Department of Women’s and Children’s health

Is peripartum depression just another depression?

LICENTIATE THESIS

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Abstract

Depressive symptoms in pregnancy are common, reported by approximately 20% of pregnant women worldwide. Of these, around 4-7% fulfill the criteria for major depressive episode (MDE).

The prevalence rates of MDE seem no different from those in non-pregnant women of childbearing ages, or may even be lower. Further, the clinical presentation of depressive symptoms in women of childbearing age does not differ depending on whether women are pregnant, postpartum or outside the peripartum period. For this reason, some researchers argue that peripartum depression is just another depression, merely occurring at a stressful point in life.

Antenatal depression and antidepressant treatment have been associated with an increased risk of poor pregnancy outcomes, such as preterm birth, impaired placental function, decreased fetal body and head growth. Nevertheless, little is known about the biological mechanisms behind these complications and more research is needed to elucidate the underlying pathways.

In this thesis we have studied 1) attentional bias in antenatal and postpartum depression, with or without antidepressant treatment and 2) peripheral inflammatory markers in pregnancy (depressed, SSRI-treated, healthy controls).

The title for this thesis is: Is peripartum depression just another depression? Based on the findings we have obtained thus far, the answer would be no. One argument would be that, as presented in study I, women who suffer from antenatal and postpartum depression do not display the typical attentional bias to negative words that is characteristic of depressive states in the non-pregnant population. Whether this is due to protective mechanisms of pregnancy or due to features that distinguish antenatal and postpartum depression from non-peripartum depression remains to be demonstrated.

Secondly, study II describes that women with antenatal depression had significantly lower levels of peripheral inflammatory markers than healthy pregnant controls. Hypothetically, this could be due to dysregulated switch to the anti-inflammatory pro-M2 milieu that characterizes normal third trimester pregnancy. These findings are clearly at odds with the literature in non-pregnant samples, where depression has been associated with increased levels of proinflammatory cytokines, but should be interpreted in the context of pregnancy-induced changes in inflammatory response.

Moreover, treatment for antenatal depression is not as straightforward as it is in non-pregnant patients. When considering treatment, the expecting mother has to be aware of the risk-benefit profile for herself and the child. While antidepressant therapy clearly improves the mood of treated women, our findings do not indicate that antidepressant treatment has any positive impact on their inflammatory profile.
List of Papers

This Licentiate thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I  Edvinsson, Å., Skalkidou, A., Hellgren, C., Gingnell, M., Ekselius, L., Willebrand, M., Sundström Poromaa, I. Different patterns of attentional bias in antenatal and postpartum depression. *Submitted*

II Edvinsson, Å., Bränn, E., Hellgren, C., Freyhult, E., White, R., Kamali-Moghaddam, M., Olivier, J.D., Bergquist, J., Boström, A.E., Schiöth, H.B., Skalkidou, A., Cunningham, J.L., Sundström Poromaa, I. Lower inflammatory markers in women with antenatal depression brings the M1/M2 balance into focus from a new direction. *Submitted*
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<tr>
<td>DSM</td>
<td>The Diagnostic and Statistical Manual of psychiatric disorders</td>
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<td>MDE</td>
<td>Major depressive episode</td>
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<td>SSRI</td>
<td>Selective serotonin reuptake inhibitor</td>
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<td>PrA</td>
<td>Pregnancy-related anxiety</td>
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<tr>
<td>HPA</td>
<td>Hypothalamus-pituitary-adrenal</td>
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<tr>
<td>CRH</td>
<td>Corticotropin releasing hormone</td>
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<tr>
<td>GABA</td>
<td>Gamma-aminobutyric acid</td>
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<tr>
<td>Th1</td>
<td>Type 1 T helper</td>
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<td>Th2</td>
<td>Type 2 T helper</td>
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<td>TNF</td>
<td>Tumor necrosis factor</td>
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<td>IL</td>
<td>Interleukin</td>
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<td>TGF</td>
<td>Transforming growth factor</td>
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<td>MCP</td>
<td>Monocyte chemoattractant protein</td>
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<td>WM</td>
<td>Working memory</td>
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<td>NIRS</td>
<td>Near-infrared spectroscopy</td>
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<td>PFC</td>
<td>Prefrontal cortex</td>
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<td>TCA</td>
<td>Tricyclic antidepressants</td>
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<td>NICE</td>
<td>National Institute for Health and Care Excellence</td>
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<tr>
<td>qPCR</td>
<td>Quantitative real-time polymerase chain reaction</td>
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<tr>
<td>BASIC</td>
<td>Biology, Affect, Stress, Imaging, Cognition</td>
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<tr>
<td>EPDS</td>
<td>Edinburgh Postnatal Depression Scale</td>
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<tr>
<td>MADRS</td>
<td>Montgomery-Åsberg Depression Rating Scale</td>
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<tr>
<td>STAI</td>
<td>Spielberger State-Trait Anxiety Inventory</td>
</tr>
<tr>
<td>MDD</td>
<td>Major depressive disorder</td>
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<tr>
<td>M.I.N.I</td>
<td>Mini International Neuropsychiatric Interview</td>
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<tr>
<td>LDA</td>
<td>Low-density array</td>
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Introduction

Peripartum depression is a new concept introduced by the fifth edition of The Diagnostic and Statistical Manual of psychiatric disorders (DSM-5). Previously, in the DSM-IV, the specifier used for depression in relation to childbirth was major depressive episode (MDE) with postnatal onset, which effectively discarded all depressive episodes that started during pregnancy.

From a clinical perspective, the peripartum onset specifier was welcomed, as around 50% of episodes of postpartum depression have an onset of symptoms already before delivery (1). However, the DSM-5 specifier retained the four-week onset window in the postpartum period, which continues to be questioned. Numerous reports suggest that many depressive episodes start between two and six months postpartum, and that the peak in postpartum onset occurs within the first three months postpartum (2, 3).

Further criticism of the DSM-5 criteria also includes the failure to incorporate distinct onset specifiers for pregnancy and postpartum (4), with the argument that such specifiers would stimulate and refine research on the etiology of MDEs during these time-points. Obviously, circulating hormone levels, stress, sleep patterns and immune system functioning differ between pregnancy and the postpartum period, and distinct specifiers could have shed light on the roles that these factors play in the onset of mood disorders in relation to child birth (5).

Yet another concern with the absence of pregnancy and postpartum onset specifiers in the DSM-5, is the strong connection between bipolar disorder and the risk of postpartum depression (and psychosis) (6-8), which appear less concerning in women with antenatal onset. With potentially different etiologies, different clinical profiles, and different responses to treatment between the antenatal and postpartum MDEs, unless further researched, diagnostic inaccuracies and inappropriate treatments will continue to be prevailed. For these reasons, this thesis has not used the concept of peripartum depression, but instead separated the antenatal depression from the postpartum depression.

Nevertheless, the new definition of peripartum depression is, according to DSM-5 criteria, an MDE with onset during the entire pregnancy or in the four weeks following delivery. The symptoms can be divided into core symptoms: depressed mood and loss of interest or pleasure in usual activities; and additional symptoms such as change in appetite or weight, change in sleep and activity, fatigue or loss of energy, feelings of guilt or worthlessness, diminished ability to think or concentrate, presence of suicidal thoughts, plans or attempts. The symptoms must have affected the patients to such degree that they impaired her way of functioning at home, at work, at school or in another important way most of the day and nearly every day, for at least two weeks. Thus, the clinical presentation of depressive symptoms in women of childbearing age does not differ depending on whether women are pregnant, postpartum or outside the peripartum period (9). For this reason, some researchers argue that peripartum depression is just another depression, merely occurring at a stressful point in life (9).

Antenatal depression

Depressive symptoms in pregnancy are common, reported by approximately 20% of pregnant women worldwide (3). Of these, around 4-7% fulfill the criteria for MDE (10-13). Again, in favor of those arguing that peripartum depression is no different from other types of depressive episodes, the prevalence rates of MDE seem no different from those in non-pregnant women of childbearing ages (14), or may even be lower (9). Furthermore, several lines of evidence suggest that pregnancy is a protective period for the most severe forms of depression. Women are less inclined to endorse
Depressive symptoms in pregnancy (9), the risk of suicide is extremely low in pregnancy, even in comparison with the postpartum period (15), and pregnancy has also been shown to protect against psychiatric admission (16).

Even so, being depressed during pregnancy is associated with other concerns. Most importantly, the depression will reduce quality of life and work performance of pregnant women at an important stage of life. Women with antenatal depression more often are in need of prolonged sick leaves during pregnancy, and they have increased number of health care contacts, especially in relation to fear of childbirth. Planned Caesarean section and epidural analgesia during labor are also more common in women with antenatal depression (17, 18).

Secondly, antenatal depression has been associated with an increased risk of poor pregnancy outcomes, such as preterm birth, impaired placental function and decreased fetal body and head growth (19). These effects appear more profound in low-income countries, whereas intermediate and minor effects are noted in the US and European countries, respectively (19). Nevertheless, little is known about the biological mechanisms behind these complications and more research is needed to elucidate the underlying pathways (20).

Finally, treatment of antenatal depression is associated with a number of concerns, and the decision to continue or initiate antidepressant treatment should be individualized. Initiation of antidepressant treatment in pregnancy is rare (21) presumably due to concerns about adverse effects. Most women who use antidepressants during pregnancy were not pregnant at treatment initiation, and will have to decide whether to continue treatment or not when pregnancy is detected. Almost 75% of pregnant women who had selective serotonin reuptake inhibitor (SSRI) prescribed before pregnancy discontinued treatment prior to pregnancy, when discovering pregnancy, or in the first trimester (21, 22). A further decrease in the use of SSRIs is noted throughout pregnancy (23).

Comorbid depression and anxiety

Perinatal anxiety disorders are less commonly studied than antenatal depression but are also a health issue for both the pregnant woman and the fetus. A systematic review by Goodman et al. 2014, found high comorbidity between antenatal depression and any anxiety disorders (24). In the Swedish setting, approximately 24% of women with antenatal depression also suffer from any anxiety disorder, such general anxiety disorders, panic disorder, obsessive-compulsive disorder or social phobia (11). However, according to the review by Goodman, the prevalence rates of these anxiety disorders in pregnancy were variable between studies, and no precise estimates were possible to obtain (24). In addition, some researchers have introduced the concept pregnancy-related anxiety (PrA), which is characterized by fear and worry related to pregnancy (25). Anxiety in pregnancy, general or pregnancy-related (PrA), has often been seen as a feature of depression rather than being an independent syndrome (26). However, Huizink et al. (2004) showed that general anxiety and depression only can explain a small amount of the variance in PrA scores which support PrA's independence from depression (27). Regardless of the exact prevalence of comorbid depression/anxiety disorder in pregnancy it is of most importance to take the comorbidity into account due to different symptom profiles and difficulties in finding the best treatment.

What leads up to antenatal depression?

Common risk factors for antenatal depression include a history of depression, neurotic personality traits, life experiences, unplanned or unwanted pregnancy, present/past pregnancy complications, poor relationship/lack of partner and poor social support (28, 29). In addition, genetic vulnerability, the hormonal changes during pregnancy, the inflammatory load, and stress are also bound to shape the individual risk for depression during pregnancy, as discussed below.

Several of the hormonal alterations in pregnant women have, perhaps rightfully, been held responsible for impairing women’s mental health during the peripartum period. The foremost-cited
endocrine mediators in the development of antenatal depression include the pregnancy hypercortisolism, the decreased hypothalamus-pituitary-adrenal (HPA) axis reactivity during pregnancy (30-34), and potentially a decreased availability of neuroactive progesterone metabolites.

In women, cortisol concentrations continuously increase throughout pregnancy, only to drop abruptly after delivery of the placenta (35). Furthermore, unlike the negative feedback that cortisol exerts on hypothalamic corticotropin releasing hormone (CRH), hypercortisolism stimulates further CRH production by the placenta, leading to a more than 100-fold increase in CRH plasma levels and thus elevated maternal cortisol levels (36). Following delivery, and the expulsion of the placenta a transient HPA axis suppression, lasting 4-6 weeks, is seen in newly-delivered mothers (37). However, although more than 40 studies on the HPA axis markers have been published on maternal peripartum depression, the findings can at best be described as inconsistent (31). Studies from our group have for instance described no difference in cortisol reactivity between depressed and non-depressed pregnant women, no difference in evening cortisol levels, no difference in cortisone to cortisol ratio, but higher CRH levels in women on antidepressant treatment during pregnancy (30, 31, 33, 34). Interestingly, the cortisol to cortisol ratio, potentially a marker of placental 11-β-hydroxysteroid dehydrogenase type 2 (the placental enzyme that protects the fetus from excess exposure to maternal cortisol), is associated with birth weight in women with psychiatric morbidity but not in healthy controls. This finding suggests that while HPA axis markers are not different in women with antenatal depression, their influence on offspring health is greater than in healthy pregnant women (33).

During pregnancy, progesterone levels are also increased by approximately 50-fold (38). Importantly, progesterone is metabolized into neuroactive steroids, among which allopregnanolone and pregnanolone are the two neurosteroids most studied. Neurosteroids potentiate the gamma-aminobutyric acid A (GABA$\text{A}$) receptor, where they increase hyperpolarization and act in a similar manner to barbiturates and benzodiazepines (39). As GABA is the major inhibitory transmitter in the central nervous system, acute administration of allopregnanolone has sedative, anxiolytic, and anti-convulsant properties but may also negatively influence cognitive function (40, 41). While preliminary analyses suggested lower allopregnanolone levels in women with antenatal depression (42), other studies have shown unchanged levels, or higher levels in patients with prenatal anxiety (43, 44). Yet, the snap-shot of peripheral levels may not always reflect the events that take place in the brain, and indeed, a study from our group has indicated that neurosteroid-sensitive GABA$\text{A}$ receptors are up-regulated in pregnancy (45).

**Inflammation**

During normal pregnancy, the immune system undergoes numerous changes to protect the woman from pathogens while at the same time avoiding alienation of the semi-allogeneic fetus (46). During the early phase of pregnancy, a successful implantation depends on a proinflammatory microenvironment. The Type 1 T helper (Th1) cell response in the early phase of pregnancy is followed by a shift to Type 2 T helper (Th2) cells to control endocrine and immune interactions (47-49). Pregnancy-induced changes in progesterone, estradiol, leukemic inhibitory factor, and prostaglandins are likely to be partially responsible for the Th1/Th2-switch (50, 51).

Recently, the role of peripheral and central macrophages (microglia) in initiating and regulating proinflammatory and anti-inflammatory states have come into focus, with repercussion for pregnancy (52). Macrophages are plastic cells that can switch from the classic proinflammatory M1 state with associated elevated levels of tumor necrosis factor alpha (TNF-$\alpha$), interleukin (IL)-6 and IL-1$\beta$ to an alternative M2 state. The M2 macrophages are induced by IL-4 and IL-13, and produce IL-10, IL-4, and transforming growth factor beta (TGF-$\beta$) (53, 54). M2 macrophages are involved in wound healing and tissue remodeling tasks, with additional contributions to the metabolic performance and the endocrine signaling of the tissues (53). Early pregnancy is characterized by an increase in M1 macrophages, however, once the placenta is developed, a shift to a predominantly pro-M2 milieu occurs preventing fetus rejection until parturition (55). Finally, immediately prior to delivery, a last
inflammatory phase, characterized by high levels of proinflammatory cytokines in both cervical tissue (56-58) and circulating blood (59), is noted.

A bidirectional communication between the immune system and the central nervous system is essential for normal brain functions, such as initiating and regulating stress responses, emotions and behavior (60). Sickness behavior induced by proinflammatory cytokines resembles major depressive disorder, and interferon alpha (INF-α) treatment induces major depressive disorder in 25% of patients, suggesting a causal mechanism (61, 62). In non-pregnant subjects, peripheral proinflammatory markers such as IL-6, IL-1β, IFN-α, TNF-α, and the chemokine monocyte chemoattractant protein 1 (MCP1)/chemokine (C-C motif) ligand 2 (CCL2) are increased in the blood and cerebrospinal fluid of a subgroup of patients with mood disorders versus healthy controls when assessed both at baseline and after exposure to stressors (52, 63, 64). Similarly, the current knowledge indicates that shifts toward M1 macrophages in the M1/M2 balance may be related to depression development in the non-pregnant population (52, 60, 65).

While the inflammatory response has been extensively studied in obstetric complications such as preterm birth and preeclampsia (66, 67), studies on the role of peripheral inflammatory markers in antenatal depression are thus far few. In addition, the extent of evaluated markers has, with few exceptions, been limited to IL-6, IL-10, IL-1β, and TNF-α (68-71), and most data has been derived from the beginning of the second trimester. Thus far, findings can be described as inconsistent with unchanged, increased or decreased levels of cytokines and other inflammatory markers in women suffering from antenatal depression (68-71).

A review from 2016 by Leff-Gelman et al. suggests a predominant Th1/Th17 proinflammatory activity responsible for changes in monoaminergic systems, immune function, neurosecretory activity, and placental function, associated with preterm labor (72).

Cognitive bias

From a psychological perspective, biased information processing in attention, memory and interpretation is proposed to be one of the central cognitive dysfunctions found in patients with major depressive disorder (73). According to cognitive theories, depression is caused and maintained by emotional processing bias and by deficits in cognitive control when negative information is processed (73, 74). Emotion processing biases in depressed subjects include a tendency to interpret stimuli as more negative than they are. This can be measured by emotion discrimination tasks, where subjects for instance are asked to recognize facial expressions of happiness, sadness, anger, fear, surprise, and disgust. Typically, the depressed patient is more prone to identify neutral faces as depressed, and less often correctly characterize the happy faces (75, 76).

The most commonly cognitive deficit described in depressed patients is memory impairment. The association between depression and memory impairment are especially noted in contexts when attention is not obstructed by the task (77), when increased cognitive effort is required (78), and when attention is easily distracted by personal concerns and other task irrelevant thoughts (79). Moreover, deficits in working memory (WM) in depressed patients have also been described, although findings are inconsistent (73). Other cognitive deficits include concentration difficulties and distractibility. However, there is also a debate whether the cognitive dysfunction seen in depressed patients is solely due to biases in processing emotional stimuli, or whether they represent a more general cognitive deficit that also would affect the processing of non-emotional material.

Attentional bias is considered a core cognitive process for development, and maintenance of a depressive episode. Attentional bias is most frequently studied by the emotional Stroop test (80). In this task, subjects are asked to name the colors of words with different emotional contents while ignoring the meaning of the word. Attentional bias is typified by longer latency (emotional interference) to name the color of affectively valenced words as compared with neutral ones. Enhanced attention toward threatening or general negative cues has relatively consistently been reported in patients with depressive disorders (81-83). Attentional bias is influenced by depression severity, with greater effect sizes noted in samples with clinical depression than in samples merely
reporting depressed mood (82). In addition, more impaired attentional bias has been reported in subjects with comorbid anxiety (84, 85). Findings in relation to antidepressant treatment are less conclusive; while some studies have indicated that attentional bias is improved with treatment (86), others have demonstrated remaining cognitive deficits in remitted patients (82, 87-90).

Research along the lines of these cognitive theories is, by comparison, relatively scarce in pregnancy and peripartum depression. Some small studies on cognitive function in pregnant women point at deficits in some specific areas like learning and memory (91). One longitudinal study on memory and cognition in pregnancy reported a statistically non-significant trend towards memory impairment in pregnant cases, seen in one out of four cognitive tests (92). However, several studies revealed that pregnant women often consider their memory impaired, while they cognitively perform at the same level as non-pregnant controls (93-95). Regarding peripartum depression, disrupted attentional processing of infant emotion has been reported in early-pregnant women with depressive symptoms (96). Similarly, increased selective attention to fearful faces has been described in distressed pregnant women, suggesting heightened sensitivity to danger cues during pregnancy (97). By use of near-infrared spectroscopy (NIRS) the same research group also reported on increased prefrontal cortex (PFC) activity during processing of fear-relevant stimuli in second-trimester pregnant women (98). However, all of these studies have relied on subjective reporting of depressed mood and studies in women with antenatal depressive disorder are lacking.

The great majority of postpartum depression studies have, on the other hand, explored mother-infant interactions, which has been important for the understanding of short- and long-term consequences for the children of affected mothers (99). Findings from this line of research suggest that mothers with postpartum depression are more likely to identify infant negative emotions and that they are biased towards recognition of negative infant emotional expressions (100). Also, while infant cry stimuli typically activate ventral striatal reward networks in healthy postpartum women (101), this response is diminished in depressed mothers (102-105). However, studies that have employed stimuli that are unrelated to motherhood are rare. Postpartum depression has been associated with worse recognition of negative facial expressions (75), diminished response to negative social and non-social stimuli (106), and impaired working memory performance (107).

Antidepressant treatment

Selective serotonin reuptake inhibitors (SSRIs) are the most widely prescribed antidepressants in most countries (108). The SSRI substances available for prescription in Sweden are citalopram, sertraline, escitalopram, fluoxetine, paroxetine, and fluvoxamine. SSRIs inhibit the reuptake of the neurotransmitter serotonin (5-HT) by blocking the serotonin transporters on the pre-synaptic cell. Subsequently levels of serotonin will increase in the extracellular cleft of the synapsis available to bind to the postsynaptic receptors (109).

In 2015, about 11% of all Swedish women in childbearing age (15-44 years) were prescribed antidepressant medication (The National Board of Health and Welfare in Sweden, 2016). A majority of these women were not pregnant at treatment initiation, which potentially would place them in a situation where they would have to make a decision whether to continue treatment or discontinue in case of pregnancy.

When treating pregnant women with any types of drugs, safety issues are important. Compared to tricyclic antidepressants (TCA), selective serotonin reuptake inhibitors (SSRIs) are considered relatively safe to use in pregnancy as they have few adverse side-effects and a good efficacy (110). Therefore, SSRIs are the most commonly prescribed antidepressants to pregnant women, with up to 3% of the European and 4-10 % of the North American pregnant women using these medications (111-113). In Sweden, citalopram and sertraline are the two most commonly prescribed SSRIs in pregnancy (23). However, potentially due to concerns about adverse effects from antidepressant use, 75% of the pregnant women who had SSRI prescribed before pregnancy discontinued treatment prior to pregnancy, when discovering pregnancy, or in the first trimester (21, 22). A further decrease in the
use of SSRIs is noted throughout pregnancy, with a user prevalence of 2.7% at conception, and 2.1%, 1.7% and 1.3% respectively in the first, second and third trimester (23). Initiation of antidepressant treatment in pregnancy is rare (21).

The decision to continue (or discontinue) SSRI treatment in pregnancy should be individualized. According to UK National Institute for Health and Care Excellence (NICE) 2015 guidelines, antidepressants should not routinely be prescribed to patients with mild depression, because of the poor risk-benefit ratio. Instead, for women with mild to moderate depression, psychological interventions are first line treatment. Pharmacological treatment is only recommended for women with moderate to severe depression if the woman has expressed a preference for medical treatment, declines psychological treatment or did not respond to psychological treatment. However, the NICE guidelines also recommend to consider pharmacological treatment in women with a history of severe depression that show mild symptoms of depression during pregnancy. When antidepressant treatment is prescribed, NICE stresses that the drugs with lowest risk profile and at the lowest effective dose should be used. Furthermore, single drug treatment is preferred above multiple drug treatment. The NICE guidelines also mention the risk of neonatal adaptation syndrome in babies exposed to paroxetine and venlafaxine.

The effects of SSRIs on pregnancy outcome and fetal development

SSRIs cross the placental barrier and are also found in the amniotic fluid and cord blood (114-117). For this reason, these drugs have the potential to influence fetal outcomes. However, when studying the risk of SSRI use in pregnancy and its’ adverse effects on the offspring, the underlying effects of the depression itself, and the socioeconomic features that often are associated with psychiatric morbidity need to be taken into account as well. By using a comparison group of untreated depressed women the risk of confounding by indication is minimized. The most elegant epidemiological studies have incorporated a sibling design, by which socioeconomic confounders can be controlled for (118). Moreover, most epidemiological studies rely on the prescription of medication, which may result in misclassification bias due to compliance issues.

Neonatal adaptation syndrome, characterized by jitteriness, convulsions, respiratory distress, hypoglycemia, and feeding problems was early on described in infants exposed to SSRI in utero (20, 119). Using the Neonatal Abstinence Score as outcome, a Swedish study recently described the prevalence of neonatal maladaptation in relation to SSRI exposure. Overall, 3% of infants exposed to SSRI developed severe abstinence symptoms, which is slightly lower than previously reported in international studies (120). In addition to abstinence symptoms, SSRI-exposed fetuses run a higher risk of requiring neonatal care, and neonatal seizures are more often reported (121-123). Further, these children are at increased risk of developing pulmonary hypertension, especially when exposed to SSRIs in late pregnancy (124-128).

SSRI use during pregnancy has also been associated with preterm birth (22, 129-131), low birth weight or small for gestational age, fetal growth restriction, reduced fetal head growth (but not the body) and poor fetoplacental function (112, 121, 122, 132-134). However, results are conflicting, and many researchers have pointed out that the associations may be driven by the depression itself or by other conditions related to mood disorders (i.e. smoking, obesity, drug abuse), rather than the SSRI treatment (131, 135, 136). Regarding preeclampsia, studies show an association with SSRI treatment, where the risk is further increased among women who continue treatment into the second trimester (132, 137-139). Previously, an association between SSRI use and miscarriage has been claimed (140, 141). However, a well-designed Danish study did not find an association between SSRI treatment and miscarriages when adjusted for underlying psychiatric disorders (142). Of importance, no increased risk of stillbirth, neonatal- and postneonatal mortality was found in pregnant women on SSRI treatment (143).

Other adverse, but less frequent, effects of antenatal SSRI exposure are congenital malformations. Omphalocele, anencephaly and craniosynostosis have been associated with SSRI exposure in utero (144-147). Also, cardiac malformations have been reported in infants of SSRI-treated mothers (136,
148), although these results are in conflict with studies that did not find, or found small differences, in the risk of malformations in infants of SSRI-treated vs. non-treated mothers (149, 150). On the other hand, there is also a study that showed an increased risk of cardiovascular birth defects among untreated depressed mothers (151), stressing out that the mechanisms underlying the depression might be involved in the increased risk for cardiac malformations. Although not all studies replicate the increased risk for cardiovascular birth defects, the risk for other major malformations was increased by SSRI exposure during pregnancy (152). Importantly, a recent, large Nordic cohort study with a sibling design found no increase in overall cardiac birth defects among infants exposed to SSRIs or venlafaxine in utero. Although a higher proportion of septal defects and right ventricular outflow tract defects were seen in antidepressant-exposed infants, no association was noted in the sibling-controlled analyses (118).

The effect of SSRIs on placental function

Due to the difficulties in establishing causal relationships between SSRI use and adverse perinatal outcomes, additional studies on the biological effects of SSRI are needed. These studies will shed light on the biological mechanisms that these types of antidepressants may influence, which potentially strengthen (or weaken) the epidemiological findings at hand.

SSRI treatment and antenatal depression have been suggested to alter gene expression in the fetal side of the placenta. A pilot microarray study in our group revealed 108 genes that were differentially expressed in the placenta of women with antenatal depression and 109 genes that were differentially expressed in the placenta of women that used antidepressants during their pregnancy. Validation in a larger group of antenatally depressed women, antidepressant users during pregnancy and healthy controls, confirmed that ROCK2 and C12orf39 were differentially expressed in both the depression- and the antidepressant treatment group, whereas ROCK1, GCC2, KTN1, and DNM1L were only confirmed to be differentially expressed in the placenta of antidepressant-treated women (153), indicating that the results found in the gene expression of the placenta of women using antidepressants during pregnancy were more robust compared those of antenatally depressed women.

Neurotrophic growth factor (NGF) is involved in neuronal cell survival and differentiation and altered placental NGF levels have been associated with pregnancy complications (154-156). Kaihola et al., (2015) described SSRI-induced changes in the NGF signaling pathway of the placenta. Immunohistochemical stainings revealed NGF protein levels to be increased in both trophoblasts and endothelial cells in placentas from SSRI-treated women compared with those from untreated depressed women and healthy controls. Moreover, increased levels of ROCK2 and Raf-1, signaling proteins downstream in the NGF signaling pathway, were seen in stromal cells of placentas from SSRI-treated women when compared to healthy controls. Moreover, a tendency towards increased ROCK2 levels in trophoblasts and endothelial cells of SSRI-treated women was found. SSRI-treated women also displayed higher levels of phosphorylated ROCK2 in all placental cell types in comparison with untreated depressed and healthy controls. These findings might point towards altered placental function in women on treatment with SSRI, which may have relevance for the development of preeclampsia (157). However, further studies in this area are needed.
Aims

The overall aim for this thesis is to investigate cognitive and biological factors that put susceptible women at risk for depression during pregnancy. In addition, the biological consequences of antenatal depression, as well as antidepressant use during pregnancy are investigated.

The specific aims are:

I To investigate attentional bias in women with antenatal and postpartum depressive disorders by use of the emotional Stroop task.

II To assess peripheral inflammatory markers in healthy women, women with antenatal depression, and in women using SSRI during pregnancy. A secondary aim was to evaluate the usefulness of these markers for the purpose of diagnosing antenatal depression.

III To discriminate between the effects of antenatal depression and the use of SSRIs on placental gene expression by analyzing genes previously known for their involvement in major depression, but importantly, also have a known role for placental function. (ongoing)

IV To assess the inflammatory profile in pregnancy and postpartum by investigating the levels of peripheral inflammatory markers in healthy pregnant women and healthy postpartum women, some of which have paired blood samples. (ongoing)
Material and methods

Study population

All included studies were undertaken as parts of the Biology, Affect, Stress, Imaging, Cognition (BASIC) project. The BASIC study is a population-based, longitudinal study on psychological wellbeing during pregnancy and the postpartum period in Uppsala County, Sweden. The study is conducted at the Department of Obstetrics and Gynecology at Uppsala University Hospital. All women attending the routine ultrasound examination in gestational week 16-18 are invited to participate. Upon invitation, written information is given and a written consent is obtained from women who choose to participate. Exclusion criteria are (1) inability to adequately communicate in Swedish, (2) protected identity, (3) pathologic pregnancies as diagnosed by routine ultrasound, and (4) age less than 18 years. In Uppsala, all routine ultrasound examinations are performed at Uppsala University Hospital and 97% of pregnant women participate. Moreover, the delivery ward of the Hospital is the only available within the county.

The BASIC-project was initiated in 2009, after brief pilot study. As of June 2016, 4568 women have been included in the study, with a participation rate of about 22%. Women contribute to the study at: gestational week 17 (blood samples and a web-based questionnaire including the Edinburgh Postnatal Depression Scale (EPDS), other psychometric measures and demographic data), gestational week 32 (EPDS, psychometric measures and demographic data), delivery (maternal and umbilical cord blood samples, cerebral-spinal fluid samples, amniotic fluid, uterus and placenta samples) and at 6 weeks (EPDS, psychometric measures and demographic data), 6 months (psychometric measures, demographic data and mother-infant bonding questionnaire), and 1 year postpartum (psychometric measures and infant temperament assessment). Women with high EPDS scores (≥12) or with answers indicating suicidal ideation are being contacted by a study doctor, assessed and referred when appropriate.

In addition, two sub-studies (one in late pregnancy and one in the early postpartum period) were performed between January 2010 and May 2013. Both of these studies specifically included women with EPDS score ≥ 12 and a random sample of women with EPDS scores < 12 at gestational week 32 or postpartum week 6. Pregnant women were assessed in gestational week 35-39 (according to the ultrasound-estimated date of delivery) and postpartum women 6-14 weeks after delivery. Exclusion criteria for pregnant women were serious pregnancy-related complications (preeclampsia, intrauterine growth restriction, or gestational diabetes). Exclusion criteria for postpartum women were serious complications or disorders in the offspring requiring extended neonatal care. Treatment with antidepressant drugs was not an exclusion criterion; any ongoing medication was recorded. Women who participated in any of these two sub-studies visited the research laboratory at the Department of Women’s and Children’s Health, Uppsala University. The visits were scheduled between 8 AM and 3 PM, with the majority starting either at 9 AM or at 1 PM, and all women had been fasting at least 90 minutes before blood samples were drawn. In both sub-studies, presence of ongoing primary anxiety and depressive disorders were established by use of the Mini International Neuropsychiatric Interview (M.I.N.I) (158). The interview also included questions on previous depressive episodes. In women who were on treatment with SSRI but where the diagnostic interview failed to indicate the reason for treatment, no attempts were made to ascertain the reason for treatment initiation. All women filled out the Edinburgh Postnatal Depression Scale (EPDS) (159, 160), the Montgomery-Åsberg Depression Rating Scale (MADRS-S) (137) and the Spielberger State-Trait Anxiety Inventory (STAI), and blood samples were collected. In addition, cognitive tests (memory, attentional bias (Stroop test)) and psychophysiology tests (startle response and pre-pulse inhibition) were performed. For all women,
socio-demographic variables, and medical and psychiatric history, were derived from the BASIC questionnaires administered during pregnancy. All participating women were also interviewed about alcohol use, smoking, and medication in the preceding three months. Information regarding height and first trimester weight, visits made to specialized care for fear of childbirth, concomitant somatic disorders, antidepressant treatment, pregnancy complications, delivery outcome and neonatal care was collected from the medical records. In addition, women were asked about sleep duration in the night preceding the test session.

Placental tissue was collected as part of the BASIC project between April 2010 and September 2013, and all in all, 957 placental samples have been biobanked. Placental tissue samples were obtained immediately following delivery. Two basal plate biopsy specimens of the maternal-fetal interface, approximately 1 cm in thickness, were excised from the central part of the placenta in a way that each sample contained the decidua basalis and villous placenta. Areas involving calcification or infarcts were avoided. The tissue samples were briefly washed in sterile phosphate-buffered saline (PBS) and immediately frozen and stored at −70 °C.

The studies in this thesis are based on the BASIC project. Study I and II are both nested case-control studies where the cases and controls are assessed based on their outcome and selected within the BASIC cohort that runs prospectively. In study I we are not studying causality in terms of exposures giving rise to an outcome. Instead we are investigating changes in cognitive function that can be seen in depressed cases. In study II the inflammatory markers are considered to be the exposure and antenatal depression the outcome. Study III, which is not presented at this stage will make use of the placental tissue samples of the BASIC cohort.

The study procedures are in accordance with ethical standards for human experimentation and the study was approved by the Regional Ethical Review Board of Uppsala, Sweden, and the procedures were in accordance with the Helsinki Declaration of 1975 (revised in 2008).

Study I

234 pregnant and 202 postpartum women had participated in the BASIC sub-studies by May 2013. Of these, 201 pregnant and 173 postpartum women had performed the Stroop test. Among these, 24 pregnant and 14 postpartum women with solely anxiety disorders were excluded. In addition, two postpartum women were excluded because they misunderstood the instructions of the Stroop task, thus leaving 177 pregnant and 157 postpartum women available for analyses. Among these, 40 suffered from antenatal depression and 33 from postpartum depression. Among pregnant women, 15 were on treatment with antidepressants, and corresponding number for postpartum women were 8.

In this study women were categorized as depressed if they fulfilled criteria for major or minor depressive disorder or persistent depressive disorder (PDD), previously known as dysthymia, according to M.I.N.I.

Study II

Two hundred and fifty-eight pregnant women participated in this sub-study to the BASIC pregnancy cohort. Of these, 160 were healthy pregnant controls, 59 had antenatal depression and 39 women were on treatment with SSRIs.

Blood samples for this study were compiled from two different sources within the BASIC framework; i) from a late pregnancy sub-study (n=205) or ii) from blood samples collected in conjunction with a planned Caesarean section (n=53).

Out of 234 pregnant women who participated in the BASIC sub-study in late pregnancy, blood samples for 216 women were available. For the purpose of the present study, women with anxiety-only disorders (n=11) were excluded, leaving 205 available blood samples to use. Women with an ongoing minor or major depressive episode (n=23) according to M.I.N.I, or a prior episode in combination with at least one EPDS score of 13 or more during pregnancy (n=31), were considered to
have experienced a depressive episode during pregnancy (n=54). Remaining women were considered healthy controls (n=124) or were on treatment with SSRI (n=27). Serum concentrations of cortisol and cortisone were available for the 120 healthy controls, 48 women with antenatal depression and 26 women on SSRI treatment.

In addition, healthy controls (n=36), depressed cases (n=5) and women on SSRI treatment (n=12) were also sampled among the BASIC study participants who underwent an elective Caesarean section at Uppsala University Hospital. The morning before the Caesarean section, which is typically performed in gestational week 38, fasting blood samples were collected. In this part of the study, depressed cases were defined as women who had discontinued antidepressant use early in pregnancy and had EPDS scores ≥ 17 at some point during pregnancy. Exclusion criteria were serious pregnancy-related complications such as preeclampsia, intrauterine growth restriction, and gestational diabetes. In addition, all twin pregnancies were excluded.

**Methods**

**Stroop test**

The Stroop task was designed using the E-Prime psychology software tool (Psychology Software Tools Inc, Sharpsburg USA) and contained two blocks, each containing five unique words from each word category: neutral, positive, negative, and negatively valenced obstetric words. Each word was presented once in each color: blue, green, yellow and red, resulting in 80 word presentations per block. Subjects were asked to identify the color of the word while ignoring the meaning and press the colored keyboard key that corresponded to that color. Time to response was registered when the participant pressed the correctly colored keyboard letter on the computer.

The neutral, positive, and negative words were matched for number of syllables and linguistic frequency. The negative words, part of which have been used previously (161, 162), were selected in order to be emotionally relevant for the depressed group. We also included a set of negatively valenced obstetric words, which were matched against the other word categories for number of syllables. The obstetric words had lower linguistic frequency than the other word categories, but in this population of pregnant and postpartum women, we considered them to be more familiar than in the general population. The obstetric words were chosen based on low valence and high arousal to be comparable with the negative words. All words were validated in an independent sample of 40 pregnant women, by use of a self-assessment manikin scale ranging from 1 (low valence, low arousal) to 9 (high valence, high arousal) (163).

**Inflammatory markers**

Stored plasma/serum samples were collected from -70°C freezers and thawed on ice before being transferred to 96-well plates, each consisting of 90 samples and 6 controls. None of the samples used in this study had previously been thawed. Moreover, all samples were analyzed using the same batch of reagents, with cases and controls evenly distributed within the plates. Analyses of the relative levels of 92 inflammatory proteins were performed at Clinical Biomarker Facility at the SciLifeLab Uppsala, using Proseek Multiplex Inflammation (Olink Bioscience, Sweden), which is based on proximity extension assay (PEA) technology (164, 165). In brief, for each inflammatory protein, when a pair of DNA oligonucleotide-labeled antibody probes binds to a common target protein the DNA oligonucleotides in proximity hybridize to each other allowing a proximity-dependent DNA polymerization to form an amplifiable DNA molecule. The newly formed DNA template is subsequently amplified and quantified using BioMark™ HD real-time PCR platform (Fluidigm, South San Francisco, CA, USA). The assay has sensitivity down to fg/mL and detects relative protein values
that can be used for comparison between groups, but not for absolute quantification. The plasma sample (1 μL) was mixed with 3 μL incubation mix containing 92 pairs of probes, each consisting of an antibody labeled with a unique corresponding DNA oligonucleotide. The mixture was first incubated at 4°C overnight. Then, 96 μL extension mix containing PEA enzyme and PCR reagents was added, and the samples were incubated for 5 min at room temperature before the plate was transferred to the thermal cycler for an initial DNA extension at 50°C for 20 min followed by 17 cycles of DNA amplification. A 96.96 Dynamic Array IFC (Fluidigm, South San Francisco, CA, USA) was prepared and primed according to the manufacturer’s instructions. In a new plate, 2.8 μL of sample mixture was mixed with 7.2 μL detection mix from which 5 μL was loaded into the right side of the primed 96.96 Dynamic Array IFC. The unique primer pairs for each protein were loaded into the left side of the 96.96 Dynamic Array IFC, and the protein expression program was run in Fluidigm Biomark reader according to the instructions for Proseek.

Each plate was run with three negative controls (buffer) and three interplate controls. Every sample was also spiked in with two incubation controls (green fluorescent protein and phycoerythrin), one extension control and one detection control. Normalization of data was performed in GenEx software using Olink Wizard, providing normalized protein expression (NPX) data on a Log2-scale where a high protein value corresponds to a high protein concentration. In brief, the NPX is calculated in three steps from the quantification cycle (Cq) values generated in the real-time PCR: i) ΔCq_sample = Cq_sample – Cq_extension control, ii) ΔΔCq = ΔCq_sample – ΔCq_interplate control, iii) NPX = Correction factor – ΔΔCq_sample. The extension control is subtracted from the Cq-value of every sample in order to correct for technical variation and the interplate control is subtracted to compensate for possible variation between runs. Finally, the NPX is calculated by normalization against a calculation correction factor.

Cortisol and cortisone

The tandem mass spectrometry used for determination of maternal cortisol and cortisone levels has been described previously (33). A brief description of the method is found in the manuscript of study II.

Statistics

For detailed description of statistical analyses, please see the individual manuscripts. The emotional interference scores of paper I were modeled in three-way repeated measures ANOVAs with word category (positive, negative, negatively valenced obstetric words) as within-group variable, and group (women with depression vs. controls), and antidepressant use (use vs. non-use) as between-group variables. Antidepressant treatment was modeled as a separate variable as we assumed this condition would represent a greater disease burden. Because of the inherent differences in life situation and hormone levels between the pregnant and postpartum stages, separate analyses were conducted in each perinatal group. Only sphericity-assumed F- and p-values are presented.

In paper II, the primary analyses of the inflammatory markers across all three groups were made with likelihood ratio tests performed on adjusted multinomial logistic regression models, weighted to the provided population proportions of antenatal depression (10%) and SSRI use (3%), respectively. In these analyses, adjustments were made for age (continuous), body mass index (continuous), smoking (yes/no), days left to parturition (continuous), fasting status (overnight fast or 90-minute fast), preeclampsia or hypertension (yes/no), and pre-pregnancy inflammatory or rheumatoid disorder (yes/no). To reduce the risk of false discoveries caused by multiple testing, we used Bonferroni correction to adjust the p-values, and adjusted p-values less than 0.05 were considered significant. Significant inflammatory markers were subjected to follow-up analyses by multivariable logistic regression, using the same adjustments as above. Correlation analyses were made by Spearman Rank
correlation. Diagnostic performance of the inflammatory markers for detection of antenatal depression was evaluated using random forest classification in ten five-fold cross-validations.
Results

Study I

Women with antenatal depression were significantly younger, less educated, and more often had a pre-pregnancy psychiatric history than the pregnant controls. Women with postpartum depression also more commonly reported a pre-pregnancy psychiatric history, and they were less often breastfeeding in comparison with the postpartum controls. Co-morbid anxiety disorders were common in women suffering from both antenatal and postpartum depression, present in approximately half of cases.

No perinatal stage (i.e. pregnancy vs. postpartum) by emotional word category interaction was present, $F(2,259) = 3.33$. Furthermore, parity $F(1,259) = 0.97$, fear of childbirth $F(1,135) = 1.16$, breast-feeding $F(1,122) = 0.02$ or sleep deprivation $F(1,259) = 1.34$ did not influence the emotional interference scores for any of the affectively valenced words.

In pregnancy, no significant difference in emotional interference scores were noted between depressed women and healthy women $F(2,173) = 2.32; p = 0.2$, Figure 1A. However, pregnant women on antidepressant therapy had a greater emotional interference by negatively valenced obstetric stimuli ($p < 0.05$) than non-treated pregnant women, Figure 2. Women with ongoing postpartum depression displayed shorter reaction times to positive and negative stimuli than to neutral words ($p < 0.05$) in comparison with the healthy controls, Figure 1B. In addition, the self-rated depression scores were significantly negatively correlated with the emotional interference scores by positive and negative stimuli, i.e. with increasing MADRS or EPDS scores an increased attentional facilitation was noted.
Figure 1. Emotional interference scores (mean ± SD) in (A) healthy pregnant women (n = 149) and women with antenatal depression (n = 28), and (B) healthy postpartum women (n = 131) and women with postpartum depression (n = 26). No differences in emotional interference scores were noted between women with antenatal depression and pregnant controls. Women with postpartum depression displayed attentional facilitation by positive and negative words. *p < 0.05, independent t-tests.
Figure 2. Emotional interference scores (mean ± SD) in pregnant women using (n = 15), or not using antidepressant therapy (n = 162). Women on antidepressant treatment displayed greater emotional interference by negatively valenced obstetric stimuli than non-treated pregnant women. *p < 0.05, independent t-test.
Study II

The three groups; healthy controls, women with antenatal depression, and women using SSRI did not differ in terms of marital status, parity, smoking and alcohol use, or self-reported sleep disturbances. Similarly, prevalence of inflammatory diseases and obstetric complications, such as preeclampsia or hypertension, were similar between groups. However, women with antenatal depression were significantly younger, and had completed fewer years of education than controls. Their blood samples were less often taken in conjunction with a Caesarean section (and consequently following an overnight fast), and approximately one week earlier (in relation to delivery) than the other two groups. SSRI users had shorter gestational length than women with antenatal depression, and higher pre-pregnancy body mass index (BMI) than the women in the other two groups.

The adjusted multinomial regression analyses indicated that 23 of the inflammatory markers were significantly different between healthy pregnant women, depressed pregnant women and SSRI users. Post hoc multivariable logistic regression analyses of the surviving inflammatory markers are displayed in Table 1. As seen in Table 1, these differences were driven by significantly lower levels of inflammatory markers in women with antenatal depression and women on SSRI treatment in comparison with controls. No difference in any of the inflammatory markers was observed between women with antenatal depression and those who were using SSRI. Notably, for each investigated inflammatory marker, lower levels were found in the two depressed groups. Top three inflammatory factors that were down-regulated in women with antenatal depression were TNF-related apoptosis-inducing ligand (TRAIL), p=0.000001, macrophage colony-stimulating factor 1 (CSF-1), p=0.000004, and fractalkine (CX3CL1), p=0.000005. Corresponding inflammatory markers in SSRI users were CSF-1, p=0.000011, vascular endothelial growth factor A (VEGF-A), p=0.000016, and IL-15 receptor subunit alpha (IL-15RA), p=0.000027.

None of these inflammatory markers had sufficient discriminatory power as a diagnostic marker for antenatal depression. Performance measures for models based on the 74 inflammatory proteins with no more than 50% missing values yielded an average error rate of 0.24, a sensitivity of 0.32, a specificity of 0.92, and an area under the curve (AUC) of 0.69. In the permuted model, the average error rate was 0.28 (range 0.26 – 0.30), which gives an estimated probability of <0.001. We also built models based on single inflammatory markers. For each model the mean sensitivity and specificity over 50 test sets was computed and the probability of miss (1-sensitivity) and probability of false alarm (1-specificity) for each of these proteins are plotted in Figure 3. As for the overall model, the individual inflammatory markers all suffered from low sensitivity to detect cases with antenatal depression.

The majority of the inflammation markers that were significant in the adjusted multinomial regression model displayed a negative correlation with self-reported depression in late pregnancy. Furthermore, the inflammatory markers also correlated with the self-reported EPDS scores obtained in gestational week 17 and 32, Table 2. The majority of the inflammation markers were significantly negatively correlated with cortisol or the cortisol to cortisone ratio in healthy controls. In contrast, among women with antenatal depression (with or without treatment) this pattern was not found, Table 3.
Table 1. Protein expression levels in healthy pregnant women, women with antenatal depression, and women on SSRI treatment. Data displayed as mean ± SD. Post hoc comparisons by use of multivariable logistic regression analyses.

<table>
<thead>
<tr>
<th>Inflammatory marker</th>
<th>Healthy pregnant</th>
<th>n</th>
<th>Antenatal depression</th>
<th>n</th>
<th>SSRI treatment</th>
<th>adjusted p&lt;sup&gt;a&lt;/sup&gt; depressed vs. control</th>
<th>adjusted p&lt;sup&gt;a&lt;/sup&gt; SSRI use vs. control</th>
<th>adjusted p&lt;sup&gt;a&lt;/sup&gt; SSRI use vs. depressed</th>
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<td>Mean</td>
<td>SD</td>
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<td>SD</td>
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<td>0.69</td>
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*Adjusted for age, body mass index, smoking, days left to parturition, fasting, preeclampsia or hypertension, and pre-pregnancy inflammatory or rheumatoid disorder.
Figure 3. Probability of false alarm plotted against the probability of miss for the detection of antenatal depression. For clarity reasons, not all protein names are displayed in the figure.
Table 2. Spearman rank correlation between significant inflammatory markers and self-rated depression and anxiety.

<table>
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<tr>
<th>Inflammatory marker</th>
<th>EPDS Gestational week 17 (n=244)</th>
<th>EPDS Gestational week 32 (n=248)</th>
<th>MADRS Gestational week 36 (n=182)</th>
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<td>$p$</td>
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Table 3. Spearman rank correlations between significant inflammatory markers and levels of cortisol, cortisone and the quotient of cortisone and cortisol.

<table>
<thead>
<tr>
<th>Inflammatory marker</th>
<th>Healthy controls n = 120</th>
<th>Antenatal depression and SSRI treatment n = 74</th>
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<tr>
<td></td>
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Discussion

Methodological considerations

The BASIC study is a population-based project where all women in Uppsala County, who attend their routine ultrasound in gestational week 16-18, are approached for participation in the study. The BASIC project has the strength of being a large study that includes many subjects and various characteristics and background data at an individual level. The longitudinal properties of the project give the ability to observe individual differences over time and discuss causality. From a psychiatric perspective, the use of a structured interview, the Mini International Neuropsychiatric Interview (M.I.N.I), for assessment of depressive and anxiety disorders, gives greater reliability in terms of case status than self-reported depressive symptoms. Self-rated EPDS scores are however used as outcomes for the greater part of the BASIC study, in line with other large-scale epidemiologic studies.

Nevertheless, the study also has limitations and the most important is the selection bias. As mentioned in the method section, there are some exclusion criteria; non-Swedish speaking, age less than 18 years, severe pathological pregnancies, and protected identity. By including only women who are able to communicate in Swedish the proportion of native Swedes in the study is greater than in the rest of the population. Thus, important perspectives from immigrant women, and other women who have yet not learned Swedish will be lost in this project.

The overall participation rate in the BASIC project is around 22%. Drop-out analyses have shown that the study population is overrepresented of highly educated, somewhat older women, pregnant with their first child, in comparison with the Uppsala County population in general (166). From previous studies in the field, it is known that non-participants usually are more depressed than participants (167). Inclusion of these women would possibly have made the study results more robust, but it should also be acknowledged that for the biological aims of this thesis, selection bias is less likely to play a role.

In addition, the cohort design also introduces a substantial degree of heterogeneity in terms of depression severity, duration of depressive episodes, time-point for onset of depression, comorbidity with anxiety and other psychiatric disorders, and use of antidepressant treatment. Any attempt to select certain groups for a specific study question, for instance women postpartum onset of depression without prior history of depressive episodes, will inevitably reduce the number of available cases to study. Examples of this problem is noted in study I, where pregnant women with SSRI treatment displayed a different response in the Stroop task in comparison with the untreated pregnant women, presumably not as a result of treatment but potentially as a marker of more severe underlying depression. Clearly, this hypothesis should have been tested in the postpartum women as well, but the number of SSRI-treated postpartum women was too low, leading to problems with power.

Even though the BASIC project is longitudinal, the studies presented in this thesis are both cross-sectional. In study II the cross-sectional design makes it difficult to draw conclusions about causation and the temporal order of events. It would have been favorable with longitudinal blood samplings, where temporal changes in M1/M2 inflammation markers could have been followed over time in relation to cortisol levels and development of depression. However, it should be mentioned that within the BASIC frame-work such studies are underway; paired blood samples are available for a subset of women, and the inflammatory status in pregnancy may also be used for prediction of postpartum depression (Bränn et al, submitted manuscript).

Depression is a difficult phenotype to study. The depression diagnosis is based on a combination of several symptoms, and is associated with a variety of different factors like poor socioeconomic status, drug abuse, anxiety, stress, trauma, and severe medical conditions. Any study on diagnostic markers or biological factors will suffer from the imprecise diagnosis. For that reason, much of the genetic
research in psychiatry has geared towards finding relevant endophenotypes for the psychiatric disorders, for instance specific symptoms or other traits. The idea is that a specific phenotype has a closer relationship to the biological processes that give rise to psychiatric illness than the DSM-based diagnostic categories. In this thesis, this problem is illustrated by the failure to find any diagnostic inflammatory marker for antenatal depression. While specificity was high, the sensitivity for detection of cases with antenatal depression was 32%. The search for diagnostic biomarkers for MDD is still in its infancy and is complicated by the symptom-based diagnostic procedures, the multifactorial pathophysiology and the inaccessibility of the brain (168). However, some progress regarding biomarkers for depression in the non-pregnant population has been made (168), and recent studies have shown a number of inflammatory markers to be accurate and reliable predictors of treatment response in depressed subjects (169, 170).

Attentional bias in pregnancy and postpartum

In study I women with pre-pregnancy depression who continued antidepressant treatment throughout pregnancy displayed enhanced attentional bias to negatively valenced obstetric words, whereas untreated pregnant depressed women did not differ from controls and postpartum depressed women displayed attentional facilitation.

Biased information processing in attention, memory and interpretation is proposed to be one of the central cognitive dysfunctions found in patients with major depressive disorder (MDD) (73). According to cognitive theories, depression is caused and maintained by emotional processing bias and by deficits in cognitive control when negative information is processed (73, 74). However, there is a debate whether the cognitive dysfunction seen in depressed patients is due to biases in processing emotional stimuli, or characterized by more general cognitive deficits that also affect the processing of nonemotional material. Little support can be found to establish pervasive depressive deficits in general cognitive functioning. Research point at depression related deficits in the control of attention instead of limited processing capacity. When the task can capture the attention of a depressed participant, and there is no room for rumination, deficits are not found. To be able to focus and pay attention the participant needs to push away all thoughts irrelevant to the task. The review by Gotlib and Joormann supports this idea of affective interference and suggestion that the cognitive control is limited in depressed individuals (73).

Regarding memory, previous research showed that in structured situations depressed persons have the same ability to perform as nondepressed, unlike unconstrained situations where depressed people have impaired ability of initiative (171).

Hence, these performance deficits in emotionally neutral tasks are probably not a generalized deficit in depressed individuals, but more likely due to depression-related difficulties in disregarding irrelevant information.

Previous research has suggested that attentional bias is influenced by depression severity and comorbid anxiety; greater effect sizes are noted in samples with clinical depression than in samples with depressed mood, and similarly, in cases with comorbid anxiety than in cases with depression-only (82). Yet, even though all cases in this study were diagnosed with a depressive disorder, and almost 50% had comorbid anxiety, we were unable to confirm the hypothesis of attentional bias in peripartum depression, with the exception of women who continued antidepressant therapy in pregnancy.

We speculated that the 25% of women who continue antidepressant treatment during pregnancy would be the ones in greatest need of treatment, thus having the greatest, or most long-term, psychiatric morbidity (21). While these women also displayed the expected attentional bias, in line with our hypothesis, it should be stressed that this finding does not unambiguously suggest greater depression severity. It has been suggested that attentional bias to emotional content also may be a trait phenomenon, i.e. being found in remitted patients (90, 172), although findings are mixed (82). In line with the trait hypothesis, women on antidepressant treatment reported lower scores of self-rated
depression than the depressed women who managed without pharmacological treatment, suggesting the majority were in remission when tested.

Women with untreated antenatal depression had no attentional bias and women with postpartum depression presented with attentional facilitation, i.e. shorter reaction times to the positively and negatively valenced words than to the neutral words. In addition, postpartum depression severity was significantly inversely correlated with the emotional interference scores. Clearly, these findings suggest that peripartum depression is not characterized by the typical cognitive deficits found in non-peripartum depressed cases. Presumably, the attentional facilitation may be the result of an explicit strategy to override the tendency for negatively valenced stimuli to interfere with color naming, most commonly noted in nonclinical high-trait anxiety participants (80). This is an important coping strategy, which may facilitate exit from the vicious circle of emotional processing bias and deficient cognitive control, which typifies the depressive episode (80). However, it should be noted that the signature of override, i.e. the generally faster performance during the entire task was not present among the untreated postpartum depressed women.

Moreover, while our findings are counterintuitive in light of the cognitive models for non-pregnant depression (73, 74), it has previously been suggested that postpartum depression may be distinguished from major depressive disorder by emotion processing patterns that map onto specific cognitive and emotional impairments (75). For instance, women with postpartum depression performed worse on emotion recognition tasks, both in comparison with healthy controls (happiness and fear) and in comparison with non-peripartum depressed women (disgust and anger) (75). They were also less responsive to negative stimuli, with lower ratings of intensity and reactions to negative pictorial stimuli, (106), in contrast with the affective reactivity found in non-peripartum depressed women (74), and in contrast with the negatively biased interpretations of emotional stimuli found in healthy postpartum women (173). In addition, it has also been suggested that they tend to avoid or limit exposure to distressing infant stimuli (99). Our findings are also in line with previous reports on disrupted attentional processing of infant emotion in pregnant women with depressive symptoms (96).

Thus, in addition to the emotion processing patterns that may distinguish women with peripartum disorder from non-postpartum depressed women (75, 106), we add to the growing literature suggesting that peripartum depression not only is characterized by time of onset, by demonstrating that these women do not display one of the typical cognitive deficits of depression, i.e. attentional bias to negative stimuli.

Peripheral inflammatory markers in antenatal depression

The major finding of study II was that women with antenatal depression and women on treatment for depression (or anxiety) had significantly lower levels of peripheral inflammatory markers than healthy pregnant controls, for as many as 23 markers. These findings are at odds with the literature in non-pregnant samples, where depression has been associated with increased levels of proinflammatory cytokines (52, 61-64). Furthermore, none of the markers typically associated with non-pregnant depression such as IL-6, IL-1β (not included in the panel), IFN-α, TNF-α (discarded due to lack of protein expression in more than 50% of the samples), or MCP-1/CCL2, were found to be altered in our cohort of women with antenatal depression (52, 63, 64). In fact, VEGF-A, which is typically elevated in non-pregnant subjects with MDD (174), was lower in women with antenatal depression.

However, these results must be examined in the context of normal pregnancy and its associated alterations in immune and inflammatory responses. The majority of the inflammatory markers under investigation in this study are significantly elevated in pregnancy compared with the postpartum period (Bränn et al, in preparation), suggesting a depression-induced deviation from the normal inflammatory response in pregnancy. Hypothetically, antenatal depression may be associated with an incomplete switch to the pro-M2 milieu that characterizes the second and third trimester of pregnancy (55). Although proinflammatory M1 markers do not differ between groups, the M1/M2 balance is shifted toward M1 signaling, which, in agreement with current hypotheses for depression in non-
pregnant individuals, may contribute to the development of antenatal depression in vulnerable women (52, 60, 65).

In line with this reasoning, among the top down-regulated peripheral inflammatory markers, TRAIL, CSF-1, CX3CL1, VEGF-A, and IL15Ra, several have been associated with M2 macrophages. For instance, TRAIL which is a type II transmembrane protein involved in tumor growth suppression and in the regulation of both the innate and adaptive immune responses (175-177), has recently been shown to be secreted by M2 macrophages (178). Similarly, CSF-1, a proinflammatory protein also known as macrophage-CSF, stimulates the differentiation and development of M2 macrophages (179). Fractalkine/CX3CL1 is a membrane-bound or soluble chemokine expressed by neurons. Its' receptor CX3CR1 is found in the healthy brain, present on microglia (180, 181). Fractalkine has also been shown to induce M2 polarization of macrophages (54), and has a number of non-immune mechanisms, which may be of relevance in depression. For instance, fractalkine inhibits serotonergic neurotransmission by enhancing GABA the activity on serotonergic neurons (182). Besides the well-known angiogenic effects of VEGF-A, it also has neurotrophic and neuroprotective roles in the central- and peripheral nervous system (183), which may be of relevance for depression (184). Furthermore, VEGF is linked both to TGF beta and M2 macrophages in tumor models (185). Finally, interleukin-15 receptor alpha (IL15Ra) is one of three subunits of the receptor complex that binds the cytokine IL-15. IL-15 is expressed in most type of cells and plays an important role in immune cell functions, muscle and bone growth, and adiposity. IL15Ra knockout mice show antidepressant-responsive depressive-like behavior, reduced anxiety, and impaired memory, suggesting an antidepressive effect of IL15 signaling (186, 187).

In addition, several of the top down-regulated peripheral inflammatory markers have distinct actions in pregnancy. Fractalkine, VEGF-A, and IL-15Ra play important roles in blastocyst implantation and trophoblast invasion (188-192), and CSF-1 has been associated with preeclampsia (193). VEGF-A is expressed in the human placenta throughout gestation, and is known to regulate placental angiogenesis and maternal spiral artery remodeling (191, 192). However, the role of VEGF-A in adverse pregnancy outcomes such as preeclampsia, small-for-gestational-age infants, preterm birth and recurrent miscarriage seems unclear (194). Thus, the findings of the present study strengthen the role of inflammation as the mechanism behind the association between antenatal depression and its associated adverse pregnancy outcomes.

No differences in any of the inflammatory markers were noted between women with antenatal depression and those who were using SSRI. However, while in vitro experiments on murine cell lines indicate that SSRI treatment can reduce M1 activation in microglia (195), SSRI treatment in humans does not consistently reduce proinflammatory cytokine levels (196). In fact, one study reported on increased levels of proinflammatory cytokines, IFN-γ and IL-1β, and reduced levels of antiinflammatory cytokines, IL-10 and IL-13, in non-pregnant MDD patients after one-year treatment with SSRI (197). In addition, the actions of conventional antidepressants are undermined by proinflammatory cytokines, which may contribute to therapy resistance in non-pregnant MDD patients (170, 198). While it is important to consider that SSRI use in pregnancy may also be a representation of more severe psychiatric morbidity, the similar inflammatory response between women with antenatal depression and women on antidepressant treatment could strengthen the hypothesis that altered inflammatory response may be a common physiological pathway for the adverse outcomes associated with both antenatal depression and SSRI treatment, such as preterm birth and low birth weight (55, 199).

Is peripartum depression just another depression?

The clinical presentation of depressive symptoms in women of childbearing age does not differ depending on whether women are pregnant, postpartum or outside the peripartum period (9). For this
reason, some researchers argue that peripartum depression is just another depression, merely occurring at a stressful point in life (9).

The title for this thesis is: Is peripartum depression just another depression? Based on the findings we have obtained thus far, the answer would be no.

One argument would be that, as presented in study I, women who suffer from antenatal and postpartum depression do not display the typical attentional bias to negative words that is characteristic of depressive states in the non-pregnant population. Whether this is due to protective mechanisms of pregnancy or due to features that distinguish antenatal and postpartum depression from non-peripartum depression remains to be demonstrated.

Secondly, study II describes that women with antenatal depression had significantly lower levels of peripheral inflammatory markers than healthy pregnant controls. These findings are clearly at odds with the literature in non-pregnant samples, where depression has been associated with increased levels of proinflammatory cytokines (52, 61-64), but should be interpreted in the context of pregnancy-induced changes in inflammatory response.

Moreover, treatment for antenatal depression is not as straightforward as it is in non-pregnant patients. When considering treatment, the expecting mother has to be aware of the risk-benefit profile for herself and the child. While antidepressant therapy clearly improves the mood of treated women, our findings do not indicate that antidepressant treatment has any positive impact on their inflammatory profile. Women who decide continuing SSRI treatment during pregnancy can be presumed being in greatest need of treatment. Ones could speculate that these women had a severe depressive episode when starting treatment, and treated for a longer time. It is possible that earlier attempts to stop treatment have failed and possibly these women suffer from the most severe form of depression.

However, obviously the best way to answer the question of this thesis would be to compare pregnant and non-pregnant women with depression. While this is not possible within the BASIC study, attempts to directly compare the BASIC subjects with non-pregnant depressed women are underway.
Conclusion

Women who suffer from antenatal and postpartum depression do not display the typical attentional bias to negative words that is characteristic of depressive states in the non-pregnant population. Whether this is due to protective mechanisms of pregnancy or due to features that distinguish antenatal and postpartum depression from non-peripartum depression remains to be demonstrated.

Women with antenatal depression and women on treatment for depression have lower levels of a number of peripheral inflammatory markers than healthy pregnant controls. Hypothetically, this could be due to dysregulated switch to the pro-M2 milieu that characterizes normal third trimester pregnancy.

Further longitudinal research is needed to confirm the presumably dysregulated M2 shift and whether this is causing the development of antenatal depression or whether it is an outcome due to the depression.

None of the inflammatory markers under investigation had sufficient discriminatory power as a diagnostic marker for antenatal depression.

Finally, no difference in any of the inflammatory markers was noted between women with antenatal depression and those who were using SSRI. This finding strengthens the notion of a common physiological pathway for the adverse outcomes associated with antenatal depression and SSRI treatment.
Future perspectives

Antenatal depression and placental function

The aim of study III is to discriminate between the effects of antenatal depression and the use of SSRIs on placental gene expression by analyzing genes previously known for their involvement in major depression, but importantly, also have known role for placental function. Placental tissue has been collected from 47 healthy controls, 25 untreated depressed women and 45 SSRI-treated women within the BASIC project, and RNA isolated from the fetal side of the placenta. Downstream qPCR using low-density arrays (LDA) was done for 44 genes involved in HPA axis function, growth factors, monoamines, hormone receptors, rate limiting enzymes, GABA and neurosteroids.

Among the analyzed genes; Serotonin receptor 1A (HTR1A), Nerve growth factor (NGF), Neuropeptide Y receptor Y2 (NPY2R) and Aromatic l-amino acid decarboxylase (AADC) were differentially expressed between the groups (controls, depressed, SSRI-treated) (p ≤ 0.05).

For HTR1A and NPY2R a higher gene expression was seen in the depressed group compared with the controls. For AADC and NGF a higher gene expression was seen for the SSRI-treated than for non-treated.

The results thus far reveal that untreated antenatal depression may have an impact on the placenta function via an alteration of the gene expression of the two genes HTR1A and NPY2R. With the exception of AADC and NGF, no differences were noted between placental gene expression in women with antenatal depression and women on treatment with SSRI. Notably, only a few genes, out of the 44 in the array, were differentially expressed between the groups. Validation of the results from the LDA cards by protein analyses; i.e. immunohistochemistry, is ongoing.

Preliminary results show protein expression of HTR1A and NPY2R in placental trophoblasts of healthy women.

Inflammation profile in healthy pregnant and postpartum women

In working with the inflammatory markers in women with antenatal depression it became apparent that reports on the normal physiology for many of the studied inflammatory markers were lacking. For this reason, in study IV, we will describe the levels of peripheral inflammatory markers in healthy pregnant women and healthy postpartum women, some of which have paired blood samples.

Preliminary data suggest that the levels of peripheral inflammatory markers in healthy pregnant women are elevated compared with levels in postpartum women. For instance, 60 out of 76 inflammatory markers (16 excluded due to more than 50% missing values) under investigation in this study are significantly elevated in pregnancy compared with the postpartum period in women included only once (pregnancy or postpartum).

This suggests a pregnancy-induced inflammatory response in healthy pregnant women that is impaired in depressed pregnant cases where the inflammatory marker levels are lower than in controls. This might appear due to an incomplete switch to the pro-M2 milieu that characterizes the second and third trimester of pregnancy.
References


The Mini distortions erud H. The effect of antenatal depression and selective psychiatry. 2010;56(2):119


Homogenous 96

substance P

mediated abortion. Am J Repro


