Biological Aspects of Peripartum Depression

ÅSA EDVINSSON
Peripartum depression affects around 12% of women in pregnancy and postpartum, and about 2–3% of European pregnant women use antidepressants, mostly selective serotonin reuptake inhibitors (SSRIs). An increased risk of poor pregnancy outcomes has been described in women with antenatal depression and SSRI treatment during pregnancy. The biological mechanisms behind these complications are not fully understood and here we investigated several biological correlates of peripartum depression, and discriminated between the effects of antidepressant treatment and depression itself.

In Paper I, attentional biases in pregnant and postpartum women were studied by using the Emotional Stroop Task, measuring reaction times to different stimuli. The major finding was shorter reaction times in postpartum depressed women, for emotionally valenced stimuli, which can be interpreted as emotional numbing.

In Paper II, peripheral inflammatory markers were assessed by proximity extension assay technology in depressed, SSRI-treated and healthy pregnant women. Lower levels of 23 markers were found in women with antenatal depression, independent of treatment, compared with healthy controls. These findings suggest a dysregulated switch to the anti-inflammatory M2 milieu characterizing a normal third trimester.

In Paper III, normal changes in inflammatory markers across pregnancy and postpartum were assessed in healthy pregnant and postpartum women. The majority (41) of the 50 markers that differed between groups were lower postpartum. These results clearly reflect the change in the immune system in pregnancy to postpartum transition.

In Paper IV, placental gene and protein expression were investigated and nominally significant findings were noted for serotonin receptor 1A (HTR1A) and neuropeptide Y2 receptor (NPY2R), where women with untreated depression displayed higher gene expression than healthy controls. Protein expression analyses revealed higher levels of HTR1A in placentas from SSRI-treated women, compared with healthy controls and women with untreated depression. This suggests possible involvement of HTR1A in the effect of antenatal depression on the placenta.

Overall, peripartum depression is associated with altered cognitive-emotional processing, lower levels of several mostly anti-inflammatory markers, and altered placental gene and protein expression. However, we found no major differences between untreated and treated depression.

Keywords: Peripartum depression, antenatal depression, postpartum depression, antidepressant treatment, selective serotonin reuptake inhibitor, SSRI, pregnancy, postpartum, attentional bias, Emotional Stroop Task, inflammatory markers, proximity extension assay, placenta, gene expression, TaqMan low-density array, protein expression, immunohistochemistry, HTR1A, NPY2R

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To my family
List of Papers

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


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Contents

Introduction ................................................................................................... 11
   Peripartum depression ................................................................. 11
   Antenatal depression ................................................................. 12
      Risk factors of antenatal depression ....................................... 13
      Effects of antenatal depression on pregnancy outcome, fetal
development and offspring ......................................................... 14
      Co-morbid antenatal depression and anxiety ............................ 15
   Antidepressant treatment during pregnancy .............................. 16
      Serotonin in pregnancy ........................................................... 17
      Effects of SSRIs on pregnancy outcome, fetal development, and the
      offspring ............................................................................. 18
   Postpartum depression ................................................................. 20
   Attentional bias .............................................................................. 20
   Inflammation, immunity and their roles in pregnancy ................. 22
   Inflammation in depression .......................................................... 25
   Inflammation in antenatal depression .............................................. 26
   Placental function ........................................................................... 26
      The effects of antenatal depression and SSRIs on placental tissue ....27

Aims ......................................................................................................... 29

Material and Methods .................................................................................... 30
   Study population and design .......................................................... 30
      Paper I ....................................................................................... 34
      Paper II ...................................................................................... 34
      Paper III .................................................................................... 35
      Paper IV .................................................................................... 35
   Methods ............................................................................................ 36
      Depression and anxiety assessment tools .................................... 36
      Emotional Stroop test .................................................................. 37
      Blood samples .............................................................................. 37
      Proximity extension assay ......................................................... 38
      Cortisol and cortisone ............................................................... 39
      TaqMan Low-Density Arrays ....................................................... 39
      Immunohistochemistry .............................................................. 40
Abbreviations

ANOVA  Analysis of variance
BASIC  Biology, Affect, Stress, Imaging, Cognition
CBT    Cognitive behavioral therapy
CCL11  C-C motif chemokine 11/Eotaxin
CCL28  C-C motif chemokine 28
CRH    Corticotropin releasing hormone
CS     Caesarean section
CSF-1  Macrophage colony-stimulating factor 1
CX3CL1 C-X3-C motif chemokine ligand 1/Fractalkine
DSM    The Diagnostic and Statistical Manual of Mental Disorders
EPDS   Edinburgh Postnatal Depression Scale
GABA   Gamma-aminobutyric acid
GW     Gestational week
HPA    Hypothalamic-pituitary-adrenal
HTR1A  5-hydroxytryptamine (5-HT/serotonin) receptor 1A
IFN    Interferon
IL     Interleukin
IL-15RA IL-15 receptor subunit alpha
LAP    TGF-beta-1 Latency-associated peptide Transforming growth factor beta-1
LIF-R  Leukemia inhibitory factor receptor
LOD    Limit of detection
MADRS  Montgomery-Åsberg Depression Rating Scale
MCP    Monocyte chemoattractant protein
MDD    Major depressive disorder
MDE    Major depressive episode
MHC    Major histocompatibility complex
M.I.N.I. Mini International Neuropsychiatric Interview
NGF    Nerve growth factor
NICE   National Institute for Health and Care Excellence
NK cell Natural killer cell
NPY2R  Neuropeptide Y2 receptor
NPX    Normalized protein expression
<table>
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<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>PDD</td>
<td>Persistent depressive disorder</td>
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<td>PP</td>
<td>Postpartum</td>
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<td>PPD</td>
<td>Postpartum depression</td>
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<td>PrA</td>
<td>Pregnancy-related anxiety</td>
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<td>qPCR</td>
<td>Quantitative real-time polymerase chain reaction</td>
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<td>SD</td>
<td>Standard deviation</td>
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<td>SERT</td>
<td>Serotonin transporter</td>
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<td>SNRI</td>
<td>Serotonin norepinephrine reuptake inhibitor</td>
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<td>SSRI</td>
<td>Selective serotonin reuptake inhibitor</td>
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<td>STAI</td>
<td>Spielberger State-Trait Anxiety Inventory</td>
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<td>TCA</td>
<td>Tricyclic antidepressant</td>
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<td>TGF</td>
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<td>Th1</td>
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<td>T regulatory cell</td>
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<td>TaqMan Low-Density Array</td>
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<td>TGF</td>
<td>Transforming growth factor</td>
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<tr>
<td>TNF</td>
<td>Tumour necrosis factor</td>
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<tr>
<td>TRAIL</td>
<td>TNF-related apoptosis-inducing ligand/ Tumor necrosis factor ligand superfamily member 10</td>
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<tr>
<td>TRANCE</td>
<td>TNF-related activation-induced cytokine/ Tumor necrosis factor ligand superfamily member 11</td>
</tr>
<tr>
<td>TWEAK</td>
<td>TNF-related weak inducer of apoptosis/ Tumor necrosis factor ligand superfamily member 12</td>
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<tr>
<td>VEGF-A</td>
<td>Vascular endothelial growth factor A</td>
</tr>
<tr>
<td>11β-HSD2</td>
<td>11-β-hydroxysteroid dehydrogenase type 2</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine</td>
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Introduction

Peripartum depression

Women have long suffered from depression during pregnancy, but it was not until recently that this was acknowledged in the Diagnostic and Statistical Manual of Mental Disorders (DSM). By the fifth edition of DSM (DSM-5), the diagnosis of peripartum depression was finally introduced, and this diagnosis comprises depression during both pregnancy and postpartum [1]. Previously, in the DSM-IV, the specifier used for depression in relation to childbirth was major depressive episode (MDE) with postpartum onset [2], which rejected all depressive episodes arising during pregnancy.

The importance of acknowledging peripartum depression is evidenced by its relatively high prevalence. According to a recent systematic review, prevalence rates of depression in the peripartum period are estimated at 11.9%, with 13.1% in low- and middle-income countries and 11.4% in high-income countries [3].

From a clinical perspective, the peripartum onset specifier was welcomed, since up to 50% of cases of depressive episodes postpartum show onset of symptoms before delivery [1, 4]. However, in DSM-5, the specifier used for postpartum depression is still onset within four weeks following delivery, which continues to be questioned. Numerous reports indicate that many depressive episodes start between two and six months postpartum, with the majority within the first three months [5, 6].

An additional criticism of the DSM-5 criteria is the absence of distinct onset specifiers for pregnancy and the postpartum period [7], which would further motivate research on the aetiology of MDEs at these times. Pregnancy and the postpartum period differ in many aspects, most prominently in terms of circulating hormone levels, stress responsivity, sleep patterns and immune system function, and distinct onset specifiers could highlighted these factors and their roles in the development of mood disorders in relation to childbirth [8].

Yet another concern regarding the absence of pregnancy- and postpartum-onset specifiers in DSM-5 is the strong association between bipolar disorder and the risk of postpartum depression (and psychosis) [9-11], which appear less concerning in women with onset in pregnancy.

With the potentially different aetiologies, clinical profiles, and treatment responses between antenatal and postpartum depression, the risk of misdiag-
nosis and inappropriate treatment will remain in future, unless more researched. For these reasons, this thesis covers antenatal depression and postpartum depression separately.

However, the definition of peripartum depression, according to DSM-5 criteria, is an MDE [12] with onset during pregnancy or in the four weeks following delivery. The core symptoms are depressed mood and loss of interest or pleasure in usual activities, and additional symptoms are as follows: 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, and presence of suicidal thoughts, plans or attempts. The symptoms must have affected the patients to such a 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 picture of depressive symptoms in women of childbearing age does not differ whether the women are pregnant, postpartum or outside the peripartum period [13]. However, peripartum depression occurs at a stressful point in life, and due to the potentially adverse consequences for the child and family, needs to be addressed promptly, without delay [14].

Antenatal depression

Depression during pregnancy is common, and found in approximately 12% of pregnant women worldwide [3]. Around half of these women fulfil the criteria for MDE [15-18]. The prevalence of depression during pregnancy may also differ among the trimesters. In a study by Bennet et al., the prevalence of depression has been estimated to be 7.4% in the first trimester, 12.8% in the second, and 12.0% in the third [19]. Although, Gavin et al. found the prevalence of antenatal depression to attenuate during pregnancy, with a prevalence of 11.0% in the first trimester, and 8.5% in the second and third trimesters [6], both studies showed differences in the prevalence of depression across the trimesters.

The overall prevalence of MDE in pregnancy does not seem to differ from that in non-pregnant women of childbearing age [20], or may even be lower [13]. Furthermore, pregnancy is suggested to be a protective period as regards the most severe forms of depression. The risk of suicide is extremely low [21], and pregnancy is considered to protect against psychiatric readmission [22]. Nonetheless, being depressed during pregnancy is associated with reduced quality of life and work performance at an important stage of life. Women with antenatal depression are more often in need of prolonged sick-leave during pregnancy, and with an increased number of healthcare contacts, especially in relation to fear of childbirth. Antenatal depression is also relatively common in connection with planned Caesarean section (CS) and epidural analgesia during labour [23, 24]. Moreover, antenatal depression
Risk factors of antenatal depression

While psychosocial risk factors are of utmost importance for the overall understanding of antenatal depression onset and course, the present thesis is devoted to biological risk factors. Among these, genetic vulnerability, hormonal changes during pregnancy, the inflammatory load, stress susceptibility and responsiveness are also bound to shape the individual risk of depression during pregnancy.

Hormonal alterations in pregnant women have, perhaps rightfully, been held responsible for affecting women’s mental health during the peripartum period. The most studied endocrine mediators in the development of antenatal depression include hypercortisolism and decreased hypothalamic-pituitary-adrenal (HPA) axis reactivity during pregnancy [31-35]. In pregnant women, cortisol levels steadily increase throughout pregnancy, with a drastic fall after delivery of the placenta [36]. Further, unlike the negative feedback that cortisol exerts on hypothalamic corticotropin-releasing hormone (CRH), hypercortisolaemia stimulates further CRH production by the placenta, leading to a massive increase in CRH plasma levels followed by elevated maternal cortisol levels [37]. After delivery of the placenta transient HPA axis suppression occurs for 4–6 weeks, seen in newly-delivered mothers [38]. Similar alterations in the HPA axis have previously been described in non-pregnant subjects with major depression [39-41]. However, after much research on HPA axis markers in peripartum depression, the findings can at best be described as inconsistent [32]. Studies from our group, for instance, have revealed no difference in cortisol reactivity between de-
pressed and non-depressed pregnant women, no difference in evening corti-
sol levels, no difference in the cortisone to cortisol ratio, but higher CRH
levels in women on antidepressant treatment during pregnancy [31, 32, 34,
35].

During pregnancy, levels of the steroid hormone progesterone are also in-
creased, by as much as 50-fold [42]. Progesterone is metabolized into neuro-
active steroids, among which allopregnanolone and pregnanolone are well-
studied. The neurosteroids bind to the gamma-aminobutyric acid A
(GABA\textsubscript{A}) receptor, and act in a similar manner to barbiturates and benzodi-
azepines [43]. As GABA is the major inhibitory transmitter in the central
nervous system, acute administration of allopregnanolone has sedative, an-
xiolytic, and anti-convulsant properties but may also negatively influence
cognitive function [44, 45]. While preliminary analyses indicated lower allo-
pregnanolone levels in women with antenatal depression [46], other studies
have revealed unchanged levels, or higher levels in patients with prenatal
anxiety [47, 48]. Our research group has provided evidence that genetic vari-
ation in the rate-limiting enzyme of allopregnanolone synthesis may be asso-
ciated with the course of depressive symptoms throughout pregnancy [49],
and that neurosteroid-sensitive GABA\textsubscript{A} receptors are up-regulated in preg-
nancy [50].

Effects of antenatal depression on pregnancy outcome, fetal
development and offspring

Antenatal depression is a disorder with a broad range of risk factors. Com-
mon risk factors of antenatal depression include a history of depression, neu-
rotic personality traits, obesity, life experiences, unplanned or unwanted
pregnancy, present or past pregnancy complications, poor relationship or
lack of partner, domestic violence, poor social support, smoking and sub-
stance abuse [23, 51-53]. However, several of these risk factors, for example
obesity, smoking, drug and alcohol abuse, and domestic violence, are also
associated with outcomes related to antenatal depression [54]. Therefore, it
is often difficult to separate what is due to the depression \textit{per se}, and what is
due to its associated factors, when studying the effect of antenatal depression
on pregnancy outcome, fetal development and offspring. In addition, contin-
ued depression after pregnancy may also influence neonatal and child out-
comes, further complicating the picture.

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 [25-27]. Moreover, elevated risks of
pre-eclampsia and several neonatal complications have been observed in
depressed mothers [25, 26, 55, 56].
Antenatal depression is associated with several suboptimal outcomes in the offspring. A recent study by Osborne et al. revealed an association between women with maternal depression and stress responses in pregnancy and sub-optimal neurobehavioral function and increased cortisol reactivity to stress in the offspring [57]. Additionally, several other studies describe associations between maternal stress and depression and disrupted fetal neurobehavioral development and affected cognitive, emotional and behavioural outcomes throughout childhood [58-60]. Another study on long-term consequences of maternal mood on offspring’s behaviour suggested an intrauterine effect of maternal mood on children’s attention and emotional problems. However, when adjusting for paternal mood and parental mood postpartum, the relationship between children’s behaviour and antenatal depression was attenuated [61]. Recent studies by Rifkin-Graboi et al. suggest prenatal mother-to-offspring transmission of vulnerability to the development of depression and anxiety, with alterations in the amygdala in the offspring of mothers suffering from these conditions [62, 63].

Co-morbid antenatal depression and anxiety
Perinatal anxiety disorders are less well studied than antenatal depression, but are also a health issue for both the pregnant woman and the child [64]. A systematic review by Goodman et al. revealed high co-morbidity of antenatal depression and anxiety disorders [65]. In a Swedish setting, approximately 24% of women with antenatal depression also suffer from anxiety disorders, such as general anxiety disorder, panic disorder, or social phobia [16]. However, according to the review by Goodman et al., the prevalence rates of anxiety disorders in pregnancy vary between studies, and no precise estimates could be obtained [65]. A systemic review published in 2017, however, revealed the overall pooled prevalence of co-morbid anxiety symptoms and moderate to severe depressive symptoms across the three trimesters to be 6.3%. The overall prevalence of co-morbid self-reported anxiety traits and depressive symptoms across the three trimesters was 8.1%. The overall prevalence of a clinically diagnosed co-morbid anxiety and depression disorder across the three trimesters was 9.3% and that of a co-morbid generalized anxiety disorder and depression was 1.7% [66]. Moreover, some researchers have introduced the concept of pregnancy-related anxiety (PrA), which is characterized by fear and worry related to pregnancy [67]. Anxiety in pregnancy, general or PrA, has often been seen as a feature of depression rather than being an independent syndrome [68]. However, Huizink et al. revealed that general anxiety and depression can explain only a small fraction of the variance in PrA scores, which supports the independence of PrA from depression [69]. Regardless of the exact prevalence of co-morbid depression/anxiety disorder in pregnancy it is important to account for co-
morbidity due to different symptom profiles and difficulties in finding the best treatment.

Antidepressant treatment during pregnancy

Selective serotonin reuptake inhibitors (SSRIs) are the most widely prescribed antidepressants in most countries [70]. The SSRI substances available for prescription in Sweden are citalopram, sertraline, escitalopram, fluoxetine, paroxetine and fluvoxamine. The action of SSRIs is inhibition of the reuptake of the neurotransmitter serotonin (5-HT) by blocking the serotonin transporters (SERTs) on pre-synaptic nerve cells. As a consequence, serotonin levels will increase in the extracellular cleft of the synapses, available to bind to postsynaptic receptors [71] (Figure 1).

The prevalence of antidepressants prescribed to women in Sweden is increasing every year. In 2017, about 12% of all Swedish females of childbearing age (15–44 years) were prescribed antidepressant medication, with the majority using SSRIs (9%) [72].

When treating pregnant women with any type of drug, safety issues are of utmost importance. Unlike tricyclic antidepressants (TCAs), SSRIs are considered relatively safe to use in pregnancy as they have few adverse side-effects and good efficacy [73]. At present, around 3% of European and 4–10% of North American pregnant women use SSRIs [55, 74-76]. In Sweden, citalopram and sertraline are the most commonly prescribed SSRIs in pregnancy [77]. However, potentially due to concerns about adverse effects, 75% of pregnant women prescribed with SSRIs before pregnancy discontinue treatment prior to pregnancy, when discovering pregnancy, or in the first trimester [78, 79]. A further decrease in SSRI use is noted throughout pregnancy, with a user prevalence of 2.7% at conception, 2.1% in the first trimester, 1.7% in the second trimester and 1.3% in the third trimester [77]. Initiation of treatment with antidepressants in pregnancy is rare [79].

The decision to continue or discontinue SSRI treatment in pregnancy should be individualized. According to NICE guidelines [30], 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 treatments. 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 has not responded to psychological treatment. However, NICE guidelines also recommend considering pharmacological treatment in women with a history of severe depression who show mild symptoms of depression during pregnancy. When antidepressant treatment is prescribed, NICE stresses that drugs with the lowest risk profile and at the lowest effective dose should be used. Furthermore,
single-drug treatment is preferred over multiple-drug treatment. NICE guidelines also mention the risk of neonatal adaptation syndrome in infants exposed to paroxetine and venlafaxine.

Figure 1. The effect of SSRI in the synaptic cleft (Olivier et al., 2013. Frontiers in Cellular Neuroscience, vol. 7:73, p. 1-15).

Serotonin in pregnancy
Serotonin has been described as having functions in utero driving fetal development [80]. In the placenta, the switch from placental serotonin to fetal production of serotonin takes place during fetal development and disruption in this process may affect the fetal brain in the long run [81]. As serotonin is present in the early placenta and seems to be of maternal origin, it is suggested to have growth-stimulating and regulatory properties in fetal neurodevelopment [81-84]. This is supported by the hypothesis that an imbalance of the serotonin signalling system might be part of the development of some neuropsychiatric disorders, such as anxiety, affective disorders, autism and schizophrenia [85-88].
Effects of SSRIs on pregnancy outcome, fetal development, and the offspring

SSRIs can cross the placental barrier and are also found in the amniotic fluid and cord blood [89-93]. For this reason, these drugs have the potential to influence fetal outcomes, and directly influence fetal neurodevelopment [94]. However, when studying the risk of SSRI use in pregnancy and its effects on the offspring, the underlying effects of depression itself, and socioeconomic features that might be associated with psychiatric morbidity also need to be accounted for. By using a comparison group of untreated depressed women the risk of confounding by indication is reduced, but rarely eliminated. The most well-designed epidemiological studies have incorporated a sibling design, where genetic factors and socioeconomic confounders can be controlled for [95]. Moreover, most epidemiological studies rely on the prescription of medication, which may result in misclassification bias due to compliance issues in patients.

The first risk described in relation to SSRI use in pregnancy was neonatal adaptation syndrome (NAS). This is characterized by jitteriness, convulsions, respiratory distress, hypoglycaemia, and feeding problems in infants exposed to SSRIs in utero [26, 96]. A Swedish study has been carried out to assess the prevalence of neonatal maladaptation in relation to SSRI exposure in utero, using the Neonatal Abstinence Score. Overall, 3% of infants exposed to SSRIs developed severe abstinence symptoms, which is slightly lower than previous findings [97]. In addition to abstinence symptoms, SSRI-exposed fetuses run a higher risk of requiring neonatal care, with more neonatal seizures reported [98-100]. Furthermore, these children are at increased risk of developing pulmonary hypertension, especially when exposed to SSRIs in late pregnancy [101-105]. A recent meta-analysis [106] revealed an increased risk of persistent pulmonary hypertension of the newborn (PPHN) in offspring of mothers exposed to SSRIs or serotonin norepinephrine reuptake inhibitors (SNRIs) in pregnancy.

SSRI use during pregnancy has also been associated with shorter gestational length [31], preterm birth [78, 107-109], low birth weight or small-for-gestational-age infants, fetal growth restriction, reduced fetal head growth, and poor fetoplacental function [55, 98, 99, 110-112]. However, results are conflicting, and many researchers have pointed out that the associations may be driven by depression per se [57] or by other conditions related to mood disorders (e.g. smoking, obesity, drug abuse) [54], rather than SSRI treatment [109, 113, 114]. Regarding pre-eclampsia, studies have shown an increased risk in SSRI-treated pregnant women [115], where the risk is further increased among women who continue treatment into the second trimester [110, 116-118]. Previous research revealed an association between SSRI use and miscarriage [119, 120]. However, a well-designed Danish study did not reveal an association between SSRI treatment and miscarriage when adjusted
for underlying psychiatric disorders [121]. Importantly, no increased risks of stillbirth, neonatal- or post-neonatal mortality have been found in pregnant women on SSRI treatment [122].

Other adverse but less common effects of antenatal SSRI exposure are congenital malformations. Cardiac malformations have been reported in infants exposed to SSRIs in utero [114, 123], although these results are in conflict with studies that did not reveal, or revealed only small differences in the risk of malformations in infants of SSRI-treated vs. non-treated mothers [124, 125]. Moreover, there are also reports on an increased risk of cardiovascular birth defects among untreated depressed mothers [126], stressing that the mechanisms underlying depression might be involved in the increased risk of cardiac malformations. Of importance is the fact that a large Nordic cohort study with a sibling design did not reveal an increase in overall cardiac birth defects among infants exposed to SSRIs (venlafaxine) in utero. Although a higher proportion of septal defects and right-ventricular outflow tract defects were seen in infants of antidepressant-treated mothers, no association was noted in the sibling-controlled analyses [95].

Although not all studies have revealed an increased risk of cardiovascular birth defects, the risk of other major malformations has been reported to be increased by SSRI exposure during pregnancy [127]. Omphalocele, anencephaly and craniosynostosis have been associated with SSRI exposure in utero [128-131]. Additionally, a recent cohort study revealed an increased risk of cardiac, musculoskeletal, craniofacial, digestive and respiratory defects as well as craniosynostosis in infants exposed to serotonin inhibitor drugs (SSRI, SNRI and some TCAs) in utero [132]. These results further stress the importance of more research in this area.

Regarding long-term effects of SSRI use, there is an ongoing randomised, placebo-controlled trial named MAGDALENA that aims to answer whether differences in cognitive development in children exposed to SSRIs (sertraline) vs depression per se, in utero, exist [133].

Similarly, the effect of maternal SSRI treatment on the development of autism spectrum disorder (ASD) in offspring is still not fully understood. In a systematic review and meta-analysis published in 2017 it was concluded that there is a link between maternal SSRI treatment and autism in offspring but the results were inconsistent and the strongest association was seen when mothers were treated before conception. Moreover, when adjusting for previous maternal depression the association tended to become attenuated, but nevertheless, in some of the included studies the result remained significant [134].

The literature on the risk that the offspring of SSRI-treated mothers might develop attention deficit hyperactivity disorder (ADHD) is just as conflicting [135]. Two systematic reviews have been published, where one concluded that the current body of evidence suggests that SSRI treatment during pregnancy may affect the neurobiology, behaviour and neurodevelopment of
offspring in such a way that the risk of disorders such as ADHD may be increased. However, it was also suggested that these results might be confounded by maternal psychopathology per se [135]. In the other systemic review it was concluded that there is no strong evidence for a causal relationship between antidepressant treatment in pregnancy and ADHD in the offspring [136].

Postpartum depression

Postpartum depression (PPD) is defined as onset of mood symptoms within the first four weeks following childbirth. However, in clinical practice as well as in research, PPD is often described in terms of mood disorder in the first year after childbirth [137]. PPD should not be mixed up with postpartum blues (PPB), a transient condition affecting many women shortly after childbirth, and persisting for a few days [51, 138]. PPB includes symptoms such as lability, irritability, and tearfulness [138], possibly due to the dramatic drop in hormone levels within the first days after delivery [139]. PPD is a common complication and the prevalence is estimated to 7–30% across low-, middle- and high-income countries [140]. In a recent systematic review an overall incidence of PPD of 12% was reported [141]. The risk of developing PPD is substantially higher among women who have previously experienced depression, before or during pregnancy [4, 142, 143]. Other risk factors besides a history of depression are stressful life events, poor social or partner support, low self-esteem, low socioeconomic status, unplanned or unwanted pregnancy, prolonged nausea during pregnancy and pregnancy or delivery complications [144-149]. The prevalence tends to be higher in studies concerning depressive symptoms rather than clinical diagnoses, or when depression is measured by self-reporting scales instead of structured interviews [19]. Several biological factors such as HPA dysregulation, inflammatory processes and genetic vulnerabilities have been described as risk factors of the development of postpartum depression [8, 150], but these will not be covered in detail in this thesis. Recently, a study by Bränn et al. indicated elevated levels of inflammatory markers in postpartum depressed women, which suggests a halted adaptability of the immune system in such women [151].

Attentional bias

Biases in attention, interpretation and memory are considered central in the cognitive alterations found in patients with major depressive disorder [152]. Cognitive theories describe depression as a result of emotional processing biases and by deficits in cognitive control when negative information is pro-
cessed, typically seen in depressed subjects as a bias towards negative information and/or troubles disengaging attention from negative material [152, 153]. Emotion-processing biases in depressed persons involve the interpretation of stimuli as more negative than they are for others. This bias can be assessed by way of emotion-recognition tasks, where participants, for instance, are inquired to recognize facial expressions, where the depressed subject is more prone to identify neutral faces as depressed, less often classifying happy faces as happy [154, 155]. The affective interference theory describes depressed persons as being preoccupied with the processing of emotional material, which will affect their performance negatively in tasks where they should ignore the emotional stimuli (task-irrelevant stimuli) and respond to other parts of the material. In contrast, they will perform appropriately when the processing of emotional stimuli is part of the task (task-relevant stimuli) [156]. However, it is not fully understood whether the cognitive alterations seen in depressed subjects are exclusively due to biases in processing emotional material, or if they represent a more general cognitive deficit with repercussions as regards the processing of non-emotional material.

The emotional processing bias in attention found in depressed patients, attentional bias, is usually studied by means of the emotional Stroop test [157]. This task includes valenced and neutral words in different colours, and the subject is asked to ignore the meaning of the word while naming the colours of the word. Attentional bias is represented by longer reaction times (greater emotional interference) to name the colour of affectively valenced words vs. neutral ones. The literature reports increased attention toward threatening or negative stimuli in patients with depressive disorders [158-160]. However, there is also inconsistency, where no attentional bias has been found in depressed cases [161]. Attentional bias seems to be influenced by depression severity, with more pronounced biases described in subjects with clinical depression than in subjects reporting only a depressed mood [159]. In addition, more impaired attention has been reported in subjects with co-morbid anxiety [162, 163], and anxiety on its own [164]. Studies on cognitive dysfunction in relation to antidepressant treatment show somewhat inconclusive results, with indications of improvement of attentional bias with treatment [165], and, in contrast, remaining cognitive deficits in remitted patients [159, 166-169].

Research regarding cognitive deficits in depression in the peripartum period is relatively scarce. Among the few existing studies in this area, a study by Pearson et al. has revealed that early pregnant women, experiencing depressive symptoms, exhibit biased attentional processing of infant emotional stimuli, suggesting a reduced uncontrolled preferential processing of distressed infants [170]. Cognitive behavioural therapy (CBT) has seemed to normalize the disrupted attentional processing seen in depressed mothers [171]. In another study, increased attention to fearful faces was noted in dis-
tressed pregnant women, suggesting heightened sensitivity to threats during pregnancy [172]. The same research group also demonstrated increased activity in the prefrontal cortex in pregnant women processing fear-relevant stimuli [173]. However, these studies were based on subjective reporting of depressed mood, and studies in women with antenatal depressive disorder are fewer.

The majority of postpartum depression studies in this field have explored mother–infant interactions, which are important for better understanding of short- and long-term consequences for the offspring [174]. Findings suggest that postpartum depressed mothers are more prone to identify negative infant emotions and more biased towards recognition of negative infant emotional expressions [175]. Also, while infant-cry stimuli typically activate ventral striatal reward networks in healthy postpartum women [176], this response is diminished in depressed mothers [177-180]. However, studies concerning stimuli unrelated to motherhood are rare in the postpartum period. Some studies, however, have shown that postpartum depression is associated with poor recognition of negative facial expressions [154], with diminished responses to negative social and non-social stimuli [181].

Inflammation, immunity and their roles in pregnancy

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 [182]. The non-specific innate immune system has long been described as playing a more prominent role during pregnancy, while the specific adaptive immune system is described as suppressed [183, 184]. However, lately, research has indicated a balance between the innate and adaptive systems taking place, regulatory functions being of utmost importance [182, 185].

The innate immune system is the first defence against foreign pathogens. Following a damaged first barrier (skin and mucosal membranes) inflammatory substances such as histamines and prostaglandins, and complement proteins and cytokines are released to attract immune cells. The first cells to arrive at the target area are neutrophils, which most commonly destroy the pathogen via phagocytosis [186]. Following neutrophils, macrophages reach the area, digest the pathogen and present their antigens at the major histocompatibility complex (MHC-II) on their surface. Activation of macrophages by lipopolysaccharides (LPSs) on bacteria or the cytokine interferon-\(\gamma\) (IFN-\(\gamma\)) released by T-cells, leads to M1 macrophages with pro-inflammatory properties. Another activation pathway includes stimulation by interleukin-4 (IL-4) and IL-13, leading to M2 macrophages with anti-inflammatory properties [187] (Figure 2, Table 1). Further, natural killer (NK) cells recognize infected cells that have escaped cytotoxic T-cells via
depressed expression of MHC-I on the surface [188]. Inflammatory molecules such as IFN-γ and tumour necrosis factor-α (TNF-α) are released by the NK cells and kill the infected cell by a cytotoxic action [189].

**Figure 2. Activation of M1- vs. M2 macrophages.**

The adaptive immune system involves humoral and cellular defence [190]. The former includes (bone marrow-derived) B-cells with their surface receptors recognizing, binding and destroying antigens. The antigen is degraded and presented at the B-cell MHC-II complex. B-cells are sub-grouped into memory cells and plasma cells. Memory cells stay in the body waiting for new attacks the next time the same pathogen invades. Plasma cells start producing antibodies straight away [191].

The cellular defence system involves T-cells, produced in the thymus and developed into several types: memory T-cells, helper T-cells (CD4+), cytotoxic T-cells (CD8+), regulatory T-cells and NK T-cells [192]. Helper T-cells express the surface protein CD4 (CD4+) and bind to cells expressing antigens at the MHC-II complex. The CD4+ cells can be divided into types such as Th1, Th2, Th17 and T regulatory cells (Tregs). Differentiation towards Th1 is promoted by IFN-γ and IL-12, and differentiation towards Th2 by IL-4 and IL-2. Th1 releases cytokines such as IFN-γ and IL-2, and Th2 produces cytokines such as IL-4, IL-13 and IL-10 [193]. Differentiation towards Tregs by TGF-β and the following production of TGF-β, IL-10 and IL-35 has a suppressive effect on T-cells, and Tregs therefore have a protective role in the control of autoimmunity [194]. However, this division into innate and adaptive immunity is not that simple and straightforward as it might appear. Recently a review described crosstalk between neutrophils and T- and B-cells, and modulatory properties of neutrophils on adaptive immune responses [195].
In pregnancy, decidual NK cells and macrophages in the uterus act by promoting inflammation, vascular remodelling and trophoblast invasion, which in turn facilitate implantation and placentation. Decidual NK cells are also described as immunoregulatory and possible inducers of Tregs, which in turn play important roles in ensuring immune tolerance toward the semi-allogeneic fetus [196, 197].

During early pregnancy, successful implantation depends on a pro-inflammatory microenvironment. The Th1 cell response in the early phase of pregnancy is followed by a shift to Th2 cells to control endocrine and immune interactions [198-200]. Pregnancy-induced changes in progesterone, estradiol, leukaemic inhibitory factor, and prostaglandins exert influences on the immune system and are likely to be partially responsible for the Th1/Th2 switch [185, 201, 202]. Moreover, Tregs dampen the alloreactive T-cells by production of IL-10 and TGF-β, which is vital for the maintenance of pregnancy, and fetal alloantigen tolerance [203].

Lately, the role of peripheral and central macrophages (microglia) in initiating and regulating pro-inflammatory and anti-inflammatory states has come into focus, with repercussions for pregnancy [204, 205]. Macrophages are plastic cells that can switch from the classic pro-inflammatory M1 state with associated elevated levels of TNF-α, IL-6 and IL-1β to an alternative M2 state. M2 macrophages are induced by IL-4 and IL-13, and produce IL-10, IL-4, and TGF-β [206, 207] (Figure 2, Table 1). M2 macrophages are involved in wound healing and tissue remodelling tasks, with additional contributions to the metabolic performance and endocrine signalling of the tissues [206]. 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 [208]. Finally, immediately prior to delivery, a last inflammatory phase is noted, characterized by high levels of pro-inflammatory cytokines in both cervical tissue [209-211] and circulating blood [212].
### Table 1. M1- and M2 macrophage properties.

<table>
<thead>
<tr>
<th>Subtypes</th>
<th>M1</th>
<th>M2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inducers</strong></td>
<td>IFN-γ, LPS, GM-CSF, oxidative, fatty acid, HMGB1</td>
<td>IL-4, IL-10, IL-13, TGF-β, M-CSF, AMP, GC</td>
</tr>
<tr>
<td><strong>Transcription factors</strong></td>
<td>NF-κB, STAT1, IRF1, IRF5, HIF-1α, KLF6</td>
<td>STAT3, STAT6, IRF4, KLF4, PPARγ, eMaf, cMyc</td>
</tr>
<tr>
<td><strong>Cytokines</strong></td>
<td>NO, TNF-α, IL-1β, IL-6, IL-12, IL-23</td>
<td>IL-10, TGF-β</td>
</tr>
<tr>
<td><strong>Chemokines</strong></td>
<td>CXCL9, CXCL10, CXCL11</td>
<td>CCL17, CCL18, CCL22</td>
</tr>
<tr>
<td><strong>Metabolic enzymes</strong></td>
<td>iNOS, gp91phox and p22phox, ferritin, CP, DMT-1, Narmp-1</td>
<td>Arg-1, Arg-2, ODC, SMO, HO-1, Fpn, TR</td>
</tr>
<tr>
<td><strong>Cell marker</strong></td>
<td>CD80, CD86, TLR2, TLR4, MHC II</td>
<td>CD206, CD163, CD209, CD301, Fizzl, Ym1/2</td>
</tr>
<tr>
<td><strong>Functions</strong></td>
<td>Pro-inflammatory, microbicidal activity, clearance of pathogen</td>
<td>Anti-inflammatory, immune regulators, tissue repair</td>
</tr>
</tbody>
</table>

### Inflammation in depression

Communication between the immune system and the central nervous system is vital for normal brain functions, such as initiating and regulating stress responses, emotions and behaviour [213]. The association between inflammation and depression has long been described in the literature. In 1991 the theory of macrophages being associated with depression was published by Smith and colleagues [214]. The cytokine theory, in which cytokines are thought to play a key role in the inflammatory-derived development of depression, has been described in several studies [215-217]. However, the mechanisms behind inflammation being a key player in the development of depression in humans are not fully unravelled.

The theory that inflammatory events may contribute to depression is strengthened by the fact that sickness behavior induced pro-inflammatory cytokines resembles major depressive disorder, and IFN-α treatment in hepatitis C induces major depressive disorder in 25% of patients, suggesting a causal mechanism [218, 219]. In non-pregnant subjects, peripheral pro-inflammatory markers such as IL-6, IL-1β, IFN-α, TNF-α, and the chemokine monocyte chemoattractant protein 1 (MCP1)/chemokine (C-C motif) 2 (CCL2) are found to be increased in the blood and cerebrospinal fluid of a subgroup of patients with mood disorders compared with healthy controls, when assessed both at baseline and after exposure to stressors [204, 216, 220]. In relation to this, the existing literature indicates that a shift toward M1 macrophages in the M1/M2 balance may be related to the development...
of depression in the non-pregnant population [204, 213, 221]. Moreover, the indoleamine-2,3-dioxygenase (IDO)/kynurenine (KYN) pathway of tryptophan (TRY) metabolism has come into focus within immunological and psychiatric research. IDO, the enzyme that converts TRY to KYN [222], and kynurenine pathway metabolites [223], have been described to be correlated to depression. In addition, the pathway has been described as important in the immune system in pregnancy [224].

Inflammation in antenatal depression

While the inflammatory responses in obstetric complications such as preterm birth and pre-eclampsia are well studied [225, 226], few studies exist on the role of peripheral inflammatory markers in antenatal depression. Most studies are based on assessment of a limited number of markers such as IL-6, IL-10, IL-1β and TNF-α [227-230] from the beginning of the second trimester, or even earlier [231]. 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 [227-230]. However, differences in pro-inflammatory changes across time and a more pro-inflammatory third trimester have been described in women with elevated levels of symptoms of depression and/or anxiety [232]. For instance, in a study by Fransson and co-workers, associations between negative emotions and maternal serum IL-6 and IL-8 were noted [212]. Of relevance in connection with the poor pregnancy outcomes associated with antenatal depression, a review by Leff-Gelman et al. suggests predominant Th1/Th17 pro-inflammatory activity to be responsible for changes in monoaminergic systems, immune function, neurosecretory activity, and placental function, associated with preterm labour [233].

Placental function

The placenta is a transitory organ that acts as a bridge between the maternal and fetal circulations. The fetal side of the placenta contains chorionic villous trees with fetal vessels and stroma covered by a cytотrophoblast and a syncytiotrophoblast layer. Feto-maternal exchange occurs between the villi, in the intervillous space, where the maternal blood bathes the villous trees [234]. Stem-like villi called anchoring villi penetrate into the maternal part of the placental tissue, the decidua basalis, which is developed from and attached to the uterine wall. Placental dysfunction, leading to insufficient blood supply or affected transport of oxygen and nutrients, can heavily influence pregnancy outcome and lead to low birth weight, preterm birth, and birth defects [235-237]. The risk of developing conditions such as pre-
eclampsia is also increased in mothers with placental dysfunction. In addition, previous pre-eclampsia is associated with placental abnormalities in a current pregnancy [238].

Steroid hormones such as oestrogens, progestagens, androgens and glucocorticoids together with their precursor cholesterol, are all of utmost importance for the maintenance of a normal pregnancy and fetal development. The placenta is involved in biosynthesis and metabolism of steroids as well as the exchange of steroids between the mother and the fetus. Glucocorticoid transport in the maternal-fetal interface needs to be highly regulated to assure normal fetal growth and maturation. To control for high levels of maternal glucocorticoids the placental enzyme 11β-HSD2 converts cortisol to the inactive form cortisone [239]. Placental transport is of great importance when studying pregnancy and fetal development, and several factors such as blood flow, contact area, and placental metabolism have an impact on the transport process. Different mechanisms that drive the exchange of compounds in the maternal-fetal interface are active (primary and secondary) transport, passive transport, and facilitated diffusion [240].

The effects of antenatal depression and SSRIs on placental tissue

The relationships between SSRI use and adverse perinatal outcomes are not fully unravelled and additional research on the biological effects of SSRIs is needed. Such studies will highlight the biological mechanisms that these antidepressants may influence, which potentially strengthen or weaken the present epidemiological findings.

SSRI treatment and antenatal depression have been suggested to alter gene expression on the fetal side of the placenta. A pilot microarray study in our group revealed 108 genes that were differentially expressed in the placentas of women with antenatal depression and 109 genes that were differentially expressed in the placentas of women who used antidepressants during their pregnancies. 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 groups, whereas ROCK1, GCC2, KTN1 and DNM1L were confirmed to be differentially expressed only in the placentas of antidepressant-treated women [241], indicating that the results found in the gene expression of the placentas of women using antidepressants during pregnancy were more robust compared with 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 [242-244]. Kaihola et al. (2015) described SSRI-induced changes in the NGF signalling pathway of the placenta. Immunohistochemical staining 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, signalling proteins downstream in the NGF signalling pathway, were seen in stromal cells of placentas from SSRI-treated women when compared with 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 women and healthy controls. These findings might point towards altered placental function in women on treatment with SSRIs, which may be of relevance as regards the development of pre-eclampsia [245].

Another finding regarding antenatal depression and gene expression in the placenta is an association between antenatal depression and increased negative affect in the child at six months of age, in connection with lower levels of placental 11β-HSD2, NR3C1 and NR3C2 [246]. A recent study by Claubault et al. indicated that SSRIs are not cytotoxic to placental trophoblasts at clinically relevant doses. However, effects on trophoblast differentiation (syncytialization) were noted, especially as regards sertraline. Affected syncytialization, eventually leads to altered trophoblast homeostasis, which in turn may affect maternal-fetal exchange, and the hormonal balance essential for a healthy pregnancy and fetal development [247]. Another study by the same research group revealed that fluoxetine induces placental CYP19 (aromatase) in BeWo choriocarcinoma cells, while norfluoxetine, the main metabolite of fluoxetine, inhibits placental CYP19 in BeWo cells. In addition, norfluoxetine alters oestrogen production, and oestrogens, in turn, regulate the expression and activity of SERTs. This suggests a possible disruption of oestrogen synthesis regulation in trophoblasts by fluoxetine and norfluoxetine [248]. Recently the SSRI fluoxetine and its active metabolite norfluoxetine have been found to alter enzyme activity and oestrogen synthesis in a cell-culture model of the feto-placental unit [248]. However, further studies are needed to elucidate the effect of SSRIs on the placenta, and distinguish it from the effect of depression itself.
Aims

The overall aim of this work was to investigate different biological correlates in depressed pregnant women, and to further discriminate between the effects of antidepressant treatment and the untreated depression itself on these biological correlates.

The specific aims were:

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 women using SSRIs during pregnancy.

III. 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.

IV. To compare placental gene and protein expression in healthy women, women with antenatal depression and women on antidepressant treatment during pregnancy.
Material and Methods

Study population and design

All included studies were undertaken as parts of a large population-based, longitudinal cohort study named BASIC (Biology, Affect, Stress, Imaging and Cognition in pregnancy and the puerperium), which is aimed at increasing our knowledge of pathophysiological processes underlying peripartum depression. The study is being conducted at the Department of Obstetrics and Gynaecology, Uppsala University Hospital, and all women attending routine ultrasonography examination at gestational weeks (GWs) 16–18 are invited to participate. Upon invitation, written information is given and written consent is obtained from women who choose to participate. Exclusion criteria are (1) inability to communicate adequately in Swedish, (2) protected identity, (3) age less than 18 years, and (4) blood-borne infectious diseases. In Uppsala, all routine ultrasonography examinations are performed at Uppsala University Hospital and 97% of pregnant women participate. Moreover, the delivery ward of the hospital is the only one available within the county. Thus, invitation to participate in the study is population-based.

The BASIC project was initiated in 2009, after a brief pilot study. As of March 2018, around 6200 pregnancies and 5300 women have been included in the study, with a participation rate of about 21%. Women contribute to the study at i) GW 17 (web-based questionnaire including the Edinburgh Postnatal Depression Scale (EPDS), other psychological measures and demographic data), ii) GW 32 (EPDS, psychological measures and demographic data), iii) delivery (maternal and umbilical cord blood samples, umbilical cord samples, cerebral-spinal fluid samples (amniotic fluid, uterus and placenta samples previously collected)), iv) at six weeks (EPDS, psychological measures, demographic data and mother-infant bonding questionnaire), and vi) at one year postpartum (psychological measures and infant temperament assessment).

In addition to the above, 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 scores of $ \geq 12$ and a random sample of women with EPDS scores of $< 12$ at GW 32 or postpartum week six. Pregnant women were assessed around GW 38 (according to the ultrasonographically estimated date of delivery) and
postpartum women around eight weeks after delivery. 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 a.m. and 3 p.m., with the majority starting either at 9 a.m. or at 1 p.m., and all women had been fasting for at least 90 minutes before blood samples were drawn. In both sub-studies, the presence of ongoing primary anxiety and depressive disorders was established by use of the Mini International Neuropsychiatric Interview (M.I.N.I.), version 5.0.0 [249]. The interview also included questions on previous depressive episodes. In women who were on treatment with SSRIs but where the diagnostic interview failed to indicate the reason for treatment, no attempts were made to ascertain the reason for treatment initiation. For assessment of symptoms of depression and anxiety the women filled out the EPDS [250, 251], the Montgomery–Asberg Depression Rating Scale, self-rated version (MADRS-S) (137) and the Spielberger State-Trait Anxiety Inventory (STAI). In addition, cognitive tests (memory, attentional bias (Stroop test)) and psychophysiological tests (startle response and pre-pulse inhibition) were performed, and blood samples were collected. For all women, sociodemographic 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, the 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 a total of 957 placental samples were 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 (Figure 3) are based on material from the BASIC project, and the participants were selected on the basis of the presence or absence of depressive symptoms (or clinical depression). While the BASIC study is a prospective cohort investigation, the individual studies in this thesis are observational and most often based on cross-sectional sampling.

In Paper I, with an observational study design, we investigated changes in cognitive function in women with antenatal and postpartum depression. These changes may be caused by the depression, or, alternatively, lead to the
development of depression. Owing to the design of the study, causality cannot be determined.

In Paper II, which also is an observational study, inflammatory markers were considered to represent exposure, and antenatal depression the outcome. The latter assumption was based on extensive literature on the relationship between inflammation and depression.

The study described in Paper III had a descriptive observational design, where inflammatory markers were investigated in late pregnancy and early postpartum. Sampling was predominantly cross-sectional, but longitudinal for a smaller fraction of the women from whom we had paired samples.

Paper IV was an observational study, investigating differences in placental gene expression between groups of women (SSRI-treated, untreated depressed, non-depressed).

The study procedures are in accordance with ethical standards for human experimentation. The study was approved by the Regional Ethical Review Board in Uppsala, Sweden (Dnr 2009/171, approval July 1, 2009), and the procedures were in accordance with the Helsinki Declaration of 1975 (revised in 2008).
**Figure 3.** Overview of Papers (I-IV) included in the thesis. DEMO = Demographic data. Boxes marked with blue frames refer to the main sources of data/sampling for each study, and, in the right column, the number of included subjects per study.
Paper I

In total, 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 solely with anxiety disorders were excluded. In addition, two postpartum women were excluded because they misunderstood the instructions in the Stroop task, thus leaving data on 177 pregnant and 157 postpartum women available for analyses. Among these women, 40 suffered from antenatal depression and 33 from postpartum depression. Among pregnant women, 15 were on treatment with antidepressants, and the corresponding number of postpartum women was eight.

In this study women were considered to experience a depressive episode 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., or ongoing use of antidepressants.

Paper II

Two hundred and fifty-eight pregnant women participated in this sub-study of the BASIC project. 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 the late-pregnancy sub-study (n=205) and ii) from blood samples collected in conjunction with a planned Caesarean section (CS; n = 53). Out of 234 pregnant women who participated in the BASIC sub-study in late pregnancy, blood samples from 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). The remaining women were considered to be healthy controls (n = 124) or were on treatment with SSRIs (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 elective CS at Uppsala University Hospital. The morning before CS, which is typically performed in GW 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 of ≥ 17 at some
point during pregnancy. Exclusion criteria were serious pregnancy-related complications such as pre-eclampsia, intrauterine growth restriction, and gestational diabetes. In addition, all twin pregnancies were excluded.

Paper III

As in Paper II, in this study we used blood samples obtained from two different sources within the BASIC framework. Two hundred and ninety women were included in this sub-study and donated a total of 312 blood samples; 198 in late pregnancy (129 in the psychophysiological sub-study and 69 from the CS group) and 114 in the postpartum period. Twenty-two women had donated samples in both pregnancy (19 in the psychophysiological sub-study and three in the CS group) and postpartum.

First, blood samples from women at GW 38 (as in Paper II) or at eight weeks postpartum, or both, were used. In line with the aim of investigating healthy pregnant and postpartum women in this study, pregnant women with an ongoing minor or major depressive episode according to M.I.N.I. (late-pregnancy visit) (n = 20), or an EPDS score of 13 or more during pregnancy (GW 32, late-pregnancy visit (~GW 38), CS) (n = 57), were excluded. Postpartum women with an ongoing minor or major depressive episode according to M.I.N.I. (late pregnancy and/or postpartum visit) (n = 18), or an EPDS score of 13 or more during pregnancy (GW 32, late-pregnancy visit (~GW 38), CS) and/or an EPDS score of 12 or more postpartum (week six, week eight) (n = 28), were excluded. Women from whom we had paired samples with an ongoing minor or major depressive episode according to M.I.N.I. (late pregnancy and/or postpartum visit) (n = 10), or an EPDS score of 13 or more during pregnancy (GW 32, late-pregnancy visit (~GW 38), CS) and/or an EPDS score of 12 or more postpartum (week six, week eight) (n = 6), were excluded. Moreover, SSRI users, twin pregnancies, cortisone users and pre-eclamptic women were excluded in this study.

Paper IV

For this study, placental samples from 47 healthy controls, 25 women with untreated antenatal depression and 45 women on antidepressant treatment were used. General exclusion criteria for all groups were maternal age > 42 years, alcohol use during pregnancy, any pregnancy complication that would influence, or be a sign of compromised placental function, such as pre-eclampsia, gestational diabetes, pre-pregnancy diabetes, intrauterine growth restriction, offspring born small for gestational age, and gestational age < 35 weeks at delivery. The definition of “depressed” in this study was a diagnosis of depression according to M.I.N.I. (n = 19), or an EPDS score of ≥ 12 in weak 17 and 32, together with a diagnosis of previous major depression according to M.I.N.I. or according to medical records (n = 6). The definition
of “treated” was antidepressant treatment during at least half the pregnancy, according to medical records. The control subjects had a maximum EPDS score of 11, and no on-going/earlier psychiatric disease according to medical records.

Methods

Depression and anxiety assessment tools

Mini-International Neuropsychiatric Interview (M.I.N.I.)

(Supplementary material 1, section A. Depression, and B. Dysthymia, version 5.0.0).

In all papers, the structured Mini-International Neuropsychiatric Interview (M.I.N.I.), version 5.0.0 [249] was used for assessment of an ongoing or previous episode of major depression, minor depression, and PDD, according to DSM-IV and the 10th revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10). To be classified as having a major depressive episode (MDE) according to M.I.N.I., five or more symptoms have to be fulfilled in section A1–A3 that have been persisting for at least two weeks. Regarding a minor depressive episode, two to four symptoms have to be fulfilled in section A1–A3 that have been persisting for at least two weeks. To be classified as suffering from PDD or dysthymia, two or more symptoms from section B3 have to be fulfilled together with statement B1, and, moreover, the symptoms of depression have to have caused significant distress or impaired the ability to function at work, socially, or in some other important way. M.I.N.I. exhibits a specificity of 84% and a sensitivity of 95% for MDD [252].

The Edinburgh postnatal depression scale (EPDS)

(Supplementary material 2, the Swedish version of the EPDS)

In addition to the above, the Swedish version of the Edinburgh Postnatal Depression Scale (EPDS) [250] was used for assessment of depressive symptoms and depression severity in this work. The EPDS is a self-reported screening tool with a relatively low sensitivity of around 70%, but a higher specificity of approximately 90% [252, 253]. The tool includes 10 statements on mood for the past seven days, each scored from zero to three, which gives a total maximum score of 30. The validated cut-offs for peripartum depression in the Swedish setting are a score of 13 or higher in pregnancy [254], and a score of 12 or higher in the postpartum period [255].
The Montgomery-Åsberg Depression Rating Scale, self-rated version (MADRS-S)

The self-rated version of the Montgomery–Åsberg Depression Rating Scale (MADRS-S) was used for assessment of depressive symptoms and depression severity in this work. The instrument consists of nine statements, scored from zero to six, and concerns the previous three days [256].

Emotional Stroop test

In Paper I, the reaction times to affectively valenced and neutral words were assessed by using the Emotional Stroop Task, the E-Prime psychology software tool (Psychology Software Tools Inc., Sharpsburg USA). The test contained two blocks, each containing five unique words from each word category: neutral, positive and negative, and negatively valenced obstetric words. Each word was presented once in each colour: blue, green, yellow and red, resulting in 80 word presentations per block. Subjects were asked to identify the colour of the word while ignoring the meaning and press the coloured keyboard key that corresponded to that colour. Time to response was registered when the participant pressed the correctly coloured 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 [257, 258], 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 on the basis of 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 one (low valence, low arousal) to nine (high valence, high arousal) [259].

Blood samples

Maternal blood for the analysis of peripheral inflammatory markers and cortisol and cortisone (Papers II and III) was obtained at the visits made to the clinic around GW 38 and postpartum week eight, and for the CS women prior to the section. For the analysis of antidepressant serum concentrations (Paper IV), maternal blood was drawn at the delivery ward prior to the woman’s delivery. Blood samples were centrifuged at 1500 relative centrifugal force (RCF) for 10 minutes and the sera were stored at -70 °C.
Proximity extension assay

A more detailed description of the method can be found in Papers II and III. Stored plasma 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, and all samples were analysed using the same batch of reagents. Analyses of the relative levels of 92 inflammatory proteins in the plasma were performed at the Clinical Biomarker Facility at SciLifeLab Uppsala, using a Proseek Multiplex Inflammation I panel (Olink Bioscience, Sweden) (Supplementary material 3), which is based on proximity extension assay (PEA) technology [260, 261] (Figure 4). In brief, when a pair of DNA oligonucleotide-labelled antibody probes binds to the protein of interest, the DNA oligonucleotides in close proximity will hybridize and form an amplifiable DNA molecule by proximity-dependent DNA polymerization. The newly formed DNA amplicon is subsequently detected and quantified using a 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 Cq value of every sample was normalized against an extension control (correction for technical variation), an interplate control (correction for variation between runs) and a calculation correction factor. Normalization of data was performed with 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. The limit of detection (LOD) was assessed on the basis of the mean value of triplicate negative controls from each run. In Papers II and III, inflammatory markers with NPX values above LOD in less than 50% of the samples were excluded from further analyses. Missing values were replaced by LOD/√2 [262].
Figure 4. Proximity extension assay method.

Cortisol and cortisone

Tandem mass spectrometry was used for determination of maternal cortisol and cortisone levels as described previously [34]. A brief description of the method can be found in Paper II.

TaqMan Low-Density Arrays

A more detailed description of the method can be found in Paper IV. The gene expression analysis in Paper IV was performed by means of a quantitative real-time qPCR method using custom-designed TaqMan low-density arrays (TLDAs) (Applied Biosystems, Foster city, CA, USA) for 44 genes previously described within the pathophysiology of depression, and also expressed in the placenta (Supplementary material 4). These genes represent: i) monoaminergic pathways, including genes such as sodium-dependent serotonin transporter (SLC6A4), aromatic l-amino acid decarboxylase (AADC), and monoamine oxidase A (MAOA), all involved in monoamine transport to the fetus [81, 263]; ii) hypothalamic–pituitary–adrenal (HPA) axis function, including genes such as corticotropin-releasing hormone (CRH) and neuropeptide Y (NPY), which drive the maternal HPA axis during pregnancy [31], and 11β hydroxysteroid dehydrogenase types 1 and 2 (HSD11B1 and 2), responsible for shuttling of cortisol to the fetus [264]; iii)
other hormonal systems, including oxytocin (OXT) and enzymes involved in progesterone metabolism, such as 5α-reductase types I and II (SRD5A1 and 2) and 3α-hydroxysteroid dehydrogenase type III (AKR1C4) [46, 49]; iv) growth factors, including vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF), and nerve growth factor (NGF) [241, 245], and finally, v) genes involved in placental drug metabolism and transport. Four genes, GAPDH, TOP1, YWHAZ and ACTB were included in the arrays as reference genes (Supplementary material 4).

Each TaqMan LDA consisted of 384 wells and eight ports (48 wells/assays per port). The 117 samples required 15 TLDAs in total. Samples were run as singletons, and the amount of cDNA in each loading port was equivalent to 100 ng of mRNA. The arrays were run according to the manufacturer’s protocol with an ABI Prism 7900HT Sequence Detection System and ABI Prism 7900HT SDS software version 2.4 (Applied Biosystems). Each assay included a forward primer, a reverse primer, and a TaqMan® MGB probe with the reporter FAM™ and the quencher MGB-NFQ. As negative control, water was used. Real-time RT-PCR was run with thermal cycling conditions of 2 min at 50 °C and 10 min at 95 °C, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing and extension at 60 °C for 1 min. Manual confirmation of threshold detection was conducted for quality-control purposes. We utilized Ct number as input for our variability analysis among tissue samples for each target. Results for each target in TID analysis were quantified concurrently using the same baseline and threshold for a target gene in order to limit inter-plate errors in the analysis. By using NormFinder, GeNorm algorithms and GenEx software (MultiD Analyses) [265], we identified GAPDH and YWHAZ as the most stable combination of genes to use for normalization in data analysis. Normalization of the data includes subtraction of the mean Ct values from the best combination of reference genes from the mean Ct value for each gene in each group (ΔCt).

Immunohistochemistry

A more detailed description of the method can be found in Paper IV. Based on availability of placental tissue (embedded in paraffin blocks) from women included in the gene expression analysis, placental protein expression was determined in 37 healthy controls, 13 women with untreated depression and 21 women on antidepressant treatment. The primary antibodies used were anti-HTR1A (PA5-28090, rabbit, Thermo Fisher Scientific) and anti-NPY2R (PA1-41576, rabbit, Thermo Fisher Scientific), at dilutions of 1:500 and 1:250, respectively. As a negative control we used 0.1% BSA in PBS. The secondary antibody used in this study was a biotinylated goat anti-rabbit antibody (Vector labs BA-1000). As detection method we used a colorimetric system including an enzyme, horseradish peroxidase (HRP) (dilu-
tion 1:400, 1 h at room temperature, Vector labs A-2004), and a substrate, DAB (3, 3’-diaminobenzidine) (Dako). The enzyme HRP catalyses oxidation of the substrate DAB, resulting in a brown colour in the sample. Mayers’ haematoxylin was applied for staining of the cell nuclei. The immunohistochemically stained placental tissue sections were analysed in terms of staining pattern, distribution, intensity (0–3), and the proportion (%) of stained cells. Two independent scorers carried out the scoring in a blinded manner in terms of case/control status. Intensity was set at negative (0), weak (1), medium (2) or strong (3). The tissue samples were further evaluated by calculating an H-score (histo-score), based on the staining intensity and proportion of stained cells. This takes into account different staining intensities within the same tissue sample, and is assigned using the following formula: 

\[
1 \times (\% \text{ of cells with intensity score 1}) + 2 \times (\% \text{ of cells with intensity score 2}) + 3 \times (\% \text{ of cells with intensity score 3})
\]

The final score will thus range from 0 to 300 [266, 267].

**Antidepressant concentration measurement**

A more detailed description of the method can be found in Paper IV. Serum samples from 41 women in the antidepressant group in Study IV were available for concentration measurements. Citalopram, escitalopram, sertraline, and fluoxetine and its active metabolite norfluoxetine were analysed using liquid chromatography–mass spectrometry by methods described previously [268]. In brief, the substances were extracted from serum by liquid–liquid extraction, separation on C18 columns, and quantified on an Agilent MSD 1100 LC-MS system (Agilent Technologies, Palo Alto, CA, USA). Internal standards were used. Together with the unknown patient samples, each analytical series contained seven calibrators covering therapeutic, subtherapeutic, and toxic concentrations. In addition, six quality-control samples with representative target concentrations were included. The limits of quantification were 5 nmol/L for sertraline and 10 nmol/L citalopram, escitalopram, fluoxetine and norfluoxetine. Accuracy was controlled routinely with external control samples, and precision was calculated from the quality-control samples. In general, the inter-assay coefficients of variation were less than 10%. The methods were linear in the concentration ranges concerned.

**Statistics**

For detailed description of statistical analyses, please see the individual papers.

The emotional interference scores (Paper I) were modelled in five-way repeated measures ANOVAs with word category (positive, negative, negatively valenced obstetric words) as a within-group variable, and perinatal
stage (pregnancy vs. postpartum), group (women with depression vs. controls), antidepressant use (use vs. non-use), and anxiety (yes vs. no) as between-group variables. Antidepressant treatment was modelled 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, three-way ANOVAs, were conducted in each perinatal group with word category as a within-group variable, and depression, and antidepressant use as between-group variables. When the ANOVAs yielded a significant group by word-category interaction, the interaction was evaluated by post hoc independent and paired t-tests and confirmed by the Mann–Whitney U-test. Statistical analyses were performed by use of IBM SPSS Statistics 20.0. Values of \( p < 0.05 \) were considered statistically significant.

In Paper II, inflammatory markers with NPX values higher than the limit of detection (LOD) for at least 50% of the women were used, leaving 74 markers for statistical analysis. For handling of values below LOD, these were replaced by LOD/\( \sqrt{2} \) [262]. Primary analyses of the inflammatory markers across all three groups were carried out 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%). 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), pre-eclampsia 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 using Spearman’s rank correlation. Statistical analyses were performed using the statistical package R 3.2.4 and IBM SPSS version 24.0. Values of \( p < 0.05 \) were considered statistically significant.

In Paper III, inflammatory markers with NPX values over LOD for at least 50% of the women during pregnancy and postpartum were further analysed, leaving 70 markers for statistical analysis. For handling of values below LOD), these were replaced by LOD/\( \sqrt{2} \) [262]. Inflammatory markers were assessed between pregnancy and postpartum using the Linear Mixed model. The analysis was adjusted for age, pre-pregnancy BMI, use of antibiotics at blood sampling, and chronic inflammatory or rheumatic diseases. Bonferroni correction was applied to counteract the problem of multiple comparisons. The results from the Linear Mixed model were validated by using Wilcoxon paired tests in the subset of 22 women who had donated a blood sample during both pregnancy and the postpartum period. All statisti-
Analyses were performed using IBM SPSS statistics version 24.0. Values of $p$ below 0.05 were considered statistically significant.

In Paper IV, statistical comparisons between groups were performed by one-way ANOVA or the Kruskal–Wallis test, depending on the distribution of the individual variable. When ANOVA was significant, post hoc tests were used: Tukey’s HSD test with normally distributed variables and the Mann–Whitney $U$-test with skewed variables. Correction for multiple testing was performed according to Bonferroni. Statistical analyses were performed using IBM SPSS version 24.0. Values of $p < 0.05$ were considered statistically significant.
Summary of results

Paper I

In pregnancy, no significant differences in emotional interference scores were noted between depressed and non-depressed women ($p > 0.05$) (Figure 5A). However, pregnant women on antidepressant treatment showed a greater emotional interference by negatively valenced obstetric stimuli ($p < 0.05$) than non-treated pregnant women. Women with ongoing postpartum depression displayed shorter reaction times to positive and negative stimuli than to neutral words ($p < 0.05$) in comparison with non-depressed postpartum women (Figure 5B). In addition, self-rated depression scores were significantly negatively correlated with the emotional interference scores arising from positive and negative stimuli, i.e. with increasing MADRS or EPDS scores a decreased emotional interference score was noted.

A number of women accounted for two data points in the omnibus ANOVA (represented in both pregnancy and postpartum groups, n = 22), and a mixed-design ANOVA model might have been more suitable. Therefore, we also analysed the data using a mixed-design ANOVA model and the results from the omnibus ANOVA, used in this paper, did not substantially differ from the original analysis (data not shown).
Figure 5. Emotional interference scores (mean ± standard deviation (SD)) in (A) non-depressed pregnant women (n = 149) and women with antenatal depression (n = 28), and (B) non-depressed postpartum women (n = 131) and women with postpartum depression (n = 26). In this context, euthymic women on antidepressant treatment were regarded as non-depressed. No differences in emotional interference scores were noted between women with antenatal depression and non-depressed pregnant women. Women with postpartum depression displayed less emotional interference to positive ($p = 0.028$) and negative ($p = 0.022$) words, compared with non-depressed postpartum women. *$p < 0.05$, independent $t$-tests.
Multinomial logistic regression revealed 23 inflammatory markers to be significantly different between groups after Bonferroni correction for multiple testing. Post hoc multivariable logistic regression analyses of the remaining inflammatory markers are displayed in Table 2. As seen in the table, 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 SSRIs. Notably, for each investigated inflammatory marker, lower levels were found in the two depressed groups. The top three inflammatory factors that were down-regulated in women with antenatal depression were tumour necrosis factor ligand superfamily member 10/TNF-related apoptosis-inducing ligand (TRAIL), macrophage colony-stimulating factor 1 (CSF-1), and fractalkine/CX3CL1 (C-X3-C motif chemokine ligand 1). Corresponding inflammatory markers in SSRI users were CSF-1, vascular endothelial growth factor A (VEGF-A), and IL-15 receptor subunit alpha (IL-15RA). The majority of the inflammation markers were significantly negatively correlated with cortisone or the cortisone to cortisol ratio in healthy controls. In contrast, among women with antenatal depression (with or without treatment) this pattern was not found (Table 3).
Table 2. 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>Antenatal depression</th>
<th>SSRI treatment</th>
<th>adjusted p* depressed vs. control</th>
<th>adjusted p* SSRI use vs. control</th>
<th>adjusted p* SSRI use vs. depressed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean  SD</td>
<td>Mean  SD</td>
<td>Mean  SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRAIL</td>
<td>159  10.71 0.58</td>
<td>10.31 0.63</td>
<td>10.39 0.69</td>
<td>0.000001</td>
<td>0.003298</td>
<td>0.176027</td>
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<tr>
<td>CSF-1</td>
<td>159  11.48 0.41</td>
<td>11.20 0.47</td>
<td>11.16 0.41</td>
<td>0.000004</td>
<td>0.000011</td>
<td>0.853314</td>
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<tr>
<td>CX3CL1</td>
<td>159  7.52 0.68</td>
<td>7.06 0.81</td>
<td>6.96 0.73</td>
<td>0.000005</td>
<td>0.000071</td>
<td>0.803664</td>
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<tr>
<td>CST5</td>
<td>159  7.55 0.71</td>
<td>7.10 0.63</td>
<td>7.05 0.55</td>
<td>0.000033</td>
<td>0.000134</td>
<td>0.340899</td>
</tr>
<tr>
<td>DNER</td>
<td>159  9.41 0.59</td>
<td>9.12 0.60</td>
<td>8.92 0.62</td>
<td>0.000017</td>
<td>0.000080</td>
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<tr>
<td>VEGF-A</td>
<td>159  14.74 0.45</td>
<td>14.48 0.55</td>
<td>14.39 0.45</td>
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<td>0.000016</td>
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<td>STAMBP</td>
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<td>4.85 1.40</td>
<td>4.43 1.46</td>
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<td>0.000050</td>
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<td>CD5</td>
<td>159  4.52 0.58</td>
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<td>0.000267</td>
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<td>CD244</td>
<td>159  7.68 0.66</td>
<td>7.31 0.69</td>
<td>7.20 0.57</td>
<td>0.000089</td>
<td>0.000050</td>
<td>0.687181</td>
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<td>TNFRSF9</td>
<td>159  7.83 0.58</td>
<td>7.41 0.67</td>
<td>7.48 0.55</td>
<td>0.000029</td>
<td>0.001092</td>
<td>0.819474</td>
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<tr>
<td>LTA (TNFB)</td>
<td>159  4.33 0.68</td>
<td>3.97 0.63</td>
<td>3.94 0.61</td>
<td>0.000021</td>
<td>0.003959</td>
<td>0.748542</td>
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<td>IL-10RB</td>
<td>159  9.13 0.56</td>
<td>8.76 0.71</td>
<td>8.72 0.54</td>
<td>0.000048</td>
<td>0.000478</td>
<td>0.970432</td>
</tr>
<tr>
<td>CD40</td>
<td>159  12.26 0.91</td>
<td>11.73 0.92</td>
<td>11.52 0.80</td>
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<td>0.000039</td>
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<td>IL-15RA</td>
<td>159  1.29 0.26</td>
<td>1.14 0.30</td>
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<td>hGDNF</td>
<td>158  2.75 0.55</td>
<td>2.44 0.56</td>
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<td>ST1A1</td>
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<td>0.94 0.41</td>
<td>0.95 0.45</td>
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<td>ADA</td>
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<td>6.60 0.76</td>
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<td>uPA</td>
<td>159  15.98 0.50</td>
<td>15.71 0.58</td>
<td>15.66 0.66</td>
<td>0.000223</td>
<td>0.001607</td>
<td>0.953152</td>
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<td>AXIN1</td>
<td>159  4.64 2.04</td>
<td>3.70 1.84</td>
<td>3.13 1.69</td>
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<td>0.000284</td>
<td>0.669153</td>
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<td>SLAMF1</td>
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<td>IL-17C</td>
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<td>0.000773</td>
<td>0.000368</td>
<td>0.755932</td>
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</tbody>
</table>

*Adjusted for age, BMI, smoking, days left to parturition, fasting, preeclampsia or hypertension, and pre-pregnancy inflammatory or rheumatoid disorder.
Table 3. Spearman’s 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>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cortisol</td>
<td>cortisone</td>
</tr>
<tr>
<td></td>
<td>rho</td>
<td>p</td>
</tr>
<tr>
<td>TRAIL</td>
<td>-0.253</td>
<td>0.005</td>
</tr>
<tr>
<td>CSF-1</td>
<td>-0.056</td>
<td>0.540</td>
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<tr>
<td>CX3CL1</td>
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<td>0.390</td>
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<tr>
<td>CST5</td>
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<td>0.075</td>
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<td>CD244</td>
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<td>TNFRSF9</td>
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<td>LTA (TNFB)</td>
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<td>IL-10RB</td>
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<td>IL-15RA</td>
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<td>IL-17C</td>
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<td>0.060</td>
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</tbody>
</table>
Paper III

Following correction for multiple testing (Bonferroni), 50 inflammatory markers were significantly different between pregnancy and postpartum (Tables 4 and 5). Of these markers, 41 were higher in pregnancy (Table 4), while the remaining nine were higher in the postpartum period (Table 5). Among the 22 participants who contributed with samples in both late pregnancy and postpartum, all but two markers (chemokine (C-X-C motif) ligand 9 (CXCL9) and cluster of differentiation 6 (CD6)) were confirmed to be significant in the Wilcoxon test. The top three markers with the greatest decrease in the postpartum period were leukemia inhibitory factor receptor (LIF-R), latency-associated peptide transforming growth factor beta-1 (LAP TGF-beta-1), and C-C motif chemokine 28 (CCL28) (Figure 6A). Other well-studied markers that were also decreased in the postpartum period included IL-6 and IL-10. The top three markers that were increased in the postpartum period were tumour necrosis factor ligand superfamily member 11/TNF-related activation-induced cytokine (TRANCE), tumour necrosis factor ligand superfamily member 12/TNF-related weak inducer of apoptosis (TWEAK), and C-C motif chemokine/Eotaxin (CCL11) (Figure 6B).
Table 4. Markers with higher NPX values in pregnancy. Data are presented as number of samples, NPX mean, SD and mean difference (postpartum - pregnancy), as well as linear mixed model-derived \( p \)-values and Bonferroni-corrected \( p \)-values for the difference between postpartum and pregnancy NPX values.

<table>
<thead>
<tr>
<th></th>
<th>Pregnancy</th>
<th>Postpartum</th>
<th>Mean difference</th>
<th>Linear mixed model*</th>
<th>Linear mixed model Bonferroni ( p )-value*</th>
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<td>n</td>
<td>Mean (SD)</td>
<td>Mean difference</td>
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<td>114</td>
<td>5.29 (0.35)</td>
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<td>114</td>
<td>1.89 (0.73)</td>
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<td>FGF21</td>
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<td>2.52 (1.28)</td>
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</tr>
<tr>
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<td>114</td>
<td>2.71</td>
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</tbody>
</table>

*Adjusted for age at partus, pre-pregnancy BMI, antibiotics at blood sampling, and chronic inflammatory or rheumatic disease.*
Table 5. Markers with higher NPX values in the *postpartum* period. Data are presented as number of samples, NPX mean, SD and mean difference (postpartum - pregnancy), as well as linear mixed model-derived *p*-values and Bonferroni-corrected *p*-values for the difference between postpartum and pregnancy NPX values.

<table>
<thead>
<tr>
<th></th>
<th>Pregnancy</th>
<th></th>
<th>Postpartum</th>
<th></th>
<th></th>
<th>Mean difference</th>
<th>Linear mixed model <em>p</em>-value*</th>
<th>Linear mixed model Bonferroni <em>p</em>-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td></td>
<td></td>
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<tr>
<td>TRANCE</td>
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<td>0.62</td>
<td>114</td>
<td>3.23</td>
<td>0.59</td>
<td>1.06</td>
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<td>114</td>
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<td>0.25</td>
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<td>0.53</td>
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<td>0.66</td>
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<td>IL-12B</td>
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<td>0.62</td>
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<td>0.41</td>
<td>0.42</td>
<td>0.000</td>
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<td>0.57</td>
<td>114</td>
<td>2.99</td>
<td>0.41</td>
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<td>CXCL9</td>
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<td>5.62</td>
<td>0.81</td>
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</table>

*Adjusted for age at partus, pre-pregnancy BMI, antibiotics at blood sampling, and chronic inflammatory or rheumatic disease. *Not confirmed in Wilcoxon’s paired test.
Figure 6. NPX values for the top markers LIF-R, LAP TGF-beta-1 and CCL28, in the individual paired samples from pregnancy to postpartum (A). NPX values for the top markers TRANCE, TWEAK and CCL11, in the individual paired samples from pregnancy to postpartum (B).
Overall, following correction for multiple testing, none of the examined genes were differentially expressed between healthy controls, women with untreated depression and women on antidepressant treatment (Table 6). Nominally significant findings were noted for HTR1A (5-hydroxytryptamine (5-HT/serotonin) receptor 1A), NGF and NPY2R (neuropeptide Y2 receptor) (Table 6). These findings remained for HTR1A and NPY2R when analyses were restricted to women with detectable concentrations of antidepressant drugs at delivery (median ΔCt for HTR1A of 9.1 (IQR 7.9–11.8), p = 0.043 and median ΔCt for NPY2R of 10.6 (IQR 8.4–12.4), p = 0.019). Post hoc analyses revealed that women with untreated depression had higher gene expression of HTR1A (p = 0.039) and NPY2R (p = 0.008), versus healthy controls, whereas women on antidepressant treatment had similar expression of HTR1A and NPY2R as healthy controls (Figure 7). Further, post hoc analyses revealed that placental gene expression of NGF was higher in women with antidepressant treatment than in healthy controls (p = 0.038; Table 6, Figure 7). However, this finding was of only borderline significance when analyses were restricted to women who had detectable levels of antidepressant drugs at delivery (median ΔCt for NGF of 8.3 (IQR 7.8–8.9), p = 0.056). The genes with nominally significant differences between the groups were selected for determination of placental protein expression. NGF was not included in the protein analyses, as this has previously been reported by our group [245]. The immunohistochemical staining of HTR1A had an intensity of medium-strong in placental trophoblasts and endothelial cells, and medium in stromal cells, whereas the staining of NPY2R displayed medium intensity in the trophoblasts and weak intensity in endothelial and stromal cells (Figure 8).
Table 6. Gene expression in placental tissue from healthy controls, women with untreated depression and women on antidepressant treatment, sorted according to the degree of statistical significance.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Healthy controls (n = 47)</th>
<th>Women with untreated depression (n = 25)</th>
<th>Women on antidepressant treatment (n = 45)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Median ΔCt (IQR)</td>
<td>n</td>
</tr>
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<td>NPY2R</td>
<td>40</td>
<td>12.3 (10.1 – 13.3)</td>
<td>20</td>
</tr>
<tr>
<td>NGF</td>
<td>47</td>
<td>8.8 (8.1 – 9.5)</td>
<td>25</td>
</tr>
<tr>
<td>HTR1A</td>
<td>34</td>
<td>10.8 (9.6 – 12.3)</td>
<td>21</td>
</tr>
</tbody>
</table>

Figure 7. Placental gene expression (ΔCt ± SD) in women with antenatal depression, women on antidepressant treatment, and healthy controls. Women with untreated depression had higher gene expression (lower ΔCt) of HTR1A (p = 0.039) and NPY2R (p = 0.008) than healthy controls. Gene expression of NGF was higher in women with antidepressant treatment than in healthy controls (p = 0.038).
HTR1A staining intensity in trophoblasts differed between groups (Table 7), and the difference remained significant in analyses restricted to the treated women with detectable antidepressant serum levels, compared with healthy controls and untreated depressed women ($p = 0.043$). The highest HTR1A expression was noted in women on antidepressant treatment, both in comparison with healthy controls ($p = 0.011$) and compared with women with untreated depression ($p = 0.016$) (Figure 9). No difference in endothelial and stroma cell expression of HTR1A was noted between groups. Similarly, no difference between groups in placental NPY2R protein expression was confirmed.
<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n = 37)</th>
<th>Women with untreated depression (n = 13)</th>
<th>Women on antidepressant treatment (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HTR1A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Trophoblasts       | 238 (206 – 263)          | 225 (213 – 244)                       | 263 (238 – 288)                        | 0.017  
| Endothelial cells  | 225 (175 – 238)          | 225 (169 – 263)                       | 225 (206 – 263)                        | 0.284  
| Stroma cells       | 175 (138 – 213)          | 175 (156 – 194)                       | 175 (175 – 231)                        | 0.258  
| **NPY2R**          |                          |                                        |                                          |  
| Trophoblasts       | 188 (156 – 213)          | 213 (163 – 225)                       | 200 (163 – 238)                        | 0.743  
| Endothelial cells  | 63 (50 – 63)             | 63 (50 – 113)                         | 50 (50 – 81)                           | 0.351  
| Stroma cells       | 50 (50 – 63)             | 63 (50 – 113)                         | 50 (50 – 81)                           | 0.370  

Table 7. Placental protein expression of HTR1A and NPY2R in healthy controls, women with untreated depression and women on antidepressant treatment.
Figure 9. Protein expression (mean H-score ± SD) in placental trophoblasts in women with antenatal depression (n = 13), women on antidepressant treatment (n = 21), and healthy controls (n = 37). The highest HTR1A expression was noted in women on antidepressant treatment, both in comparison with healthy controls ($p = 0.011$) and compared with women with untreated depression ($p = 0.016$). No difference between groups in placental NPY2R protein expression was confirmed.
Discussion

Methodological considerations

Study population and study design
The BASIC project is a longitudinal, population-based cohort study in which all pregnant women in Uppsala County who attend routine ultrasonography at around GW 16–18 are invited to participate. The major strengths of the BASIC project are the large study population and the fact that numerous characteristics and background data are available at an individual level. The longitudinal properties of the project make it possible to observe differences over time and, from that, draw conclusions about causal relationships. The BASIC study also has limitations, and the most important is selection bias. As mentioned above (Methods), some of these limitations are introduced by the exclusion criteria for the study: non-Swedish speaking, age less than 18 years, protected identity, and blood-borne infections. By including only women who are able to communicate in Swedish the proportion of native Swedes in the study is greater than in the general population. Thus, important perspectives concerning immigrant women, and other women who have not yet learned Swedish will be lost in this project.

The overall participation rate in the BASIC project is around 21%. The study population consists of slightly higher educated, slightly older women, pregnant with their first child, compared with the Uppsala County population in general [269]. However, variables such as maternal BMI, gestational length and offspring birth weight are similar to those in the general population [270]. From previous studies in the field, concerning attrition, it is known that individuals, who choose not to participate in research studies, are usually more depressed than participants [271]. Given a similar selection bias for the BASIC project, participation of these more severely depressed women would possibly have made the study results more robust.

The cohort design introduces 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, which mirrors the population. However, when selecting certain groups for a specific study question, for instance women with postpartum onset of depression without a prior history of depressive episodes,
the number of available cases to study will inevitably be reduced. Examples of this problem are noted in Paper I, where pregnant women on antidepressant treatment displayed different responses 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 postpartum women on antidepressant treatment was too low, leading to problems with statistical power.

Self-rated EPDS scores are used to measure depressive symptoms for the greater part of the BASIC study, in line with other large-scale epidemiological studies. However, as most women included in the present work had participated in two of the BASIC sub-projects, their depression diagnoses had been established by use of a structured psychiatric interview, the Mini International Neuropsychiatric Interview (MINI). Generally, highly structured psychiatric interviews are considered the gold standard in studies on depressive and anxiety disorders, as they provide greater reliability in terms of case status than self-reported depressive symptoms. However, for the different projects (and also within the same projects) different definitions of depression had to be used in order to optimize power. Preferably, all women should have been interviewed to ensure the best possible phenotyping.

Even though the BASIC project is longitudinal, the studies presented in this thesis are mainly cross-sectional. In Paper II, the cross-sectional design makes it difficult to draw conclusions regarding causation and the temporal order of events. It would have been advantageous to have had longitudinal blood sampling, where temporal changes in M1/M2 inflammation markers could have been followed over time in relation to cortisol levels and development of depression. However, in Paper III paired blood samples were available for a subset of women, which brought a partly longitudinal design to the study.

Experimental considerations

The highly sensitive PEA method used in Papers II and III has the capacity to measure a large number of proteins and samples simultaneously. Cytokines are difficult to measure in the circulation due to their low concentrations, and PEA requires only one microlitre of serum, plasma or any other material. The PEA method also has a high specificity owing to the paired probes that both need to bind to the target protein in close proximity to give rise to amplification and a signal. We trust that the highly sensitive and specific method we used will have provided more robust results than in previous studies, most of them being based on immunoassays [272].

In Paper IV, where we studied placental tissue, the great heterogeneity of that tissue must be mentioned. This could of course have influenced the results, but we tried to minimize the problem by having a relatively large sam-
ple of placentas from obstetrically healthy women, matched in terms of age and BMI to the cases. The exposure in the placenta study was antenatal depression and treatment with antidepressant therapy. Use of antidepressant treatment relied on self-reports by the women in the BASIC questionnaires and was confirmed by review of the medical records. However, there is always a risk of misclassification, as women may choose not to report antidepressant use to their midwife or obstetrician, or may have discontinued treatment without this being noted in the medical records. This risk was reduced in Paper IV by validating the SSRI treatment with serum concentration measurements of the actual drug. Moreover, the various types, doses, half-lives and duration of antidepressant treatment may also influence expression in the placenta, and additionally affect the results of the serum concentration measurements. Finally, sensitivity analyses were performed, including only women in the treatment group who had detectable serum levels of the drug.

Isolation of RNA from placental tissue is generally difficult due to degradation. To avoid this problem, it is important to be fast when taking care of the placenta at delivery, and freeze the tissue immediately. Specific protocols were developed in the BASIC study to ensure rapid handling of tissue, and in most cases we were able to ascertain high-quality RNA from the placental samples we wanted to investigate. Moreover, a modified protocol with two-step lysis is preferred, which was implemented in Paper IV. With the addition of a beta-mercaptoethanol step to the usual lysis protocol, the RNases will be denatured while still keeping the RNA intact.

When selecting primers and probes for the analyses there are several important things to consider, in order to obtain the best possible results from the experiment and to be able to trust the results. However, when running large experiments with many samples and assays, and there is no possibility to design new primers, and instead we relied on the manufacturer’s library and validation. In Paper IV, each primer was used in all three groups, and the risk of bias is therefore spread equally across the sample. Also, four reference genes were selected on the basis of high stability in placental tissue [273, 274], and the gene expression data were normalized against the expression of the most stable combination of reference genes, according to NormFinder.

Immunohistochemistry is an established validating tool, giving the ability to study all the different cell types in the tissue. In an immunohistochemistry setting it is important to have target-specific primary antibodies. With insufficient specificity, there is a substantial risk of obtaining false-positive and also false-negative results. A superior method for isolating specific polyclonal antibodies is affinity chromatography, which was used for purifying antibodies employed in Paper IV. High specificity is also of great importance as regards the secondary antibodies. Low specificity may result in binding of
the antibody to other material and structures within the sample, therefore ending up with background and false-positive staining.

Scoring of the immunohistochemically stained tissue in Paper IV was carried out manually using a light microscope, and by calculating an H-score for each stained placenta and antibody. H-scoring is a semi-quantitative assessment tool based on both staining intensity and the proportion of stained cells. This is a subjective method, but to minimize bias it was performed by two independent scorers and in a blinded manner in terms of case/control status.

**Attentional bias in peripartum depression**

The major finding in Paper I was that pregnant and postpartum depressed women did not display any attentional bias to affectively valenced words. However, the depressed postpartum women displayed shorter reaction times to both positively and negatively valenced words than to neutral ones.

In the literature, depression is described as a result of emotional processing bias and deficits in cognitive control when processing negative information [152, 153]. However, there is not yet consensus on whether the described cognitive dysfunction in depressed patients is due to processing bias of emotional stimuli, or by more general cognitive deficits that also involve processing of non-emotional stimuli. Studies indicate that depressed cases exhibit more of a deficit in the control of attention, rather than being limited in their processing capacity [152]. Attention deficits cannot be found in depressed subjects when the task can capture the person’s attention, and there is no room for rumination. The participant needs to set aside all task-irrelevant thoughts to be able to focus and pay attention to the actual task [152]. A review by Gotlib and Joormann supports the idea of affective interference and the suggestion of limited cognitive control in depressed individuals [152].

Attentional bias has been described as being more pronounced in subjects with clinical depression than in subjects with depressed symptoms, and, similarly, in cases with co-morbid anxiety than in cases without anxiety [159]. Even though the cases in this study were considered to fulfil a diagnosis of depressive disorder, and almost half of them had co-morbid anxiety, no attentional bias in peripartum depression was revealed, with the exception of women who continued antidepressant medication in pregnancy. Research on attentional bias in patients with MDD and patients with anxiety disorder, has revealed attentional bias to task-irrelevant stimuli in the anxiety-only patients, and attentional bias to task-relevant stimuli in both patient groups [164]. Attentional bias might be more efficient in spotting anxiety-related cognitive deficits.
The shorter reaction times to positive and negative words seen in depressed postpartum women in Paper I can be interpreted in different ways. Shorter reaction times to negative stimuli could possibly be a result of an explicit strategy to override the tendency for negatively valenced stimuli to interfere with colour naming, most commonly noted in nonclinical high-trait anxiety participants [157]. This describes a coping strategy, which may facilitate exit from the vicious circle of emotional processing bias and poor cognitive control that characterizes the depressive episode [157, 275]. However, the term override generally means faster performance throughout the entire task, which would have been noted as faster reaction times to neutral words as well. The postpartum depressed women also displayed shorter reaction times to the positive words; potentially indicating emotional numbing, which is a functional impairment that may have consequences for the child’s development and well-being. Our findings, together with others, stress the need to identify these women and treat the depression as quickly as possible to ensure recovery, support for the family, and normal child development [276].

Other cognitive and emotional deficits noted in women with postpartum depression include worse performance in emotion recognition tasks compared with healthy controls when recognizing happiness and fear, and compared with non-peripartum depressed women as regards disgust and anger [154]. Women with postpartum depression are also less responsive to negative stimuli [181], in contrast to the affective reactivity seen in non-peripartum depressed women [153], and in contrast to the interpretation of negative stimuli as more negative for non-depressed postpartum women [277]. Moreover, it has been suggested that postpartum depressed women are more prone to avoid or minimize exposure to distressing infant stimuli [174]. Our findings are also in line with previous reports on disrupted attentional processing of infant emotion in pregnant women with depressive symptoms [170].

As a secondary finding in Paper I, women on antidepressant treatment during pregnancy displayed attentional bias only to obstetrically threatening words, in line with what has been described in depressed cases, for negatively valenced words. One could speculate that women who continue antidepressant treatment during pregnancy are the ones in greatest need of treatment, and the ones with the most severe psychiatric morbidity [79]. However, this finding in the antidepressant group does not necessarily suggest greater depression severity. Investigators have also reported on attentional bias to emotional content in remitted patients [169, 278], although findings are mixed [159]. In this study, women on antidepressant treatment had lower scores of self-rated depression than the untreated depressed women who managed without medicine, suggesting that the majority of the treated women were in remission when tested.

Beyond the proposed differences in emotion processing patterns between women with peripartum depression and non-peripartum depressed women
we demonstrate that women with peripartum depression do not exhibit the typical cognitive deficits of depression, i.e. attentional bias to negative stimuli.

**Peripheral inflammatory markers in peripartum women**

In Papers II and III, peripheral inflammatory markers were assessed by proximity extension assay (PEA) technology in women in the peripartum period. In Paper II, 23 inflammatory markers were found to be significantly lower in women with antenatal depression and in women with antidepressant treatment, than in healthy pregnant controls. The results found in healthy pregnant controls need to be compared with peripheral inflammatory markers of healthy postpartum women because of the known associated alterations in immune and inflammatory responses during pregnancy and in the pregnancy to postpartum transition.

In Paper III, where healthy pregnant and postpartum women were examined, the majority of the inflammatory markers investigated were found to be significantly elevated in healthy pregnant women (41 out of 50) compared with healthy postpartum women, suggesting the results in Paper II to represent a depression-induced deviation from the normal inflammatory response in pregnancy. This is further supported by the negative correlation between the inflammatory marker levels and cortisone, and the cortisone to cortisol ratio in healthy pregnant women, reported in Paper II.

To determine whether inflammatory markers have pro- or anti-inflammatory properties is not a straightforward task, as many of the markers have diverse roles in different cell-signalling pathways, tissues, and physiological conditions. The inflammatory profile is influenced by cytokine level, the target cell, the type of activation, the nature of produced cytokines, the time of action, and the different cytokines involved [279]. However, there are some typical pro-inflammatory markers such as TNF-α, IL-6 and IL-1β, and some markers such as IL-4, IL-10 and TGF-β with a more prominent anti-inflammatory profile [279].

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 altered in depressed pregnant women in Paper II [204, 216, 220]. In fact, VEGF-A, which has previously been found to be elevated in non-pregnant subjects with MDD [280], was lower in women with antenatal depression (Paper II).

Previous research on the broad range of inflammatory markers that we studied in the peripartum period is scarce. The well-studied marker TNF-α was excluded in Paper III due to low expression, and IL-1β was not included in the panel [281-283]. Even though the literature is limited, some studies
show results similar to ours (Paper III), such as higher levels of CXCL10 in pregnant women compared with postpartum women, and the opposite for CXCL9 [284]. Moreover, decreasing levels of IL-10 from pregnancy to postpartum have been reported in an *in vitro* model of IL-10 production [285]. Regarding IL-6, previous research has shown inconsistent results. In line with our findings in Paper III, IL-6 has been reported to decrease [283], but also to increase, which is at odds with our results [281], or to exhibit no significant difference [282] from pregnancy to postpartum. One study revealed a non-significant difference in IL-8 levels from pregnancy to postpartum [283], while another study revealed a U-shaped curve for IL-8, with increasing levels postpartum [281]. The described inconsistency in levels of inflammatory marker levels in the peripartum period could partly be explained by different time points of sampling.

The results in Paper II suggest that antenatal depression may be associated with an incomplete switch to the pro-M2 milieu that characterizes the second and third trimesters of pregnancy [208] (Figure 10). Although pro-inflammatory M1 markers did not differ between groups, the M1/M2 balance is suggested to shift toward M1 dominance, which, in agreement with present hypotheses on depression in non-pregnant individuals, may contribute to the development of antenatal depression in vulnerable women [204, 213, 221]. In line with this theory, studies in non-pregnant populations have shown increased levels of pro-inflammatory cytokines in depressed cases [204, 216, 218-220]. In pregnancy, women with higher self-reported depressive symptoms exhibit a more pro-inflammatory third trimester than healthy women [232].

![Figure 10](image)

*Figure 10. Antenatal depression may be associated with an incomplete switch to the pro-M2 milieu that characterizes the second and third trimester of pregnancy.*
In the group of top down-regulated inflammatory markers among depressed and antidepressant-treated cases (Paper II; TRAIL, CSF-1, CX3CL1, VEGF-A and IL-15RA), several have been associated with M2 macrophages. TRAIL, a type II transmembrane protein involved in tumour growth suppression and in regulation of both innate and adaptive immune responses [286-288], has been found to be secreted by M2 macrophages [289]. Similarly, CSF-1, also known as macrophage-CSF, acts in a regulatory manner by maintaining homeostasis between local macrophages and dendritic cells, and it further stimulates the differentiation and development of M2 macrophages [290, 291]. Fractalkine/CX3CL1 is a chemokine expressed by neurons, and its receptor CX3CR1 is present on microglia in the healthy brain [292, 293]. Fractalkine has also been shown to stimulate M2 polarization of macrophages [207]. In addition, fractalkine inhibits serotonergic neurotransmission by enhancing the GABA activity of serotonergic neurons [294], which might be of relevance in depression. VEGF-A, which most often is described in terms of its angiogenic properties, also acts in a neurotrophic manner and has neuroprotective roles in the central- and peripheral nervous system [295], which could be of relevance in depression [296]. Also, VEGF has been described to be related to both TGF-β and M2 macrophages in tumour models [297]. IL-15RA is one of three subunits of the receptor that binds the cytokine IL-15, which in turn is expressed in most cell types and plays a diversity of roles, for example within immune cell function. IL-15RA knockout mice display antidepressant-responsive depressive-like behaviour, reduced normal anxiety, and impaired memory. In addition, IL-15RA knockout mice fail to respond to IL-15 treatment compared with wild-type mice, suggesting an antidepressive effect of IL15 signalling [298, 299].

No differences in inflammatory marker levels were noted between women with antenatal depression and those on SSRI treatment. While in vitro experiments on murine cell lines suggest that SSRI treatment decreases M1 activation in microglia [300], findings on reduction in pro-inflammatory cytokine levels following SSRI treatment in humans vary according to type of medication and cytokine [301]. A study in non-pregnant MDD patients following one-year treatment with SSRIs, demonstrated elevated levels of the pro-inflammatory cytokines IFN-γ and IL-1β, and reduced levels of the anti-inflammatory cytokines IL-10 and IL-13 [302]. In addition, several pro-inflammatory cytokines interact with the effect of antidepressants, which may contribute to treatment resistance in non-pregnant MDD patients [303, 304]. 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 an altered inflammatory response may be a common physiological pathway as regards the adverse outcomes associated with both antenatal depression and antidepressant treatment, such as preterm birth and low birth weight [208, 305].
a study by Osborne et al., depressed pregnant women presented with higher levels of some pro-inflammatory markers and cortisol, and delivered their infants earlier than healthy pregnant women. The offspring of depressed mothers exhibited suboptimal neurobehavioural function and increased cortisol responses to stress. Moreover, the levels of inflammatory markers and cortisol in the depressed mothers were correlated with stress response in the offspring [57].

In Paper III, the three markers with the greatest decrease from pregnancy to postpartum were LIF-R, LAP TGF-beta-1 and CCL28. LIF-R acts as a receptor for the cytokines LIF and OSM (also significantly decreased in the postpartum period), both members of the IL-6 family [306]. LIF has anti-inflammatory features and stimulates T regulatory cells (Tregs) [307] and exerts pleiotropic actions via LIF-R on pituitary corticotropic cells, macrophages, blastocysts, embryos, and in the placenta [308]. The LAP component of LAP TGF-beta-1 is important for several functions of TGF-beta-1 such as its efficient secretion, prevention of binding to cell-surface receptors, and extracellular availability [309, 310]. Previous studies have shown both LAP TGF-beta-1 and TGF-beta-1 to have immunosuppressive properties [311, 312]. In addition, TGF-beta-1 has been found to induce Tregs and it possibly suppresses autoimmunity [313]. CCL28 (mucosa-associated epithelial chemokine (MEC)) is a chemokine that destroys microorganisms, acts in an immunomodulatory capacity, and is suggested to interact with both innate and adaptive immunity [314]. CCL28 expressed by epithelial cells is able to recruit T-lymphocytes, for example Tregs, in response to a microbial or pro-inflammatory signal [315], and therefore is suggested to have anti-inflammatory properties at these sites.

Among the markers with the greatest decrease from pregnancy to postpartum (Paper III), several have been reported to exert actions during pregnancy. LIF-R and its cytokine LIF are both in reproduction [308, 316], and LIF has been found to play an important role in implantation, embryo development, and, by way of maintaining sufficient levels, preserving early pregnancy [308, 317]. Moreover, high levels of LIF-R [318, 319] and low levels of LIF [319] have previously been reported in normal pregnancy. Also, TGF-beta-1 has been found to be involved in important processes in pregnancy, such as trophoblast invasion and proliferation, vascularization, and tolerance to the semi-allogeneic fetus [320]. CCL28 has the capacity to induce apoptosis in decidual stromal cells (DSCs), and elevated levels of CCR3 and CCR10 (receptors of CCL28), and pro-inflammatory cytokines have been observed in DSCs from spontaneous abortion [321].

The three markers showing the largest increase from pregnancy to postpartum were TRANCE, TWEAK and CCL11. TRANCE (also known as RANKL) is a member of the tumour necrosis factor (TNF) superfamily (TNFSF11) [322]. It is secreted by T-cells, and acts in a regulatory manner on osteoclastogenesis and bone remodelling [323]. Less sleep is often de-
scribed in postpartum women, and a study on mice revealed elevated levels of TRANCE in mice with sleep deprivation [324]. Further, in line with the finding in Paper III of higher levels of TRANCE postpartum, a study on mice indicated that TRANCE is involved in the function of the mammary glands and in lactation [325]. TWEAK, another member of the tumour necrosis factor (TNF) superfamily (TNFSF12), has been described in connection with chronic inflammation, angiogenesis and fibrosis [326], and is expressed by leucocytes, monocytes, dendritic cells and NK cells [327].

Notably, the two markers programmed cell death 1 ligand 1 (PD-L1) and artemin (ARTN) were excluded from further analyses in the study as a result of almost undetectable levels in the postpartum period, which may represent a drastic decrease in these two markers from pregnancy to postpartum. PD-L1 exhibits regulatory functions within immune homeostasis and promotes Tregs for maintenance of immune tolerance in pregnancy [328]. ARTN may have important roles in early embryo development and in pregnancy owing to its location within the maternal reproductive tract and in the embryo [329].

By unravelling the inflammatory changes the pregnant body undergoes, both in the context of perinatal stage and in psychiatric mood [282], a better understanding of pregnancy and postpartum complications thought to be related to immune function and inflammation, such as depression, pre-eclampsia [330] and preterm birth [212] can be obtained. There are also studies linking maternal inflammation in pregnancy to the newborn brain and behaviour relevant to psychiatric disorders [331]. In this specific study, higher levels of maternal IL-6 in blood were associated with larger newborn amygdala volume and lower impulse control at two years of age [331]. Moreover, inflammatory status in pregnancy may also be used for prediction of postpartum depression [332]. In addition, maternal inflammation in pregnancy has been described to stimulate serotonin production in the placenta, resulting in increased serotonin in the fetal forebrain, with altered serotonergic axon growth as a result [333].

Effect of antenatal depression and antidepressant treatment on placental tissue

Following correction for multiple testing, no differences in placental gene expression between women with untreated antenatal depression, women with antidepressant treatment, and healthy controls were found (Paper IV). However, given the potential risks of depression and antidepressant treatment on pregnancy outcome and the potential consequences for the child, we were motivated to report nominally significant results. These findings consisted of higher gene expression of HTR1A and NPY2R in placentas from women with untreated depression, followed by stronger immunohistochemical staining
for the protein HTR1A in placentas from women on antidepressant treatment compared with controls. We also noted nominally higher expression of the NGF gene in the treatment group compared with controls. This finding is in line with previous data from our group, where protein analyses revealed higher expression of NGF in both trophoblasts and endothelial cells in placentas from women who had used antidepressant treatment during pregnancy compared with placentas from untreated depressed women and healthy controls [245]. Overall, both results may indicate an effect of SSRIs on NGF signalling in the placenta.

5-HT and its receptor 5-HT1A (HTR1A) have previously been identified in placental tissue [334], and the expression of serotonin receptors in the placenta has been described as important for the maintenance of normal fetal brain development [94, 335]. Alterations in the expression of serotonin receptors in the placenta have been associated with maternal stress [335] and infant neurodevelopment [336]. While 5-HT1A has previously been described in the trophoblasts of human placenta [334], at present no findings on the role of this receptor in placental function have been published. It should be mentioned that the observed differences in placental HTR1A gene and protein expression, reported in Paper IV, were not accompanied by any differences in gene expression between groups as regards serotonin or norepinephrine transporters, or enzymes associated with serotonin synthesis or degradation.

5-HT receptors have most frequently been studied in the CNS, where they can appear as both heteroreceptors and autoreceptors [337-339]. When serotonin binds to the autoreceptor, further release of serotonin is inhibited, thus 5-HT1A autoreceptor stimulation leads to less serotonin in the synaptic cleft [340]. Higher levels of the 5-HT1A autoreceptor have been described in major depression [341-344]. Moreover, this inhibition of serotonin release mediated by these autoreceptors is thought to be an important element in the delayed effect of SSRI treatment [340, 345]. While presynaptic 5-HT1A autoreceptors may delay the effect of SSRI treatment, stimulation of postsynaptic 5-HT1A receptors appears to be beneficial for antidepressant action [346]. A study by Olivier et al. has demonstrated altered 5-HT1A signalling in rats exposed to fluoxetine in utero, which may partly explain the neurodevelopmental changes observed in these animals [347].

Neuropeptide Y2 receptor (NPY2R) has previously been described as having appetite-inhibiting properties [348, 349]. In addition to food intake, members of the NPY family are involved in psychomotor activity, regulation of endocrine secretion, energy homeostasis, and effects on the cardiovascular system [348]. Studies have also demonstrated increased levels of NPY in women with pre-eclampsia, suggesting its involvement in placental function [350]. NPY has been identified in the brain and placenta in rats [351]. Also, members of the NPY family have been identified in the primate brain, where NPY2R activation is believed to contribute to anxiety [352]. Our results,
however, revealed unchanged protein expression in the placenta in relation
to the studied exposures. Thus, the finding reported in Paper IV, i.e. higher
gene expression of \textit{NPY2R} in placentas from women with untreated depres-
sion, needs replication before any interpretations can be made.

Serum concentration measurements revealed detectable levels of an anti-
 depressant drug in 28 out of 41 women tested (Paper IV). Even though se-
rum concentrations validate the medical reports of ongoing use of the drug, it
should be stressed that a non-detectable SSRI in serum at delivery does not
necessarily mean non-exposure of the drug to the placenta during pregnancy.
Many women are aware of neonatal complications from SSRI use during
pregnancy [96], and some choose to discontinue medication as delivery ap-
proaches to decrease the risk of such complications [77, 353]. When anal-
yses were restricted to women with detectable levels of SSRIs, the results
remained the same (Paper IV). Moreover, there were also no differences in
placental gene expression between women with detectable and non-
detectable antidepressant serum concentrations.

Previous studies have described associations between mental illness in
pregnancy, impaired placental function, and poor pregnancy outcomes such
as preterm birth and low birth weight [26, 95, 354], but the underlying bio-
logical mechanisms and pathways are not fully unravelled, and more re-
search in this area is needed.

The nominally significant findings reported in Paper IV may be interpret-
ed as both reassuring and worrying for depressed pregnant women in need of
treatment for their condition. While the findings, at the gene level, could be
interpreted as normalization of placental gene expression by treatment of
antenatal depression, it is contradicted by the finding in HTR1A at the pro-
tein level. The genes investigated in this study were primarily selected on the
basis of previous findings associated with depression. This might have con-
tributed to the findings for HTR1A and NPY2R, where elevated gene ex-
pression was present only among women with untreated depression, whereas
antidepressant-treated women showed similar gene expression levels as
healthy controls. However, if we instead had selected the genes on the basis
of their association with SSRI treatment, we might have revealed a different
picture.

Some of the genes we studied have an impact on long-term fetal health,
such as those for HSD11B1 and 2, which control the shuttling of cortisol to
the fetus. While maternal stress is described to affect this placental enzyme
[355-357], we found no evidence that either antenatal depression or antide-
pressant treatment had such effects. However, gene expression does not
equal function, and previous studies from our group have demonstrated that
the maternal cortisone to cortisol ratio, as a functional measure of this en-
zyme, is positively associated with birth weight among the infants of women
with psychiatric morbidity [34].
This study (Paper IV) has demonstrated differential gene expression of serotonin receptor 1A and NPY2R, both of which currently are of unclear relevance as regards placental function. A previous microarray study demonstrated a multitude of genes that are differentially expressed in women on antidepressant treatment [241], and absence of clear findings in Paper IV should not be interpreted as the full picture, or that SSRI use during pregnancy is without harm.

Is the risk-benefit ratio of antidepressant use altered by the findings in this work?

Part of the aim of the current work was to elucidate the biological consequences of using antidepressant treatment during pregnancy. Overall, the findings of these studies suggest that antenatal depression per se may be associated with substantial changes in biology, and that treatment of depression has little effect, either in terms of normalization or worsening of the findings.

In Paper I, no cognitive alterations in terms of attentional biases in the depressed subjects were found. However, in the pregnant group an attentional bias to negatively valenced obstetric words was seen in the SSRI-treated women. However this finding has to be interpreted with caution owing to the relatively small sample size, and the risk of confounding by indication. Further, it is unclear whether an attentional bias for obstetric, but not other negatively valenced words, represents a risk or a benefit of SSRI-treatment.

In Paper II, both women with untreated and treated antenatal depression displayed lower levels of a number of inflammatory markers in comparison with the healthy controls. However, no differences in protein levels were noted between the untreated depressed women and the women who had received antidepressant treatment for their condition. Both groups differed compared with healthy controls, which could strengthen the theory of a common physiological pathway for the adverse outcomes associated with both these exposures. For instance, both untreated antenatal depression and antidepressant treatment have been associated with increased risks of preterm birth and low birth weight [25, 55, 98, 99]. Clearly, future studies should aim to assess if the inflammatory pattern noted in Paper II is also associated with these perinatal risks. As of now, none of the studies in this thesis had sufficient power to elucidate such associations.

In Paper IV, the results of gene expression analysis revealed higher expression of the genes HTR1A and NPY2R in placentas from untreated depressed women vs. healthy controls. However, protein expression analysis revealed higher expression of HTR1A for the SSRI-treated women compared with untreated depressed women and controls. As we have not been able to find any information in the literature on the potential roles of HTR1A and
NPY2R in terms of placental function, the overall clinical relevance of these findings remains unclear. Nevertheless, given the potential risks of depression and antidepressant treatment on pregnancy outcome and the potential consequences for the child, we were motivated to report the nominally significant results found in this study.

Overall, we found no major differences between groups as regards antidepressant treatment and untreated depression in each study. Although some small differences were noted in Paper IV, in the absence of a clear-cut role of these proteins in the placenta, these findings are hard to interpret and further research is needed.
Conclusions

I. This study demonstrated that women who suffer from antenatal and postpartum depression do not display the typical attentional bias to affectively valenced stimuli that is characteristic of depressive states in the non-pregnant population. Instead, women with postpartum depression displayed signs of emotional numbing, which may have repercussions for long-term child development and well-being.

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

III. This study revealed that levels of 41 markers decreased from late pregnancy to the postpartum period, while nine markers increased. These results clearly reflect the tremendous change in the immune system in the pregnancy to postpartum transition. Several of the top proteins that were higher in pregnancy than postpartum have anti-inflammatory and immune-modulatory properties promoting pregnancy progress.

IV. The nominally significant finding of differentially expressed HTR1A, both at the gene and the protein level that was revealed in this study suggests the involvement of HTR1A in the effect of antenatal depression on biological mechanisms in the placenta. More research is needed to elucidate the role of depression and antidepressant treatment on placental gene and protein expression, especially as regards HTR1A, and, further, the effect on the fetus.
Summary remarks and future perspectives

To summarize my work on depression and antidepressant treatment in the peripartum period, I would like to emphasize that the overall findings suggest no major differences in inflammatory markers and placental function between women who used or did not use antidepressant treatment for their depression during pregnancy. My findings are in line with a recent review by Mitchell et al., addressing the overall findings regarding the effect of antidepressant treatment and antenatal depression per se on pregnancy outcomes [358]. Also, a recent paper on the effect of antenatal depression and antidepressant treatment on expression of imprinted genes in the placenta and on offspring neurodevelopment did not reveal any medication-exposure differences in the studied biological mechanisms [359]. In addition, a study by Gustavsson et al. suggests that when controlling peripartum depressive symptoms, the effects of antidepressant medication in pregnancy on children’s neurobehavioural development are diminished, suggesting that treatment is a protective rather than a risk factor [360].

However, I want to stress that there are still inconsistencies in this field of research and more work has to be done before drawing final conclusions regarding the safety of SSRIs in treating peripartum depression [361]. Many studies, including ours, are riddled with problematic biases, such as confounding by indication, and the possibility of residual confounding cannot be stressed enough. The importance of an appropriate study design with longitudinal data sampling and proper control groups should be addressed and considered when planning new studies within this field. Thus, considering the multifactorial nature of this condition, and the many biases that may influence the results, additional research in this area is needed, especially regarding the causal mechanisms.

In Paper I the major finding was that women with postpartum depression displayed shorter reaction times to negative and positive stimuli, i.e. attentional bias away from emotionally valenced stimuli. We interpreted this finding as a sign of emotional numbing, which in turn could influence mother–infant interaction and have negative consequences for the child. These findings highlight the importance of identifying and treating women with postpartum depression as early as possible to ensure fast recovery.

Regarding research on cognitive aspects of peripartum depression, additional approaches such as study of the different types of cognitive outcomes
in relation to neurology and genetics would give a much broader understanding of how cognitive and biological factors interact in the development of depression in the peripartum period.

The findings reported in Papers II and III demonstrate the tremendous inflammatory changes the pregnant body undergoes, both during the pregnancy and in the postpartum period. The findings also demonstrate clear-cut deviations in the inflammatory marker profile of depressed pregnant women in comparison with healthy pregnant women, and finally, they demonstrate that antidepressant treatment seems unable to normalize the inflammatory changes in women with antenatal depression. One of the drawbacks of the study was the cross-sectional design, by which we were unable to ascertain the causal relationship between the inflammatory markers and the development of depression. The findings in Paper II strengthen the theory of inflammation being the mechanism behind the association between antenatal depression and its associated adverse pregnancy outcomes. Moreover, the similar inflammatory responses in women with antenatal depression and women on antidepressant treatment could strengthen the hypothesis that an altered inflammatory response may be a common physiological pathway for the adverse outcomes associated with both antenatal depression and antidepressant treatment, such as preterm birth and low birth weight. In the near future, efforts should be made to prospectively study women during pregnancy and postpartum, by longitudinally assessing characteristics such as psychiatric outcomes, together with a longitudinal sampling regimen. This approach would enable identification of causes, and potentially treatment options of pregnancy and postpartum complications associated with depression, such as pre-eclampsia, preterm birth, and low birth weight.

The few nominally significant findings reported in Paper IV may be interpreted both as reassuring and worrying for women who suffer from antenatal depression and need treatment for their condition. While the findings at the gene level suggest that treatment of antenatal depression normalizes placental gene expression, it is contradicted by the findings at the protein level. Small differences were seen between the treatment group and the untreated depressed group. However, the differences pull in different directions in regard to the different methods used. Further, the differences did not survive correction for multiple testing. In the future, studying placental expression of imprinted genes together with epigenetic regulation of these genes via methylation would further link the results to the possible effects on offspring.

At this stage, with the literature being inconclusive regarding the risks of antidepressant treatment in pregnancy, and with literature supporting the
importance of making decisions at an individual level based on a risk-benefit analysis of treating or not treating maternal depression [361, 362], decisions regarding treatment in pregnancy should be based on existing guidelines [30].
**Sammanfattning på svenska**

Depression är vanligt förekommande under graviditet och tiden efter förlossning, och har en sammanslagen prevalens på ca 12%. Den ungefärliga andelen gravida kvinnor i Europa som tar antidepressiv medicin är 2-3%, där selektiva serotoninåterupptagshämmare (SSRI) är de mest föreskrivna. I den senaste rapporten från socialstyrelsen så fick 12% av svenska kvinnor i barnafödande ålder antidepressiv medicin utskrivet år 2017. Majoriteten av dessa kvinnor var inte gravida vid behandlingsstart, dock skulle de behöva fatta beslutet att fortsätta eller avbryta behandlingen vid en eventuell graviditet.

SSRI-behandling anses i nuläget säkert för den gravida kvinnan, men konsekvenserna för fostret oroar fortfarande. SSRI kan passera moderkakan (placenta) och har mätts upp i navelstrångsblod och fostervatten. Graviditetskomplikationer, så som för tidig födsel och låg födelsevikt har rapportats hos kvinnor med SSRI-behandling under graviditet, men även hos obehandlade deprimerade, och det är för närvarande oklart om det är behandlingen eller depressionen i sig som orsakar dessa komplikationer. Fortsatta studier där man jämför effekterna av SSRI-behandling under graviditet och obehandlad depression under graviditet behövs för att kunna särskilja depressionens och behandlingens respektive påverkan på den gravida kvinnan, placentan, och fostret.

I delarbete II mättes nivåerna av 92 inflammationsmarkörer i blodet från gravida kvinnor med depression, med och utan antidepressiv behandling, samt från friska kontroller. Resultatet visade på 23 markörer som var signifikant skilda mellan grupperna. I depressionsgruppen och SSRI-gruppen var samtliga 23 lägre jämfört med kontrollgruppen. Bland de som skiljde sig mest från kontrollgruppen så återfanns markörer involverade i differentiering och stimulering av M2 makrofager, som är antiinflammatoriska makrofager som reparar skada i vävnad. I normal graviditet sker ett skifte från proinflammatoriska M1 makrofager i början av graviditeten, till en dragning mot M2 makrofager i större delen av graviditeten för att sedan skifta tillbaka mot M1 närmast förlössningen.

I delarbete III jämfördes samma inflammationsmarkörer som i delarbete II, men här i friska gravida och friska postpartumkvinnor. Femtio markörer skiljde sig signifikant mellan grupperna, och majoriteten, 41 stycken, var högre i graviditeten jämfört med postpartum. Resultaten från denna studie bekräftar de stora förändringar i immunsystemet och inflammationsstatus man ser under perinatalperioden, och flera av de proteiner som var högre i graviditeten än postpartum har antiinflammatoriska och immunreglerande egenskaper som främjar graviditeten.

I delarbete IV jämfördes effekterna av depression och SSRI-behandling under graviditet på placentans gen- och proteinuttryck genom att först analysera gener kända för att vara involverade i depression samt vara uttryckta i placenta, och sedan validera resultaten genom att undersöka proteinuttryck. Placentavävnad från friska kontroller, obehandlade deprimerade och SSRI-behandlade kvinnor analyserades med hjälp av kvantitativ realtids-PCR med TaqMan Low Density Arrays (LDA), för 44 gener. Generna Serotonin receptor 1A (HTR1A), Neuropeptide receptor Y2 (NPY2R), och Nerve growth factor (NGF) visade sig skilja i uttryck mellan grupperna. För HTR1A och NPY2R sågs ett högre genuttryck i den deprimerade gruppen jämfört med kontroller. Resultaten från proteinanalyserna visade på högre uttryck av HTR1A i placenta från SSRI-behandlade kvinnor, jämfört med både obehandlade deprimerade och friska kontroller. Dessa resultat kan tyda på HTR1As involvering i effekten av depression på olika biologiska mekanismer i placenta. Fler studier behövs dock för att utröna depressionens- och SSRI-behandlingens effekt på gen- och proteinuttryck i placenta, särskilt för HTR1A, och vidare påverkan på fostret.

Sammantaget visar denna avhandling att depression under graviditet och postpartumperioden är associerat med vissa kognitiva förändringar, lägre nivåer av flertalet mestadels antiinflammatoriska markörer i sen graviditet, samt delvis förändrat gen- och proteinuttryck i placenta. Vi fann emellertid inga stora skillnader mellan obehandlad och behandlad depression. Dock behövs mer forskning i ämnet och beslut gällande behandling av depression under denna period bör tas utifrån ett risk/nytta perspektiv, på individuell basis, och baserat på befintliga riktlinjer.
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References


195. Costa, S., Bevilacqua, D., Cassatella, M.A. and Scapini, P., Recent advances on the crosstalk between neutrophils and B or T lymphocytes. Immunology, 2018.


Supplementary material

1. The Mini-International Neuropsychiatric Interview (M.I.N.I.)

Extracts from section A. Depression and B. Dysthymia (version 5.0.0):

A. EGENTLIG DEPRESSIONSEPISOD

A1. Har du varit ihållande deprim erad eller nere under större delen av dagen, nästan varje dag under de senaste 2 veckorna?

A2. Har du under de senaste 2 veckorna tappat intresse för det mesta omkring dig eller inte kunnat ha riktigt nöje av sådant du vanligen brukar tycka om?

A3. Under de senaste 2 veckorna när du kände dig deprim erad eller ointresserad
   a. Hade du minskad eller ökad aptit nästan varje dag? Minskade eller ökade du oavsiktligt i vikt (d.v.s. med ± 5% av kroppsvikten, eller ± 3,5 kg för en person som väger 70 kg, på en månad)?
   b. Hade du problem med sömnen nästan varje natt (svårt att somna in, vaknade upp mitt i natten, vaknade aldeles för tidigt på morgonen eller sov aldeles för mycket)?
   c. Pratade eller rörde du dig långsammare än vanligt eller var du rastlös eller hade svårt att sitta stilla nästan varje dag?
   d. Kände du dig trött eller kraftlös nästan varje dag?
   e. Kände du dig värdelös eller hade skuldkänslor nästan varje dag?
   f. Hade du svårt att koncentrera dig eller fatta beslut nästan varje dag?
   g. Funderade du ofta på att göra dig illa, hade du tankar på att ta ditt liv eller önskade att du var död?

A4. a. Har du någonsin haft andra perioder på minst två veckor när du känt dig deprim erad eller ointresserad av det mesta och upplevt de flesta av de problem vi just talade om?
b. Var det något intervall på minst två månader när du inte var deprime-
rad och kunde känna intresse för saker och ting mellan den pågående
episoden och någon tidigare depression?

B. DYSTYMI

B1. Har du känt dig ledsen, nere eller deprimerad nästan jämt under de sen-
aste två åren?

B2. Förekom under denna tid något avbrott då du kände dig OK under två
eller fler månader?

B3. Under perioden när du kände dig deprimerad för det mesta:
   a. Förändrades din aptit avsevärt på något sätt?
   b. Hade du svårt att sova eller Sov du för mycket?
   c. Kände du dig trött eller kraftlös?
   d. Tappade du självförtroendet?
   e. Hade du svårt att koncentrera dig eller fatta beslut?
   f. Kände du hopplöshet?

B4. Har depressionssymptomen gett dig starkt obehag eller medfört att du
fungerat sämre socialt, på jobbet eller i något annat viktigt samman-
hang?
2. The Swedish version of Edinburgh Postnatal Depression Scale (EPDS)

Eftersom du nyligen har fått barn, skulle vi vilja veta hur du mår. Var snäll och stryk under det svar, som bäst stämmer överens med hur du känt dig de senaste 7 dagarna, inte bara hur du mår idag. Här är ett exempel, som redan är ifyllt:

Jag har känt mig lycklig:
    Ja, hela tiden
    Ja, för det mesta
    Nej, inte särskilt ofta
    Nej, inte alls

Detta betyder: “Jag har känt mig lycklig mest hela tiden under veckan som har gått”. Var snäll och fyll i de andra frågorna på samma sätt.

**Under de senaste 7 dagarna**

1. Jag har kunnat skratta och se tillvaron från den ljusa sidan:
   Lika bra som vanligt
   Nästan lika bra som vanligt
   Mycket mindre än vanligt
   Inte alls

2. Jag har glätt mig åt saker som ska hända:
   Lika mycket som vanligt
   Något mindre än vanligt
   Mycket mindre än vanligt
   Inte alls

3. Jag har lagt skulden på mig själv onödigt mycket när något har gått snett:
   Ja, för det mesta
   Ja, ibland
   Nej, inte så ofta
   Nej, inte alls

4. Jag har känt mig rädd och orolig utan egentlig anledning:
   Nej, inte alls
   Nej, knappast alls
   Ja, ibland
   Ja, mycket ofta

5. Jag har känt mig skrämd eller panikslagen utan speciell anledning:
Ja, mycket ofta
Ja, ibland
Nej, ganska sällan
Nej, inte alls

6. Det har kört ihop sig för mig och blivit för mycket:
Ja, mesta tiden har jag inte kunnat ta itu med något alls
Ja, ibland har jag inte kunnat ta itu med saker lika bra som vanligt
Nej, för det mesta har jag kunnat ta itu med saker ganska bra
Nej, jag har kunnat ta itu med saker precis som vanligt

7. Jag har känt mig så olycklig att jag har haft svårt att sova:
Ja, för det mesta
Ja, rätt ofta
Nej, sällan
Nej, aldrig

8. Jag har känt mig ledsen och nere:
Ja, för det mesta
Ja, ganska ofta
Nej, sällan
Nej, aldrig

9. Jag har känt mig så olycklig att jag har gråtit:
Ja, nästan jämt
Ja, ganska ofta
Bara någon gång
Nej, aldrig

10. Tankar på att göra mig själv illa har förekommit:
Ja, rätt så ofta
Ja, då och då
Knappast alls
Aldrig


### 3. Inflammation panel I (Papers II and III)

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*Excluded from the panel due to technical issues.*
## 4. Gene information (Paper IV)

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