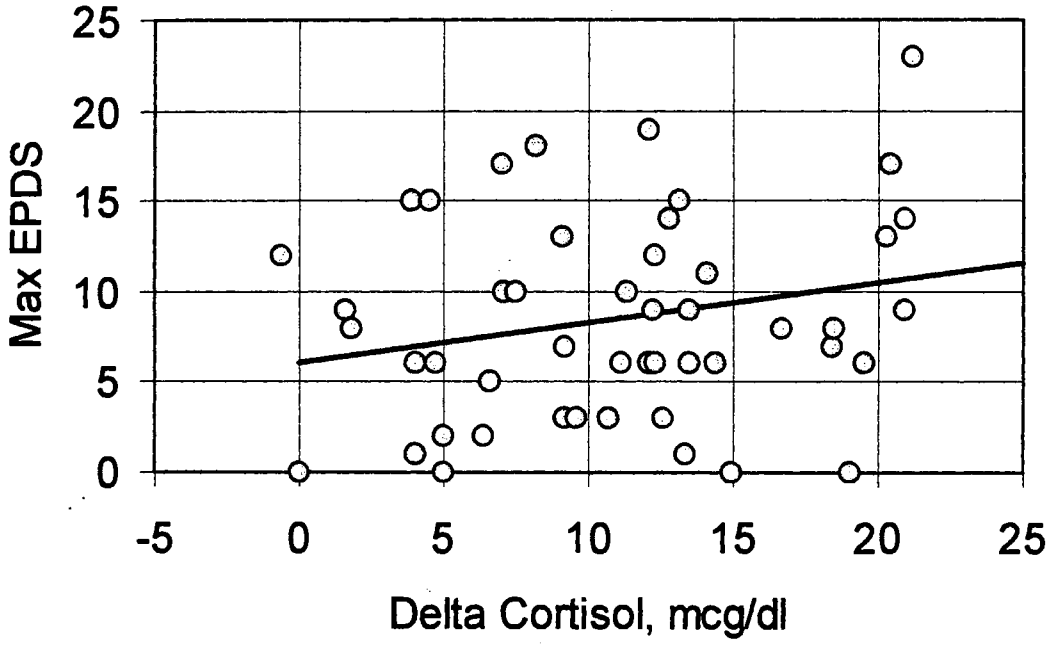

FIGURE 7

Delta Serum Cortisol Versus Maximum Score Obtained on the Edinburgh Postnatal Depression Scale (EPDS) over an 11-Week Period from One Week Postpartum Through 12 Weeks Postpartum

TABLE X

A SUMMARY OF NON-SIGNIFICANT RESULTS (P > 0.15)
OF CORRELATION BETWEEN VARIOUS SERUM
HORMONE LEVELS AND MOOD TEST* SCORES
USING SIMPLE LINEAR REGRESSION OF DIFFERENCES

Variable	N	P value
Thyroid Ab**		NA***
TSH	64	0.9985
Free T4	64	0.8355
E2	64	0.8609
E3	64	0.3415
PRL	64	0.2998
PGN	64	0.2674
Lact		NA**

*Mood test administered was the Edinburgh Postnatal Depression Scale (EPDS).

**Thyroid Ab=presence of serum antibodies against either thyroid peroxidase or thyroglobulin; TSH=serum thyroid stimulating hormone; Free T4=serum free thyroxine; E2=serum estradiol; E3=serum estriol; PRL=serum prolactin; PGN=serum progesterone; Lact=lactation

***No differences can be calculated on yes/no variables.

Information from the simple linear regression can also be used to make a prediction about the maximum EPDS score in the twelve weeks postpartum using the value of delta cortisol. The equation for the line describing this relationship is: max EPDS = 6.03 + 0.22(Δ cort). Therefore, if a woman's postpartum max EPDS was 13, her delta cortisol value should be 31.7. Normal diurnal variation in cortisol is about 10 mcg/dL (Bakerman, 1994).

Two-Tailed t-Tests

For the two-tailed t-test, participating women were divided into "depressed" and "not-depressed" according to the score they received on the EPDS with 12/13 as the cutoff (Cox et al, 1987). For each of the postpartum time points, one, four, eight, and 12 weeks, the depressed group's mean value for six different hormones was compared with the non-depressed group. There were no significant differences in any of the hormone averages between the two groups at one week postpartum. At four weeks postpartum, the depressed group had significantly higher TSH (P=0.016), progesterone (P=0.059), and estradiol (P=0.042). There were no significant differences in any of the hormone averages between the two groups at eight weeks postpartum. At 12 weeks postpartum, the depressed group had slightly significantly higher estradiol (P=0.12) (Table XI).

TABLE XI

RESULTS OF TWO-TAILED t-TESTS COMPARING AVERAGES OF SIX SERUM HORMONE CONCENTRATIONS BETWEEN DEPRESSED AND NON-DEPRESSED POSTPARTUM WOMEN AT FOUR TIME POINTS

One Week Postpartum

Hormone	Not Depressed N	Depressed N	P value	df*
TSH	18	5	0.27	21
Free T4	18	5	0.88	21
Prolactin	18	5	0.22	21
Cortisol	18	5	0.60	21
Progesterone	17	5	0.29	20
Estradiol	18	5	0.79	21

Four Weeks Postpartum

Hormone	Not Depressed N	Depressed N	P value	df*
TSH	42	9	0.016	49
Free T4	42	9	0.28	49
Prolactin	42	9	0.28	49
Cortisol	42	9	0.21	49
Progesterone	42	8	0.059	48
Estradiol	39	8	0.042	45

Eight Weeks Postpartum

Hormone	Not Depressed N	Depressed N	P value	df*
TSH	28	3	0.54	29
Free T4	28	3	0.82	29
Prolactin	28	3	0.73	29
Cortisol	28	3	0.66	29
Progesterone	28	2	0.73	28
Estradiol	27	3	0.61	28

12 Weeks Postpartum

Hormone	Not Depressed N	Depressed N	P value	df*
TSH	29	3	0.59	30
Free T4	29	3	0.45	30
Prolactin	29	3	0.94	30
Cortisol	29	3	0.34	30
Progesterone	27	2	0.76	27
Estradiol	28	3	0.12	29

*df=degrees of freedom

Chi Square

Lactation

For this statistical test subjects were asked whether or not they were breastfeeding at times 1, 4, 8, and 12 weeks. If the answer was "yes" at any time point, the variable was "yes". If the subject never breastfed, the variable was "no". The decision was made to assign all women who breastfed their infants for any period of time a "yes", because if subjects had been separated on the basis of how long they lactated, numbers would have been so low in each category that it would have been difficult to obtain any statistically meaningful information.

Although the accepted standard of significance in this type of statistical test, as in most others, is $P < 0.05$, for the purposes of this investigation and due to the difficulty of detecting slight differences in small populations, any P values less than 0.15 will be regarded as "marginally statistically significant." Differences that are biologically or scientifically significant do not necessarily meet the margin required for statistical rigor. The breastfeeding group had a marginally statistically significant lower incidence of depression than the non-breastfeeding group ($P = 0.1223$) (Figure 8).

In addition to the statistical analysis mentioned above, average hormonal levels at the four postpartum time points for both breastfeeding and non-breastfeeding women are included for completeness (Table XII).

Figure 8. Lactation (reported yes/no at any postpartum time) versus Edinburgh Postnatal Depression Scale (EPDS) scores indicative of postpartum depression (> 12) at four weeks. N = 44. P = 0.1223

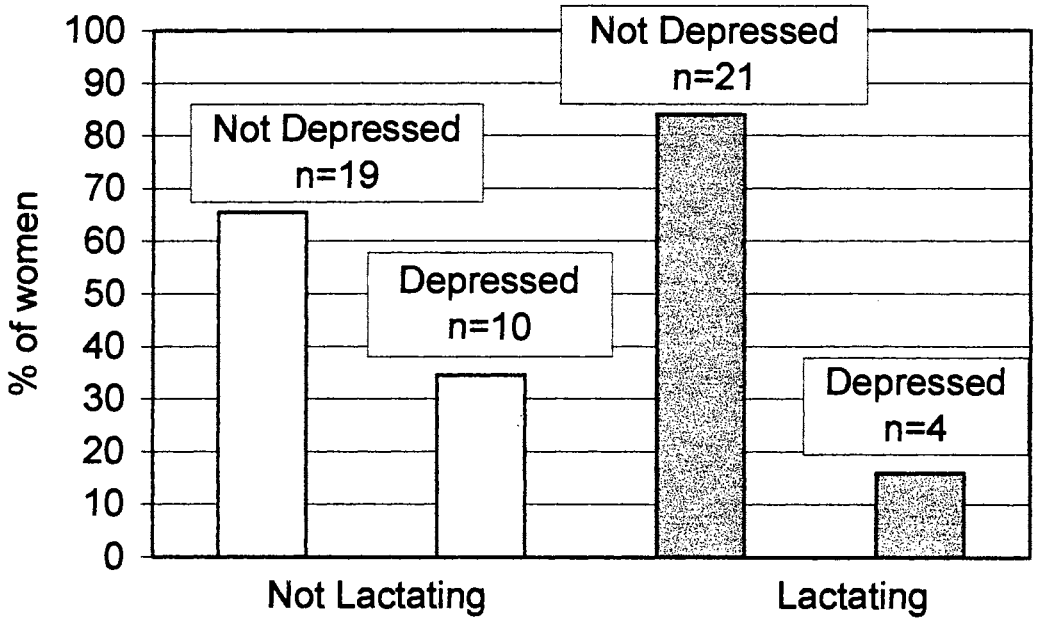

FIGURE 8

Lactation at Any Postpartum Time Versus Probable Postpartum Depression as Indicated by the Edinburgh Postnatal Depression Scale (EPDS) at Four Weeks Postpartum

TABLE XII

A SUMMARY OF AVERAGE SERUM HORMONE LEVELS AT FOUR POSTPARTUM TIME POINTS FOR BREASTFEEDING AND NON-BREASTFEEDING WOMEN

Women who breastfed for any period of time during the first 12 weeks

	TSH* Mu/mL	**Free T4** ng/dl	**PRL** ng/mL	**Cortisol** mcg/dl	**PGN** ng/dl	**E2** ng/mL
Week						
1	1.09	1.05	127.05	16.28	49.98	20.51
4	1.02	0.98	51.19	9.68	32.24	39.29
8	1.24	1.05	44.26	9.61	42.50	20.85
12	1.45	1.02	43.53	10.02	87.05	38.27

Women who did not breastfeed

	TSH* Mu/ml	**Free T4** ng/dl	**PRL** ng/mL	**Cortisol** mcg/dl	**PGN** ng/dl	**E2** ng/mL
Week						
1	1.46	1.08	56.63	14.94	45.87	25.18
4	1.57	1.05	13.74	8.57	119.04	109.53
8	1.52	1.06	11.82	11.08	189.40	73.67
12	1.57	1.07	14.26	10.19	98.69	49.69

*TSH=thyroid stimulating hormone; Free T4=unbound serum thyroxine; PRL=prolactin; PGN= progesterone; E2=estradiol

Anxiety and High Prenatal Progesterone

To test the hypothesis that high prenatal progesterone might predispose a woman to symptoms of anxiety after birth, progesterone scores of the 75 women who participated in this study were examined. Of those 75, 65 gave us enough data to use for this analysis. Eleven (16.9%) had prenatal serum progesterone values above the accepted high end of the normal range, 20,000 mg/Dl (Bakerman, 1994). Since the screening tool used in this study was not specifically designed to test for anxiety, subjects' prenatal scores on questions 4 and 5 of the EPDS, which seem to deal specifically with anxiety, were compared to their maximum postnatal scores on these same questions. In many of the cases, compliance was low, so there was only one postnatal EPDS score to check, and its time point varied. Even so, five of 11 women examined had higher scores on the anxiety questions in the postnatal EPDS than on the prenatal one (Table XIII). Four of the 11 had the same score, and only two women had a lower prenatal than postnatal score on the anxiety questions. Of the remaining 54 whose progesterone was not higher than the upper range of normal when they were tested in the third trimester, 28 had anxiety scores that rose sometime within the first 12 weeks postpartum. Fifteen of the 54 had the same score, and 11 had a higher prenatal than postnatal score on the anxiety questions. The results of a Chi Square test were $P = 0.5091$.

TABLE XIII

A SUMMARY OF DATA ON QUESTIONS 4 AND 5, CONCERNING ANXIETY, ON THE EDINBURGH POSTNATAL DEPRESSION SCALE

Women with high prenatal progesterone*

	N	Prenatal average EPDS questions 4 and 5	Average of postnatal max EPDS on questions 4 and 5
Scores that rose after birth	5	1.2	3.6
Scores that stayed the same after birth	4	2.0	2.0
Scores that fell after birth	2	2.0	1.0

Women with prenatal progesterone below maximum normal value

	N	Prenatal average EPDS questions 4 and 5	Average of postnatal max EPDS on questions 4 and 5
Scores that rose after birth	28	1.5	3.2
Scores that stayed the same after birth	15	1.3	1.3
Scores that fell after birth	11	3.3	1.4

*High prenatal progesterone is defined as any serum value above the reported upper limit of normal, 20,000 mg/Dl (Bakerman, 1994). Differences in the two above groups were not significant when evaluated by Chi square, P=0.5091.

Visual Analog Scales

Visual Analog Scales (VAS), published by Cox et al in 1983, were given to the volunteers when they came to the clinic a week or two before delivery, so that the women could take the scales with them to the hospital, then complete the remainder at home after dismissal (Appendix C). The same six statements comprising the scales were to be evaluated and completed with a pencil dot on the line every day at approximately the same time for 21 days postpartum.

Thirty-eight women returned the scales, and of those 38, 34 also took the EPDS test at four weeks postpartum. A simple linear regression test was run for each of the six statements of the VAS to see if the average length in centimeters of the line segment created by placing a pencil dot on the scale could predict the EPDS score at four weeks postpartum. A decision was made not to use the entire three-week period during which VAS data was collected to determine a mean. Instead, only the last seven postpartum days of the VAS test, 15-21, were used. It was thought that some lability of mood that is normal in the very early postpartum might prevent the VAS mean computed over all 21 days from reflecting accurately the mood state closer in time to the four-week EPDS assessment.

Responses to all six statements of the VAS (Appendix C) correlated significantly with four-week EPDS scores, with coefficients of determination ranging from 0.19 for statement one to a high of 0.63 for statement six (Table XIV). In other words, the average length of the line segment created by making a pencil mark to evaluate statement six of the VAS was the best predictor among the six VAS statements of EPDS score at four weeks postpartum, accounting for about 63% of the EPDS score variability.

TABLE XIV

A SUMMARY OF RESULTS FROM SIMPLE LINEAR REGRESSION OF AVERAGE SCORES ON STATEMENTS 1-6 OF THE VISUAL ANALOG SCALES FOR DAYS 15-21 POSTPARTUM WITH FOUR-WEEK SCORE ON THE EDINBURGH POSTNATAL DEPRESSION SCALE

Statement	N*	P**	R^2***	Slope	Y intercept
1	34	9.09×10^{-3}	0.194	-0.093	13.7
2	34	3.2×10^{-6}	0.497	0.140	4.31
3	34	4.95×10^{-7}	0.551	0.158	4.02
4	34	1.60×10^{-5}	0.446	0.122	3.97
5	34	5.19×10^{-8}	0.609	0.127	3.74
6	34	2.65×10^{-8}	0.625	0.123	3.90

*N=number of subjects reporting.
**P=probability value.
***R^2=coefficient of determination.

Only the first of the six statements is positive, and therefore the severity of the depressive symptom is inversely proportional to the length of the line segment created by placement of a pencil dot. It follows that the simple linear regression and corresponding line graph have a negative slope (Figure 9). For the other five statements, the severity of the depressive symptom is proportional to the length of the line. Therefore those corresponding regressions and line graphs have positive slopes (Figures 10-14).

Figure 9. Simple regression of average response for postpartum days 15-21 on Visual Analog Scale statement one (Appendix C) versus score obtained on the Edinburgh Postnatal Depression Scale (EPDS) at four weeks postpartum. Statement one was, "I am feeling happy and self-confident." N = 34. P = 0.0091. Slope of the line is -0.093.

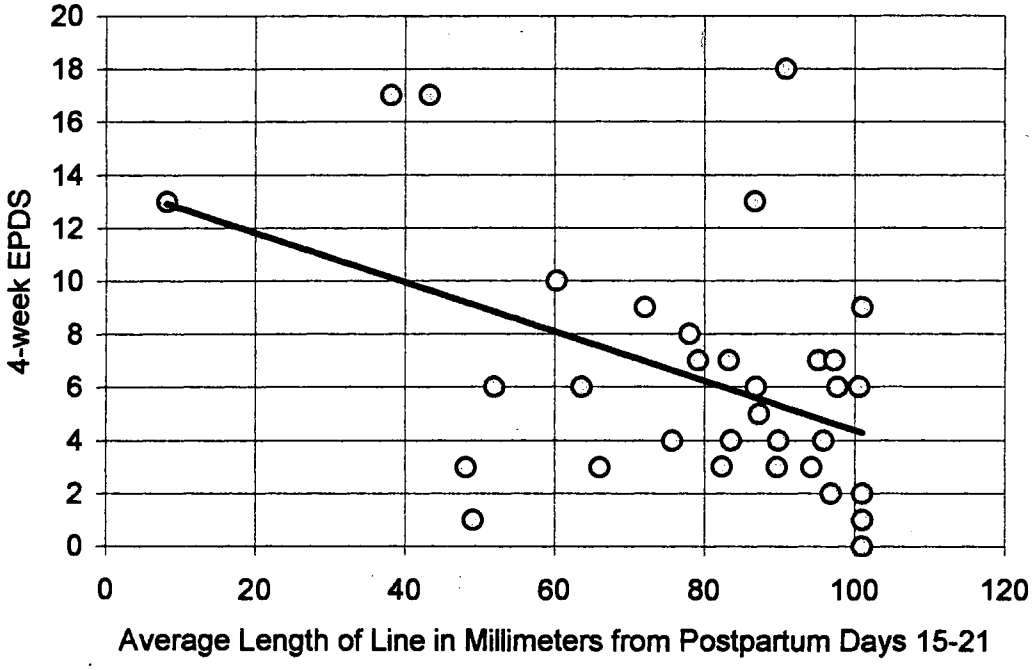

FIGURE 9

Average Response for Postpartum Days 15-21 on Visual Analog Scale Statement One Versus Score on the Edinburgh Postnatal Depression Scale at Four Weeks Postpartum

Figure 10. Simple regression of average response for postpartum days 15-21 on Visual Analog Scale statement two (Appendix C) versus score obtained on the Edinburgh Postnatal Depression Scale (EPDS) at four weeks postpartum. Statement two was, "I am feeling miserable and depressed." N = 34. P = 0.00000322. Slope of the line is 0.140.

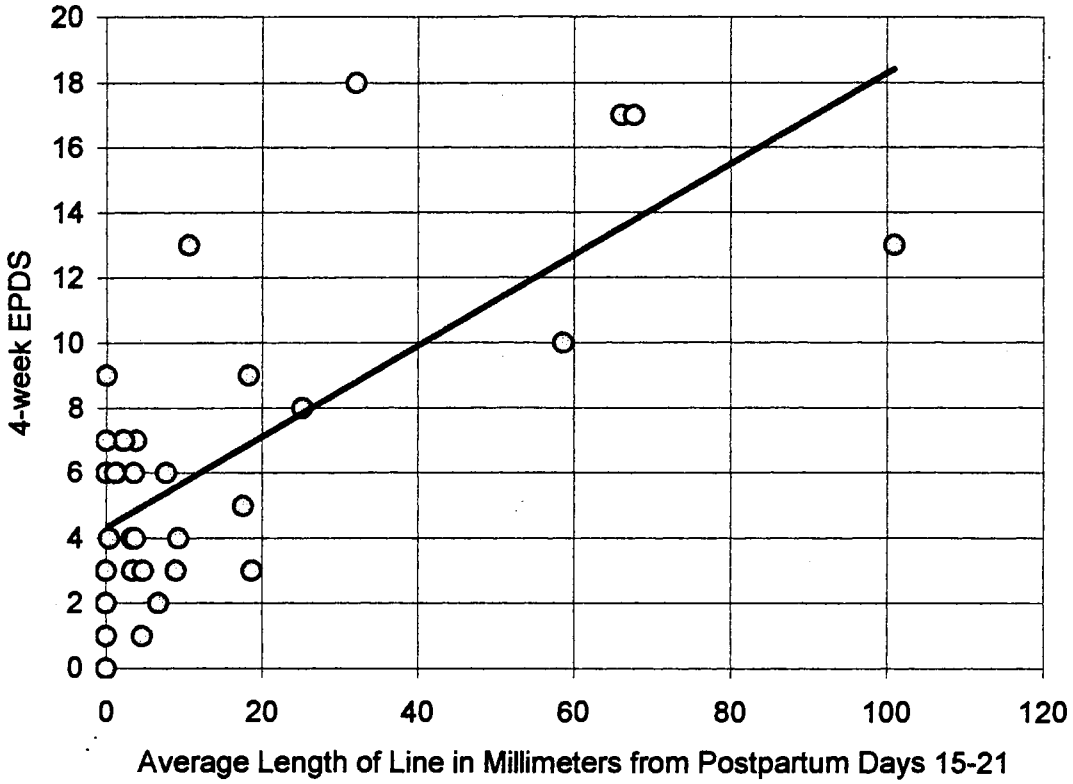

FIGURE 10

Average Response for Postpartum Days 15-21 on Visual Analog Scale Statement Two Versus Score on the Edinburgh Postnatal Depression Scale at Four Weeks Postpartum

Figure 11. Simple regression of average response for postpartum days 15-21 on Visual Analog Scale statement three (Appendix C) versus score obtained on the Edinburgh Postnatal Depression Scale (EPDS) at four weeks postpartum. Statement three was, "I have been in tears." N = 34. P = 0.000000495. Slope of the line is 0.158.

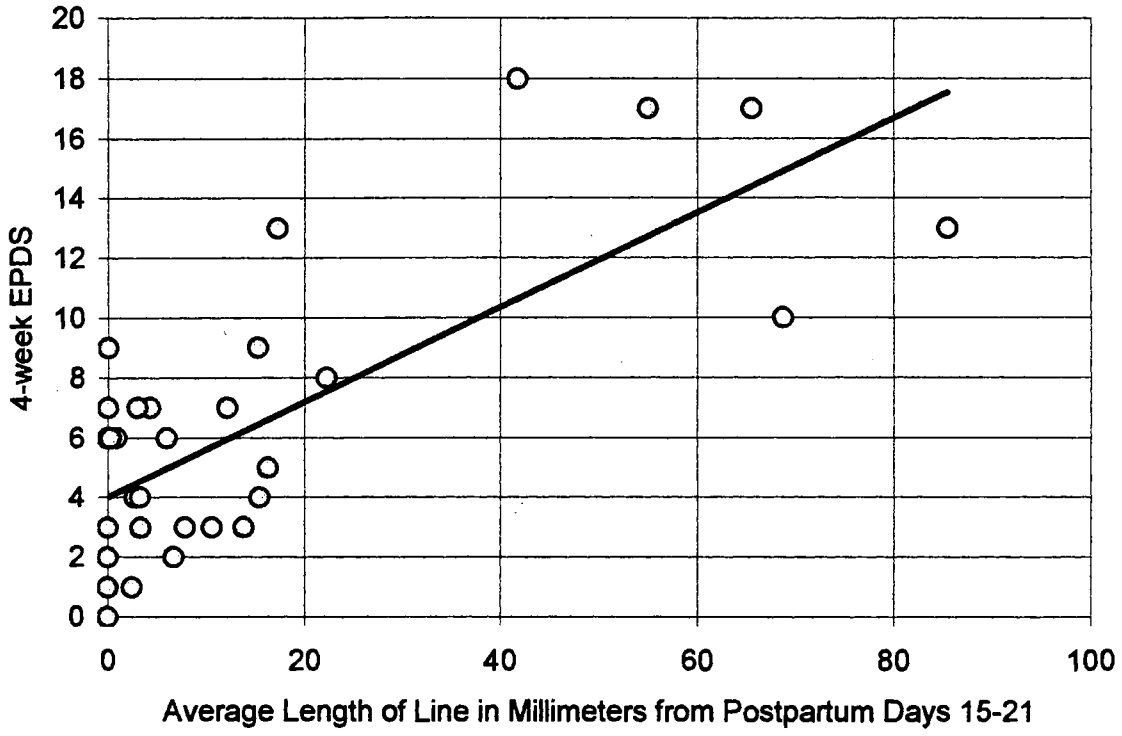

FIGURE 11

Average Response for Postpartum Days 15-21 on Visual Analog Scale Statement Three Versus Score on the Edinburgh Postnatal Depression Scale at Four Weeks Postpartum

Figure 12. Simple regression of average response for postpartum days 15-21 on Visual Analog Scale statement four (Appendix C) versus score obtained on the Edinburgh Postnatal Depression Scale (EPDS) at four weeks postpartum. Statement four was, "I am very worried and anxious." N = 34. P = 0.000016. Slope of the line is 0.122.

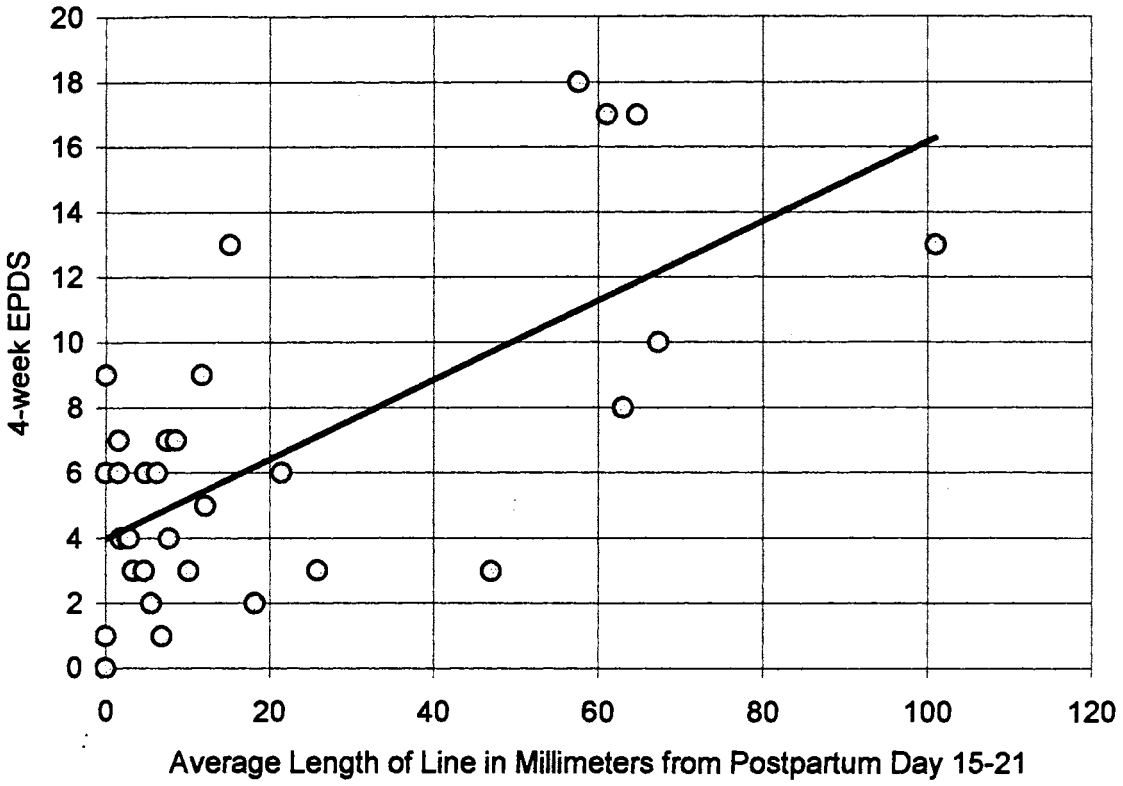

FIGURE 12

Average Response for Postpartum Days 15-21 on Visual Analog Scale Statement Four Versus Score on the Edinburgh Postnatal Depression Scale at Four Weeks Postpartum

Figure 13. Simple regression of average response for postpartum days 15-21 on Visual Analog Scale statement five (Appendix C) versus score obtained on the Edinburgh Postnatal Depression Scale (EPDS) at four weeks postpartum. Statement five was, "I am very irritable and quick-tempered." N = 34. P = 0.0000000519. Slope of the line is 0.127.

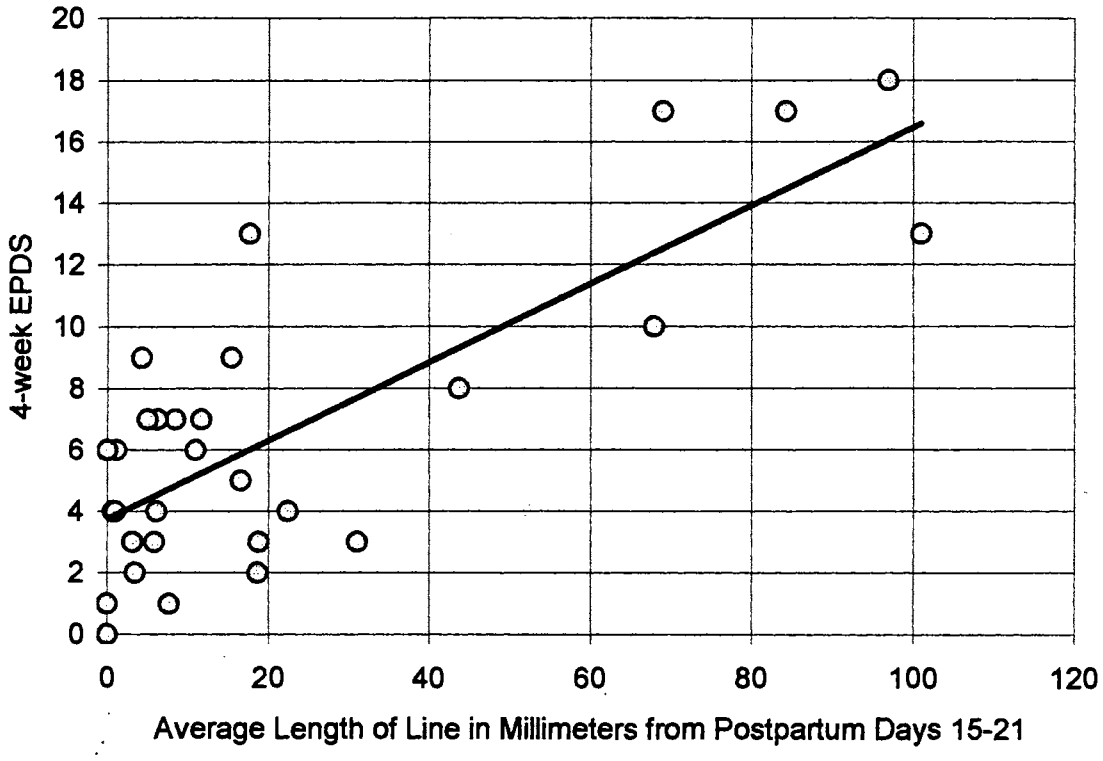

FIGURE 13

Average Response for Postpartum Days 15-21 on Visual Analog Scale Statement Five Versus Score on the Edinburgh Postnatal Depression Scale at Four Weeks Postpartum

Figure 14. Simple regression of average response for postpartum days 15-21 on Visual Analog Scale statement six (Appendix C) versus score obtained on the Edinburgh Postnatal Depression Scale (EPDS) at four weeks postpartum. Statement six was, "My spirits are going up and down like a yoyo." N = 34. P = 0.0000000265. Slope of the line is 0.123.

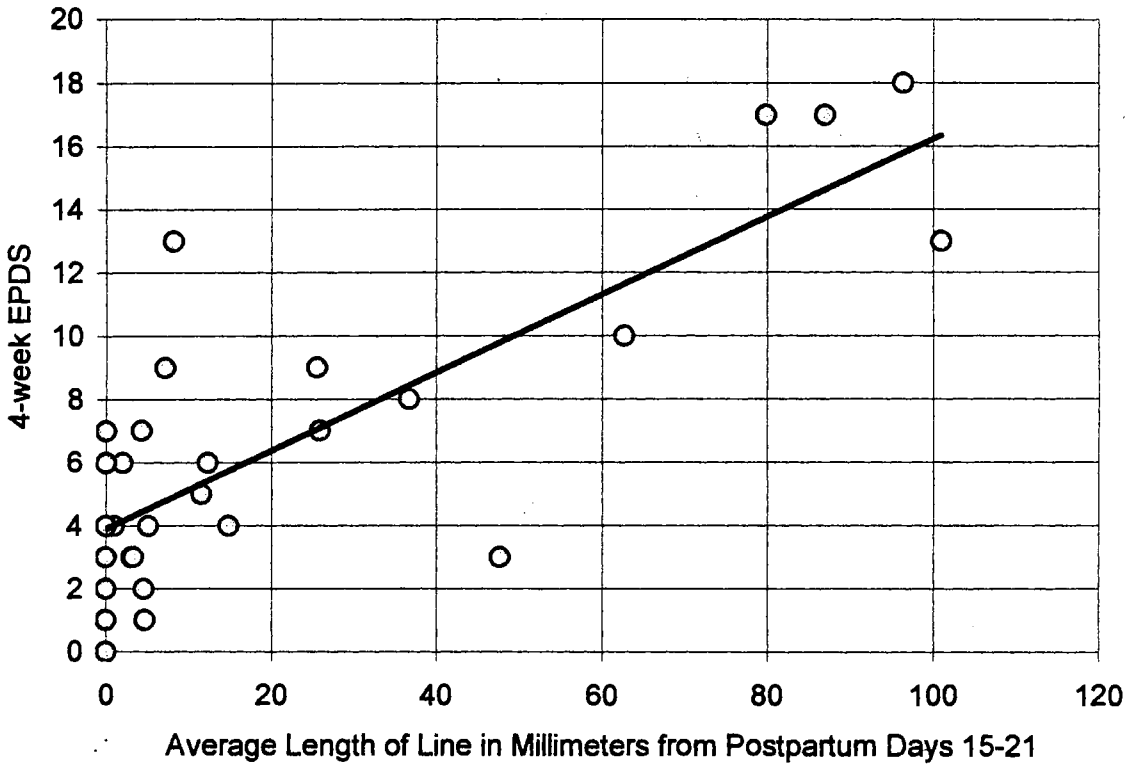

FIGURE 14

Average Response for Postpartum Days 15-21 on Visual Analog Scale Statement Six Versus Score on the Edinburgh Postnatal Depression Scale at Four Weeks Postpartum

CHAPTER FIVE

DISCUSSION AND CONCLUSIONS

Postpartum depression is a serious public health concern that is probably physiologically based in at least some cases and warrants the attention of the medical community. This research project was an attempt to examine some possible hormonal correlates of PPD that might lead to studies of potential cause and effect relationships in the future. Although the results seem to raise more questions than they answer, they appear, along with dozens of other studies, to lend credence to the idea that the problem of postpartum mood dysphoria is complex, has a variety etiologies, and can likely be treated more effectively as physicians become aware of various physiological correlates.

EPDS Score Averages and Ranges

One of the most striking features was the change in range of EPDS scores from prenatal to postnatal time points. As explained previously, by design the study did not include any women who had EPDS scores above 12 prenatally. The range of EPDS scores at the prenatal screen, then, was 0-12, with 6.0 as the average. By the four-week time period, however, that range had extended to an upper limit of 23 and included 14 women who had scores above 12 and were, therefore, likely depressed. However, the average EPDS score rose only half a point. The explanation for this result can only be that there was not only a rise in number of exceptionally high scores, but also a concomitant rise in number of very low scores. In other words, although more women were depressed postnatally in this group, more women were also reporting feeling decidedly happy and satisfied, as evidenced by an increase in low scores.

The significance of this observation becomes obvious with regard to literature that suggests that postpartum depression is not a "real" phenomenon. In August 2001, data were published from a study done in 1991-1992 that indicated that the rate of postnatal depression is no higher than depression in women at any other time in their lives, and that it actually fell in their particular group of patients from the prenatal to postnatal period. The researchers made the case that symptoms of depression are not more common or severe after childbirth than during pregnancy (Evans et al, 2001). This is debatable, as the published prevalence of major depressive disorder for women in the United States is 5-10% (Beckham and Leber, 1995). Perhaps the reason that the rate of depression in women appears to be stable in the female population whether they have recently delivered a child or not, is that while some are more prone to sadness, anxiety, or difficulties coping, there are also women who are especially resistant to depression for a period of time after they give birth. Those resistant to depression, for reasons either psychosocial or physiological in origin, may offset in statistical averages those who are at high risk of depression. The phenomenon of postpartum depression and the increased vulnerability of some women to mood dysphorias at this particular point in their lives may be masked by those who are somehow at decreased risk.

Serum Hormone Average Means

The serum hormone average means presented in Table III are, without exception, within the accepted normal range. Although in some cases an abnormally high or low value extends a hormone's range beyond normal, the averages seem to convey the notion that the samples used in this study are rather like the population of childbearing women

as a whole. This confirmation of "normal" makes it reasonable to place confidence in the statistical results that show correlations of hormone values with mood scores.

The serum hormone mean values presented in Table XII were grouped according to whether or not the volunteers were breastfeeding. Statistically analysis of this data was not a stated aim of this research project, but the trends that are readily evident are mostly to be expected. From the very first week, for example, the average levels of serum prolactin were much lower in the non-breastfeeding women than they were in the ones who claimed to breastfeed. This seems to agree with the long-established idea that suckling stimulates the anterior pituitary to release prolactin, which, in turn, promotes milk production (Guyton and Hall, 2000). In the absence of such stimulation, prolactin inhibitory hormone, also called dopamine, is released from the hypothalamus and serves to inhibit the anterior pituitary from producing and secreting prolactin.

After the first week, women who did not breastfeed had consistently higher average serum progesterone and estradiol levels than the women who breastfed. This is in agreement with the generally established notion that, in breastfeeding women, either the direct action of nervous signals sent by suckling or the prolactin that is released by the anterior pituitary in response to suckling inhibits pituitary gonadotropin production that would normally stimulate release of ovarian steroids (Guyton and Hall, 2000). Since this inhibition is not present in non-lactating women, their ovaries are free to resume normal cycling at an earlier time after giving birth.

Free T4 values do not appear to be different between the two groups of women, but a surprising finding is that, at each of the time points, TSH levels are higher in the non-nursing group. Statistical analysis of these data does not address postpartum mood

dysphoria, but investigation of serum TSH differences between nursing and non-nursing women remains a point of interest for future investigation.

Hormonal Correlates with Elevated EPDS Scores

Results indicate that third trimester levels of cortisol, progesterone, and estriol may have some predictive value with regard to tendency to develop elevated EPDS scores suggestive of depression. Although none of these substances showed significant correlation with maximum EPDS score in a simple linear regression, the multiple backward stepwise regression may give a more rigorous and reliable result, because by design, it takes into account the effects that various hormone levels have on each other.

Progesterone

The data show a possibly significant relationship between high prenatal progesterone and tendency to higher EPDS scores. However, the results are not supported and confirmed by a significant correlation between delta progesterone and higher EPDS in the simple regression of differences test. This may be due in part to the fact that the time point at which progesterone is likely to be lowest, one week postpartum, was also the time point for which there was the poorest compliance. If a larger sample of women had had their lowest progesterone level recorded, it might well have produced a correlation with maximum postnatal EPDS.

Progesterone is known to function as an anxiolytic, because it interacts with GABA-A receptors to enhance GABA's ability to bind to neuronal membranes. In fact, this mediation of receptor function is similar to that of benzodiazepines and barbiturates. In addition, progesterone's sedative effect can be attributed to this same mode of action (Reddy and Kulkarni, 1997). With this in mind, it was noted that questions 4 and 5 of the

EPDS seem to deal specifically with the new mother's feelings of anxiety. Anxiety is a common feature of clinical depression, and research has shown the prevalence of anxiety as a separate entity from PPD to be 8.7% at 14 weeks postpartum and 16.8% at 30 weeks postpartum (Stuart et al, 1998). In fact, a recent study that examined the tendency of anxiety and PPD to occur together in the same woman, concluded that the EPDS may be a good screening instrument for anxiety as well as depression. It correlates with the well-known State Anxiety Scale of the State-Trait Anxiety Inventory with $r = 0.73$ at 14 weeks and $r = 0.82$ at 30 weeks (Stuart et al, 1998).

It seems to follow that high prenatal progesterone and its anxiolytic properties might set the woman up for feelings of anxiety after the birth when the hormone is suddenly withdrawn upon delivery of the placenta. The effect may not have been seen in some previous studies because mood was not treated as a continuum, but subjects were simply grouped into the categories "depressed" and "not depressed". Recall that the current findings do not necessarily show that high prenatal progesterone predisposes a woman to depression later. They only show that high prenatal progesterone is correlated with a higher postnatal maximum EPDS score.

The results of a Chi Square test on the answers given to questions 4 and 5 of the EPDS do not support a hypothesis that withdrawal of progesterone at the time of birth leaves the women vulnerable to anxiety for some period of time. However, small sample numbers and use of only some of the questions on the EPDS for this particular inquiry make it impossible to state with certainty the true nature of the relationship between prenatal progesterone levels and anxiety.

Future research might involve testing women whose prenatal progesterone was found to be relatively high with the State-Trait Anxiety Inventory, developed by Dr. Charles D. Spielberger in 1983. This well-accepted, self-scoring, 40-item exam is considered capable of an accurate quantification of postnatal anxiety apart from its being one of just several components in postpartum depression. If anxiety is indeed found highly correlated with elevated prenatal progesterone, further investigation of Dalton's claims that natural progesterone can be a suitable prophylaxis might be in order. The tentative conclusion that may be reached from this investigation is that there is some evidence to suggest that high prenatal progesterone may serve as a useful predictor of postpartum depression, and the idea warrants further investigation. In any case, these data appear to offer at least some support for Dalton's hypothesis that progesterone withdrawal at the time of birth is a causative factor in development of postpartum depression (Dalton, 1989; Dalton, 1995).

Results of a t-test at four weeks postpartum showed significantly higher average serum progesterone in women whose EPDS scores were 13 or above than in women with EPDS scores below 13. This association between high progesterone and presumed postnatal depression seems to contradict Dalton's (1995) idea that progesterone may help alleviate PPD. However, the depressed group contained only eight women, and one of them had a progesterone value about 1,000 ng/mL higher than the average of the rest of the women in the depressed group. When the test is rerun without this data point, the probability value rises to >0.5, and the difference in average serum progesterone is not significant between the depressed and non-depressed women. This number was not reported in the results section, because simply discarding a seemingly aberrant value in a

small sample size is a highly controversial practice in statistics. Therefore, it is difficult to place much credence in the results whether the "outlying" data point is left in the sample of depressed women or discarded.

Estriol

In light of the one previous study that was found that correlated *high* prenatal estriol with greater tendency to experience postpartum blues in the first 10 days after birth (O'Hara et al, 1991), results showing that *low* prenatal estriol correlated with later increases in EPDS scores were surprising. Exact comparisons are difficult, however, since different mood measures were used, and women in the O'Hara study were not assessed at 4, 8, and 12 weeks postpartum.

Estriol is only one of two important prenatal estrogens. The ovaries and placenta also manufacture estradiol, which is much more biologically active, although it is present in lesser amounts (Wilson et al, 1998). Prenatal estradiol was assayed in this study. Unfortunately, the results were unreliable because of cross-reaction with estriol. No such cross-reaction was reported to occur by the manufacturer with the test for estriol. Therefore, usable data were only obtained for prenatal estriol, not prenatal estradiol.

Since total plasma free estrogen reflects both estriol and estradiol during pregnancy, there are at least two possible scenarios in which lower placental estriol might correlate with higher placental or ovarian estradiol. First, if low placental estriol means less competition with the more potent estradiol for estrogen receptors, then perhaps estradiol can be freer to exert its powerful effects on the CNS. Alternatively, low placental estriol could reflect increased use of the alternative pathway for the processing of steroids in the fetal adrenal and liver that leads to production of DHEAS, rather than 16-OH DHEAS.

DHEAS is the precursor of estradiol in the placenta. Should lower estriol levels correspond to high levels of the more potent estradiol, perhaps the later abrupt loss of placental estrogens at the time of birth would carry a higher risk of later depressive symptomology.

Without prenatal estradiol levels, it was not possible to attempt a correlation between high delta estrogen values and high EPDS scores. However, the literature linking low postpartum estradiol to tendency to postpartum depression is so abundant, that the links between the two are hard to deny. Data from this study are likely not an adequate basis from which to draw firm conclusions about the etiology of a link between low prenatal estriol and later tendency to mood dysphoria. However, the tentative conclusion that may be reached from these data is that low serum estriol may be a predictor for later propensity to develop postpartum depression. Given the significant P value and the lack of other investigations into this possible relationship, further research seems warranted. Confirmation of a relationship between either high or low prenatal estriol and later increased risk of developing PPD would be especially useful, because an expectant mother and her physician could have advance warning of vulnerability, rather than a mere endocrine marker present at the time the dysphoria is occurring.

Ratio of Progesterone to Estriol

In addition to correlations between absolute values of estriol or progesterone and EPDS score, there may be a relationship between the ratio of progesterone to estriol, estradiol or total estrogen and EPDS score. A changing progesterone:estrogen ratio has been explored as a possible factor in other perinatal events, such as human parturition. Levels of both estrogens and progesterone are so great in normal human pregnancy,

however, that there is an excess of both in terms of classic estrogen and progesterone receptors (Wilson et al, 1998). Therefore, unless estrogens and progesterone function by some nongenomic mechanisms, their concentrations relative to each other may not be a critical factor in parturition (Wilson et al, 1998) nor, perhaps, for mood state. However, after delivery of the placenta, progesterone:estrogen ratio might become a more important factor in light of the previously discussed effects of female sex steroids on MAO activity (Arpels, 1996). Results of the present research did not reveal any correlations between estriol or progesterone and EPDS score after the prenatal time point. However, future investigation of correlations between progesterone:estrogen and EPDS might reveal a clinically relevant relationship.

Estradiol

Results of a t-test at four weeks postpartum showed a significantly higher serum estradiol in women whose EPDS scores were 13 or above than the average serum estradiol for women with EPDS scores below 13. However, the same woman whose abnormally high progesterone value appeared to confound the progesterone t-test results had a serum estradiol value that was about 1,400 pg/mL higher than the average of the other depressed women. Further, as with the progesterone t-test results, only eight women were in the depressed group. When the test is rerun without this data point, the probability value rises to >0.35, and no differences in average serum estradiol are significant between the depressed and non-depressed women. This p value was not reported in the results section, because discarding a seemingly aberrant value in a small sample size is a highly controversial practice in statistics. Therefore, it is difficult to

place much credence in the results whether the "outlying" data point is left in the sample of depressed women or discarded.

At 12 weeks postpartum a t-test revealed that women with EPDS scores of 13 or higher had a slightly significant higher serum estradiol than women with EPDS scores below 13. No obvious outliers in the data could account for the result, but the depressed group contained only three women. It is quite possible that the difference between the two groups occurred as mere chance. Estradiol is generally believed to contribute to mood elevation and has been shown to be an effective treatment for PPD (Arpels, 1996; Sichel et al., 1995; Gregoire et al, 1996; Grinspoon, 1997).

Cortisol

The significant correlation between high prenatal cortisol levels and high maximum EPDS score is in agreement with two past studies that have linked high prenatal cortisol levels to blues and PPD (Handley, 1980; Okano and Nomura, 1992). However, Okano also found an even more significant association between high *postnatal* cortisol levels and tendency to development of blues. Since compliance in the present study was low at one week postpartum, it is not clear whether high cortisol in the early postpartum period correlated with high EPDS scores. Further, the EPDS is designed to screen for depression several weeks after birth, rather than the blues a short time after delivery. In any case, the tentative conclusion that may be reached from the present results is that high prenatal cortisol may also serve as a useful marker for later propensity to develop postpartum depression.

Most of the maternal plasma cortisol present in the third trimester is of fetal adrenal origin (Majzoub et al, 1999). It is manufactured primarily in response to CRH from the

placenta and functions to prepare fetal lungs for gas exchange and the ductus arteriosus for closure after birth. Cortisol stimulates, rather than inhibits, placental CRH production, and is hence an indicator of high CRH levels. High CRH levels probably down-regulate CRH receptor numbers in the anterior pituitary of the mother (Magiakou, et al, 1996). A transient period of hypothalamic-pituitary-adrenal suppression is likely following childbirth due to the down-regulation. In support of this notion, some research has shown that ACTH response to injection of ovine CRH in the postpartum period was more blunted in a group of depressed women than in a group of euthymic women (Magiakou, at al, 1996). Interestingly, there was no difference between these two groups in levels of cortisol response.

Postpartum depression can sometimes exhibit features of the "atypical" variety, such as irritability, hyperphagia, and hypersomnia, as seen in seasonal affective disorder and the chronic fatigue/fibromyaliga syndrome discussed earlier. This type of depression has been linked to underproduction of hypothalamic CRH (Cizza, 1997). Further, research has shown that in women, the CRH gene contains estrogen receptor-binding estrogen-responsive elements (Vamvakopoulos, 1993). Cizza believes that low postpartum estrogen levels may hinder the expression of CRH and prevent proper secretion of cortisol (Cizza, 1997). Although it should be remembered that too much CRH and cortisol can also be associated with depression (Nemeroff, 1998), not enough CRH and the subsequent underproduction of ACTH, have been suspected as biochemical markers for PPD or blues (Magiokou et al, 1996). To summarize, then, it seems plausible that women with high prenatal cortisol levels and/or particularly low postpartum estrogen so

blunt their HPA axis that postpartum CRH production does not return to normal levels for several weeks, leaving them at increased risk for PPD.

Prolactin/Lactation

The results of the Chi Square test showed a slightly significant association between not breastfeeding and tendency to become depressed. These findings would seem to imply that a crash in plasma prolactin levels within the first few weeks after birth may precipitate acute onset of postpartum dysphorias. However, the p value was above 0.10, and the results were not supported by a correlation of high prenatal prolactin with max EPDS scores or by a correlation of large delta prolactin with max EPDS scores.

Curiously, neither the multiple backward stepwise regression nor the simple linear regression found any significant relationship between maximum EPDS score and whether or not the woman was lactating. Running a Chi Square in spite of these negative results was justified, however, because lactation was one of only two independent variables tested that could be considered an "all or none" state rather than a continuum, as levels of hormones are. (The other was presence or absence of thyroid antibodies.) In addition, weak but significant relationships are not always equally revealed by various statistical tests. The authors felt that any valid and appropriate statistical instrument that showed some degree of significance between two variables should be reported for the sake of thoroughness. The slightly significant results obtained were in agreement with a study by Abou-Saleh (1998) in which women who breastfed had significantly lower EPDS scores and Present State Examination (PSE) scores (for anxiety) than their non-breastfeeding counterparts. In Abou-Saleh's study, the cut-off for depression was 11/12, instead of 12/13. If the present research had used the same criteria, it may have resulted in smaller

p values that would have appeared to lend even more support to the idea that breastfeeding somehow provides some protection against PPD. In any case, the present results suggest an inverse relationship between lactation and depression and represent an area where more research may be warranted.

TSH, Free T4, and Thyroid Antibodies

In the Multiple Backward Stepwise Regression at four weeks postpartum, high-end TSH, high free T4, and positive thyroid antibodies all correlated with high-end maximum EPDS scores, suggesting a disruption of some sort in thyroid function in women with the highest EPDS scores. Admittedly, neither high TSH nor high free T4 showed significant correlation with maximum EPDS score in a simple linear regression at four weeks. However, both the multiple backward stepwise regression and the simple linear regression with EPDS scores taken at the same time point gave significant results at four weeks for TSH and thyroid antibodies. Further, in the t-test women with EPDS scores of 13 or higher had significantly higher TSH than women with EPDS scores below 13, even though none of the women in the depressed group had a clinically significant elevated TSH value. Though not definitive, these results seem to suggest a mild, subclinical hypothyroidism, defined as the presence of normal thyroid hormone levels and an elevated TSH (Pies, 1997). Depression is a known feature of subclinical hypothyroidism, occurring in perhaps 15% of cases (Pies, 1997). Previous studies have only occasionally reported a clear relationship between PPD and TSH or T4 levels. For example, two published reports over a decade ago noted that transient postpartum hypothyroidism, as evidenced by low T4, seemed to be linked to postpartum depression (Harris et al, 1989; Pop et al, 1991).

The result from the multiple backward stepwise regression showing a correlation of high T4 at four weeks with max EPDS score is perhaps the weakest of the significant thyroid hormone data, because it is not supported by results from either the simple linear regression or the t-test. However, the association between the higher postpartum serum T4 values and greater incidence of depressive symptomology at some postpartum time point may suggest a transient, subclinical or relative "hyperthyroidism." This rise in serum T4 could be a reflection of the causative factor in some cases of postpartum depression—postpartum autoimmune thyroiditis.

It must be noted that none of the women's scores for free T4 in this study was above the maximum accepted normal value, 2.4 ng/dL (Bakerman, 1994). None of the women, therefore, could be diagnosed as clinically hyperthyroid. The point must be made, however, that the direction in which the free T4 changed suggests trends that agree with earlier statements by researchers that postpartum thyroiditis tends to progress from hyperthyroidism some 4-12 weeks postpartum to a hypothyroid phase 3-8 months postpartum. The condition is self-correcting and rarely requires thyroxine replacement therapy (McPherren Stover and Marnejon, 1995; Roti and Emerson, 1992). However, education and psychotherapy might help ease the fears or mental discomfort of the new mother during this period in which child bonding is taking place.

Note that by eight weeks postpartum, only high TSH and positive thyroid antibody tests correlated with maximum depression scores in both the multiple backward stepwise regression and the simple linear regression. As with the free T4 results, levels of TSH were nearly always below the accepted maximum normal value. However, the correlation between high-end TSH values and high end EPDS scores coordinates well

with the expectation that frank postpartum hyperthyroidism is usually followed by hypothyroidism due to postpartum thyroiditis. By this time, apparently, most of the women with positive thyroid antibodies have experienced a plunge in their T4 levels, while TSH remains elevated due to lack of negative feedback by T4 and/or stimulation by autoimmune antibodies against TSH receptors (Dayan and Daniels, 1996). Incidentally, the reader may recall that autoantibodies against the TSH receptor were not assayed in this research project.

Any linkage that exists in this study between thyroid autoantibodies or hormones and maximum EPDS score becomes undetectable by 12 weeks postpartum. This finding could suggest that detectable abnormalities in levels of T4 and TSH, as well as presence of autoantibodies, are well on their way to self-resolution by this time, as suggested by McPherren Stover and Marnejon (1995). However, most of the women in this project who screened positive for postpartum depression prior to 12 weeks opted to go on appropriate medication and were dismissed from the study. A likely explanation for failure to find a correlation at this time point, then, is that the subjects whose plasma assays were most likely to show such a relationship were the least likely to remain in the study.

Visual Analog Scales

The highly significant correlations between each of the six statements of the VAS and EPDS scores at four weeks postpartum seem to suggest a relatively stable mood state over the two-week period from postpartum day 15 to postpartum day 28. These results support O'Hara's report that women who met VAS blues criteria and/or the Handley Blues criteria were more likely to experience postpartum depression measured at

postpartum week nine than women who did not meet these criteria (O'Hara et al, 1991). However, O'Hara et al were assessing the first ten days postpartum retrospectively at week nine. It is possible that women who were clinically depressed at week nine remembered their first postpartum days in a more negative light than they would have if they had not been depressed at the time of the retrospective assessment.

There may be a potential clinical significance of the finding that mood and blues symptoms in the third postpartum week are predictive of EPDS score at four weeks postpartum. Since mood appears to remain relatively stable during this time period, perhaps the EPDS could be used to diagnose postpartum depression with an acceptable degree of accuracy earlier than the four to five weeks postpartum recommended by the authors (Cox and Holden, 1994). Such an early diagnosis would make it possible to begin treatment quicker and, hopefully, shorten the time of suffering.

Limitations of the Study

Small sample size, missing data associated with poor compliance, and significant attrition limit the confidence that can be placed in the results, particularly in areas where the probability values are not smaller than 0.05. In addition, the EPDS, although an excellent screen for depression, is not a definitive diagnosis, and at best any one score only reflects the feelings of a participant over a week-long period, far short of the minimum of two weeks required to differentiate true PPD from a milder and more transient dysphoria. Finally, in some cases, the maximum postpartum EPDS score used occurred before the hormonal level with which it correlated, making the goal, a prediction of risk for depression based on serum hormone values, impossible.

Ethical considerations also limited this study of postpartum depression. When a patient received an EPDS score of 13 or above, the physician was notified, a diagnosis of depression was confirmed, and the patient was typically given treatment options, including antidepressant medication. Since antidepressants may interfere with the relationship between hormones and mood, it was necessary to dismiss from the study patients who elected to take them. It became impossible, then, to know how long the depression would have lasted on its own or what the results of serum hormone assays and/or EPDS tests would have looked like for those women at later time points in the study. Further, the eight and 12-week time points presumably had fewer women with elevated EPDS scores than if depressed volunteers diagnosed at four weeks had remained in the study. Therefore, it became more difficult to detect correlations between hormones and elevated mood scores as time progressed.

There are at least two ways to get serum hormone data and EPDS scores from depressed women at eight and 12 weeks without encountering the ethical dilemma described above. The first is that some women's postpartum depression might not develop until the second or third postpartum month, and they would therefore not be dismissed from the study at some earlier time point. The second is that women who suspect they might have postpartum depression could be recruited from the community between six and 12 weeks after they give birth.

Summary and Recommendations

To summarize, this research agrees with former findings that positive thyroid antibody status is the best indicator among thyroid hormones, autoantibodies, and thyrotropic hormones, of tendency to develop elevated EPDS scores suggestive of depression.

However, elevated TSH and T4 levels may also serve as markers of a risk for postpartum depression, especially if they are used as an adjunct to positive thyroid antibody tests. Although thyroid dysfunction is far from the only possible endocrine causative factor in the genesis of postpartum depression, it is likely often a significant component in PPD's pathogenesis. Results also point to the need for further investigation into a possible correlation of prenatal serum estriol with later depressive symtomology.

In conclusion, given the variety of serum hormone correlates with PPD across the literature and in the present data, it is the considered opinion of the author that PPD has several unrelated etiologies, and indeed may represent several distinct maladies, each of which has depression as a common symptom. The challenge in the future, then, is to carefully identify each of the conditions, hormonal and otherwise, that can result in PPD and work to develop effective screening tests and treatments. Only with clear knowledge of PPD's causes will it be possible to appropriately combat this emotionally painful and sometimes devastating condition.

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APPENDIX A

EDINBURGH POSTNATAL DEPRESSION SCALE

How Are You Feeling?
As you have recently had a baby, we would like to know how you are feeling now. Please underline the answer which comes closest to how you have felt in the past 7 days, not just how you feel today.

Here is an example, already completed:

I have felt happy:
Yes, most of the time
Yes, some of the time
No, not very often
No, not at all

This would mean: "I have felt happy some of the time" during the past week. Please complete the other questions in the same way.

IN THE PAST SEVEN DAYS

1. I have been able to laugh and see the funny side of things:
   As much as I always could
   Not quite so much now
   Definitely not so much now
   Not at all

2. I have looked forward with enjoyment to things:
   As much as I ever did
   Rather less than I used to
   Definitely less than I used to
   Hardly at all

*3. I have blamed myself unnecessarily when things went wrong:
   Yes, most of the time
   Yes, some of the time
   Not very often
   No, never

4. I have felt worried and anxious for no very good reason:
   No, not at all
   Hardly ever
   Yes, sometimes
   Yes, very often

*5. I have felt scared or panicky for no very good reason:
Yes, quite a lot
Yes, sometimes
No, not much
No, not at all

*6. Things have been getting on top of me:
Yes, most of the time I haven't been able to cope at all
Yes, sometimes I haven't been coping as well as usual
No, most of the time I have coped quite well
No, I have been coping as well as ever

*7. I have been so unhappy that I have had difficulty sleeping:
Yes, most of the time
Yes, sometimes
Not very often
No, not at all

*8. I have felt sad or miserable:
Yes, most of the time
Yes, quite often
Not very often
No, not at all

*9. I have been so unhappy that I have been crying:
Yes, most of the time
Yes, quite often
Only occasionally
No, never

*10. The thought of harming myself has occurred to me:
Yes, quite often
Sometimes
Hardly ever
Never

Response categories are scored 0, 1, 2, and 3 according to increased severity of the symptom. Items marked with an asterisk are reverse scored (i.e. 3, 2, 1 and 0). The total score is calculated by adding together the scores for each of the ten items. Users may reproduce the scale without further permission providing they respect copyright (which remains with the *British Journal of Psychiatry*) by quoting the names of the authors, the title and the source of the paper in all reproduced copies.

APPENDIX B
PATIENT ENROLLMENT INFORMATION

Name: _____
Last First Middle

Date of birth: _____
Month Day Year

Are you expecting more than one baby?
Yes No

Do you expect to deliver vaginally?
Yes No

Marital status: (circle one)
Married Separated Divorced Widowed Single

Was this pregnancy planned?
Yes No

Is this your first child?
Yes No

If no, please give number of children before this pregnancy:

Did you experience feelings of sadness or "the blues" for a few days during the first two weeks after delivering any previous children?
Yes No

Did you feel depressed for more than one week during the first three months after delivering any of your previous children?
Yes No

If yes, please check or circle the symptoms you experienced.
_____ poor appetite or increased appetite with weight loss or weight gain
_____ sleep difficulty or sleeping too much
_____ loss of energy or tiredness
_____ feelings of nervousness and agitation or feelings of being slowed down or unable to get going
_____ excessive guilt
_____ inability to think or concentrate or trouble making decisions
_____ loss of interest or pleasure in usual activities, including social contact or sex
_____ recurrent thoughts of death or suicide, or any suicidal behavior

Have you ever been diagnosed with depression?
Yes No

Have you ever taken prescription medication to treat depression?
Yes No

Have you ever been diagnosed with bipolar disorder (manic depression)?
Yes No

Do you have any close family members who have been diagnosed with depression or bipolar disorder (manic-depression)?
Yes No

If yes, please list and give your relationship to each one.

When you are not pregnant, do you regularly experience premenstrual syndrom (PMS); that is, do you tend to become markedly depressed and/or irritable before you start your period?
Yes No

Do you drink alcohol?
Yes No

If yes, please state how many drinks per day. (A drink is defined as one 12-ounce can of beer or a six-ounce glass of wine or two ounces of hard liquor.)

Are you currently taking any medications prescribed by your doctor?
Yes No

If yes, please list medications below:

Are you using over the counter drugs?
Yes No

If yes, please list medications below:

Are you currently using illegal drugs?
Yes No

After the birth of the child, the following questions will be asked each time blood is drawn:

Name: _____
Last First Middle

Do you drink alcohol?
Yes No

If yes, please state how many drinks per day. (A drink is defined as one 12-ounce can of beer or a six-ounce glass of wine or two ounces of hard liquor.)

Are you currently taking any medications prescribed by your doctor, or are you using over the counter or illegal drugs?
Yes No

If yes, please list medications below (don't forget to mention birth control pills):

Are you breast-feeding your baby at this time?
Yes No

If yes, are you supplementing with formula or baby food?
Yes No

If yes, please state the approximate number of bottles of formula or jars of baby food you are giving the baby per day:
Bottles (4 oz):_____ per day Jars of baby food:_____ per day

Have you experienced any stressful life events such as marital separation, death in the family, severe health problems or the like since the birth of your child?
Yes No

Are you sleep-deprived at this time?
Yes No

If yes, estimate how many hours, on average, sleep you are getting each day?

I understand that if the results of my test indicate that I may be experiencing some form of depression, my doctor will be notified. I further understand that I may be terminated from the study for any of the following reasons: failure to completely or properly fill out the questionnaire, complicated or abnormal delivery, Cesarean delivery, delivery of more than one baby or use of prescription or illegal drugs or alcohol. My information will be kept strictly confidential.

_____ _____

signature date

APPENDIX C

VISUAL ANALOG SCALES

Read each statement below. Decide whether each statement describes how you have been feeling in the past 24 hours. Place a dot somewhere along each line to show how much you agree with the statement above it.

1. I am feeling happy and self-confident.

Not at all — Better than I have ever felt before

2. I am feeling miserable and depressed.

Not at all — Worse than I have ever felt before

3. I have been in tears.

Not at all — All the time

4. I am very worried and anxious.

Not at all — Worse than I have ever been before

5. I am very irritable and quick tempered.

Not at all — Worse than I have ever been before

6. My spirits are going up and down like a yoyo.

Not at all — More so than I can ever remember

APPENDIX D

INTERNAL REVIEW BOARD FORM

Oklahoma State University College of Osteopathic Medicine

Institutional Review Board

Memo

To: Gary Watson, Ph.D., Associate Professor of Biochemistry

Sarah Breese-McCoy, M.S. (Ph.D. candidate)

From: Dianne Miller-Hardy, Ph.D., J.D.

Chairman, Institutional Review Board

Date: January 3, 2000

Re: **Correlation of Postpartum Depression with Various Plasma Hormone Levels**

The OSU Institutional Review Board has received a copy of the above protocol and consent form containing the recommended changes to the consent form. The protocol now meets complete approval of the OSU-IRB and the investigators are free to begin their research. If you have any questions regarding this memo, please don't hesitate to phone me at extension 8406.

Protocol # 9899015

APPENDIX E

INFORMED CONSENT FORM

INFORMED CONSENT FORM

Title of Project: **Correlation Between Plasma Hormone Levels and Postpartum Mood State**

Investigators: **Sarah J. McCoy, M.S.**
Graduate Student
Department of Research
(918) 561-8241

Gary H. Watson, Ph.D.
Associate Professor of Biochemistry
Department of Research
(918) 561-8241

This is to certify that I, _____, hereby agree to participate as a volunteer in a scientific investigation as part of an authorized research program of OSU-COM under the supervision of Sarah McCoy.

The purpose of this research is to search for a physical, hormonal cause for postpartum depression that occurs within the first three months after an infant is born.

The procedures to be followed are:

- I will be asked to answer several questions during screening to assure my meeting all the criteria for admission into the study.
- I will be asked to voluntarily submit a urine sample for drug screen analysis.
- Approximately 20 mLs (3 tubes) of blood will be drawn from me at 35 weeks of pregnancy and 5 times following the normal, vaginal birth of a normal, healthy infant at time of delivery, 1 week, 4 weeks, 8 weeks, and 12 weeks postpartum.
- The blood will be drawn at my obstetrician's office by medical personnel on staff there or at the hospital.
- At the time the blood is drawn, I may be asked to complete a short standardized questionnaire that is designed to screen for depression.

I understand that I may expect the following physical discomfort during the course of this research:

- Mild pain or minor bruising of the arm associated with having blood drawn.

I understand that this research will result in the following benefit to me:

A payment schedule has been established by which I will receive payment for taking part in this study.

- After completion of my part in this study (e.g. all samples collected and forms completed) I will receive a maximum of $120.00. If I fail to make all blood draws, $20 per draw will be subtracted from the total amount shown above.

Beyond the reimbursement stated above, there are no direct benefits to me. After the completion of this study a summary of outcomes can be requested through my primary care physician.

I understand that my results will be coded such that my identity will be protected at all times and publication of results will not identify me in any way.

I understand that I am free to refuse to participate in any procedure and to refuse to answer any question at any time, and am free to withdraw my consent, and to withdraw from the research at any time without penalty. However, I will only be paid for the times my blood is actually drawn and I fully complete the questionnaire.

I understand that the research investigators named at the beginning of this form, and the Director of the Office of Research and Sponsored Programs, OSU-COM (918) 561-8241 will answer any of my questions about the research procedures, my rights as a subject, and research-related injuries at any time.

If I have questions about my medical treatment or if I feel I have an injury from participation, I should call my primary care physician for assistance or advice.

Additional information on the limits and restrictions of monetary compensation can be obtained by calling Dr. Gary Watson at 561-8241.

I understand that by agreeing to participate in this research and signing this form, I do not waive any of my legal rights, nor are the investigators, sponsor, the institution or its agents free from liability for negligence.

APPROVED

JAN 1 0 2001

OSU-COM
INSTITUTIONAL REVIEW BOARD

I have read this consent form and freely consent to participate in the study. I will receive a copy of this consent form.

Subject _____ Date _____

I certify that I have explained all elements of this consent form to the subject or legal representative and answered all questions asked before requesting they sign it.

Investigator or their representative who is requesting consent _____ Date _____

Witness _____ Date _____

APPROVED

JAN 1 0 2001

OSU-COM

REVIEW BOARD

VITA 2

Sarah J. McCoy

Candidate for the Degree of

Doctor of Philosophy

Dissertation: CORRELATION OF POSTPARTUM MOOD DYSPHORIAS WITH VARIOUS PLASMA HORMONE LEVELS

Major Field: Biomedical Sciences

Biographical:

Personal Data: Born in Tulsa, Oklahoma, June 22, 1963, daughter of C. F. (Nick) Breese and C. JoAnn Breese. Married to Thomas F. McCoy September 1, 1986. Mother of four children, Ellen Elizabeth, Philip Thomas, Davis Colgate, and Mark Nicholas.

Education: Graduated from Owasso High School, May, 1981; received Bachelor of Science degree in Health Science from the University of Tulsa, in Owasso, Oklahoma, May, 1985. Received Master of Science degree in Organismic Biology from University of Tulsa, May, 1988. Completed the requirements for the Doctor of Philosophy degree with a major in Biomedical Science at Oklahoma State University College of Osteopathic Medicine in December, 2001.

Experience: Employed as adjunct instructor at Tulsa Community College, 1985-1996; 1998-1999; 2001, and Rogers State University as associate instructor 1996-1998. Taught zoology, general biology, anatomy, physiology, human anatomy & physiology, college algebra, chemistry I, chemistry II, basic biochemisty, general physical science, basic physical science, basic biology and basic chemistry. Guest lecturer at OSU College of Osteopathic Medicine in Biochemistry and Physiology 1998-2001.