Physiological Stress Reactivity in Late Pregnancy

CHARLOTTE HELLGREN
Abstract


During pregnancy, the basal activity is increased in both of our major stress response systems: the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis. At the same time, the reactivity towards stressors is reduced. These alterations sustain maternal and fetal homeostasis, and are involved in the regulation of gestational length. Although the feto-placental hormone synthesis produces the main endocrinological changes, also the central nervous system undergoes adaptation. Together, these profound adjustments have been suggested to make women’s mental health more vulnerable during pregnancy and postpartum period. The aim of this thesis was to examine factors connected to physiological stress responses during the late pregnancy in relation to pain, labour onset, emotional reactivity, and mental health.

The first study examined the pain and sympathetic response during cold stress, in relation to time to delivery. Women with fewer days to spontaneous delivery had lower sympathetic reactivity, while no pain measure was associated with time to delivery.

In the second study, acoustic startle response modulation was employed to study emotional reactivity during late gestation, and at four to six weeks postpartum. The startle response was measured by eye-blink electromyography, while the participants watched pleasant and unpleasant pictures, and positive and negative anticipation stimuli. A significant reduction in startle modulation by anticipation was found during the postpartum assessment. However, no startle modulation by pleasant, or unpleasant, pictures was detected at either time-point.

The serum level of allopregnanolone, a neurosteroid implied in pregnancy-induced hyporeactivity, was analysed in relation to self-reported symptoms of anxiety and depression. Although the participants reported low levels of depression, the women with the highest depression scores had significantly lower levels of serum allopregnanolone. There was no correlation between allopregnanolone and anxiety scores.

In the fourth study, the cortisol awakening response was compared between women with depression during pregnancy, women with depression prior to pregnancy, and women who had never suffered from depression. No group differences in cortisol awakening response during late pregnancy were found.

The results are in line with the previously described pregnancy-induced hyporesponsiveness, and add to the knowledge on maternal stress hyporeactivity, gestational length, and maternal mental health.

Keywords: acoustic startle response, allopregnanolone, antenatal, anxiety, cold pressor test, cortisol, cortisol awakening response, depression, electrodermal, estradiol, hypothalamic-pituitary-adrenal axis, postpartum, pregnancy, progesterone, skin conductance, stress, sympathetic nervous system

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“nature, who delights in muddle and mystery, so that even now (the first of November 1927) we know not why we go upstairs, or why we come down again”

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


III Hellgren C., Åkerud H., Skalkidou A., Bäckström T., Sundström-Poromaa I. Low serum allopregnanolone is associated with symptoms of depression in late pregnancy. *Submitted*.

IV Hellgren C., Åkerud H., Skalkidou A., Sundström-Poromaa I. Cortisol awakening response in late pregnancy in women with previous or ongoing depression. *Submitted*.

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### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<td>AUC</td>
<td>Area under the curve</td>
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<tr>
<td>AVP</td>
<td>Vasopressin</td>
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<tr>
<td>BDNF</td>
<td>Brain-derived neurotrophic factor</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<td>CAR</td>
<td>Cortisol awakening response</td>
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<td>CRH</td>
<td>Corticotropin releasing hormone</td>
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<tr>
<td>DSM-IV</td>
<td>Diagnostic and statistical manual of mental disorders, 4th edition</td>
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<tr>
<td>EMG</td>
<td>Electromyography</td>
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<tr>
<td>EPDS</td>
<td>Edinburgh postnatal depression scale</td>
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<tr>
<td>dB</td>
<td>Decibel</td>
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<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
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<tr>
<td>HPA axis</td>
<td>Hypothalamic-pituitary-adrenal axis</td>
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<td>hPL</td>
<td>Human placental lactogen</td>
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<tr>
<td>IAPS</td>
<td>International affective picture system</td>
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<tr>
<td>MADRS-S</td>
<td>Montgomery-Åsberg depression rating scale – self assessment</td>
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<td>MHPG</td>
<td>3-methoxy-4-phenyl glycol</td>
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<td>MINI</td>
<td>Mini international neuropsychiatric interview</td>
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<tr>
<td>pCRH</td>
<td>Placental corticotropin releasing hormone</td>
</tr>
<tr>
<td>POMC</td>
<td>Pro-opiomelanocortin</td>
</tr>
<tr>
<td>PVN</td>
<td>Paraventricular nucleus of the hypothalamus</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
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<tr>
<td>SPSS</td>
<td>Statistical package for the social sciences</td>
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<tr>
<td>SSRI</td>
<td>Selective serotonin reuptake inhibitor</td>
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<tr>
<td>STAI-S, -T</td>
<td>Spielberger state trait anxiety inventory (-state, -trait)</td>
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<tr>
<td>VAS</td>
<td>Visual analogue scale</td>
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Introduction

Important endocrinological alterations in normal pregnancy and puerperium

During pregnancy, the placenta produces increasing amounts of steroid hormones, peptides, and other factors which reset the maternal homeostasis [1]. The endocrine adaptations for childbirth and nursing result in the suspension of ovulation and the development and growth of the uterus, the placenta, and the foetus. In order to achieve this, readjustments of maternal immunology, stress physiology, and metabolism are required. The changes involve hormones and neurotransmitters which are known to have roles in mental health and disease. Several of the hormonal alterations have been suggested to alter women’s mood, cognition, and prevalence of psychiatric disorders during pregnancy and the postpartum period [2-4]. However, it is important to bear in mind that the majority of childbearing women maintain a good mental health at this turbulent time in their lives; the complex pattern of neuroendocrine change must consequently be rich in elements which protect against mood disturbances.

It is primarily the placenta and the developing fetal endocrine system which govern the endocrinology of pregnancy. The feto-placental unit releases increasing amounts of hormones into the maternal bloodstream as it grows - a supply that is physically removed at delivery. A number of the maternal endocrine changes therefore follow a common trail. Oestrogens, progesterone, human placental lactogen (hPL), testosterone, corticotrophin releasing hormone (CRH), and cortisol all rise continuously through the 40 weeks of pregnancy, followed by a drastic drop at parturition. [1, 5-9].

All maternal organ systems have to adapt; this leads to well known pregnancy side-effects like fatigue and sensitivity to infections, and less known effects, like enlarged adrenal glands [10, 11]. Sometimes the adaptations cause unwanted complications, such as hypertension [12]. The hormonal changes of pregnancy and childbirth also have effects on our major stress systems; the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis.
Physiological and neuroendocrinological changes in pregnancy

The feto-placental hormone production regulates several pregnancy specific changes. Human placental lactogen and cortisol act to increase glucose and fatty acids in the circulation to meet the fetal energy demand [1]. Placental production also increases circulating levels of leptin, an appetite and energy expenditure regulating hormone with possible involvement in mood disorders [13]. Another important metabolic alteration is slightly decreased levels of free thyroid hormones [14, 15]. Thyroid hormones enable tissue energy consumption and clinical hypothyroidism is well known to cause depressive symptoms [15]. The net effect of the metabolic changes is a gradual increase of around 30% of the pre-pregnancy maternal basal metabolic rate [14, 16].

The high oestrogen level stimulates the renin-angiotensin system to increase maternal blood volume by around 40% [17]. As sympathetic nervous system tone increases and parasympathetic tone decreases, maternal heart rate and blood pressure also change. Some women develop a pathologically high sympathetic tone and pregnancy-induced hypertension [12].

Regulation of the maternal immunology is another important function of the steroid hormone surges [17]. As the foetus is perceived as non-self, the maternal immune system must be tranquil enough to allow the foetus to thrive, but it is still crucial to maintain a good defence against pathogens. Cortisol is routinely called immunosuppressive, but cortisol, rather, changes the balance between the two main components of the immune system. Thus, in parallel with the hypercortisolism of pregnancy, there is an up-regulation of the generalised inflammatory response, (i.e. monocytes and granulocytes) while there is a down-regulation of the antigen-specific component (i.e. T-cells) [11]. The placental tissue produces a plethora of cytokines which have been shown to interact with placental hormone synthesis. Cytokine signaling has also been implied in uterine contractions and rupture of membranes [18].

There are few studies on neurotransmitter adaptations inside the human central nervous system during pregnancy. However, analyses of cerebrospinal fluid (CSF) from women undergoing elective caesarean section show significantly lower levels of γ-amino-butyric acid (GABA), as well as the norepinephrine metabolite 3-methoxy-4-phenyl glycol (MHPG), than CSF from non-pregnant women [21]. Indirect evidence of central nervous changes also include decreased maternal serum levels of brain-derived neural growth factor (BDNF), both before and after delivery, and low BDNF is correlated with decreased serotonin levels in serum at both time-points [22]. A reduced availability of serum tryptophan, a serotonin precursor, is also evident during pregnancy [23]. Low GABA, serotonin, and BDNF are all considered bio-
markers for depression [21, 22], while most studies report CSF MHPG concentrations to be equal in control and depressed populations [24]. The indirect human evidence is supported by studies in rodents, where pregnancy has been found to decrease serotonin, GABA, and norepinephrine content in brain tissue [25-27].

Physiological and neuroendocrinological changes in the postpartum period

The serum concentrations of feto-placental hormones decline fast after parturition, and instead of abnormally high steroid hormones, postpartal oestrogen, progesterone, and cortisol levels are below the normal non-pregnant/non-postpartal range [6, 8, 9]. Low oestradiol and progesterone concentrations persist when breast-feeding is continuous and prolactin is high [1]. The low HPA reactivity of pregnancy is still reduced during the hypocortisolism of lactation [28]. Ovulatory cycles with regular oestrogen and progesterone levels, and normal HPA axis reactivity, resume within a few weeks to months of non-lactation [29] [28]. Serum prolactin returns to normal within a few months after breast-feeding cessation [28]. Other hormones, like leptin, return to normal within weeks after delivery and do not depend on lactation status [6, 30].

To prepare for lactation is an important goal for the endocrine changes during pregnancy. Prolactin starts increasing during early pregnancy [31], induced by oestrogens [1]. CSF and plasma levels of prolactin in pregnant women at term are around ten times higher than in non-pregnant women [21]. When progesterone is withdrawn at parturition, the build-up of prolactin can induce milk production [31]. Furthermore, plasma prolactin crosses the blood brain barrier [32] and is responsible for decreasing postpartum stress responses, not only in the HPA axis activity, but also in the oxytocin “anti-stress” system [33, 34]. Prolactin is also immunomodulatory [35]. The immunological state during the postpartum period is characterized by an initial inflammatory response to delivery, and an exaggerated response has been postulated to increase the risk for postpartum depression [36].

The posterior pituitary peptide oxytocin is responsible for the ejection of milk in response to suckling. Oxytocin levels are higher in plasma, but not in CSF in pregnant compared to non-pregnant women [21]. Average plasma oxytocin does not increase from pregnancy levels in the postpartum period [37], although it transiently rises at nursing [38]. Oxytocin signalling within the brain is important for well-being and social attachment and has therefore been researched in relation to puerperal mood [39]. Since oxytocin is a negative regulator of the HPA axis and plasma levels rise with nursing or merely holding an infant, oxytocin is believed to support maternal calm during the postpartum and lactation period [39]. However, the associations between
oxytocin, nursing, bonding, and emotional calm are not obvious, as oxytocin release within the brain seems to be independent of suckling [40] and peripheral oxytocin does not seem to readily cross the blood-brain barrier [21, 41].

Several studies have described the normal postpartum as a depression-like state with reference to neuroendocrinological parameters. Serum indicators of serotonergic activity in healthy women a few days after delivery are low [42, 43], although the serotonergic indices seem to be restored to late pregnancy values at six weeks postpartum [43]. A few brain imaging studies have also been made in different stages of the postpartum period. For instance, magnetic resonance spectroscopy examination has indicated decreased cortical GABA concentrations in healthy women several months into the postpartum period [44], while a positron emission tomography study has revealed elevated monoamine oxidase A at two weeks postpartum, i.e. increased breakdown of serotonin, norepinephrine, and dopamine [45].

The stress response system and its regulation during pregnancy and puerperium

Both the HPA axis and the sympathetic nervous system are organized via signals from the hypothalamus, particularly the paraventricular nucleus (PVN) [46]. The PVN receives input from several other parts of the brain and the coordinated sympathetic (and parasympathetic) output from the PVN travels as a nerve signal to the target organs, including the adrenal medulla [47]. The sympathetic nervous system thus responds within seconds when increased attention or action is required, and reverts rapidly as the stimulus disappears [47]. The response includes cardiovascular changes, as well as increased sweat gland activity [1]. The stimulation of the adrenal medulla releases epinephrine into the circulation. Epinephrine increases heart rate, relaxes smooth muscles in the respiratory tract, and improves energy availability [1].

The HPA axis reacts relatively slowly to stressful input. An increase in CRH and vasopressin (AVP) from the paraventricular nucleus of the hypothalamus causes a rise in adrenocorticotropic hormone (ACTH) secretion from the anterior pituitary, which in turn activates the adrenal cortices to release cortisol [1]. The peak in plasma and free salivary cortisol can be observed about 25 minutes after a laboratory stressor and baseline cortisol is not reached until about an hour after the stressful stimulus is ended [48]. Cortisol directs the metabolism towards increased acute energy usage and is immunomodulatory [1].

ACTH is not secreted in its bioactive form, but is cleaved from a larger polypeptide called pro-opiomelanocortin (POMC) [1]. Another POMC
cleavage product is β-endorphin, an analgesic opioid hormone, i.e. a balancing element integrated in the HPA axis response [1].

The maternal HPA axis

Optimal fertility is dependent on a balanced hypothalamic-pituitary adrenal axis. Increased levels of glucocorticoids have a direct inhibitory effect on reproductive tissues and will, at the same time, inhibit hormone production at all levels of the hypothalamic-pituitary-gonadal axis [49]. This means that long-term stress, or highly stressful life events, can impair fertility through amenorrhea or reduced sperm count [50].

Feto-placental production causes the baseline levels of HPA axis hormones to become elevated during pregnancy [8, 12, 51] so that in the third trimester, cortisol levels are around three times non-pregnant levels [8]. In contrast, the reactivity to acute stressful stimuli is dampened in late pregnancy [51-53].

The long-term continuous increase of steroid hormones requires an uncoupling of the regular negative feedback systems. This is achieved by the feto-placental unit, which produces large amounts of oestrogens, progesterone, and CRH - among numerous other substances - to ultimately cause its own expulsion [54]. Instead of the negative feedback cortisol has on hypothalamic CRH production, cortisol has a positive feedback on placental CRH production, which in turn increases pituitary ACTH release [10, 55] (Figure 1). The maternal adrenals are enlarged during pregnancy, reflecting the increased production of cortisol [10]. Importantly, a substantial amount of cortisol is also produced by the fetal adrenal during the very last weeks of gestation, probably as a signal for the maturation of fetal organs, as well as for the timing of parturition [56]. The feto-placental unit also synthesises the other HPA axis hormones ACTH and β-endorphin [57].

The altered HPA feedback is further evidenced by the fact that pharmacological cortisol suppression by dexamethasone is reduced during late pregnancy and during the first weeks postpartum [58, 59].

Exogenous oestrogen decreases the sympathetic response to mental stress in healthy men [60] and perimenopausal women, and also decreases the HPA axis responsiveness in perimenopausal women [61]. Thus, also the increase in oestrogen may play a role in pregnancy-induced hyporesponsiveness.

While the baseline cortisol levels return to normal within a couple of days after parturition [62], the HPA axis hyporesponsiveness lingers in breastfeeding women [63]. It has been suggested that the lowered stress reactivity postpartum is important for maternal wellbeing [64] but an increased risk for immediate postpartum mood symptoms has also been found in relation to a higher degree of HPA axis dampening [65]. However, as in non-pregnant populations, the associations between HPA-measures and psychological variables during pregnancy have been contradictory. For example, higher,
lower, as well as equal serum CRH in depressed, as compared to non-depressed pregnant women have been reported [66-69].

Rats also display an attenuated HPA axis response to stress during pregnancy and lactation, including decreased negative feedback by glucocorticoids [70]. The progesterone metabolite allopregnanolone has been suggested to contribute to the decreased HPA axis reactivity through increased opioid inhibition in the brainstem, resulting in reduced excitatory noradrenergic signals to the PVN [71]. Via GABAergic inhibition, allopregnanolone may also suppress PVN CRH neurons [71]. After parturition, the pregnancy-induced opioid inhibition ends, but HPA responsiveness is maintained during lactation, perhaps due to central prolactin and oxytocin responses to suckling [72].

The maternal autonomic nervous system

In parallel with HPA axis changes, the hormonal changes of pregnancy and childbirth also affect our other major stress system: the sympathetic nervous system (SNS). In the third trimester, the basal muscle sympathetic nerve activity is almost doubled [12], while the skin conductance reactivity to a mental stressor, as well as to relaxation, is dampened [51, 73]. Diastolic blood pressure responses to physiological and psychological stressors are also reduced in the second trimester compared to before pregnancy [53]. In contrast, heart rate reactivity to a psychosocial stressor seems to be comparable in third trimester pregnant and non-pregnant women [74]. Salivary alpha-amylase, an indicator of autonomic nervous system activation, is another example of a stress response that is attenuated during pregnancy; the salivary alpha-amylase response to psychosocial stress is lower in second trimester pregnant women than in non-pregnant women, and even lower in the third trimester [75]. Correspondingly, the parasympathetic activation is decreased during rest [51]. A study describing cardiovascular and epinephrine and norepinephrine responses to a cold pressor test in late pregnancy did not find a correlation between the physiological responses and the subjective pain measurements [76, 77]. However, whether the altered pain experience in late pregnancy is influenced by the dampened sympathetic reactivity is not clear.

Basal plasma epinephrine and norepinephrine levels do not appear to be generally altered during normal pregnancy, but there are several reports of reduced reactivity to physiological stressors [78]. In the postpartum period, lactation seems to reduce basal plasma levels of norepinephrine, but not impede sympathomedullary reactivity, as breastfeeding women have the same norepinephrine response to exercise as mothers of bottle-fed infants [28].
Figure 1. Schematic illustration of the endocrine and nervous pathways which mediate physiological responses to stimuli requiring increased vigilance (‘stress stimuli’). Based on illustrations in [3, 79]. a) Stress response mechanisms in non-pregnant humans. b) Stress response regulation under the influence of placental CRH production, which is not submitted to the negative feedback which usually controls circulating cortisol levels. Placental CRH is instead positively regulated by cortisol. Roman numerals indicate the foci of the studies included in this thesis.
**Spontaneous labour onset**

Although a human pregnancy is ideally 40 ± 2 weeks long, counting from the first day of the last menstrual period, there is a large variability [80]. Five to ten percent of all singleton births are pre-term, i.e. before 37 weeks of gestation, but only around half of the pre-term births are preceded by spontaneous labour [81, 82]. Post-term pregnancy, when spontaneous labour has failed to initiate two weeks after the estimated date of delivery, precedes around 9% of all births in Sweden [83].

Several factors which contribute to labour onset have been identified. Uterine stretch, local prostaglandin release, fetal HPA axis activation, and oxytocin signalling are known to be involved, but the key which ultimately initiates labour at term is still unknown [57]. One theory is the “placental clock” hypothesis [84], which states that a sudden increase in bio-available placental corticotropic hormone (pCRH) in late pregnancy determines gestational length by augmenting the prostaglandin and oxytocin signals which mediate uterine contractions. Maternal levels of pCRH have some predictive value regarding the risk of prematurity but when it comes to the more exact timing in term pregnancy pCRH is not of any practical use [85]. In non-primate mammals, a sudden drop in progesterone precedes parturition, while in humans and other primates the high progesterone levels are produced by the placenta and remain until after the delivery. The initiation of human labour has therefore been hypothesized to depend on functional progesterone withdrawal, through altered binding or expression of progesterone receptors [86]. The actions of progesterone may also be antagonized by the late pregnancy surge in fetal cortisol production [87]. Oestrogens also seem to be important; lower oestradiol and, especially, higher oestriol/oestradiol ratio predicts successful response to post-term labour induction [88].

Different types of prostaglandins are involved both in inhibiting and mediating uterine contractions and are also needed for cervical ripening [57]. Prostaglandin synthesis is in turn induced by oxytocin production within the uterus, which increases before parturition [57]. The contraction-induced stretch on the lower uterine segment during labour stimulates oxytocin release, which enhances the contractions and additional oxytocin release in a positive feedback loop.

Involvement of the sympathetic nervous system in the regulation of labour is supported by the fact that lumbar sympathetic blockade reduces labour duration [89]. There is also a marked reduction in adrenergic innervation of the uterus during pregnancy, a process which may have a role in the regulation of uterine contractions [90]. Whether the attenuation of sympathetic reactivity during pregnancy is involved in regulation of labour onset at term is not known.

Given the critical evolutionary significance, it is likely that labour initiation does not depend on one single factor, but that redundancy has evolved.
This is supported by the finding that oxytocin signalling is not requisite for successful parturition in mice [91, 92].

The reason behind spontaneous pre-term labour onset is often unknown. Especially in gestational week 33 and earlier, spontaneous labour is often associated with intrauterine infection [93]. The presence of bacteria induces maternal cytokines and fetal cortisol production and inhibits prostaglandin breakdown so that a premature prostaglandin peak occurs [93]. Other aetiologies include placental abruption, and abnormal uterine tension from poly-hydroamnion (excess amniotic fluid), or multiple foetuses [93].

Primiparity and genetic predisposition increases the risk for post-term pregnancy, but the aetiology is often unknown [88]. The most common reason for post-term pregnancy has been suggested to be faulty pregnancy dating, and a high maternal body mass index (BMI) has also been identified as a risk factor [88, 94]. However, a high BMI is also a risk factor for incorrect pregnancy dating. In conclusion, much is known about the molecular mechanisms of labour regulation, but the levels of understanding are insufficient for predicting labour onset.

Pain sensitivity during pregnancy and in the postpartum period

Pain tolerance and/or pain thresholds are augmented in women during late pregnancy [76, 95, 96]. Experimental studies on human pregnancy-induced analgesia are few and differ largely in methodology; they also differ in which pain modality is studied (pressure, thermal etc.) and whether it is the pain tolerance, endurance or perceived pain intensity that is of interest. In general, there seems to be a lack of longitudinal studies and studies with large sample sizes. The regulation of opioid signalling in the spinal cord during human pregnancy is naturally difficult to assess. The established theory of pregnancy-induced analgesia is consequently based on rodent and pig models [97, 98]. Sows display pregnancy-induced analgesia a couple of days before parturition. The effect persists immediately postpartum and can then be blocked by an opioid antagonist [98]. The increase in pain tolerance in rats is also considered to depend mainly on alterations in opioid signalling; it has been shown that the dynorphin content increases selectively in the lumbar spinal cord in rats in late pregnancy, and that methionine-enkephalin levels rise in the entire spinal cord [99]. Interestingly, some aspects of lower epidural and spinal analgesia requirements seem to be established in pregnant women, although this phenomenon has been explained by mechanical reduction in the spinal and epidural spaces [100]. The late pregnancy rise in plasma β-endorphin has been suggested to contribute to relieve the stress and pain associated with the process of labour [101, 102]. The human placenta
produces small amounts of the opioid receptor agonists β-endorphin and dynorphin [57], but the increased systemic levels are due to HPA axis activity. Baseline pregnancy levels of β-endorphin have previously been reported not to influence pain tolerance or pain intensity in response to a cold pressor test [77].

Mood, cognition and mental health during pregnancy

The finding that physiological stress reactivity is progressively blunted during pregnancy is robust across experimental designs. On the other hand, the psychological reactivity to stress during the course of pregnancy remains elusive. The relatively few longitudinal subjects on the matter do not reveal any clinically significant changes in positive or negative affect [103], depression [51], or anxiety [104] in healthy pregnant women. However, it has been argued that the psychological reactivity to highly stressful events, such as an earthquake, is lower during late pregnancy [105]. Less psychological distress in response to an experimental stressor in the third, than in the second trimester, has also been reported [106].

The popular concept of ‘pregnancy brain’ or ‘placenta brain’, i.e. that pregnant women become increasingly forgetful, unfocused, and emotionally labile, has little support in scientific literature [107]. Several smaller studies have reported deterioration in some aspects of memory function during pregnancy [4]. One of the rare longitudinal studies on cognitive function indicates only a statistically non-significant trend towards deterioration in one out of four tests of memory and cognition during pregnancy [107]. However, there are some reports that pregnant women perceive their memory or other type of mental performance to be deteriorated during pregnancy, while they perform as well as non-pregnant controls [51, 108, 109]. An increase in subjective feelings of confusion and fatigue during pregnancy has been reported [51], but longitudinal studies about emotional processing are lacking. Since subjective ratings of emotionality during pregnancy and postpartum may be biased by cultural beliefs about the effects of pregnancy, it is valuable to also use objective measures.

Rat mothers display increased aggression and a decrease in anxiety-like behaviours during both pregnancy and lactation [110]. It is also suggested that learning and memory are improved by pregnancy, lactation, and mere pup care [111]. Enhanced spatial memory function even appears to be maintained a year after lactation [112].

Antenatal and postpartum depression and anxiety

Although there are studies pointing to a high risk for onset or exacerbation of mood disorders in the puerperal period [113], the prevalence of depression
and anxiety may not be drastically higher at this time than during pregnancy or in the general female population [114-117]. Around 6-12% of all women are estimated to suffer from depression during pregnancy [113, 118] and the same figures are often used when estimating the frequency of postpartum depression [116, 119, 120]. However, by definition a postpartum depression starts after the delivery and few studies have pre-pregnancy or antenatal longitudinal data. One study suggests that 45% of women who are screen-positive for depression at six weeks after delivery, were also screen-positive prior to their pregnancy [121]. According to the strict definition, postpartum depression has its onset within four weeks after delivery [122], but the term is often used for depressive episodes which are ongoing at some point during the year after delivery. It is also debated whether antenatal depression and postpartum depression differ in symptomatology from major depressive disorder in any other aspect than timing [123].

Perinatal anxiety disorders are less studied than perinatal depression, despite similar prevalence and high comorbidity. A meta-analysis indicated possible increases in new onset of panic disorder and generalised anxiety disorder during the postpartum period, compared to during pregnancy or in the general population, but largely stable courses of pre-existing anxiety disorders [124].

Regardless of the absolute prevalence and symptoms of depression and anxiety disorders during pregnancy and postpartum period, they present unique implications for pharmacological treatment and bonding with the infant, thus affecting the patient differently than depressive episodes during other times in life.

Previous depression is a major risk factor for postpartum depression [116], and perhaps an even more important risk factor for antenatal depression [123]. Socioeconomic factors such as lack of partner support, financial difficulties, or other life events are also very strong predictors for perinatal depression and anxiety [116, 125]. Still, during late pregnancy and the postpartum period, the biological systems known to be part of the depressive symptomatology are under a great strain [42-44, 65]; and a vulnerability to depression could theoretically be more evident during this period.

Stress response regulation in depression and anxiety

The heterogeneous aetiology, symptomatology, and treatment response patterns in patients with depression or anxiety are likely to prevent neuroscience from finding a single mechanism, or a single valid biomarker, for either depression or anxiety. However, alteration in HPA axis function is a frequent finding in major depression [126-128]. The general understanding is that major depression patients have a hyperactive HPA axis, and consequently a
higher cortisol production. A 2012 meta-analysis estimates the clinical significance to be modest, based on low overall effect sizes [129].

In generalised anxiety disorder and panic disorder, the cortisol output is thought to be exhausted by the chronic stress, and consequently be maintained at a low level [130], but possibly with higher response to awakening [131].

Like the other HPA axis products, β-endorphin has also been investigated in relation to mood, although the results are inconclusive. In 2010, Yim and colleagues described a higher β-endorphin increase profile in women who were healthy during pregnancy, but experienced depression in the postpartum period [132].

The autonomic nervous system is also affected by mental illness. The sympathetic nerve activity is higher in major depression [133], and heart rate variability is lower [134]. There are several conflicting reports of autonomic function alterations in anxiety disorder patients, including increased and decreased basal levels and reactivity, and decreased habituation [135].

It is widely recognised that vulnerability to depression is partly heritable, and genetic variations in monoamine, brain-derived neurotrophic factor, and HPA related genes, which increase susceptibility, have been identified [136-139]. There is also evidence that some biological markers of depression, including neurosteroid profile and cortisol reactivity, seem to linger in remitted patients [128, 140, 141]. Altered HPA axis reactivity may also precede the onset of a first depressive episode or a relapse [127]. These observations underline the importance of investigating the potential consequences of previous depression on endocrinological function during pregnancy.

The skin conductance response

In study I, skin conductance response was used as a measure of sympathetic reactivity, during a cold pressor test (see below). The first studies coupling changes in skin conduction to experimental stimuli were performed in 1888 by the French neurologist Charles Féré [142]. Since then, the technique has been used in a wide range of psychological and physiological experiments as well as in “lie detector” tests [51, 143, 144]. The skin conductance level depends on the activity of the eccrine sweat glands, which reflects the output of the sympathetic nervous system. The skin conductance response is not influenced by surrounding normal indoor temperature range or by local blood flow [142]. Eccrine sweat glands are distributed over most parts of the human skin but are most abundant on palmar and plantar skin, where skin conductance is predominantly measured. Studies on, for example, attention, emotion, and pain have therefore employed the skin conductance response as a simple and non-invasive measure of sympathetic activity [142, 145].
During rest, skin conductance as a function of time is typically a negative slope with one to three non-specific peaks per minute. Within a few seconds of the perception of a sensory stimulus that calls for attention, a signal from the prefrontal cortex, via sympathetic innervation, causes the sweat glands to fill up. This will transiently render the skin more conductive, which is referred to as the skin conductance response. A more intense or more interesting stimulus will be followed by a larger response – larger peaks, more frequent peaks and slower recovery [142]. The classical use of skin conductance in psychological experiments is to look at the latency, amplitude, and rise-time of individual peaks following transient exposure to discrete stimuli, such as pictures or words. Alternatively, skin conductance level or peak frequency can be used during continuous stimuli exposure, such as a stressful task [142]. Peak frequency has been used as primary outcome in studies of clinical pain and is related to subjective pain ratings [145-147]. Other ways to quantify skin conductance activity are skin conductance area under the curve, or rate of change. The rate of skin conductance change denotes the slope of a given time portion of the skin conductance curve, while the area under the curve is a function of the peak frequency and amplitude over time [148].

The cold pressor test

The cold pressor test is frequently used as an experimental stressor and/or as a pain threshold assessment [76, 149-151]. The test typically implies that the subject is asked to submerge a hand or foot into cold water or crushed ice until the cold-induced pain becomes intolerable, while the physiological response of interest is concurrently measured. Alternatively, ice can be applied to the subjects’ forehead [152]. Several researchers have applied the cold pressor test in pregnant and postpartum women. The findings indicate that pregnancy increases the pain tolerance [76] and attenuates the cortisol response to a cold pressor test [151].

The acoustic startle response and its modulation

In study II, we attempted to assess emotional regulation through affective modulation of the acoustic startle response. The acoustic startle response is a defensive reflex response to a sudden aversive stimulus and is widely used in psychopharmacological animal research [153]. The human startle response can be experimentally assessed by measuring the contraction of musculus orbicularis oculi, the eye blink, in response to auditory or tactile stimuli. In rodents, the startle reflex is measured as whole body twitch. The startle reflex is a simple brainstem reflex but it is modulated by impulses from several
other parts of the brain, such as the amygdalae. The amygdalae are nuclei within the limbic system which are known to be activated by emotional stimuli, especially fear and emotional faces [153, 154]. The baseline startle response is significantly increased in post-traumatic stress disorder [155, 156], generalised anxiety disorder [157], specific phobias [158, 159], and obsessive compulsive disorder [160, 161].

Anxiety, fear, and negative affect (threat of shock, aversive images) potentiate the startle magnitude, through activation of the amygdalae. Conversely, startle can be attenuated by positive affect (reward, pleasant images) [157, 162]. In addition, the startle reflex is potentiated during the anticipation of highly emotional images compared to neutral images [163, 164]. Several anxiety disorders are associated with an altered affect-modulated startle response [155, 157, 165]. Depressed patients respond with lower modulation by affective images, as well as by anticipation stimuli [166, 167]. Interestingly, short term SSRI treatment also results in reduced affective startle modulation in healthy subjects [168]. Furthermore, tryptophan depletion has been reported to decrease the startle magnitude [169]. There is also a bi-phasic effect of cortisol on baseline startle in humans, but cortisol does not appear to alter the emotional modulation [170].

Some of the neurotransmitter systems which are believed to affect the startle response, such as GABAergic, opioid, and noradrenergic signalling [171], may be altered during pregnancy and the postpartum [71]. However, baseline startle response has previously been shown to be comparable in women in the third trimester of pregnancy and postpartum women [172]; but no study examining affective modulation of the startle response in pregnant or postpartum women has been published before.

Allopregnanolone – in stress, mood, and pregnancy

Study III focuses on correlates of pregnancy levels of allopregnanolone, (3α-hydroxy-5α-pregnan-20-one). Allopregnanolone is a neuroactive steroid, i.e. a steroid with central nervous system activity which is also synthesised within the brain [173]. Allopregnanolone exerts its function via allosteric binding to GABA A-receptors which enhances the GABAergic signalling [174]. As GABA is the major inhibitory transmitter in the central nervous system, allopregnanolone has sedative, anxiolytic, and anti-convulsant properties [173].

A functionally relevant amount of allopregnanolone is synthesised in the brain [175], but the main source of brain and serum allopregnanolone in non-pregnant women is the corpus luteum and adrenal cortex [176, 177]. The allopregnanolone synthesis in the adrenal cortex is stimulated in parallel with cortisol secretion, and thereby provides a negative regulation, or balance, to the HPA axis stress response.
Patients with major depression have been found to have low levels of allopregnanolone [178-180]. On the contrary, currently remitted women with major depression or bipolar disorder have higher serum allopregnanolone concentrations, regardless of treatment and co-morbidity [181]. Another study showed no difference in allopregnanolone between post-menopausal women with or without menopause-associated mood alterations [182].

The functional role of allopregnanolone in mood disorder pathophysiology is corroborated by the finding that successful treatment with selective serotonin reuptake inhibitors (SSRI) increases plasma and cerebrospinal fluid allopregnanolone [179, 180], although there are conflicting results [178].

There are also reports of low serum allopregnanolone in mild mood disturbance, such as the postpartum blues, which is a transient episode of depressed mood during the first week after childbirth [183]. In postmenopausal women, allopregnanolone has been shown to be related to negative mood in an inverted u-shaped curve, where levels of 1.5-2 nmol/l are associated with the highest magnitude of negative mood symptoms while concentrations below and above are associated with lower levels of negative mood [184].

During pregnancy, maternal serum concentrations of allopregnanolone rise to more than ten times the maximum menstrual cycle levels [185, 186], due to feto-placental synthesis. The fatigue experienced by many women during the first trimester has been proposed to be an effect of allopregnanolone [187]. An allopregnanolone serum concentration equivalent to that in the third trimester is sedative if intravenously administered to non-pregnant women [188], suggesting that tolerance develops during pregnancy [187].

A major issue when studying neuroactive substances in humans is accessing central nervous system levels of the hormone of interest. Allopregnanolone crosses the blood-brain barrier, and although cerebrospinal fluid concentrations are around ten times lower, they are in strong correlation with serum levels of the steroid [189]. It can therefore be assumed that the variations in serum allopregnanolone concentration during pregnancy are reflected in the brain. More importantly, allopregnanolone serum levels were correlated with brain tissue levels in a human post mortem study [190].

In rats, exogenous allopregnanolone suppresses stress responses through up-regulation of opioid signalling in the brainstem, mimicking the effects of pregnancy [71]. Rodent studies further suggest that chronic stress, and the consequent HPA axis overload, can cause a depletion of serum and brain levels of allopregnanolone. Without the balancing effect of an adequate allopregnanolone response, the HPA axis function is thought to deteriorate further. In a series of experiments, Evans and colleagues have confirmed that exogenous allopregnanolone can protect rats against development of the behavioural deficits induced by the social isolation model of depression. Moreover, the continuous allopregnanolone supplementation also prevented the decline in hippocampal cell proliferation seen after social isolation [191].
Only one small study has investigated pregnancy allopregnanolone levels in clinically depressed pregnant women, and found no difference compared to controls [192]. However, the relationship between serum allopregnanolone and depressive symptoms outside the clinical range has not been studied.

The cortisol awakening response

In study IV, the cortisol awakening response was used as a test of stress reactivity. The physiological secretion of cortisol is relatively high during morning hours, decreases during the day, and begins to slowly rise again a couple of hours after midnight [193]. Although ACTH and cortisol start to rise in the hours before waking, a marked increase in free cortisol (50 – 160%) is seen between the time of awakening and 30 – 45 minutes thereafter [194]. This increase, the cortisol awakening response was first described in 1997 [195]. Since the ACTH response to awakening is not as rapid, the cortisol response is thought to depend on a sympathetically mediated increase in the adrenal splanchnic cortex sensitivity to ACTH [196]. The function of the awakening response is not established, although it has been suggested that it has a role in the transition from sleep inertia to alertness [196]. Despite the uncertain mechanism behind the cortisol awakening response, it has spurred a lot of research interest due to reports of its associations with mental health, as well as cardiovascular health, bone density, and perceived stress [194]. Interestingly, the cortisol awakening response has been reported to be increased not only in currently depressed, but also in formerly depressed individuals, rendering it a potential vulnerability marker for, or scar after, depression [128]. The cortisol awakening response is also slightly higher in women than in men [197].

Although the absolute saliva cortisol concentrations are increased in late pregnancy, a distinct cortisol awakening response is still present in gestational weeks 32-36 [198]. However, in analogy with the reduced stress reactivity to other stimuli [51, 151], Buss and colleagues have found a decrease in cortisol awakening response between the 17th and 31st week of pregnancy and also found an association between this dampening and the timing of delivery [199]. The cortisol awakening response has been reported to not be affected by antenatal symptoms of depression, but these studies did not take previous depressive episodes into account [200, 201].
Aims

I: to assess pain threshold and sympathetic nervous system response to cold pain in relation to onset of labour in healthy pregnant women

II: to compare startle response modulation, during anticipation and during viewing of affective images, across late pregnancy and puerperium of healthy women

III: to determine if the serum level of the neurosteroid allopregnanolone is correlated to self-reported symptoms of depression and anxiety in late pregnancy

IV: to compare the cortisol awakening response in late pregnancy in women with ongoing depression with currently healthy women, with and without a history of depression
Materials and Methods

Paper I-III

Ninety-nine pregnant women were recruited via advertisement and primary care midwives, and visited the laboratory at the Department of Women’s and Children's Health in gestational week 36-40. The inclusion criteria were age >18 years, and an uncomplicated singleton pregnancy. The sequence of procedures was: informed consent, venous blood sampling, interview about obstetric history and medication, psychiatric interview, self-report questionnaires, startle modulation test, and cold pressor test. For study II, 31 women returned four to six weeks after delivery for a second visit, which included blood sampling, questionnaires, and a second startle modulation assessment.

Psychiatric evaluation by self-report questionnaires and structured interview

There are several tools available to screen for psychiatric symptoms or obtain psychiatric diagnoses for research purposes. Two screening instruments to assess depressive symptoms which are commonly used in clinical practice in Sweden are the Montgomery-Åsberg Depression Rating Scale – Self-rated version (MADRS-S) and the Edinburgh Postnatal Depression Scale (EPDS). MADRS-S concerns the past three days and consists of nine items (e.g. appetite, pessimism, zest for life) rated from 0 to 6 [202]. MADRS-S is primarily designed to detect change and is therefore often used in clinical trials of antidepressants [203, 204]. No validated MADRS-S cut-off score is published for the detection of minor or major depression, but a score of 13 (90th percentile) or more was considered as elevated depressive symptoms in study III.

The EPDS was developed for use in the postpartum period, and therefore avoids questions on somatic symptoms which can be confounded with normal postpartum symptoms [205]. The EPDS contains ten items which can be rated on a scale from 0 to 3, based on the past seven days. The Swedish version of the EPDS has been validated during pregnancy [206] and during two to three months after delivery [207] with the suggested cut-off scores of ≥13 and ≥12 respectively to detect mild depression with positive predictive values of 54% and 59%.
A questionnaire commonly used to evaluate anxiety is the Spielberger State-Trait Anxiety Inventory (STAI) [208]. The state and trait versions, STAI-S and STAI-T, assess current anxiety level and anxiety prone personality respectively. For both the STAI-S and -T, a cut-off score of >40 gives around 80% sensitivity and specificity for detecting anxiety disorders during pregnancy [114].

Most self-report questionnaires do not cover all criteria required for a psychiatric diagnosis, for example differential diagnoses and duration of symptoms. Therefore, a structured interview has higher specificity for detecting depression or anxiety disorders. The Mini International Neuropsychiatric Interview (MINI) is a structured interview based on DSM-IV criteria [209].

**Paper I**

Ninety-three women were included in the analysis of skin conductance activity during a cold pressor test in gestational week 36-40. The skin conductance measurement from one woman was lost due to a technical error. Further, exclusion from analyses was warranted by current treatment with SSRI (n=2), or ongoing major depressive disorder or primary anxiety disorders detected by MINI (n=3). Furthermore, no included women reported use of analgesics within 24 hours of the test session. The cold pressor test consisted of one minute of holding the dominant hand in 35 ± 1 °C water (baseline), followed by maximum one minute in 0 ± 1 °C water (cold pressor).

Skin conductance was assessed via electrodes on the non-dominant hand with a Med-Storm skin conductance algesimeter (Med-Storm Innovation, Oslo, Norway). The skin conductance measurements were: area under the curve (AUC), peaks per second, and rate of change. The measurements were extracted for the first 30 seconds of baseline and cold pressor respectively (Figure 2). Pain threshold, cold endurance, and pain ratings were also measured. Information on the number of days to delivery, and whether the onset was spontaneous, was obtained from medical records.

In addition to the data published in paper I, plasma levels of β-endorphin were also analysed. This was done using a radioimmunoassay using the EUIRA-beta-endorphin kit (Euro-Diagnostica, Sweden) as described in [101].

Cold pressor test outcomes, including skin conductance measurements, were compared between women with less than two weeks and women with 14 days or more left to spontaneous delivery using Mann-Whitney U-tests. Linear relationships between days left to delivery and the skin conductance reactivity measures were also explored using linear regressions. SPSS Statistics for Windows, version 17 (SPSS Inc, Chicago), was used for statistical analyses in paper I and II.
Figure 2. Med-Storm skin conductance monitoring system screenshot from one cold pressor test (CPT), with skin conductance (SC, μSiemens) on the y-axis, and time (s) on the x-axis. Curly brackets indicate periods of warm and cold water, and the 30 s time windows from which data were extracted for analysis. The slope of the dashed line illustrates the extracted SC rate of change during the CPT. The area under the curve was calculated in respect to the ground (not shown). The peaks and troughs are indicated by red and blue dots respectively.

Paper II

The acoustic startle response modulation, by affective pictures and by anticipation, was electromyographically assessed in 31 healthy women during gestational weeks 36-39, and again at four to six weeks postpartum. Inclusion in study II required valid startle data from the startle test performed during pregnancy, and no current SSRI use or ongoing MINI diagnosis.

During the test, the women were seated in front of a computer screen showing a 15 minute slideshow consisting of five conditions: completely black slides (control condition), green slides (positive anticipation), red slides (negative anticipation), and pleasant and unpleasant affective image slides. Pleasant images were always immediately preceded by a green slide to signal positive anticipation, whereas red slides preceded the negative pictures. The pictures were obtained from the international affective picture system (IAPS) [210] and different picture series were used at the two visits to avoid habituation. A total of 48 acoustic startle probes (105 dB sound pulses)
were delivered binaurally by headphones. Twenty probes were programmed to occur during the control condition, and seven probes during positive anticipation stimuli, negative anticipation stimuli, pleasant image stimuli, and unpleasant image stimuli respectively. The acoustic probes and the electromyographic recording of the eye blink startle responses were controlled by a commercial startle system (SR-HLAB, San Diego Instruments, San Diego, CA, USA). All women provided affective ratings of the pictures. Valence was rated on 10-cm visual analogue scales ranging from positive (0.0 cm) to negative (10.0 cm). Arousal ratings ranged between calm (0.0 cm) and aroused (10.0 cm). Serum levels of oestradiol and progesterone were obtained at both sessions.

Z-scores of the respective startle magnitudes were compared between sessions, within subjects in a repeated measures ANOVA. Differences in picture ratings, depression scores, and anxiety scores between pregnancy and postpartum states were compared with Mann–Whitney U-tests.

Paper III

Ninety-six women were included in Study III, two women were excluded due to current SSRI medication, and one did not provide a blood sample. Serum allopregnanolone was purified with celite chromatography and analysed by radioimmunoassay at the Umeå Neurosteroid Research Center. Depression scores and anxiety levels were self-reported with the MADRS-S, and the STAI-S and STAI-T respectively. The cut-off for presence of depressive symptoms was set at a MADRS-S score of 13 or more, while the anxiety cut-off was 40 points for STAI-S and STAI-T.

Mann-Whitney U-tests were used for comparisons between groups, and linear regression was applied to enable control for potential confounders of the association between allopregnanolone self-rated depression scores. SPSS Statistics for Windows, version 20 (IBM Corp. Armonk, NY), was used for statistical analyses in papers III and IV.

Paper IV

Women were recruited via e-mail, and participated in a set of neuropsychological tests at the Department of Women's and Children's Health in gestational week 36-40, as a sub-study to the pregnancy cohort ‘Biology, Affect, Stress, Imaging, and Cognition in pregnancy and the puerperium’ (BASIC). All pregnant women in Uppsala County were invited to participate in BASIC at the time of their routine ultrasound screening in gestational week 16-18. Following informed consent, women were followed by web-based questionnaires at gestational week 17, gestational week 32, six weeks
postpartum, and six months postpartum. For the current study, 216 BASIC participants were recruited and attended a test session.

At the visit, the women received a cortisol awakening response sampling kit with instructions and a report sheet. Twin pregnancy, use of systemic steroid medication, and reported delay between awakening and the first sample over 5 minutes warranted exclusion from the analysis. In all, the final analysis included salivary cortisol from 134 women, from time of morning awakening and 15, 30, and 45 minutes post-awakening. Ongoing depressive episodes were screened with the MINI-interview [209]. Previous depressive episodes were assessed by the question “Have you ever experienced a period of two weeks or more when you have felt depressed or uninterested in most things?” (analogous to MINI question A4a). If the woman answered ‘yes’, the interviewer asked her to consider the time-period when she felt depressed or uninterested (or ‘the worst one’ if she had experienced several such time-periods) and asked the questions in the depression module of the MINI interview. EPDS scores were obtained at the visit and from the web questionnaires in gestational weeks 17 and 32.

The cortisol awakening response was compared between three groups: women who experienced depression during the current pregnancy, currently depression-free women with a previous experience of major or minor depression prior to the current pregnancy, and women who had never suffered from a depressive episode (controls). In the depressed group, we included women with a current depressive disorder (major or minor depression or dysthymia), or a history of major or minor depressive episode, where EPDS scores ≥ 13 at any time-point during pregnancy indicated that the prior (or last) episode had been ongoing during the present pregnancy. Women with a prior depressive episode had a history of at least one major or minor depressive episode according to the MINI interview, but not during the present pregnancy, as indicated by EPDS scores < 13 at all time-points (gestational week 17, 32 and at the visit). Control subjects were defined as having no current or prior depressive disorder, no current anxiety disorder (panic disorder, generalised anxiety disorder, or social phobia) as assessed by MINI.

Salivary cortisol concentrations were analysed by electrochemiluminescence immunoassay at the Department of Clinical Chemistry at Uppsala University Hospital. A linear mixed model with time-point as repeated measure, and group and time-point as fixed factors was applied to assess group differences in magnitude and shape of the cortisol curves.
Summary of results

Paper I

The mean number of skin conductance fluctuations (peaks) per second, the rate of change, and the area under the curve all increased significantly during the cold pressor test compared to the baseline measurement (all p-values < 0.001, Wilcoxon signed-rank tests), indicating that the cold pressor activated the sympathetic nervous system. Furthermore, the level of sympathetic activation depended on perceived pain; positive correlations were found between self-reported pain intensity (VAS-score) and skin conductance area under curve (r = 0.241, p = 0.026), and with rate of skin conductance change (r = 0.354, p = 0.001). VAS-score was also weakly correlated to the number of skin conductance fluctuations per second (r = 0.209, p = 0.055). In addition, the skin conductance rate of change was significantly higher in women with cold endurance under 60 seconds.

Skin conductance activity during the cold pressor test decreased significantly with fewer days left to spontaneous parturition in simple linear regressions (area under the curve: r = -0.313, p = 0.007 and rate of change: r = -0.319, p = 0.006). The number of days left to delivery was normally distributed, but since no skin conductance variables followed normal distribution the regressions’ residual distributions were checked for normality, and found to meet this criterion (Shapiro-Wilk W > 0.95).

In line with the negative correlation, women with fewer than 14 days left to parturition (median) had significantly lower skin conductance response than women with two to four weeks left. The difference in skin conductance area under curve (AUC) approached significance (Table 1).
Table 1. Results from the cold pressor test

<table>
<thead>
<tr>
<th></th>
<th>All women, spontaneous/induced labour (n = 93)</th>
<th>Two weeks or more from spontaneous parturition (n = 40)</th>
<th>Less than two weeks from spontaneous parturition (n = 39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAS-score (mm)</td>
<td>55.7 ± 20.3</td>
<td>59.6 ± 18.6</td>
<td>52.6 ± 19.4</td>
</tr>
<tr>
<td>SC AUC (µSs)a</td>
<td>205.1 [0 – 1375.4]</td>
<td>250.6 [0.49 – 1375.4]</td>
<td>133.5 [0.00 – 744.2]</td>
</tr>
<tr>
<td>Rate of SC change (µS/s)a</td>
<td>0.24 [-0.19 – 2.33]</td>
<td>0.35 [-0.10 – 2.33]</td>
<td>0.15 [-0.16 – 1.34]</td>
</tr>
<tr>
<td>SC peaks per secondb</td>
<td>0.20 [0.00 – 0.43]</td>
<td>0.20 [0.03 – 0.43]</td>
<td>0.20 [0.00 – 0.37]</td>
</tr>
</tbody>
</table>

Pain ratings are presented as mean ± SD; skin conductance (SC) variables are presented as median [range] because of non-normal distribution.

a Since the skin conductance measurements are retrieved from 30 second time windows, only 74 women who endured at least 30 seconds of the CPT are included in this analysis.

b p = 0.053 compared to subjects with more than two weeks left to parturition

** p = 0.009 compared to subjects with more than two weeks left to parturition

Meanwhile, no subjective pain measure (cold-induced pain perception threshold, cold endurance, VAS-score) or baseline skin conductance activity measure were associated with time left to delivery.

One woman did not provide a blood sample, which is why the plasma concentration of β-endorphin was obtained from 92 women. Plasma β-endorphin concentrations did not follow normal distribution and cold pressor test outcomes were therefore compared between β-endorphin quartiles using Kruskal-Wallis tests. Post-hoc Mann-Whitney tests indicated that the women with the lowest β-endorphin levels had significantly higher rates of skin conductance change compared to the other women (Figure 3), but area under curve, peaks per second, cold endurance, and pain ratings did not differ significantly.

![Figure 3. Rate of skin conductance (SC) change in 84 women across β-endorphin plasma concentration quartiles. Boxes indicate interquartile range with medians, error bars indicate total range. *p < 0.05, **p < 0.01 compared to the lowest quartile.](image)
No startle modulation by affective images was observed in either late pregnancy or postpartum (Figure 4), but the startle response during positive anticipation stimuli was lower in comparison with negative anticipation (F(1,30) = 29.96; p < 0.001) (Figure 5). A significant reduction in startle modulation range, i.e. the difference between negative anticipation and positive anticipation stimuli, was found at the postpartum assessment. Meanwhile, neither self-reported mood and anxiety symptoms nor baseline startle response was altered. There was a correlation between the startle response anticipation modulation range postpartum and the postpartal serum progesterone (r = 0.37, p = 0.038), but there was no significant correlation with postpartal oestradiol levels, or with either steroid hormone level during pregnancy.

Figure 4. Mean startle magnitude z-scores. No significant difference was seen between startle responses during pleasant and unpleasant pictures in either pregnant or postpartum state. Error bars indicate standard error of the mean.

Figure 5. The difference between startle magnitude during positive compared to negative anticipation (green vs. red screens) was significantly more pronounced during pregnancy (p<0.001) than during the postpartum (ns). This was reflected in a significant state by stimulus interaction (F(1,30) = 4.86; p = 0.035).
The women rated the unpleasant images slightly more negative and the pleasant images slightly more positive at the postpartum visit. They also indicated increased arousal for unpleasant, but not pleasant, images at their postpartum visit (Table 2).

Table 2. VAS-ratings of valence and arousal of the affective pictures (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Late pregnancy</th>
<th>Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valence, pleasant images</td>
<td>1.9 ± 0.9</td>
<td>1.6 ± 0.7 *</td>
</tr>
<tr>
<td>Valence, unpleasant images</td>
<td>7.8 ± 1.1</td>
<td>8.1 ± 1.0 *</td>
</tr>
<tr>
<td>Arousal, pleasant images</td>
<td>2.9 ± 1.8</td>
<td>2.6 ± 1.8</td>
</tr>
<tr>
<td>Arousal, unpleasant images</td>
<td>5.7 ± 1.9</td>
<td>6.4 ± 1.6 **</td>
</tr>
</tbody>
</table>

* p < 0.05; ** p < 0.01, compared with late pregnancy
Paper III

Out of the 96 participating women, ten had elevated depression scores (MADRS-S ≥ 13), and this group had significantly lower allopregnanolone serum concentration compared to women with MADRS-S scores in the lower range (39.0 ± 17.9 nmol/l vs. 54.6 ± 18.7 nmol/l), p = 0.014.

A significant negative correlation was found between self-rated depression scores and serum allopregnanolone concentration (Figure 6). The linear association between self-rated depression scores and allopregnanolone serum concentrations remained significant when adjusted for gestational length, progesterone levels, and parity. Self-rated anxiety was not associated with allopregnanolone concentrations during pregnancy and neither were postpartum depression scores.

Furthermore, allopregnanolone was in significant negative correlation with number of days to delivery in the 80 women who had spontaneous labour onset (Pearson correlation coefficient = -0.259; p = 0.020). The corresponding association was not seen in progesterone levels (Pearson correlation coefficient = -0.073; p = 0.520), although serum allopregnanolone was in significant correlation with serum progesterone (Pearson correlation coefficient = 0.530; p < 0.001).

Figure 6. Linear regression of self-reported depression score (MADRS-S) as a function of allopregnanolone serum concentrations, r = -0.220, p = 0.031.
Paper IV

The final group sizes were: current depression, n = 38; previous depression, n = 39; never depressed, n = 57. No group differences were seen in the size of the cortisol awakening response (effect of group F (2,139) = 0.68; p = 0.507), or in the shape of the response over the time-points (group by time-point interaction (F (6,354) = 0.48; p = 0.826) (Figure 7). On average, the women with depression during the current pregnancy were slightly younger than, and with a lower education level than, the two other groups. However, the lack of group differences in cortisol awakening response remained when the model was adjusted for age, educational level, and SSRI treatment.

Neither the individual cortisol values, nor the AUC values, were associated with number of days between sampling and day of delivery (all p > 0.5).

Figure 7. Cortisol awakening response in women with no depression, previous depression, and antenatal depression according to interview. Data is presented as natural logarithm of mean and SEM. There were no significant group differences in cortisol values, or interaction with time-point.
Discussion

Methodological considerations

Participant selection bias
Participants in study I-III were recruited via advertisement and thus actively volunteered to participate. This imposes a self-selection bias which resulted in a sample with lower parity and higher education in comparison with the general population of pregnant women. Although the BASIC study, which was the recruitment basis for study IV, has a population-based recruitment strategy, the participants in BASIC are also more likely to be primiparous, and to have a university education (Sundström-Poromaa, unpublished observations). Moreover, few of the participating women are born outside of Sweden. Although this has implications for the generalisability of the results, a high level of homogeneity is also a prerequisite for explorative studies with relatively small sample sizes. Both labour onset and stress system regulation during pregnancy are known to be affected by socioeconomic status, migration, and minority status [93, 211]. To account for these factors, substantially larger study populations are required. However, strategies to recruit study populations which truly represent the target population are essential for expanding the knowledge on stress system regulation during pregnancy.

Cross-sectional design
In studies I, III, and IV, we used cross-sectional designs, which only allow for comparisons between individuals. Since we have studied factors which change rapidly during pregnancy and puerperium, longitudinal designs would have increased the possibility to draw firm conclusions from the results. However, also in study II, where we had longitudinal data, the interpretation would have been facilitated if we had also performed tests pre-pregnancy and/or after the return of regular ovulation. This would require a lot of time and large starting sample sizes, as attrition due to pregnancy complications and participant drop-out could be substantial. Repeated measurements also introduce habituation.
Subjective and objective assessments of stress, pain, and affect

A recent review found a general low correspondence between measures of psychological experience and objective measurements of stress (cortisol and cardiovascular reactivity) [212]. Regarding cortisol, one reason for lack of correlation may be that the baseline ultradian pulsatility of cortisol secretion renders single cortisol analyses rather uninformative [213].

Compared to the determination of steroid hormones in plasma and saliva, the autonomous nervous system parameters, such as skin conductance, have the advantages of immediacy and high temporal resolution. The psychophysiological relevance of the skin conductance response is further indicated by the observation that both higher VAS-scores and lower cold endurance were associated with increased skin conductance reactivity in study I. Skin conductance activity have previously been shown to be associated with ratings of postoperative pain [145], and can thus be thought of as an objective measure of pain. However, any salient stimuli or emotion, positive or negative, will induce sympathetic activation. Thus, the method is better suited for the study of experimental pain.

As pain stimulus, we employed the cold pressor test. It is a relatively mild laboratory stressor which has been proven to be well tolerated by pregnant women [76]. However, we do not know how the reactivity to cold stress relates to the reactivity towards other types of stressors, such as mental arithmetic, public speech, or physical exercise. It is possible that the responses to different types of tests have different relevance for maternal well-being. However, also study designs involving repeated testing with a variety of stressors during pregnancy could be considered unethical.

Among psychophysiological reactivity measures, the startle response is unique in the sense that it can be reduced by appetitive stimuli, and increased by fear and anxiety. It has therefore been seen as an objective measure of anxiety, fear, and/or amygdala activation [157]. Fear- and anxiety-potentiation of startle is used in pharmacological animal models of anxiety, but the translation to human state and trait anxiety has been questioned by prominent startle researchers [214]. However, the lack of significant startle modulation by pleasant and unpleasant images during pregnancy and the postpartum period is surprising in relation to existing literature [162] and our previous experience. By use of the same experimental setup, we have previously observed a significant startle modulation also during pleasant compared to unpleasant images in healthy women during the follicular and luteal phase of the menstrual cycle [215]. However, also in that study, the effect of the anticipation stimuli was stronger than the effect of the affective pictures. A contributing factor could be that the images were shown for a shorter time than the anticipation stimuli, as the paradigm used in the study is primarily designed to assess anticipation. Another important issue is the quality of the affective pictures. Although they are validated to induce emotion and
arousal, this validation is made in a population of American college students, and for the most part many years ago [210]. Several demographic and contextual variables therefore differ from our study participants. The low arousal, compared the normative scores, indicated in the ratings from our participants is likely to have contributed to the weak effect of picture valence.

The picture ratings were the only subjective ratings of emotional reactivity that we obtained from the women. The mean valence ratings only changed marginally, but the slightly increased mean arousal towards unpleasant pictures during the postpartum visit are in line with a previous observation of lower affective response during late pregnancy [105].

Sympathetic stress response and labour onset

We found an association between sympathetic stress response and days to spontaneous delivery, within the last month of pregnancy in a relatively large sample of 74 individuals. Several earlier studies have shown reduced stress responses in women in late pregnancy, and in the postpartum period, compared to early pregnancy or non-pregnant controls. The studies vary considerably in study design, some are longitudinal during pregnancy [51, 106] or across pregnancy and puerperium [151, 216] and some compare separate groups of women in different trimesters and/or puerperium with non-pregnant controls [28, 75]. No previous study has, to our knowledge, shown a decrease in stress reactivity during human pregnancy within such a narrow time span. However, when it comes to pain, one study by Cogan and Spinnato [96] did repeated testing at the end of pregnancy and found a rise in pressure pain thresholds in six women during the last 16 days before parturition. Unlike Cogan and Spinnato [96], we did not find an effect of time to delivery in the subjective pain measures (pain threshold, cold endurance, and VAS-score). This discrepancy is most likely due to the cross-sectional design of our study, where the inter-individual variability in pain experience is not accounted for. It is also possible that sensation of cold pain is differently regulated in pregnancy than pressure pain. Since we had a ceiling effect, in that the majority of women endured the maximum 60 seconds of the cold pressor test, one possibility is also that an effect on cold endurance would be visible if the upper time limit was extended.

We were unable to detect any relation between baseline skin conductance measures and time to parturition. Hence, it is unlikely that our findings are secondary to an increasing sympathetic tone. While our cross-sectional study presents inter-individual differences in sympathetic nervous system reactivity, a longitudinal study design could render more insight into the patterns of pregnancy-induced stress adaptation over the course of pregnancy and puerperium. Our interpretation of the results is that the sympathetic stress re-
response is continuously decreasing during the last weeks before giving birth, while the baseline sympathetic level remains stable. Since no similar association was seen in absolute cortisol, or awakening response, in relation to time to delivery (study IV), skin conductance or sympathetic response may offer a more sensitive parameter for the investigation of stress response regulation before labour. If the relationship is present also in post-term pregnancies, skin conductance assessment would have a potential to predict the woman’s chances for having a spontaneous onset of labour, or successful response to labour induction.

**β-endorphin, pain, and sympathetic response**

High β-endorphin levels have been suggested to increase pain tolerance during pregnancy [102]. A study in our lab has also indicated an increase in analgesic requirement during labour in women with low circulating levels of β-endorphin in gestational week 37 [101]. Nevertheless, pregnancy-induced analgesia is thought to depend on signalling within the central nervous system, and plasma levels of β-endorphin cannot be seen as indicative of central opioid analgesic activity [217]. The higher sympathetic activation in women with relatively low β-endorphin that we observed during the cold pressor test could theoretically be a sign of peripheral opioid analgesia [218]. However, our results are very preliminary and need to be replicated. Only one out of three skin conductance variables was significantly elevated in the women with the lowest β-endorphin, and, in line with Saisto et al. [77], the endorphin level was not correlated with any subjective measure of pain.

**Startle modulation across pregnancy and postpartum**

We found a reduced startle modulation by image anticipation at four to six weeks postpartum, compared to during late pregnancy, in a within-subjects design. The lack of a habituation effect on modulation in the same paradigm in healthy non-pregnant women contradicts the possibility that repeated testing accounted for the attenuation during anticipation [215]. Also, the presence of an effect of emotional images in non-pregnant women [215] indicates that the emotional startle modulation in the women in the present study was reduced, in late pregnancy as well as during the postpartum period. Reduced startle modulation during anticipation, as well as by affective images, has been observed in depressed subjects [164, 167], and may therefore be viewed as indicative of emotional dysfunction or a pro-depressive state, in line with other “depression-like” alterations in CNS signalling indices found in pregnancy [21-23] and the postpartum period [42-44]. However, the subjects in this study did not suffer from depression, and neither do the majority of
postpartum women. It is therefore more likely that reduced startle modulation in the peripartum represents an adaptive change in emotional reactivity at a time of rapid neuroendocrine changes.

As mentioned in the introduction, the qualitative and quantitative differences in any type of psychophysiological reactivity between late pregnancy - with its high baseline activity, and the postpartum period - with low baseline activity, are sparsely studied, although both states are thought to be characterised by hyporeactivity. The lower extent of startle modulation by negative anticipation which was detected postpartum may thus be attributed to the factors suggested to cause postpartum hyporeactivity, i.e. central nervous system oxytocin and prolactin [33, 34]. Since we did not obtain any direct indices of oxytocin, prolactin, or HPA signalling, we can only speculate on this matter. As mentioned in the introduction, the amygdalae are proposed to be the key structure for affective modulation of startle. Breastfeeding women have reduced right amygdala activation in response to arousing pictures at three to six months postpartum, compared to nulliparous controls. However, the amygdala activation is not affected by nasal oxytocin administration in either group, suggesting that acute oxytocin concentration is not mediating this difference [219]. Instead, the relative progesterone deficiency of the postpartum may play a part, as progesterone administration has been reported to increase amygdala activation to emotional stimuli in cycling women [220]. This could be argued to be supported by the fact that we saw an association between postpartal serum progesterone and the decreased startle modulation to image anticipation in the postpartum period. However, since ovulation is suppressed during early postpartum and lactation, the variation in serum progesterone (and its neuroactive metabolites) is dependent on HPA axis activity [221]. It is therefore possible that the reduced modulation is secondary to the decreased HPA activity.

Studies comparing baseline startle in postpartal rats with diestrous rats have yielded contradictory results. One study argues lactation-induced startle attenuation [222], whereas another found an increase in startle [223]. Rats subjected to rapid progesterone withdrawal display an enhanced baseline startle response [224]. In contrast, no difference in baseline startle response was detected between pregnant and postpartum states in this study or between pregnant women and women examined on postpartal days one to seven in a previous study from our lab [172]. This highlights the importance of caution when translating animal data to human conditions, especially in the field of reproduction.
Allopregnanolone and mood during late pregnancy

We observed that women who had higher depression scores displayed significantly lower allopregnanolone serum concentrations. This was also mirrored in a negative association between late pregnancy serum allopregnanolone and self-rated depression scores.

In disagreement with our results, one previous study examining clinically depressed and euthymic pregnant women found no difference in allopregnanolone levels [192]. One explanation for the discrepant findings could be that the group sizes of Pearson-Murphy and colleagues were too small to detect the relatively modest effect of allopregnanolone suggested by our results. In addition, Pearson-Murphy and colleagues studied a population of women with first onset of clinical depression during pregnancy; our subjects represent a relatively healthy pregnant population with varying degrees of depressed mood, in most cases outside the range of clinical disease. However, the allopregnanolone precursor 5α-dihydroprogesterone (5α-DHP) was elevated in the depressed women in the Pearson-Murphy study, indicating a related neurosteroid imbalance. While the conflicting results underline the lack of direct clinical value of allopregnanolone during pregnancy, they also provide evidence for the importance of a general neurosteroid balance. Pregnancy and postpartum period entail large neuroendocrine changes, and often increased stress. It is possible that the elevation of neuroactive progesterone metabolites, with their inhibitory potential, is one of the factors which ensure that most women maintain good mental health throughout these challenges [183, 192], but is of less relevance for the development of clinical depression during pregnancy [192].

Serum concentration is not an ideal measure when studying the effect of allopregnanolone on mood, which is most likely determined by local changes in neurosteroid synthesis and GABA<sub>A</sub> receptor subunit composition within the brain [225]. However, the association we observed suggests that the massive increase in peripheral feto-placental allopregnanolone synthesis has the potential to affect the central nervous system, and to some extent protect women from negative mood symptoms. Allopregnanolone readily crosses the blood-brain barrier, therefore the serum levels of the steroid are correlated with brain tissue levels [190]. In addition, allopregnanolone is accumulated in certain brain areas in rats [226].

In non-pregnant women, allopregnanolone release is coupled to HPA-axis activity. Allopregnanolone decreases the HPA-response in pregnant rodents [71] and in addition to the direct GABA-enhancing effect, allopregnanolone administration affects the transcription of genes involved in the HPA-axis regulation in rats [227]. Thus, there is a possibility that the association seen in our study is mediated by HPA-axis regulation effects on mood [66].

Based on rodent studies [228, 229], we had expected that high serum allopregnanolone would also be a protective factor against symptoms of anxiety.
We found no such effect, perhaps because of our relatively non-anxious study population. It must also be mentioned that we asked about state anxiety (referring to the momentary anxiety at the visit), and trait anxiety (referring to anxiety prone personality). We did not obtain self-rated symptoms of anxiety in the last days, which would be more analogous to the MADRS-S depression ratings.

We found a significant association between higher serum allopregnanolone concentration and fewer days left to spontaneous delivery. Since allopregnanolone is a metabolite of progesterone, this correlation could be assumed to be secondary to the continuous placental progesterone production. However, we did not observe a correlation between progesterone levels and days to parturition. Allopregnanolone and closely related compounds have previously been implied to be involved in the timing of delivery, but the mechanism likely involves the relative proportions of several steroids [186].

One limitation to our study is the rather healthy study population; a MADRS-S score of 13 or more does not indicate a pathological level of depressed mood. Thus, future studies with larger numbers of women suffering from depressed mood are needed to prove the validity of the association with serum allopregnanolone.

Cortisol awakening response in currently and previously depressed pregnant women

In line with Shea et al. (2007), we found no difference between the controls and the women with depression during pregnancy. Furthermore, our results gave no support for the hypothesis that biological vulnerability to, or scar after, a depressive episode is visible in the cortisol awakening response of pregnant women with a history of depression, as has been shown in non-pregnant individuals [128].

A meta-analysis of differences in salivary cortisol between depressed and healthy study participants suggests that patients with depression have slightly higher absolute morning cortisol values than healthy controls, although this difference does not seem to be clinically significant [129]. The largest study on cortisol awakening response in non-pregnant individuals [128] found around 2.5 nmol/l higher absolute cortisol peak levels in subjects with previous, or ongoing, depression. Since the current study was smaller, we may not have had power to detect a difference in that size range. Thus, we cannot rule out that a small difference in cortisol awakening response may exist between women with ongoing depression and controls. Given the very high inter-individual variation, even in healthy subjects, it is unlikely that a group difference of 2-3 nmol/l is clinically relevant.
Extensive assessments of HPA axis function, such as dexamethasone suppression or ACTH-provocation are not feasible during pregnancy due to probable effects on the foetus [230]. The cortisol awakening response offers an opportunity to study cortisol reactivity without subjecting the participants to experimental stressors. However, to disentangle the effects of depression and pregnancy on individual patterns of HPA axis function, it may be necessary to consider genetic variations in HPA axis related genes [231], as well as subtypes of depression [3, 232]. It is also possible that an HPA axis vulnerability is significant and visible in the cortisol awakening response only in patients with severe depression during pregnancy. Still, one study has found significantly higher evening (12 h after awakening) cortisol at the end of the second trimester in 13 women with major depression, compared to 33 non-depressed controls [67]; this observation underlines the link between HPA axis function and depression during pregnancy.

It has been suggested that morning cortisol values are less informative, as they represent the highest levels of the day, and that their inter-individual variation, especially during pregnancy, may be limited by a “ceiling effect” [233]. However, as exemplified in this study, the inter-individual variation in cortisol awakening response is relatively large.

The strengths of this study include a careful group designation based on a structured interview concerning current depressive symptoms, as well as previous episodes, and repeated self-report questionnaires during pregnancy. The participating women were also in a narrow gestational age range, during the last weeks of term pregnancies, which should have minimised the variation caused by the increasing dampening of cortisol reactivity with gestational age. We also excluded all samples where the wake-up sample was collected more than five minutes after the reported wake up-time. However, the lack of further control over participants’ coherence to the sampling protocol must be considered a limitation.

We conclude that there seems to be no essential difference in cortisol awakening response in gestational weeks 36-40, between women with or without previous, or ongoing, depressive episodes.
Clinical implications and future perspectives

The studies in this thesis have a predominantly basic research approach and primarily concern healthy, pregnant women. The changes in psychophysiological and endocrine reactivity during pregnancy are indeed inherently interesting as they represent unique examples of the plasticity of human homeostasis. Moreover, increased knowledge of normal variation is crucial to provide a background for deviations with connections to mental health and vulnerability, as touched upon in the included studies. An interesting point is that not only exaggerated reactivity, but also very insufficient reactivity, can be indicative of dysregulation, and thus both ends of the spectrum should be analysed in relation to the middle [234].

As repeatedly stated above, there is a lack of longitudinal studies. Obviously, to have the best theoretical and statistical power for studying any changes during pregnancy and postpartum, it is necessary to obtain pre-pregnancy data, although this may be time-consuming. Also, additional studies following the same individuals over several time-points during and after pregnancy, and after the return to cycling status, are needed.

Maternal stress systems in pregnancy complications

Serious pregnancy complications with possible links to dysregulation of HPA and sympathetic activity, such as pregnancy-induced hypertension, gestational diabetes, and pre-term labour, affect many women [12, 85, 235]. Further study of reactivity measures in these patient groups may offer opportunities for prediction of these complications, or relevant outcome measures for preventive interventions.

Maternal stress systems and fetal development

The main research activity in the field of maternal stress systems is aimed at consequences for the intrauterine milieu. The blunting of stress responses during pregnancy is suggested to protect the foetus from excess cortisol and vascular constriction compromising uterine blood flow. The fetal production of cortisol is known to be essential for fetal maturation [17], but excess glucocorticoids can have adverse effects on the development of the fetal HPA-axis [236, 237], and consequently offspring health through adolescence and adulthood. The mediator of the relationship between antenatal depression, and anxiety, and later child development has therefore been suggested to be excess cortisol exposure. However, the association between maternal distress and maternal cortisol values during pregnancy is not evident; as exemplified in study IV, and by previous studies [238, 239]. In this area of study, too, intra-individual variation may give more insight [200]. Importantly, prenatal
anxiety has been linked to decreased placental capacity to protect the foetus from circulating glucocorticoids [240].

Reflections on cross species translation

Although stress and pain regulation show common features in human and rodent pregnancy, rodents and humans differ greatly in, for example, gestational length, number of offspring, placental hormone synthesis, and timing of progesterone withdrawal [57, 87], factors which potentially affect labour initiation. Rodent studies emphasise an increase in aggressive behaviour, and a decrease in anxious behaviour in both pregnant and lactating females [110], findings which are not clearly demonstrated in humans. There are also differences in the social organisation of caring for the offspring. Furthermore, corticosterone reactivity to experimental stressors in rats is in proportion with their total diurnal corticosterone variation, while human cortisol responses to experimental stressors in humans are more subtle [213]. These factors deserve thought when comparing rodent data to endocrine and behavioural parameters in humans.
Conclusions

The sympathetic stress response is continuously decreasing, even in the last weeks before giving birth at term, while the baseline sympathetic level seems to be stable during the final month. Measurement of skin conductance is a sensitive and non-invasive means to assess sympathetic stress activity, and is an interesting tool for increasing the knowledge about sympathetic reactions during pregnancy. Given the complex interactions between stress reactivity and labour initiation, this subject deserves more research attention.

Healthy women with stable anxiety and depression scores have decreased startle modulation by anticipation in the puerperium in comparison with late pregnancy. Since altered startle response modulation has previously been associated with anxiety and depressive states, this result provides a background for further studies examining emotional modulation in women who develop anxiety and/or depressive disorders during pregnancy and the postpartum period.

Our finding of relatively low serum allopregnanolone in late pregnant women with moderate depressive symptoms, compared with women with little depressive symptoms, is in line with a growing body of evidence for the importance of neurosteroid balance in mood regulation. The fact that our study population was fairly healthy and had low levels of anxiety hinders generalisation to women with ongoing psychiatric disorders.

A history of depression, or ongoing depression, is not an important determinant for cortisol awakening response during late pregnancy. Thus, pregnancy seems to smooth out, or reverse, the HPA alterations associated with depression in non-pregnant individuals. Further studies examining cortisol reactivity in relation to depression during pregnancy will be needed to consider additional determinants, like depression subtypes and HPA axis related gene polymorphisms.
Sammanfattning på svenska


Målet med denna avhandling var att undersöka några aspekter av stressreaktivitet under slutet av graviditeten i relation till förlossningsstart, emotioneLL reaktivitet och psykisk hälsa. I samtliga delstudier deltog kvinnor med högst fem veckor kvar till beräknat förlossningsdatum.

Delstudie I

Förutom den sänkta stressreaktiviteten verkar också smärtkänsligheten minska under sen graviditet, både hos människor och hos andra däggdjur. Hur smärtkänslighet upplevs är dock högst subjektivt och kan vara svårt att mäta. Hudkonduktans, det vill säga aktiviteten i de eccrina svettkörtlarna i handflator och på fotsulor, styrs helt av sympatiska nervsystemet och har föreslagits vara ett objektivt mått på smärtintensitet som kan komplettera självrapportering. I delstudie ett undersöktes smärta och sympatiskt svar vid köldstress i relation till hur många dagar kvinnorna hade kvar till förlossning. Sympatisk aktivitet (hudkonduktans) mättes medan kvinnan höll en hand i iskallt vatten. Vi mätte också hur lång tid det tog innan kvinnan kände köldsmärta samt hur lång tid hon stod ut med att hålla handen i vattnet.
Kvinnan fick sedan skatta hur stark smärtan varit. Det visade sig att det sympatiska svaret var lägre hos de kvinnor som hade färre dagar kvar till en spontan förlossningsstart, medan smärtkänslighet inte visade något samband med tid till förlossning. Detta tyder på att den sympatiska reaktiviteten minskar under de sista veckorna före förlossningen, även om detta inte helt kan bevisas i en tvärsnittsstudie. Ytterligare studier på sympatisk reaktivitet i förhållande till förlossningsstart kan kanske ge bättre metoder för att förutsäga tidpunkten för spontan förlossning.

**Delstudie II**


**Delstudie III**

Allopregnanolon är en neuroaktiv progesteronmetabolit som har uppmätts i lägre nivåer i blod och cerebrospinalvätska hos deprimerade individer än hos icke-deprimerade. Under en graviditet mer än tiofaldigas serumnivån av allopregnanolon och i djurförsök har Allopregnanolon visats mediera den
minskade HPA-axel-reaktiviteten under dräktighet och digivning. I den tredje delstudien analyserades serumnivåer av allopregnanolon i relation till självskattade ångest- och depressionssymptom under sen graviditet. Studiedeltagarna rapporterade över lag låg förekomst av ångest- och depressionssymptom, men de kvinnor som hade högst depressionsskattningar visade sig ha signifikant lägre allopregnanolonnivåer än resten av gruppen. Ångestskattningarna var däremot inte associerade med serumkoncentrationerna av allopregnanolon. Resultaten skulle kunna tyda på att den höga nivån av allopregnanolon under graviditeten till viss del skyddar mot de påfrestningar som de övriga endokrinologiska förändringarna kan utgöra för den maternella mentala hälsan.

Delstudie IV

Stresshormonet kortisol stiger hastigt vid uppvaknande och är som högst cirka 30 minuter efteråt. Detta kortisolsvar har visat sig vara större hos deprimerade patienter, och är även högt hos friska individer som tidigare varit deprimerade. Kortisolsvaret vid uppvaknande försvagas till viss del under graviditeten, i takt med att basalnivån stiger, men är fortfarande påvisbart under sen graviditet. I delstudie fyra mättes kortisolsvaret vid uppvaknande hos tre grupper av kvinnor under sen graviditet: kvinnor som lidit av depression under graviditet, nu friska kvinnor som tidigare lidit av depression samt kvinnor som aldrig drabbats av depression. Det visade sig att det inte fanns någon statistisk signifikant skillnad i kortisolsvar, eller skillnad i absolut kortisolnivå, mellan grupperna. Detta tyder på att det ökade kortisolsvar som visats under och efter en depression hos icke-gravida inte följer med under graviditeten.
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