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# Maternal Attachment and Mothers' Mood: The Impact of Social Support, Birth Experience and Hormones

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# Maternal Attachment and Mothers' Mood:

# The Impact of Social Support, Birth Experience and Hormones

Doctoral Thesis

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# General Introduction

Many women experience a worsening of their emotional well-being over pregnancy and the postpartum period. Many future mothers present an emergence and/or exacerbation of affective symptoms, such as stress and depression, during late pregnancy stages, but particularly immediately after childbirth (e.g. Manconi et al., 2024).

Simultaneously, women start forming an attachment with their unborn baby over pregnancy and strengthen their attachment to their newborn shortly after childbirth (e.g. Rossen et al., 2017). Substantial research states that the emergence of affective symptoms may negatively impact the quality and strength of maternal attachment at postpartum (Bonacquisti et al., 2020).

Women also experience marked steroid fluctuations during pregnancy and the postpartum period (e.g. Liang et al., 2020), which seem to have a tremendous impact on the emergence of postpartum affective symptoms (e.g. Parizek et al., 2014) and the development of maternal postnatal attachment (e.g. Glynn et al., 2016).

Besides hormonal and psychological factors, external factors seem to influence the mothers' emotional well-being and maternal attachment. The social support that mothers receive appears to buffer the exacerbation of affective symptoms (e.g. Liu et al., 2020) while prompting the development of a healthy maternal attachment (e.g. Martin & Brock, 2023). Conversely, a negative birth experience might have detrimental effects on the mother's emotional well-being and, in turn, negatively affect the quality of the maternal postnatal attachment in the postpartum period (e.g., Froeliger et al., 2023; Junge-Hoffmeister et al., 2022).

Despite existing research, there is limited research depicting the trajectory of affective symptoms, well-being and maternal attachment across pregnancy, especially from early to later postpartum stages, and many inconsistencies remain. Moreover, minimal research has explored whether the intensity of the negative impact of affective symptoms on maternal postnatal attachment ameliorates or intensifies over the postpartum period. There is also a scarcity of research exploring the influence of social support received at postpartum on the mothers' emotional well-being and whether the intensity of its effect changes later in the postpartum period. Regarding birth experience, most studies have explored the effects of objective aspects of birth, such as delivery type, while very little is known about the potential impact of the subjective experience of birth. The mechanism through which social support and birth experience influence affect and maternal attachment is unclear. Lastly, research exploring the hormonal effects on maternal attachment is inconsistent, and studies examining the steroid's impact on mothers’ affect have focused on a very narrow time frame, from late pregnancy to immediate postpartum, underexploring the later effects on the postpartum period.

## Study 1:

# The Interplay of Affective Symptoms, Social Support, and Birth Experience in Shaping Maternal Postnatal Attachment

# Introduction

## Affective Symptoms, Well-being and Maternal Attachment over Pregnancy and the Postpartum Period

### *Depressive Symptoms*

Many women experience varying levels of depressive symptoms during pregnancy and the postpartum period, with prevalence rates fluctuating significantly across the different gestational stages: 7.4% in the first pregnancy trimester, in the second trimester, and 12.0% in the third one (Bennet et al., 2021), and 27% at postpartum (Wang et al., 2021).

Few recent studies have tried to depict a specific trajectory of depressive symptoms over pregnancy and the postpartum period. It has been suggested that depressive symptoms tend to increase during the third trimester (Wang et al., 2023; Zikic et al., 2024) and peak in the early postpartum period (Wang et al., 2023; Zikic et al., 2024; Iwata et al., 2016). Notably, Wang et al. (2023) proposed a U-shaped trajectory, with symptoms slightly decreasing in the second and third pregnancy trimesters and rising significantly postpartum, peaking at three months after delivery. Similarly, Zikic et al. (2024) observed a significant exacerbation of depressive symptoms in the first months postpartum. However, unlike Wang et al. (2023), the authors reported that depressive symptoms started to rise in the third pregnancy trimester slightly and peaked earlier, around one month postpartum, followed by a later gradual decline from six to twelve months postpartum (Zikic et al., 2024). Likewise, Iwata et al. (2016) also confirmed that depressive symptoms increase after childbirth, peaking during the first month postpartum. Nevertheless, unlike Zikic et al. (2024), the

authors noted an earlier stabilisation, with symptoms beginning to decrease around two months postpartum and dropping entirely by six months postpartum (Iwata et al., 2016).

Besides that, some studies have stated that the rates and the trajectories of depressive symptoms vary according to the symptomatology severity. Specifically, 66.7% of women with low depressive symptoms maintain these low levels throughout the perinatal period. In comparison, 24.5% of women experience moderate symptoms; these are more prominent early in pregnancy and decline over time (Yu et al., 2020). In contrast, 8.8% of women belong to a high-persistent group, characterised by consistently elevated depressive symptoms from early pregnancy through six weeks postpartum. Another subset, approximately 10.2%, develops delayed-onset depression, with low depressive symptoms during pregnancy that sharply increase postpartum and persist through eight weeks postpartum. A smaller proportion, 1.1%, suffers from chronic depression, defined by persistently high symptoms throughout pregnancy and the postpartum period (Dekel et al., 2019).

In summary, converging evidence suggests that women experience marked fluctuation in their depressive symptoms during pregnancy and postpartum. Depressive symptoms generally decrease during pregnancy and exacerbate in the early postpartum period. Nonetheless, there are some divergences in the course that depressive symptoms follow throughout pregnancy and the postpartum period, particularly regarding the timing and duration of symptom peaks and declines, as well as the gestational starting points for analysis. Wang et al. (2023) propose a U-shaped trajectory, beginning with a slight decrease in the second and third trimesters and peaking at three months postpartum. In contrast, Zikic et al. (2024) depict a trajectory that begins later with an increase in the third pregnancy trimester, peaks earlier at one month postpartum and declines from 6 to 12 months postpartum. By contrast, Iwata et al. (2016) focus solely

on the postpartum period, observing a peak at one month postpartum and noting an earlier stabilisation, with symptoms decreasing by two months and resolving by six months postpartum. Apart from these divergencies in the depressive symptomatology trajectory, some studies have depicted different rates and trajectories depending on the severity of the depression (Dekel et al., 2019; Yu et al., 2020). Thus, it is still pendent to depict the specific trajectory of depressive symptoms over pregnancy and the postpartum period.

### *Anxiety and Stress Symptoms*

The incidence and prevalence of anxiety disorders during the perinatal period display distinct patterns of change throughout pregnancy and postpartum. Anxiety disorders are prevalent during pregnancy, with rates ranging from 13% to 21%, peaking during mid-pregnancy (24 weeks) and late pregnancy (33 weeks) (Fairbrother et al., 2015; Fairbrother et al., 2016; Barat et al., 2023). In terms of new cases, 3.9% of women develop anxiety disorders during pregnancy, indicating that some women may begin experiencing these disorders between conception and 40 weeks of gestation (Fairbrother et al., 2016).

Postpartum anxiety also shows significant prevalence and incidence. The prevalence of postpartum anxiety ranges from 11% to 17% (Fairbrother et al., 2016; Barat et al., 2021), with a peak around 4 to 6 weeks postpartum and assessments extending up to 12 weeks. The incidence of new-onset postpartum anxiety disorders is reported to range from 2.2% to 8.8% (Fairbrother et al., 2016). However, the incidence of new anxiety cases from the early to mid-postpartum period is 2.3%, which is slightly lower than the 3.9% incidence over pregnancy.

Regarding the longitudinal trajectory of anxiety, mothers present an increase in their anxiety symptoms starting from mid-pregnancy and peaking in late pregnancy. These anxiety levels remain elevated up to one month postpartum and decrease around six weeks postpartum (Liou et al., 2014).

Despite the general trajectory of anxiety, three distinct patterns emerge during pregnancy: most women (73%) experience no significant anxiety, 23.6% exhibit low-stable anxiety, and a smaller subset (3.4%) experiences moderate-ascending anxiety, which worsens throughout pregnancy (Gao et al., 2023). These anxiety trajectories remain relatively stable for most women, while a minority experience worsening symptoms, particularly in the high-stable and moderate-stable groups (Lee et al., 2021).

Unlike anxiety symptoms, stress levels gradually decrease from the first to the third trimester of pregnancy (Goletzke et al., 2017; Liou et al., 2014). However, like anxiety, stress levels rebound at 4–6 weeks postpartum (Miller et al., 2006; Liou et al., 2014). Of note, stress trajectories vary depending on the source of stress. For instance, overall stress and stress related to infant nurturing begin to decline as early as 42 days postpartum, continuing to decrease further by 3 and 6 months postpartum. In contrast, stress associated with personal needs, body changes, and sexuality ameliorates at 6 months postpartum.

In summary, pregnant women and new mothers experience diverse and dynamic trajectories of anxiety and stress throughout the perinatal period. Women experience a heightening of their anxiety symptoms during pregnancy and a decrease in the first postpartum month, while their stress ameliorates during pregnancy and exacerbates shortly after childbirth. Anxiety generally increases during pregnancy and decreases within the first month postpartum, whereas stress tends to decrease during pregnancy

but rises again after childbirth. So, this divergence between the anxiety and stress trajectories suggests that different factors may influence each affective symptom. For instance, anxiety symptoms may be more related to discomforts related to pregnancy and the fear of childbirth, whereas stress may be related to the transition into motherhood. Besides, research on stress is scarce compared to the solid evidence about anxiety. This scarcity of research on stress highlights the need for further research to properly depict the changes in stress symptoms that women experience during pregnancy and the postpartum period.

### *General Well-being*

Throughout the perinatal period, women's psychological well-being tends to fluctuate, influenced by conception methods, maternal age, and social support. Women generally experience an increase in life satisfaction from preconception to pregnancy and from pregnancy to the postpartum period, remaining stable over the postpartum period (Quick et al., 2023).

Within the first six months postpartum, various factors significantly impact quality of life. Women aged 26 to 35 are likelier to report poorer physical health than younger mothers. Sleep disturbances often lead to declines in these areas of quality of life, whereas social support—especially from a significant other—is strongly associated with better outcomes across all domains (Al Rehaili et al., 2023). Besides, women who conceive via in vitro fertilisation typically start with lower life satisfaction before pregnancy but show significant improvement by six months postpartum, continuing to rise through 24 months postpartum (Kiesswetter et al., 2023). In contrast, those who conceive naturally tend to experience a temporary decline in life satisfaction at six months postpartum, which returns to preconception levels by 12 months postpartum and

remains stable. Although in vitro fertilisation mothers often report higher stress levels before pregnancy, their stress levels gradually decrease after childbirth and align with those of the natural conception group by 12 months postpartum. By 24 months postpartum, in vitro fertilisation mothers usually experience lower worry levels than naturally conceiving mothers (Kiesswetter et al., 2023).

In summary, women generally experience improved well-being from preconception through pregnancy and into the postpartum period. However, within the first six months postpartum, deviations in well-being may occur, particularly among older women who conceived naturally, experienced poor sleep quality, or received low social support. Despite these findings, minimal research on women's well-being is still available throughout pregnancy and postpartum. Furthermore, the described trajectory is too broad and does not specify the changes in well-being across the distinct gestational and postpartum stages. Additionally, it is not very easy to interpret and compare the results from the different studies because of the use of very different instruments to measure well-being. This complex interpretation of the results underscores the need for employing a more comprehensive and representative measure of well-being to develop a more detailed trajectory during this period.

### *Sleep Quality*

Throughout pregnancy and the postpartum period, women experience significant changes in sleep patterns, with disturbances becoming more prevalent as pregnancy progresses and persisting after delivery. In the first trimester (1–13 weeks), about 50% of women experience poor sleep quality, and insomnia begins to affect a third of these women (Ojelere & Adeoye, 2024). Other studies have found that around 10–15 weeks of gestation, 33.7% of women begin reporting poor sleep quality (Manconi et al., 2024).

As pregnancy advances, sleep quality continues to decline. By 34–36 weeks of gestation, 46.2% to 62.5% of women report poor sleep quality, and the prevalence of insomnia reaches 55.7% (Manconi et al., 2024; Umeno et al., 2020). Total sleep time decreases, while nighttime awakenings and difficulty maintaining sleep increase (Mindell et al., 2015). In the third trimester, women experience more frequent nocturnal awakenings due to physical discomfort, frequent urination, and restless leg syndrome (Mindell et al., 2015).

In addition to reduced sleep quality, women's sleep timing shifts, and short sleep duration becomes particularly common as pregnancy progresses, with 65% of women sleeping fewer than 7 hours per night by the third trimester (Al-Musharaf, 2022). In addition, younger women and those with lower socioeconomic status experience worse sleep quality by the third trimester (Yang et al., 2023).

Postpartum sleep disturbances are also common. Within the first six weeks postpartum, 34.4% of women continue to report insomnia, and poor sleep quality affects 71.4% of new mothers, marking the peak of sleep disturbances (Manconi et al., 2024; Sedov & Tomfohr-Madsen, 2021). Some women maintain subclinical or clinical insomnia symptoms into the postpartum period, particularly those who experience significant sleep problems during the third trimester (Sedov & Tomfohr-Madsen, 2021).

In summary, sleep quality steadily deteriorates from early pregnancy through the third trimester, with disruptions often continuing into the postpartum period. Sleep disturbances are common, especially as pregnancy progresses, and short sleep duration becomes more pronounced in the later stages. These sleep disturbances are most severe in the final trimester and immediately postpartum (Ojelere & Adeoye, 2024; Manconi et al., 2024; Umeno et al., 2020). Despite the converging evidence about a steady

deterioration of sleep quality from early pregnancy to immediate postpartum, how sleep quality evolves as postpartum time progresses remains to be explored.

### *Maternal Attachment*

The formation of maternal attachment is a gradual process that begins early in pregnancy and reaches its peak in the early postpartum period. During early pregnancy, mothers typically start developing an emotional bond with their unborn child, which grows stronger progressively throughout pregnancy and culminates in the early postpartum period, when maternal attachment is at its most intense.

Maternal-foetal attachment typically starts in the first trimester and increases steadily through the second and third trimesters. Stronger antenatal attachment throughout pregnancy predicts better maternal-infant bonding at 8 weeks postpartum (Rossen et al., 2017).

By the second trimester, mothers with higher levels of maternal-foetal attachment are more likely to experience better bonding with their infants after childbirth. Stronger attachment at mid-pregnancy is consistently associated with positive postpartum bonding outcomes, even when accounting for psychological factors such as anxiety and depression (Petri et al., 2018).

In the third trimester, the impact of maternal-foetal attachment becomes more pronounced. Higher levels of maternal-foetal attachment at this stage are linked to improved maternal caregiving behaviours 4 months postpartum (Sacchi et al., 2021). However, the influence of maternal-foetal attachment on maternal sensitivity is not always consistent. For instance, maternal-foetal attachment during the second and third trimesters does not consistently predict sensitivity, intrusiveness, or positive regard for the child in the first year postpartum, suggesting that maternal-foetal attachment may

impact some aspects of maternal behaviour without directly predicting sensitivity (Dau et al., 2019). Additionally, mothers who present a stronger maternal-foetal attachment in the third trimester also experience fewer impairments in their maternal attachment at 12 weeks postpartum, demonstrating the predicting power of prenatal attachment over the development of postnatal bonding. Prenatal bonding explains a notable proportion of the variance in postpartum bonding quality, highlighting its importance in establishing positive mother-infant interactions (Dubber et al., 2015).

During postpartum, early mother-to-infant bonding influences later emotional availability and responsiveness between mothers and infants. Strong early maternal bonding at 8 weeks postpartum tends to predict greater emotional availability and responsiveness at 12 months postpartum (Rossen et al., 2019).

Overall, these findings highlight the progressive development of maternal attachment, which begins early in pregnancy and continues into the postpartum period. Maternal-foetal attachment formed during pregnancy plays a pivotal role in shaping maternal behaviours and improving the quality of mother-infant interactions at postpartum. Despite the solid evidence about the gradual formation of maternal attachment from early pregnancy to early postpartum, there remains a scarcity of research exploring the trajectory of the mother-infant bond during the postpartum period.

### The Influence of Affective Symptoms on Maternal Attachment over Pregnancy and the Postpartum Period

Maternal depression and anxiety have profound and lasting effects on maternal foetal attachment throughout pregnancy and postpartum mother-infant bonding. During pregnancy, depressive symptoms that emerge as early as 23-25 weeks gestation and

continue into late pregnancy are closely linked to significant bonding impairments in the early postpartum days (Ohoka et al., 2014; Kanekasu et al., 2024). When these depressive symptoms persist from pregnancy to postpartum, bonding impairments are particularly severe at 5 days and 1 month after childbirth (Ohoka et al., 2014). However, by 3 months postpartum, the influence of prenatal depressive symptoms diminishes (Kanekasu et al., 2024).

At postpartum, affective symptoms continue to impair maternal-infant bonding, particularly in the early weeks after birth. Elevated postpartum depression (Moehler et al., 2006) and anxiety symptoms (Kawai et al., 2023) at 1 month-6 weeks postpartum are strongly associated with weaker maternal attachment, including lack of affection and feelings of anger or rejection toward the infant (Kawai et al., 2023). This negative effect of depression can occur even earlier in the postpartum period, as Bonacquisti et al. (2020) observed poorer mother-to-infant bonds among mothers who experienced depressive, anxiety, and stress symptoms a few days after childbirth. Further, depressive symptoms at 1 month postpartum are also linked to lack of maternal feeling and caregiving anxiety (Tsuchida et al., 2019). These adverse effects and affective symptoms of mother-to-infant bonding extend far beyond the first month, as mothers with both current and lifetime depression at 3 to 4 months postpartum report poorer bonding compared to non-depressed mothers (Nonnenmacher et al., 2016). Even far beyond, mothers who experience postpartum depression at one and six months postpartum are at higher risk of presenting a weaker maternal attachment at 1 year postpartum, indicating a persistent impact of early postpartum depression on bonding. However, this was especially the case for mothers with chronic or persistent depression (Kasamatsu et al., 2020).

Nevertheless, depression does not consistently impair maternal sensitivity. Studies show that there is no significant difference in maternal sensitivity between mothers with and without a major depressive episode during the first 12 months postpartum, suggesting that depression affects general maternal attachment but may not necessarily impact a mother's ability to respond sensitively to her infant (Dau et al., 2019).

Overall, depression and anxiety symptoms in mothers significantly influence maternal attachment during pregnancy and postpartum, particularly during the early months after childbirth. Despite substantial evidence stating the adverse effects of affective symptoms on postnatal attachment, it remains unclear whether the intensity of this impact varies between early and mid-postpartum. Furthermore, while many studies have examined the influence of anxiety and depression on postpartum bonding, research specifically addressing the effects of stress symptoms on maternal attachment is still limited.

## The Influence of Social Support on Affective Symptoms, Well-being and Maternal Attachment during Pregnancy and the Postpartum Period

### *Affective Symptoms*

Social support is critical for mitigating stress, anxiety, and depressive symptoms among pregnant women and new mothers across pregnancy. Changes in social support throughout pregnancy significantly impact emotional health (Castelar-Ríos et al., 2022). Between gestational weeks 12 and 32, increased perceived social support is consistently linked to lower levels of stress and depression, while decreases in support often result in heightened affective symptoms (Castelar-Ríos et al., 2022).

The influence of social support is particularly evident during early and mid-pregnancy (before 15 weeks and between 15–27 weeks), where poor social support can lead to worsening symptoms of stress, anxiety, and depression (Gao et al., 2023). In contrast, strong social support buffers the emergence of affective symptoms, especially in the second and third pregnancy trimesters (Gao et al., 2023). Moreover, higher levels of social support during these periods are strongly linked to reduced anxiety related to self-care, foetal health, and childbirth concerns, particularly in the third trimester, as pregnancy-related anxiety usually peaks around childbirth (Huang et al., 2022). Similarly, partner support is key in reducing stress levels, particularly in the second and third trimesters (Martin & Brock, 2023). More than that, social support influences stress trajectories throughout pregnancy, at gestational weeks 12–14, 24–26, and 34–36, showing lower stress levels among women receiving adequate support (Goletzke et al., 2017).

Social support received during pregnancy, particularly in the third trimester, not only has an immediate impact on maternal mental health but also exerts lasting effects that extend into the postpartum period. For instance, a study by McCall-Hosenfeld et al. (2016) found that social support during the third trimester was a significant predictor of maternal depressive symptoms at both 6 and 12 months postpartum.

Social support received during the early postpartum period also plays a crucial role in regulating affect in mothers, and the source of social support appears to be a key factor. For example, a study by Liu et al. (2020) the results showed that lower levels of family support predicted increased anxiety and depressive symptoms at six weeks postpartum. Additionally, support from friends and colleagues alleviated depressive symptoms during this period (Liu et al., 2020).

Lastly, social support also shapes general well-being in the early postpartum period, where women with strong social support report higher life satisfaction and quality of life (Al Rehaili et al., 2023).

To sum up, social support ameliorates stress and depressive symptoms and enhances well-being in women during pregnancy and the postpartum period. However, research on the specific impact of postpartum social support on the emergence of affective symptoms in mothers—and whether the strength of this impact varies over the postpartum period—remains limited. Furthermore, it is still uncertain which source of social support has the most significant influence on maternal psychological well-being.

### *Maternal Attachment*

Social support strongly impacts the formation of maternal attachment during pregnancy and the postpartum period. Support from partners significantly enhances maternal-foetal bonding and strengthens maternal postnatal attachment.

Strong family support in early pregnancy, particularly during the first trimester (8-12 weeks gestation), strengthens emotional bonds with unborn children by improving pregnancy adaptation in mothers. Thus, pregnant women who receive higher levels of family support during pregnancy have more resources to adapt emotionally and behaviourally better, which, in turn, facilitates the development of stronger maternal-foetal bonds. This study highlights the critical role of early family support in promoting maternal adaptation and, consequently, fostering a strong maternal-foetal bond (Wu et al., 2024).

As pregnancy progresses, social support begins to have an impact on maternal attachment at postpartum. For instance, consistent partner support during late pregnancy (32 weeks gestation) predicts stronger prenatal bonding, which predicts better postnatal

bonding at 8 months postpartum. So, support from partners enhances maternal attachment during pregnancy and predicts a positive and healthy postnatal attachment (Cuijlits et al., 2019). Conversely, inadequate social support is associated with bonding difficulties. Mothers with lower levels of support during early pregnancy (before 25 weeks gestation) are more likely to experience bonding challenges both during pregnancy and at 1 month postpartum (Ohara et al., 2018).

Support from partners and family can also indirectly impact the mother-to-infant bond by moderating the mother's effect. Partner and family support during early pregnancy (before 20 weeks gestation), late pregnancy (third trimester), and at 6 months postpartum mediate the relationship between prenatal depressive symptoms and mothers' emotional availability to their infants at 6 months postpartum (MacMillan et al., 2021). In other words, mothers who receive high levels of support from family and partners are more emotionally available to their infants, even if they suffer prenatal depressive symptoms. This statement suggests that family and partner support is central to promoting maternal emotional availability and bonding (MacMillan et al., 2021). Similarly, Martin & Brock (2023) found out support from partners significantly reduced maternal stress during the second and third trimesters (13 to 27 weeks gestation), which was associated with fewer bonding impairments at 1 month and 6 months postpartum (Martin & Brock, 2023).

In summary, social support from partners and family received over pregnancy is pivotal in strengthening maternal attachment during pregnancy and postpartum. More into detail, support from close relationships during pregnancy helps to alleviate maternal stress and depressive symptoms, which results in stronger bonds with their infants. While substantial evidence supports the significant impact of social support during pregnancy on both prenatal and postnatal attachment, there is less research about

the influence of postpartum social support on the mother-to-infant bond and how this support evolves from early to mid-postpartum. Additionally, it remains unclear whether support from partners or family has a more pronounced effect, whether support from friends contributes meaningfully, and whether general social support or other specific supports explain the observed benefits in maternal attachment.

## The Influence of Birth Experience on Affective Symptoms, Well-being and Maternal Attachment over Pregnancy and the Postpartum Period

### *Affective Symptoms*

Birth experiences for mothers have a powerful influence on their postpartum depression, stress, and anxiety symptoms. Mothers with a perception of a negative birth experience are at higher risk of developing postpartum depression, stress, and anxiety, highlighting the importance of the subjective experience of childbirth on mothers' postpartum mental health. At 2 months postpartum, mothers who report negative memories of their childbirth are more likely to experience symptoms of postpartum depression, with an adjusted odds ratio of 2.4 (Froeliger et al., 2023). The emotional response to the birth experience is a strong predictor of early postpartum depressive symptoms. Specifically, the authors observed that negative recollections from the immediate post-delivery period (within 2 days) impacted mothers' mental health at 2 months postpartum (Froeliger et al., 2023).

Going deeper into the birth experience itself, mistreatment during childbirth—such as lack of privacy, absence of a companion, or feeling disrespected by healthcare providers—is significantly associated with a 55% higher prevalence of depressive symptoms by 4 weeks postpartum (Paiz et al., 2022). This finding highlights the

influence power of the social factors related to birth experience on the onset of postpartum depression (Paiz et al., 2022).

Further along in the postpartum period, at 3- and 4-months postpartum, childbirth experience continues to influence mothers' mental health. Negative childbirth experiences contribute to elevated depressive and anxiety symptoms (Ahmadpour et al., 2023), as well as acute stress reactions (Gürber et al., 2017). In contrast, mothers who report a more positive childbirth experience show lower levels of both postpartum depression and anxiety, emphasising that childbirth experience can also exert a protective factor against postpartum affective symptoms (Ahmadpour et al., 2023).

Another study showed that a negative birth experience could even have longer-lasting effects on mothers' mental health over the postpartum period, up to 8 months postpartum, especially on the mothers' anxiety symptoms (Bell et al., 2016). Even mothers who did not have a distinctly negative birth experience but rated their birth as unsatisfying still tended to exhibit elevated anxiety symptoms at both 2 and 8 months postpartum. However, this pattern did not extend to depression, indicating that while birth experience may have a lasting impact on postpartum anxiety, the effect on depression might be more nuanced or occur earlier in the postpartum timeline (Bell et al., 2016).

Besides, the adverse effects of a poor birth experience on the onset of postpartum depression and anxiety symptoms ultimately impact maternal stress. So, a negative birth experience is linked to higher levels of parenting stress at 3 months postpartum (Molgora et al., 2020). Specifically, this association is mediated by postpartum anxiety and depression, indicating that the initial negative emotional impact

of birth leads to increased anxiety and depressive symptoms, which subsequently heighten stress related to parenting (Molgora et al., 2020).

Overall, the subjective perception of the childbirth experience plays a key role in regulating the mothers' emotional well-being during the early postpartum period. Although substantial evidence highlights the influence of birth experiences on mothers' postpartum mental health, research specifically examining the impact of childbirth on postpartum stress symptoms is scarce, and it is even more scarce in its impact on postpartum well-being.

### *Maternal Attachment*

The influence of birth experience on maternal postnatal attachment varies based on delivery type, subjective experience, assessment timepoint, and maternal psychological factors.

On the one hand, several studies support the notion that the type of delivery is a significant factor influencing maternal postnatal attachment. For example, Moniri et al. (2023) found that while the overall subjective birth experience did not directly correlate with mother-to-infant bonding at six weeks postpartum, the mode of delivery had a notable impact. Mothers who had vaginal births reported stronger bonds with their infants compared to those who underwent caesarean sections. Similarly, Doblin et al. (2023) observed that mothers who had spontaneous vaginal delivery demonstrated a stronger association between a positive birth experience and mother-to-infant bonding at eight weeks postpartum compared to mothers who delivered by induced vaginal birth or by caesarean section. However, this effect diminished by 14 months postpartum, suggesting that the early postpartum period, particularly the first two months, is a critical window during which birth experiences greatly influence maternal attachment.

These findings collectively indicate that specific aspects of the birth experience, such as delivery type, play a meaningful role in shaping bonding during the immediate postpartum period.

On the other hand, the study of Junge-Hoffmeister et al. (2022) stated that subjective birth perceptions were a more substantial predictor of maternal attachment impairments at postpartum than objective factors related to birth, such as medical complications. Their study revealed that mothers who reported negative subjective birth experiences at approximately 24 weeks postpartum were significantly more likely to encounter severe bonding challenges. These results underscore that the way mothers interpret and emotionally process their birth experience can be a stronger predictor of the quality of maternal postnatal attachment than the obstetric characteristics of the birth itself.

Eitenmüller et al. (2022) propose an indirect mechanism through which birth experience influences maternal postnatal attachment—via its impact on postpartum depression. Specifically, they found that a negative birth experience is associated with higher postpartum depression scores, which, in turn, predict impaired bonding. This study underscores the marked mediating effect of maternal mental health over the relationship between birth experiences and mother-to-infant bonding, particularly within the first six months postpartum. Similarly, Stuijfzand et al. (2020) observed that maternal psychological distress mediates the relationship between maternal symptoms of childbirth and posttraumatic stress disorder and weaker maternal attachment at three months postpartum. More specifically, while posttraumatic stress disorder symptoms assessed at one-month postpartum predicted poorer bonding at three months, this effect disappeared after accounting for general psychological distress at one month.

In summary, the birth experience strongly influences postpartum bonding, particularly in the early months following childbirth. Although substantial evidence supports the role of birth mode in maternal postnatal attachment, research investigating the subjective experience of childbirth and its impact on bonding remains limited. Furthermore, it is unclear whether the influence of subjective birth experience operates directly or through its effect on regulating maternal affective symptoms.

## Justification of the Study

Women often experience dynamic changes in mental health symptoms and well-being throughout the perinatal period. Research has shown that mothers present diminishing of their depressive and stress symptoms, together with an improvement of their well-being and sleep quality during the second and third trimesters of pregnancy (e.g., Wang et al., 2023; Goletzke et al., 2017; Quick et al., 2023; Manconi et al., 2024). In contrast, in the early postpartum period, new mothers experience a sharp increase in their affective symptoms, coupled with a decline in their well-being and sleep quality, which tend to peak within the first few postpartum months before stabilising by six months (e.g., Wang et al., 2023; Goletzke et al., 2017; Quick et al., 2023; Manconi et al., 2024).

Despite existing research, significant inconsistencies remain in understanding the trajectory of affective symptoms and well-being across pregnancy and the postpartum period. These inconsistencies are mainly in the variability of gestational starting points for assessments and the unclear duration of symptom peaks and well-being declines during the postpartum period. Furthermore, many studies employed scales and questionnaires that measured very specific traits of well-being rather than instruments that assessed more comprehensive and generalised well-being factors. This limitation reduces the comparability of findings, limiting a complete understanding of maternal well-being. Therefore, there is a critical need for further research that examines the detailed course of affective symptoms and well-being from early pregnancy through to mid-postpartum. Future research should explore when the mothers experience improvements and worsening of their affective symptoms and well-being across the different gestational and postpartum stages and how the intensity of these changes evolves. Additionally, future studies should employ holistic and

multidimensional measures of well-being to provide a more accurate depiction of the dynamics of well-being.

Simultaneously, women form maternal attachment during pregnancy, intensifying and culminating in the early postpartum period (e.g., Rossen et al., 2017). While growing evidence highlights the gradual evolution of maternal attachment from early pregnancy to early postpartum, research on the trajectory of the mother-infant bond throughout the postpartum period remains scarce. Furthermore, most research exploring maternal postnatal attachment examined isolated maternal behaviour instead of assessing maternal attachment more broadly and comprehensively. This gap indicates the need to explore the longitudinal fluctuations of maternal attachment over the postpartum period and all aspects that represent maternal attachment.

Consistent research states that affective symptoms harm maternal postnatal attachment (Bonacquisti et al., 2020). However, despite this substantial evidence, it remains to be explored whether the intensity of these effects differs between the early and mid-postpartum periods. Additionally, while many studies have examined the effects of anxiety and depressive symptoms on maternal attachment at postpartum, there is a scarcity of research exploring the impact of stress symptoms on maternal postnatal attachment. These gaps indicate the need for further research exploring the impact of affective symptoms on maternal attachment over the postpartum period, specifically, the influence of stress symptoms.

Research has consistently highlighted the protective role of social support against postpartum depression and stress and its prompting of well-being (e.g., Barat et al., 2023). In addition to its mental health benefits, social support strongly impacts maternal attachment (Cuijlits et al., 2019). However, research on the specific impact of

postpartum social support on the emergence of affective symptoms in mothers—and whether the strength of this impact varies over the postpartum period—remains limited. Furthermore, it is still uncertain which source of social support has the most significant influence on maternal psychological well-being.

Similarly, birth experience directly impacts emotional well-being and the quality of maternal attachment (e.g., Froeliger et al., 2023; Junge-Hoffmeister et al., 2022). Although substantial evidence highlights the influence of childbirth on mothers' postpartum mental health and maternal attachment, there is a scarcity of research exploring the effects of the subjective perception of birth experience. Additionally, research specifically examining the impact of childbirth on postpartum stress symptoms is minimal, and it is even more scarce in its impact on postpartum well-being.

While few studies have found a link between the buffering effects of social support and birth experience on the mothers' affective symptoms and stronger maternal postnatal attachments (e.g. Eitenmüller et al., 2022; Martin & Brock, 2023), the specific mechanisms by which social support and birth experience influence maternal postnatal attachment are not fully understood. In other words, it is unclear whether social support and birth experiences directly enhance maternal attachment or if their alleviation of stress and depressive symptoms indirectly strengthen the mother-infant bonds.

The present study aims to uncover these gaps regarding the predictive factors of maternal postnatal attachment by exploring the dynamics and the complex interplay between affective symptoms, well-being, social support, and birth experience to impact maternal postnatal attachment from early to mid-postpartum. We will investigate how emotional well-being, in conjunction with social support and birth experience, influences the development of maternal attachment throughout the postpartum period.

## Objectives

The primary objective of this study is to investigate how mental health and well-being factors, together with social and obstetric factors, shape maternal attachment during the early postpartum period in first-time gestational mothers. Building on previous research that highlights the influence of mental health in conjunction with social and obstetric-experiential factors on fostering postnatal attachment, this study seeks to uncover the interrelations among these factors and their underlying mechanisms to influence maternal postnatal attachment. Specifically, we seek to examine whether the hypothetic impact of social support and birth experience on maternal postnatal attachment is indirect and is mediated by affective symptoms, as previous studies showed an effect of social support and birth experience on affective symptoms. We broke down these overarching goals into the following specific objectives:

*Objective 1:* Examine the fluctuations in levels of stress, depression symptoms, general well-being, and sleep quality from pre-conception through to 6 months postpartum. We will focus on the emergence of affective symptoms and the decline in well-being and sleep quality during the postpartum period.

*Objective 2:* Track changes in maternal attachment from early pregnancy through mid-postpartum, focusing on how maternal attachment strengthens or weakens over the different gestational and postpartum stages.

*Objective 3:* Investigate how and to what extent stress, depressive symptoms, and general well-being contribute to changes in maternal attachment over the postpartum period.

*Objective 4:* Examine whether perceived social support (from partners, family and friends) affects the emergence of affective symptoms and the preservation of well-being over the postpartum period.

*Objective 5:* Investigate the differentiated strength in the predictive effects of the distinct social support sources on affective symptoms across the postpartum period.

*Objective 6:* Explore whether perceived social support (from partners, family, and friends) impacts the strength and development of maternal attachment during the postpartum period.

*Objective 7*: Examine the differentiated strength in the predictive effects of the distinct social support sources on maternal attachment across the postpartum period.

*Objective 8:* Investigate whether birth experience impacts the emergence of affective symptoms and the preservation of well-being.

*Objective 9:* Explore whether birth experience affects the strength and development of maternal attachment from early to mid-postpartum.

*Objective 10:* Assess whether affective symptoms mediate the effect of social support on maternal attachment in the early postpartum period.

*Objective 11:* Evaluate whether affective symptoms and well-being mediate the effect of birth experience on maternal attachment in the early postpartum period.

# Methods

## Participants

From the ongoing longitudinal project, we recruited a sample of 322 nulliparous women without a history of previous pregnancies longer than 12 weeks and who were planning to attempt a pregnancy within the following six months, either through non-assisted means or using assisted reproductive techniques such as in vitro fertilisation and artificial insemination. Additionally, we recruited 55 nulliparous women who were partners of those planning pregnancies. To serve as a comparison group, we also recruited 61 nulliparous women who were not planning to conceive within the following two years. The control participants were selected concurrently with the future first-time mothers and were matched by age to ensure comparability across groups. All participants were between 25-45 years old and were willing to voluntarily participate in the clinical sessions according to the program established in the protocol.

Our sample size is based on the birth success rate of each conception method obtained in our previous study (Hoekzema et al., 2017), as well as in reports by the Spanish Fertility Society on assisted reproduction methods (Registro Nacional de Actividad 2015-Registro SEF [MSSSI], 2005); and in the estimation of the final sample size required to capture the effects sizes obtained in our previous study (Hoekzema et al., 2017).

### *Recruitment Institutions*

The participants were recruited through two primary channels: the gynecologic department of Hospital Clínic and an Instagram account named “BeMother”. At Hospital Clínic, recruitment focused on women undergoing in vitro fertilisation or artificial insemination treatments. The Instagram account “BeMother” served as an additional recruitment platform, targeting nulliparous women planning to conceive,

their female partners, and nulliparous women not attempting pregnancy. This dual approach allowed for a broader and more diverse recruitment of participants.

### *Exclusion criteria*

The study excluded women with a history or current diagnosis of endocrinological, immunological, or neurological pathologies, severe psychiatric disorders, or any medical condition deemed clinically significant that could interfere with the interpretation of the tests. Women receiving psychopharmacological or hormonal treatments were also excluded from the study. Additionally, women with a body mass index above 35 or below 18 were not eligible to participate.

#### *Final sample*

A total of 126 gestational mothers ($M^{Age}$= 34.29, $SD^{Age}$= 4.13), 23 non-gestational mothers ($M^{Age}$= 32.82, $SD^{Age}$= 4.31), and 33 nulliparous women ($M^{Age}$= 30.96, $SD^{Age}$= 3.88), completed all the experimental sessions. The final sample included 116 gestational mothers, 21 non-gestational mothers and 30 nulliparous women (see Table 1).

**Table 1**

*Number of participants per group and experimental session*

| | **Gestational mothers & Pregnancy weeks N=** | **Natural conception N=** | **Assisted reproduction N=** | **Non-gestational mothers N=** | **Nulliparous women N=** |
|---|---|---|---|---|---|
| **Before pregnancy** | 283 | 189 | 94 | 52 | 57 |
| **18-22 pregnancy weeks** | 174 (18.23±1.34) | 76 | 98 | 37 | 41 |
| **34-36 pregnancy weeks** | 166 (33.85±1.08) | 74 | 92 | 33 | 38 |
| **4 weeks postpartum** | 167 (39.40±1.97) | 75 | 92 | 32 | 51 |
| **6 months postpartum** | 123 | 59 | 64 | 24 | 42 |
| **All sessions completed** | 116 | 58 | 58 | 21 | 30 |

**Table 2**

*Social, demographic and obstetric data of the sample*

| | Gestational mothers | Natural conception mothers | Assisted reproduction mothers | Non-gestational mothers | Nulliparous women |
|---|---|---|---|---|---|
| **Age** | 34.30±4.14 | 31.45±3.16 | 34.96±3.89 | 32.82±4.31 | 3.97 ± 3.88 |
| **BMI at baseline** | 23.78±4.42 | 23.35±3.98 | 23.99±4.60 | 24.65±4.06 | 22.16 ± 2.93 |
| **Primary education** | 8 | 1 | 2 | 1 | 0 |
| **Secondary education** | 38 | 5 | 11 | 5 | 3 |
| **Higher education** | 275 | 76 | 90 | 49 | 59 |
| **Income 0-600€** | 0 | 0 | 0 | 0 | 1 |
| **Income 601-1200€** | 8 | 2 | 1 | 0 | 10 |
| **Income 1201-2000€** | 56 | 12 | 15 | 2 | 20 |
| **Income 2001-3000** | 91 | 17 | 31 | 18 | 15 |
| **Income 3001-4500** | 117 | 37 | 38 | 26 | 6 |
| **Income >4500** | 36 | 13 | 14 | 6 | 1 |
| **Months looking pregnancy** | 14.21±16.62 | 4.10±5.87 | 15.17±19.23 | - | - |
| **1 baby/ 2 babies** | 159/4 | 72/0 | 84/4 | - | - |
| **Vaginal delivery** | 106 | 50 | 55 | - | - |
| **Planned C-section** | 17 | 8 | 9 | - | - |

| | | | | | |
|---|---|---|---|---|---|
| **Unplanned C-section** | 32 | 11 | 21 | - | - |

### *Instruments*

*General Demographic-Social-Obstetric Questionnaire*: age, height, weight, body mass index, hormonal status, previous pregnancies, endocrinological, immunological, and neurological pathologies, psychiatric disorders, psychopharmacological treatment, hormonal treatment, use of contraceptives, months searching for pregnancy, conception method, number of babies, baby gender.

*Maternal Antenatal Attachment Scale (MAAS) and Maternal Postnatal Attachment Scale* (MPAS) (Navarro-Aresti et al., 2016): these maternal attachment scales are self-report questionnaires designed to assess the quality and the strength of a mother's emotional bond with her baby during pregnancy (antenatal) and after birth (postnatal). Specifically, the MAAS consists of multiple items that assess dimensions such as the mother's thoughts and feelings about the unborn baby, her sense of closeness to the baby, and her intentions to care for and protect the baby. The MPAS includes items that focus on the mother's feelings of joy and pleasure in her baby, the quality of interaction with the baby, and any feelings of distress or annoyance to the baby. In both scales, responses are typically rated on a Likert scale, with higher scores indicating stronger and more positive maternal attachment.

*Well-Being Index* (*WHO-5*, 1998 version; Topp et al., 2015): a brief, self-reported questionnaire designed to assess an individual's subjective psychological well-being. It consists of five statements about positive mood, vitality, and general interest in daily activities over the past two weeks. Each item is rated on a 6-point Likert scale

ranging from 0 (at no time) to 5 (all of the time), resulting in a total score ranging from 0 to 25. Higher scores indicate greater well-being.

*Stress Scale* (Cohen et al., 1983): a widely used self-report questionnaire designed to measure how individuals perceive their lives as stressful. It consists of 10 items that assess how unpredictable, uncontrollable, and overloaded respondents find their lives, focusing on feelings and thoughts during the past month. Each item is rated on a 5-point Likert scale ranging from 0 (never) to 4 (very often), with higher total scores indicating greater stress.

*Mini International Neuropsychiatric Interview* (Sheehan et al., 2000): it is a structured diagnostic interview designed to assess the presence of major psychiatric disorders according to the Diagnostic Statistical Manual-V and the International Classification of Diseases criteria. It consists of a series of standardised questions that screen for a wide range of mental health conditions, including mood disorders, anxiety disorders, substance use disorders, and psychotic disorders, among others.

*Edinburg antenatal and postnatal depression scale* (Cox, Holden, & Sagovsky, 1987): it is a widely used self-report questionnaire designed to screen for symptoms of depression during pregnancy and the postpartum period. It consists of 10 items that assess the severity of depressive symptoms experienced by the respondent over the past seven days, focusing on mood, anxiety, and anhedonia. Each item is rated on a 4-point scale (from 0 to 3), with higher scores indicating more severe depressive symptoms.

*Pittsburgh Sleep Quality Index* (Buysse et al., 1989): a self-report questionnaire designed to measure the quality and patterns of sleep over one month. It assesses seven key components of sleep, including subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications, and

daytime dysfunction. Each component is scored on a scale from 0 to 3, with higher scores indicating poorer sleep quality. The total score ranges from 0 to 21, with a score above five generally indicating significant sleep difficulties.

*Perceived Social Support Scale* (Gregory et al., 1988): it is a self-report questionnaire designed to measure an individual's perception of social support from key sources, including a partner, family, and friends. It consists of 12 items that assess the extent to which individuals feel supported in their social relationships. Each item is rated on a 7-point Likert scale, ranging from 1 (very strongly disagree) to 7 (very strongly agree), with higher scores indicating a greater level of perceived social support.

*Birth Experience Questionnaire* (Saxbe, Taline Horton, & Bryna Tsai, 2018): a self-report instrument designed to assess a mother's perception of her childbirth experience. It evaluates various aspects of the birth, including the level of pain experienced, the quality of support from healthcare providers and family members, the degree of control and involvement in decision-making during childbirth, and overall satisfaction with the birth process. The items are rated on a Likert scale, capturing both positive and negative dimensions of the birth experience.

**Procedure**

Participants were assessed at 5-time points: T1) up to 6 months before pregnancy; T2) 18-21 weeks of pregnancy; T3) 34-36 weeks of pregnancy; T4) during the first month after childbirth; T5) at 6 months following parturition. These assessments consisted of self-report online questionnaires and scales to examine psychological health and maternal attachment and collect demographic, social and obstetric data. The assessment instruments used in the study varied based on the experimental sessions and the participant groups. Across all sessions, mental health

questionnaires and scales were administered to all three participant groups. However, the gestational mothers' group exclusively completed the maternal attachment scales at all assessment points. Additionally, the perceived social support and birth experience questionnaires were administered only to gestational mothers during the postpartum assessment points.

**Statistical analysis**

The present study explored the mechanisms and timing through which emotional well-being and social and obstetric factors impact postnatal maternal attachment. To achieve this main goal, we conducted several analyses.

First, we examined the trajectories of affective symptoms, well-being, and maternal attachment over pregnancy and the postpartum period, as well as the trajectory of perceived social support over the postpartum period. For that, we employed mixed-effects linear regression analyses, using the pre-conception stage as the baseline for comparison. These linear regression analyses enabled us to account for the repeated measures nature of the data, tracking changes from pre-conception through pregnancy and into 6 months postpartum while simultaneously addressing individual variability among participants. The mixed-effects models included fixed effects to estimate the impact of different gestational and postpartum time points on levels of affective symptoms (stress and depressive symptoms), well-being, social support and maternal attachment. The fixed effects allowed us to quantify changes over time relative to the baseline pre-conception stage. The model also incorporated random effects to analyse individual participant differences, including unobserved heterogeneity and to control for within-subject variations across multiple measurements. The random effects improved the accuracy of the estimates by considering how each participant's unique baseline levels and changes could influence the outcome variables. Furthermore, these models

provided key insights into how specific gestational stages might influence the trajectory of affect symptoms and maternal attachment and highlighted specific periods when changes were more pronounced.

As a complementary analysis, we conducted simple effects analyses to examine differences in affective symptoms, well-being, and maternal attachment between mothers who conceived naturally and those who underwent an assisted reproduction method across all time points. These analyses enabled us to explore how conception methods might influence affective symptoms, well-being, and maternal attachment trajectories over pregnancy and postpartum. This approach facilitated estimating and comparing marginal means for affective symptoms, well-being, and maternal attachment at each time point while accounting for individual variability and controlling for potential confounding factors: age, time searching for pregnancy and number of babies. Within-group comparisons tracked changes over time within each conception group, identifying critical stages where affective symptoms, well-being, or maternal attachment significantly increased or decreased relative to other time points. Additionally, between-group contrasts enabled pairwise comparisons at each gestational and postpartum stage to identify significant differences in the trajectories of these variables between naturally conceiving mothers and those using assisted reproduction.

We then examined the predictive effects of affective symptoms, well-being, social support and birth experience on maternal attachment over the postpartum period. Before assessing the predictors of maternal attachment, we explored the interrelations between the predictive factors to identify their dynamics and power for influencing maternal postnatal attachment. So, we examined whether social support and birth experience independently predicted changes in affective symptoms and well-being across the postpartum period through mixed-effects linear regression models.

Additionally, we examined whether the strength of these relationships varied between early and mid-postpartum stages by adding an interaction term between social support and postpartum time (4 weeks vs. 6 months postpartum). Using mixed-effects models allowed us to account for fixed effects (such as the influence of social support on affective symptoms) and random effects (such as individual differences among mothers).

After examining the interplay between the expected predictors of maternal attachment, we conducted mixed-effects linear regression models to assess the predictive independent effects of affective symptoms, well-being, social support, and birth experience on maternal attachment over the postpartum period: 4 weeks and 6 months postpartum. These models included fixed effects to assess the effect of, for example, stress on maternal attachment. The models also incorporated random effects to examine individual differences among mothers. This approach allowed for control of repeated measures within individuals and captured variability due to unique characteristics or unobserved factors. Additionally, the model explored the interaction of the predictors with the postpartum time points to affect maternal attachment. Examining the interaction effects enables us to explore whether the strength or direction of these associations varied over the postpartum time. These mixed-effects models with time interaction provided a more detailed picture of the progress of these effects across the postpartum period.

The preliminary findings that indicated the distinct social support sources independently and strongly predicted affective symptoms and maternal attachment led us to investigate the differential effects of distinct social support sources—partner, family, and friends—on affective symptoms and maternal attachment during the postpartum period. For this investigation, we conducted multiple regression analyses at

two specific time points: 4 weeks postpartum and 6 months postpartum. We conducted multiple regression analyses for each postpartum time point separately to prevent multicollinearity caused by the addition of postpartum time as an interaction factor. This independent approach provided a more precise depiction of the distinct contributions of each social support source at each postpartum stage, as we previously observed that the predictive effects of social support were more potent at 4 weeks postpartum. In contrast, their effects were less pronounced at 6 months postpartum. Thus, conducting separate regression analyses allowed us to compare the predictive strength of social support sources at each postpartum time point and to explore potential differences in their effects between the early and later postpartum periods.

Finally, we conducted cross-sectional mediation analyses at 4 weeks postpartum to approach the main study goal. These mediation analyses examined whether the observed substantial impacts of social support and birth experience on maternal attachment were direct or indirect through the mediating effect of stress or depressive symptoms. We selected the 4-week postpartum time point because it was when the strongest associations occurred. For each mediation model, the independent variable was either social support or birth experience measured at 4 weeks, the dependent variable was the maternal attachment, and the mediators were either stress or depressive symptoms. The mediation models provided estimates of both direct and indirect effects. The direct effects represented the influence of each social support (family and friends) on maternal attachment without considering the mediators. The indirect effects captured how social support or birth experience influenced maternal attachment through the mediation of stress or depression symptoms. These mediation analyses used a nonparametric bootstrapping approach with 2000 resamples to obtain confidence intervals of the indirect effects and improve the reliability of the mediation's estimates.

# Results

## Changes in Affective Symptoms, Well-being, Maternal Attachment and Social Support from Pre-conception and into the Postpartum Period

### *Stress and Depressive Symptoms*

Gestational mothers experienced a decrease in their depressive symptoms during pregnancy. Specifically, depression significantly decreased at 18-22 weeks (*Est.* = -0.85, 95% *CI* [-1.33, -0.37], *SE* = 0.24, *t* (712.66) = -3.59, $p < .001$) and at 34-36 weeks (*Est.* = -1.37, 95% *CI* [-1.85, -0.89], *SE* = 0.24, *t* (712.66) = -5.78, $p < .001$) compared to pre-conception, while stress did not significantly change. However, gestational mothers manifested a significant increase in their stress and depressive symptoms in the postpartum period. Stress increased significantly at 4 weeks postpartum (*Est.* = 3.94, 95% *CI* [2.46, 5.42], *SE* = 0.76, *t* (711.02) = 5.19, $p < .001$) and remained high at 6 months postpartum compared to pre-conception (*Est.* = 2.58, 95% *CI* [1.09, 4.07], *SE* = 0.76, *t* (711.02) = 3.39, $p < .001$). Depressive symptoms followed a similar pattern, with a significant increase at 6 months postpartum compared to pre-conception (*Est.* = -0.18, 95% *CI* [-0.32, -0.04], *SE* = 0.07, *t* (678.32) = -2.61, $p = .01$), (see Figures 1 and 2).

A simple effects analysis also indicated that stress and depression were significantly higher at 4 weeks postpartum compared to 18-22 weeks ($M_{diff.}$ = -5.34, *SE* = 0.76, *t* (711) = -7.01 $p < .000$; $M_{diff.}$ = -0.26, *SE* = 0.06, *t* (679) = -3.82, $p = .001$) and compared to 34-36 weeks ($M_{diff.}$ = -3.49, *SE* = 0.76, *t* (712) = -4.57, $p = .000$; $M_{diff.}$ = -0.24679, *SE* = 0.0694, *t* (679) = -3.558, $p = .0037$). At 6 months postpartum, stress was still significantly higher than at 18-22 weeks ($M_{diff.}$ = -3.98, *SE* = 0.76, *t* (711) = -5.22, *p*

< .000) and at 34-36 weeks ($M_{diff.}$ = -2.12, $SE$ = 0.76, $t$ (712) = -2.78, $p$ = .043) (see Supplementary Table 1).

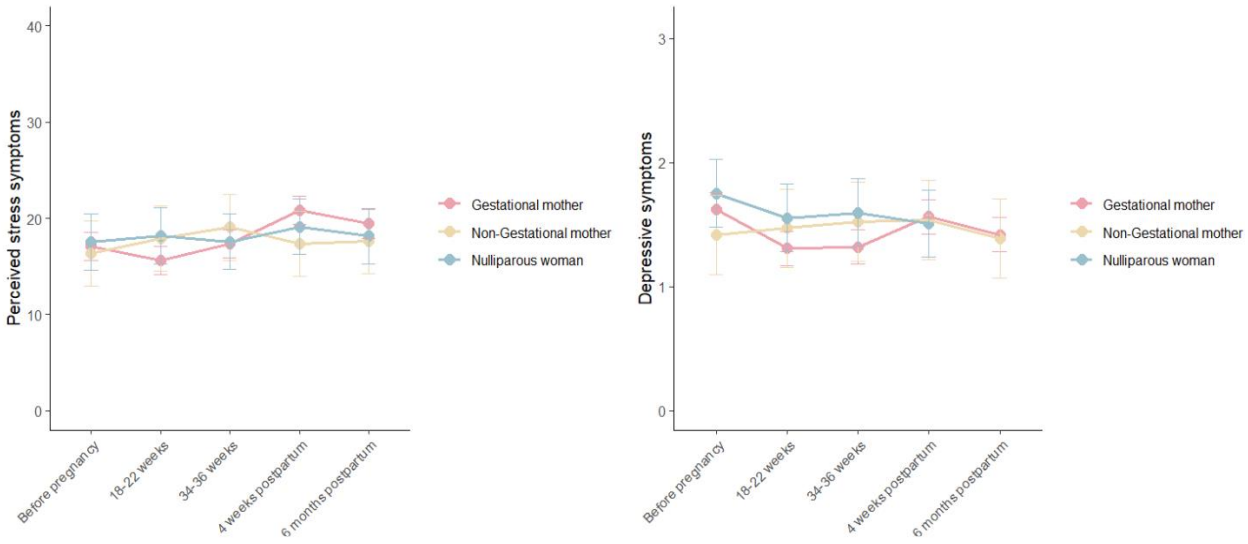
Non-gestational mothers did not experience significant changes in stress or depressive symptoms during their partner's pregnancy and postpartum period (see Figure 1 and 2 and Supplementary Table 2) (see Figure 1 and Supplementary Table 2). Moreover, non-gestational mothers displayed a tendency for depressive symptoms to increase over the postpartum period, but these changes were not statistically significant (see Figure 1 and Supplementary Table 2).

Nulliparous women did not experience any significant changes in stress or depressive symptoms over pregnancy or at 4 weeks postpartum in the group of mothers (see Figure 1 and Supplementary Table 3). It is important to note that the measures of depressive symptoms for nulliparous women at 6 months postpartum were excluded from the analysis due to data collection issues that affected the reliability of these values.

In sum, gestational mothers experienced an amelioration of their stress and depressive symptoms during pregnancy, whereas they manifested an aggravation of their affective symptoms during the postpartum period. Meanwhile, non-gestational mothers and nulliparous women did not experience any significant changes.

**Figure 1**

*Stress and depression over pregnancy and postpartum across all groups*




### *Well-being and Sleep Quality*

Aligned with the affective symptoms' trajectories, gestational mothers tended to improvement in well-being at 18-22 weeks (*Est.* = 0.69, 95% *CI* [-0.02, -1.40], *SE* = 0.37, *t* (724.78) = 1.87, *p* = 0.06), but this trend reversed, with a significant worsening in well-being at 4 weeks postpartum (*Est.* = -1.99, 95% *CI* [-2.71, -1.27], *SE* = 0.37, *t* (711.02) = -5.46, *p* < .001) compared to pre-conception (see Figure 4). The simple effects analysis showed a significant worsening of well-being at 4 weeks postpartum compared to 18-22 weeks ($M_{diff.}$ = 2.65, *SE* = 0.37, *t* (712) = 7.23, *p* < .0001) and compared to 34-36 weeks ($M_{diff.}$ = 2.03, *SE* = 0.37, *t* (712) = 5.52, *p* < .0001). Well-being remained significantly worse at 6 months postpartum than at 18-22 weeks ($M_{diff.}$ = 1.24, *SE* = 0.37, *t* (712) = 3.39, *p* = .0066). However, it improved slightly from 4 weeks to 6 months postpartum ($M_{diff.}$ = -1.40, *SE* = 0.37, *t* (711) = -3.85, *p* = .0012) (see Supplementary Table 1).

Neither non-gestational mothers nor nulliparous women presented any significant changes in their well-being (see Figure 2 and Supplementary Tables 2 & 3).

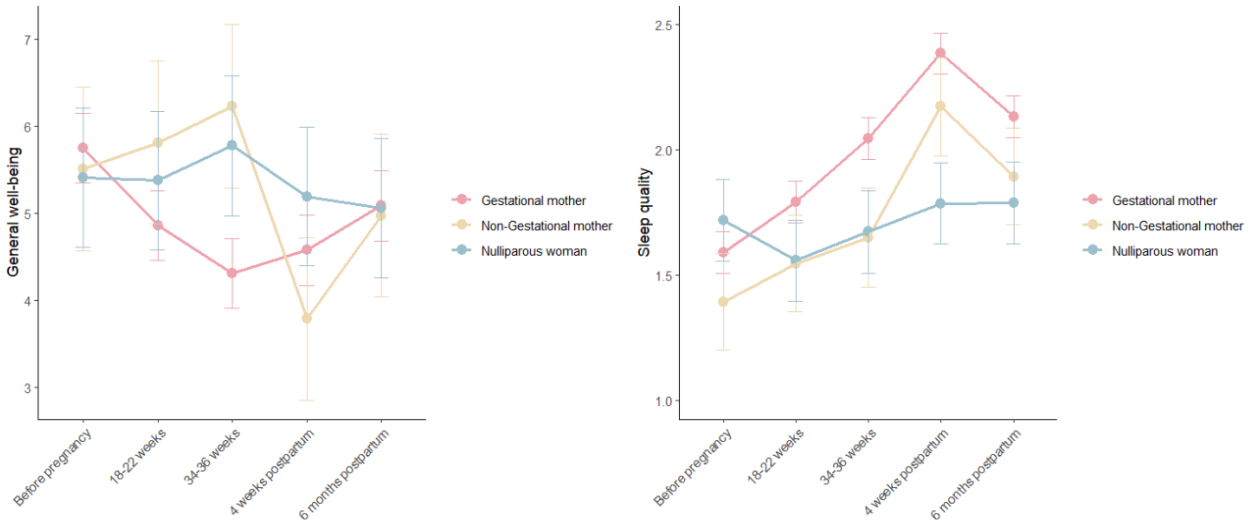
Regarding sleep quality, gestational mothers exhibited a progressive worsening beginning at 18-22 weeks (*Est.* = -0.20, 95% *CI* [-0.29, -0.11], *SE* = 0.05, *t* (700.48) = -4.20, $p < .001$), which worsened further at 34-36 weeks (*Est.* = -0.46, 95% *CI* [-0.56, -0.36], *SE* = 0.05, *t* (701.48) = -9.52, $p < .001$) and reached its lowest point at 4 weeks postpartum (*Est.* = -0.79, 95% *CI* [-0.89, -0.69], *SE* = 0.05, *t* (700.48) = -16.64, $p < .001$). By 6 months postpartum, sleep quality had slightly improved (*Est.* = -0.54, 95% *CI* [-0.64, -0.44], *SE* = 0.05, *t* (699.88) = -11.41, $p < .001$) but remained significantly worse than pre-conception levels (see Figure 2). Simple effects analysis indicated that sleep quality worsened significantly at 34-36 weeks compared to 18-22 weeks ($M_{diff.}$ = -0.26, *SE* = 0.04, *t* (700) = -5.35, $p < .000$) and continued to worsen at 4 weeks postpartum compared to 18-22 weeks ($M_{diff.}$ = -0.59, *SE* = 0.04, *t* (700) = -12.471, $p < .000$). However, it improved significantly from 4 weeks to 6 months postpartum ($M_{diff.}$ = 0.25, *SE* = 0.04, *t* (700) = 5.286, $p < .000$), (see Supplementary Table 1).

Non-gestational mothers presented a significant worsening of their sleep quality at 4 weeks postpartum compared to pre-conception ($M_{diff.}$ = -0.78, *SE* = 0.11, *t* (702) = -6.973, $p < .001$), to 18-22 weeks ($M_{diff.}$ = -0.62, *SE* = 0.11, *t* (702) = -5.60, $p < .000$), and to 34.36 weeks ($M_{diff.}$ = -0.52, *SE* = 0.11, *t* (699) = -4.64, $p < .000$). Although their sleep quality shows an improvement trend, it remains significantly worse than pre-conception ($M_{diff.}$ = -0.50, *SE* = 0.11, *t* (699) = -4.53, $p < .000$) and 34-36 weeks ($M_{diff.}$ = -0.52, *SE* = 0.11, *t* (699) = -4.64, $p < .000$), (see Figure 2 and Supplementary Table 2). Nulliparous women did not experience any significant changes in their sleep quality over time (see Figure 2 and Supplementary Table 3).

Overall, well-being and sleep quality exhibited similar trajectories in gestational mothers, who experienced decreasing well-being and worsening sleep quality from late pregnancy to early postpartum and only slightly recovered at 6 months postpartum. Attuned with their partners, non-gestational mothers reported worsening their sleep quality over the postpartum period, although their well-being did not worsen. By contrast, nulliparous women did not present significant deviations in their well-being or sleep quality over time.

**Figure 2**

*Well-being and sleep quality over pregnancy and postpartum across all groups*

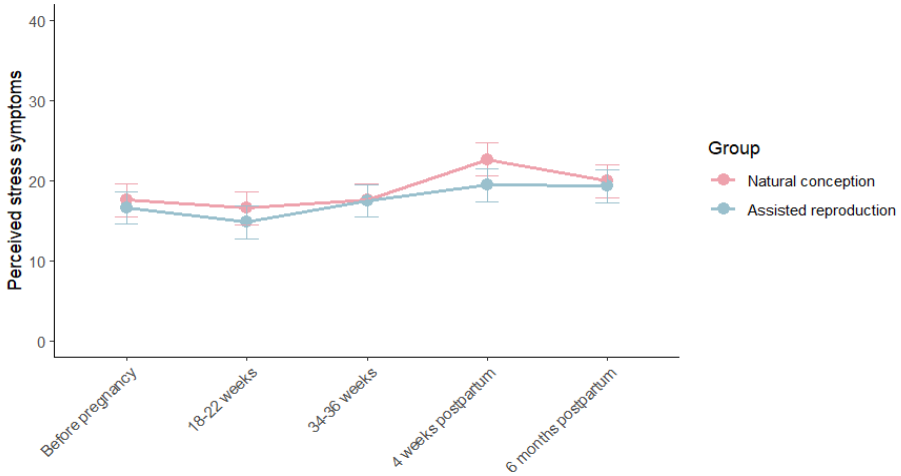


***Affective Symptoms between Natural Conception and Assisted Reproduction Groups over Pregnancy and Postpartum***

Mothers who conceived naturally reported significantly higher stress levels compared to mothers who used assisted reproduction methods in the first month postpartum *($M_{diff.}$* = 3.17, *SE* = 1.48, *t* (332) = 2.14, *p* = .03). At the same time, no significant differences were observed at the other gestational stages (see Figure 3 and Supplementary Table 4). No significant differences between groups were observed in depression, sleep quality or well-being (see Supplementary Figures 1-3 and Supplementary Tables 4-7),

**Figure 3**

*Comparisons in stress between the natural conception and the assisted reproduction groups over pregnancy and at postpartum*

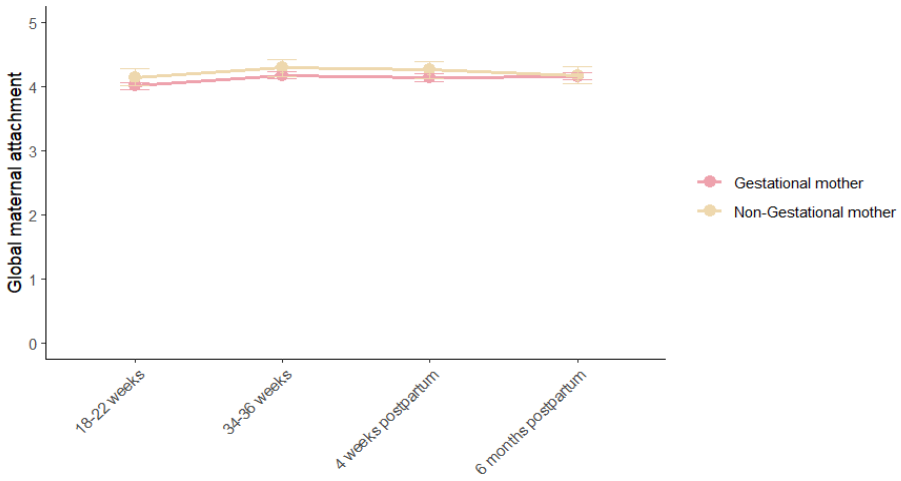


### *Global Maternal Attachment*

Gestational mothers experienced a significant strengthening of their global maternal attachment at 34-36 weeks of pregnancy (*Est.* = 0.17, 95% *CI* [0.11, 0.22], *SE* = 0.03, *t* (437.48) = 5.51, $p < .001$), compared to the baseline maternal attachment measure collected at 18-22 weeks. At 4 weeks postpartum, the mothers' global maternal attachment remained significantly stronger (*Est.* = 0.13, 95% *CI* [0.77, 0.19], *SE* = 0.03, *t* (437.17) = 4.36, $p < .001$) compared to 18-22 weeks. By 6 months postpartum, global maternal attachment remained significantly stronger (*Est.* = 0.15, 95% *CI* [0.10, 0.21], *SE* = 0.03, *t* (438.29) = 5.06, $p < .001$) than at 18-22 weeks (see Figure 4 and Supplementary Table 1 & 2). In contrast, non-gestational mothers showed no significant changes in either global maternal attachment or the quality of their maternal attachment at 34-36 weeks of pregnancy, 4 weeks postpartum, or 6 months postpartum compared to 18-22 weeks of pregnancy (See Figure 3 and Supplementary Tables 1-2)

**Figure 4**

*Global maternal attachment over pregnancy and postpartum in gestational and non-gestational mothers*

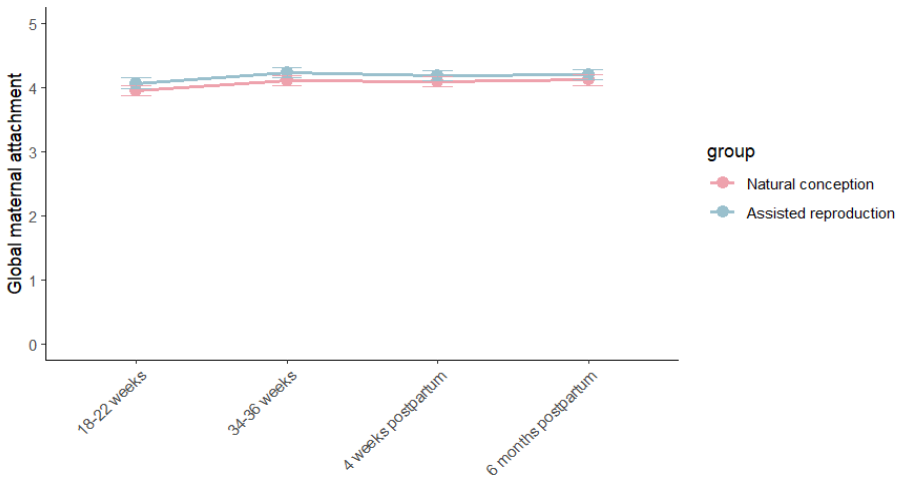



***Maternal Attachment between Natural Conception and Assisted Reproduction Groups over Pregnancy and Postpartum***

Mothers who conceived naturally reported slightly lower levels of global maternal attachment compared to mothers who used assisted reproduction methods at 18-22 weeks gestation ($M_{diff.}$ = -0.12, $SE$ = 0.0579, $t$ (299) = -2.05, $p$ = .04). At 34-36 weeks gestation, mothers from the natural conception group also referred weaker global maternal attachment ($M_{diff.}$ = -0.13, $SE$ = 0.0581, $t$ (301) = -2.17, $p$ = .03). In contrast, no significant differences were observed at 4 weeks postpartum or 6 months postpartum (see Figure 5 and Supplementary Table 3).

**Figure 5**

*Global maternal attachment between natural conception and assisted reproduction groups over pregnancy and postpartum*




***Perceived Social Support***

At 6 months postpartum, gestational mothers exhibited significant decreases in their global perceived social support, as well as their perceived social support from their partner, family, and friends, compared to 4 weeks postpartum (*Est.* = -0.28, *SE* = 0.09, *t* (146.55) = -2.97, *p* = 0.00; *Est.* = -0.26, *SE* = 0.09, *t* (146.43) = -2.96, *p* = 0.00; *Est.* = -0.34, *SE* = 0.11, *t* (146.53) = -3.18, *p* = 0.00; *Est.* = -0.36, *SE* = 0.11, *t* (146.56) = -3.40, *p* = 0.00, respectively) (see Figures 4-5 and Supplementary Table 1). In contrast, non-gestational mothers did not experience any significant changes in their different perceived social supports during the postpartum period, except for an increase in support from their partner at 6 months postpartum (*Est.* = 0.66, *SE* = 0.23, *t* (146.10) = 2.90, *p* = 0.00) (see Figures 6-7 and Supplementary Table 2).

Overall, gestational mothers reported lower overall social support, including lower support from their partner, family and friends at 6 months postpartum compared to 4 weeks postpartum, while non-gestational mothers did not report significant changes during the same period, except an increase in their partner support.

**Figure 6**

*Global perceived social support and support from partner over postpartum in gestational and non-gestational mothers*

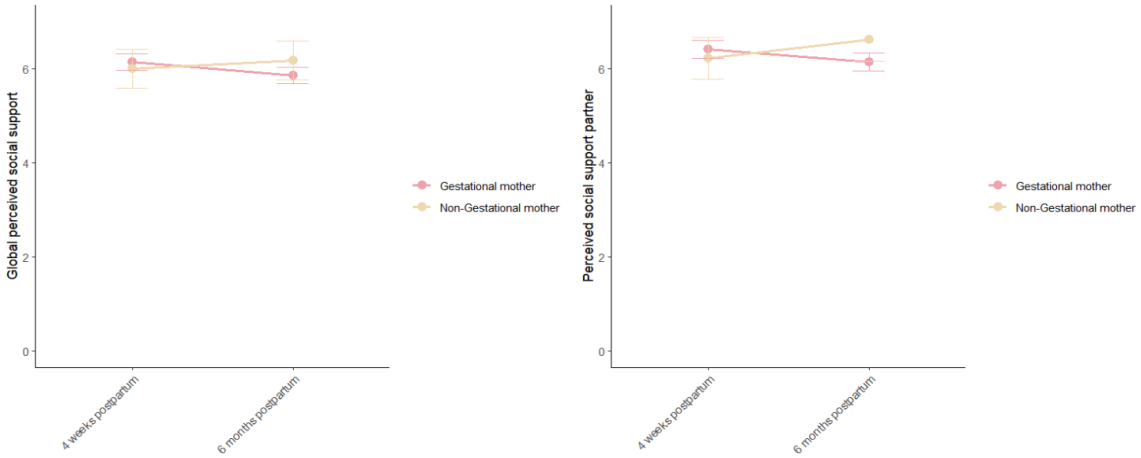
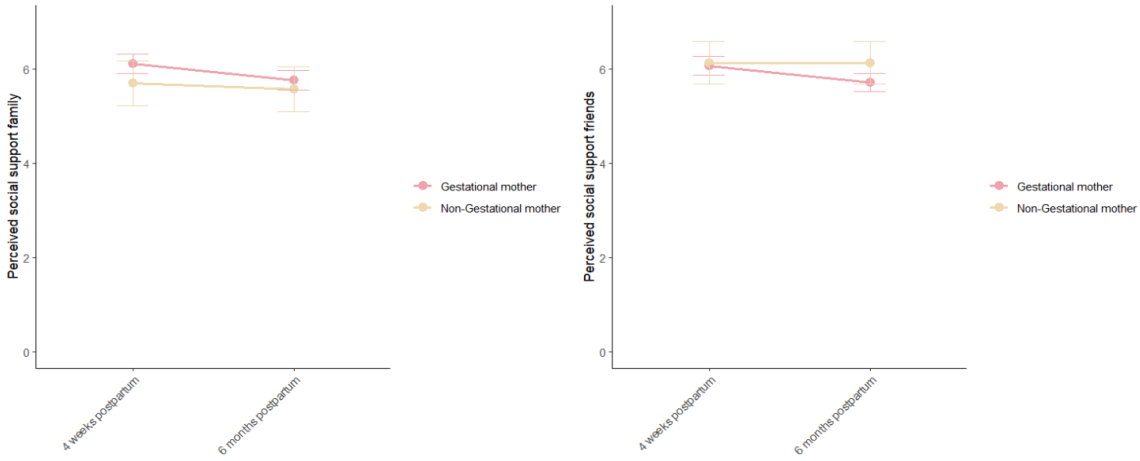


**Figure 7**

*Perceived social support from family and friends over postpartum in gestational and non-gestational mothers*




***Summary***

Gestational mothers showed significant changes in stress, depressive symptoms, well-being, and sleep quality from pregnancy through the postpartum period. Initially, the mothers' stress and depressive symptoms decreased at mid-pregnancy but increased significantly at 4 weeks postpartum and remained high at 6 months. In line with the affective symptoms' deviations, gestational mothers' well-being slightly improved at mid-pregnancy, and their sleep quality was generally good. Still, their well-being and sleep quality steadily worsened from late pregnancy and were at their worst at 4 weeks postpartum, though there were some improvements by 6 months postpartum. Non-gestational mothers did not show significant changes in stress or depressive symptoms, though their sleep quality worsened significantly at 4 weeks postpartum. Nulliparous women did not display significant changes in stress, depressive symptoms, well-being, or sleep quality.

Regarding maternal attachment, gestational mothers consistently strengthened their mother-to-infant bond starting in late pregnancy, with further improvement at 4 weeks and remaining strong at 6 months postpartum. Non-gestational mothers, however, showed minimal changes. Gestational mothers exhibited more consistent and pronounced developments compared to non-gestational mothers.

Social support patterns varied, with gestational mothers experiencing significant declines by 6 months postpartum, while non-gestational mothers generally maintained stable or slightly improved levels.

**The Impact of Affective Symptoms and Well-being on Global Maternal Attachment**

Building on these initial findings, we aimed to investigate whether the observed deterioration in mothers' emotional well-being had a measurable impact on the development of maternal attachment during the postpartum period.

By 4 weeks postpartum, global maternal attachment in gestational mothers was negatively influenced by stress (*Est.* = -0.02, 95% *CI* [-0.03, -0.01], *SE* = 0.00, *t* (221.09) = -7.84, $p < .001$), depression (*Est.* = -0.21, 95% *CI* [-0.27, -0.15], *SE* = 0.03, *t* (203.64) = -6.86, $p < .001$), and sleep quality (*Est.* = -0.30, 95% *CI* [-0.46, -0.14], *SE* = 0.08, *t* (182.68) = -3.56, $p < .001$). By 6 months postpartum, only the effects of stress (*Est.* = -0.15, 95% *CI* [-0.29, -0.01], *SE* = 0.07, *t* (136.05) = -2.12, $p = .04$) and depression (*Est.* = -0.21, 95% *CI* [-0.33, -0.09], *SE* = 0.06, *t* (133.04) = -3.62, $p < .001$) remained significant, though reduced in strength. However, the effects of sleep quality were no longer significant. This reduction in impact over time was significant for stress

(*Est.* = 0.01, 95% *CI* [0.00, 0.02], *SE* = 0.00, *t* (139.82) = 2.22, *p* = .03) and depression (*Est.* = 0.14, 95% *CI* [0.07, 0.21], *SE* = 0.04, *t* (137.06) = 3.94, *p* < .001) (see Figures 8-9 and Supplementary Table 9).

By contrast, well-being in gestational mothers had a positive effect on their global maternal attachment at 4 weeks postpartum (*Est.* = 0.05, 95% *CI* [0.04, 0.06], *SE* = 0.01, *t* (230.53) = 9.74, *p* < .001), whereas this effect weakened and lost significance at 6 months postpartum (see Figure 9 and Supplementary Table 9).

**Figure 8**

*Impact of stress and depression on global maternal attachment at postpartum*

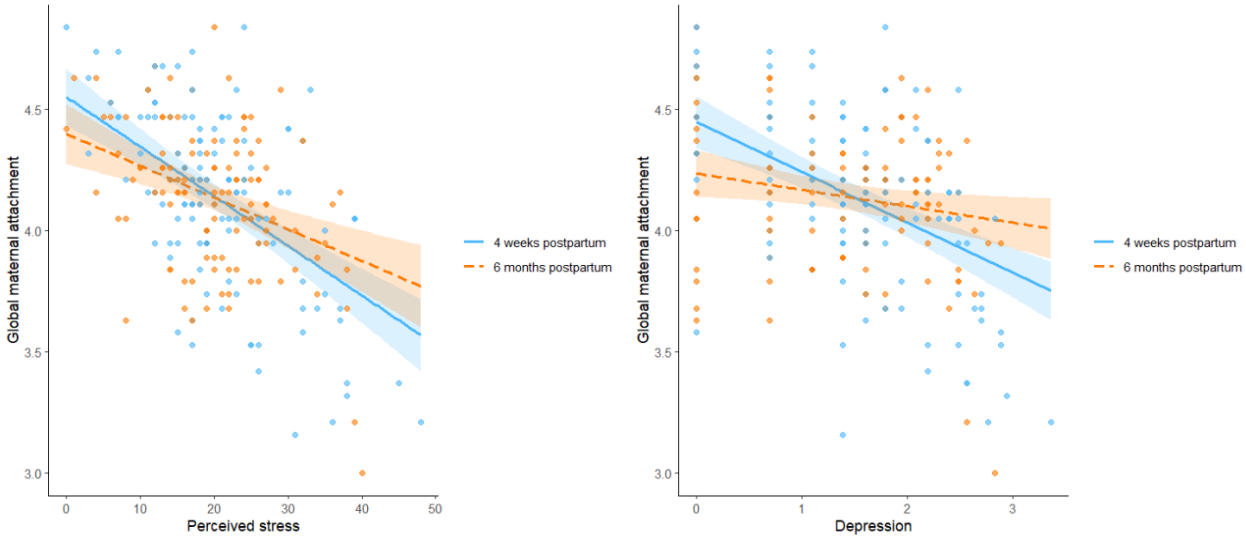
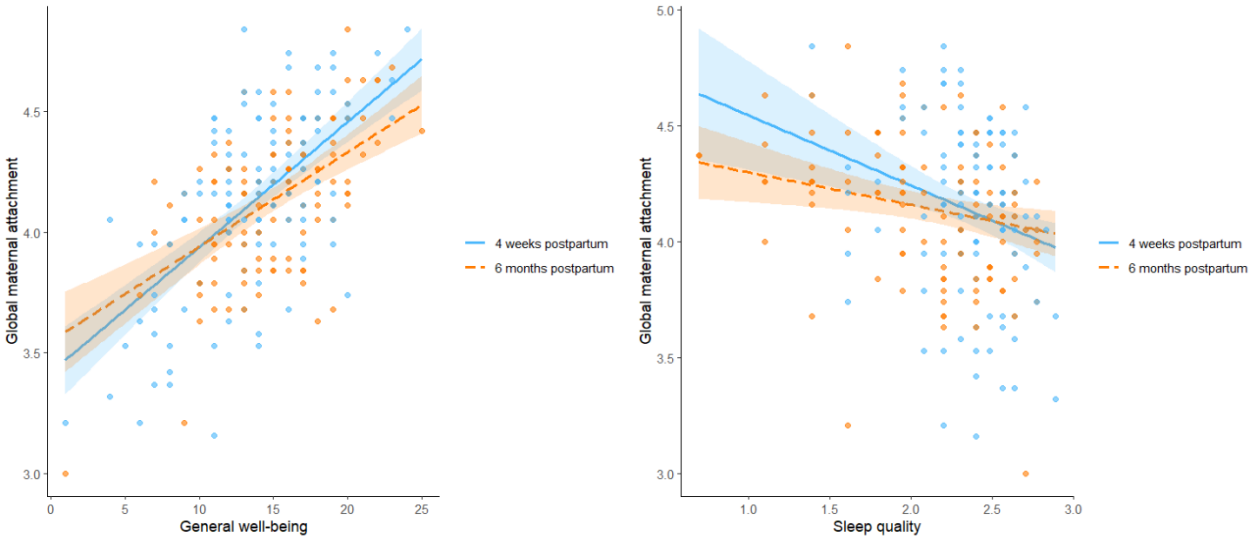


**Figure 9**

*Impact of well-being and sleep quality on global maternal attachment at postpartum*




***Summary***

Overall, maternal attachment in gestational mothers was negatively influenced by stress, depressive symptoms, and poor sleep quality at 4 weeks postpartum, while these effects weakened at 6 months postpartum. By contrast, general well-being had a positive impact, while its effect diminished at 6 months postpartum.

**The Impact of Perceived Social Support on Affective Symptoms and Maternal Attachment**

***Stress***

By 4 weeks postpartum, stress symptoms in gestational mothers were significantly reduced by global perceived social support (*Est.* = -3.03, 95% *CI* [-4.55, -1.51], *SE* = 0.77, *t* (219.02) = -3.96, $p < .001$), support from partner (*Est.* = -1.57, 95%

*CI* [-2.93, -0.21], *SE* = 0.69, *t* (239.77) = -2.26, *p* = .02), support from family (*Est.* = -2.47, 95% *CI* [-3.77, -1.17], *SE* = 0.66, *t* (222.23) = -3.75, *p* < .001), and from friends (*Est.* = -3.35, 95% *CI* [-4.67, -2.03], *SE* = 0.67, *t* (216.41) = -4.97, *p* < .001). By 6 months postpartum, however, stress was ameliorated by an even stronger effect of social support from friends (*Est.* = -12.43, 95% *CI* [-21.16, -3.70], *SE* = 4.42, *t* (162.28) = -2.81, *p* = .01), whose effect significantly increased over time (*Est.* = 1.71, 95% *CI* [0.27, 3.15], *SE* = 0.73, *t* (161.58) = 2.33, *p* = .02), (see Figures 10-11 and Supplementary Table 10).

Overall, perceived social support significantly ameliorated stress at 4 weeks postpartum, while its soothing effect diminished by 6 months postpartum.

**Figure 10**

*The impact of global perceived social support and support from partners on stress at postpartum*

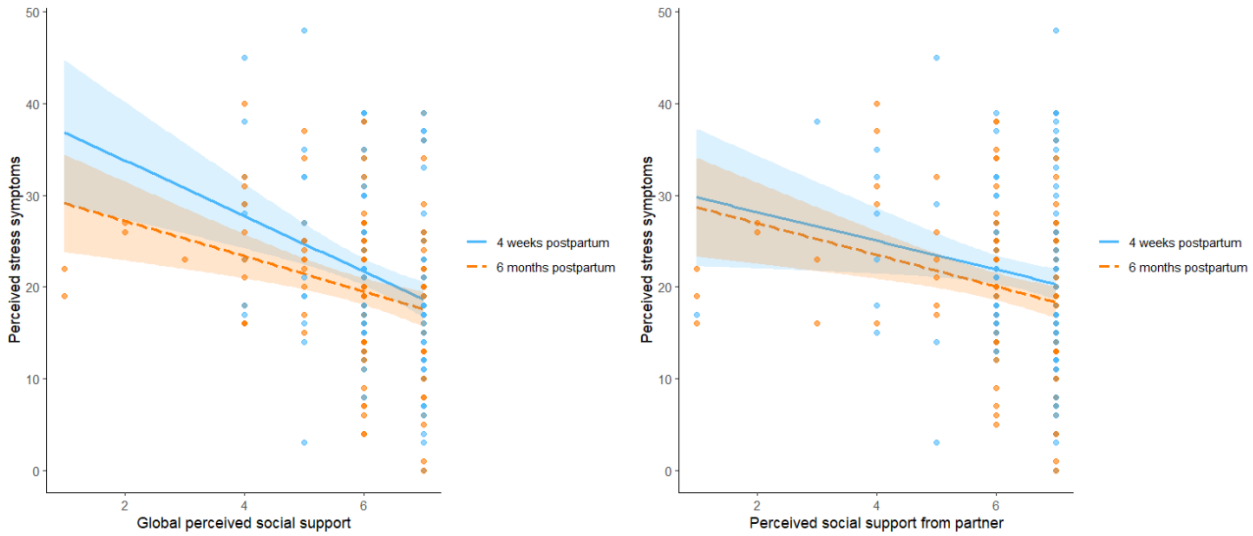
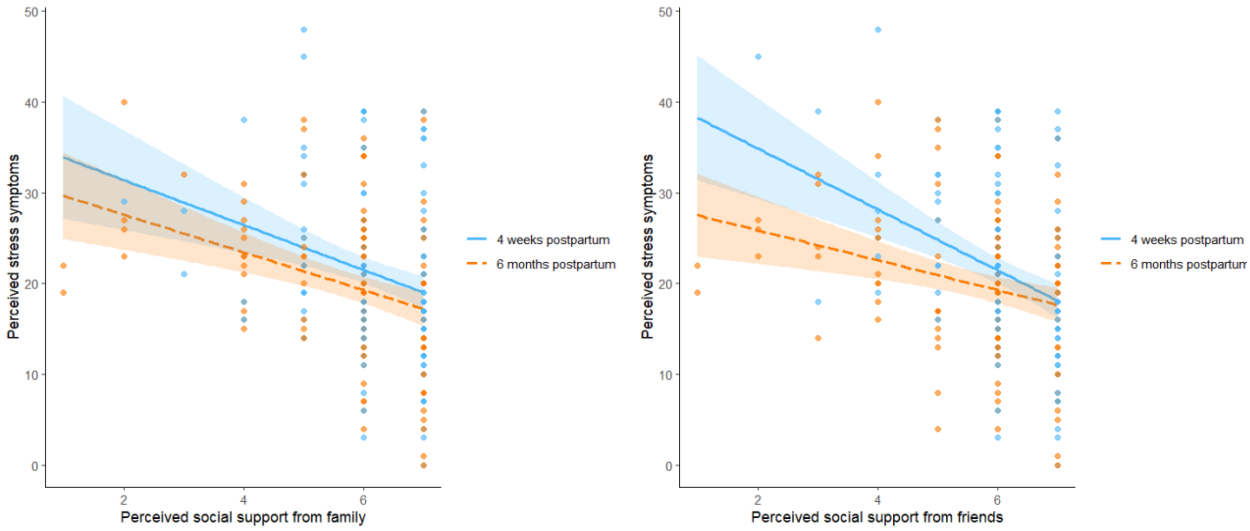


**Figure 11**

*The impact of perceived social support from family and friends on stress at postpartum*




When evaluating the relative strengths of the effects of different sources of social support on stress, the findings revealed a time-sensitive pattern in the influence of social support types. At 4 weeks postpartum, support from friends had a significant negative effect on stress ($\beta$=−3.01, $SE$ = 0.86, $t(161) = -3.51$, $p$= 0.001), accounting for the largest proportion of variance explained ($lmg$=0.09). In contrast, neither family support nor partner support significantly influenced stress at this early stage. Variance inflation factor values ranged from 1.21 to 1.60, confirming no collinearity concerns (see Figure 12 and Supplementary Table 11).

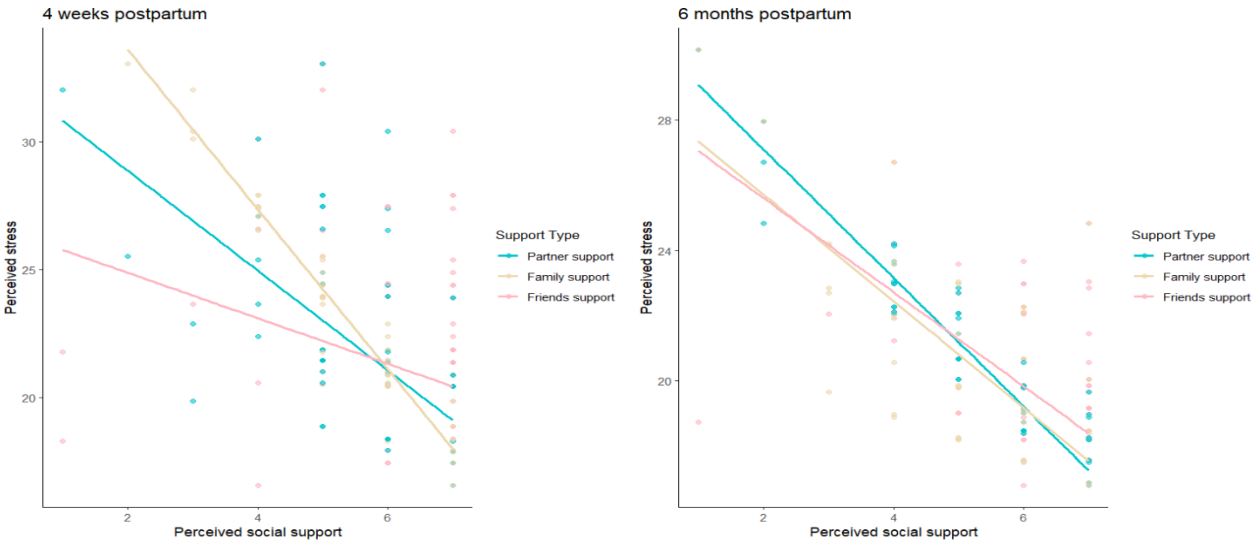
By 6 months postpartum, support from family emerged as a marginally significant predictor of reduced stress ($\beta$= −1.59, $SE$ = 0.81, $t(119) = -1.97$, $p$= 0.05), explaining the largest proportion of variance ($lmg$=0.055). However, the effects of both partner support and friends' support were not significant at this later stage. Variance

inflation factor values ranged from 1.89 to 2.21, again confirming no collinearity concerns (see Figure 12 and Supplementary Table 11).

These results highlight the strong influence of friends' support in mitigating stress at early postpartum, while family support may have a minimal impact at mid-postpartum. Conversely, partner support showed no measurable impact on postpartum stress at either time point, indicating a potentially minimal mitigating role.

**Figure 12**

*The differential effects of support from partner, family and friends on stress over postpartum*




***Depression***

At 4 weeks postpartum, depressive symptoms in gestational mothers were significantly buffered by global perceived social support (*Est.* = -0.15, 95% *CI* [-0.29, -0.01], *SE* = 0.07, *t* (217.40) = -2.14, *p* = .03), family support (*Est.* = -0.18, 95% *CI* [-0.30, -0.06], *SE* = 0.06, *t* (222.07) = -3.06, *p* = .003), and friend support (*Est.* = -0.22, 95% *CI* [-0.34, -0.10], *SE* = 0.06, *t* (219.63) = -3.32, *p* < .001). However, support from

the partner had little effect at this early stage. By 6 months postpartum, these protective effects of global support, family support, and friend support on depressive symptoms had diminished and were no longer significant. Notably, the reduction over time in the effect of family support was statistically significant (*Est.* = -0.14, 95% *CI* [-0.26, -0.01], *SE* = 0.06, *t* (153.67) = -2.11, *p* = .04). In contrast, partner support showed a significant ameliorating effect on depressive symptoms by 6 months postpartum (*Est.* = 0.83, 95% *CI* [0.01, 1.65], *SE* = 0.41, *t* (147.60) = 2.00, *p* = .05), with its impact significantly increasing over time (*Est.* = -0.16, 95% *CI* [-0.28, -0.04], *SE* = 0.06, *t* (146.44) = -2.53, *p* = .01), (see Figures 13-14 and Supplementary Table 12).

**Figure 13**

*The impact of global perceived social support and support from partners on depression at postpartum*

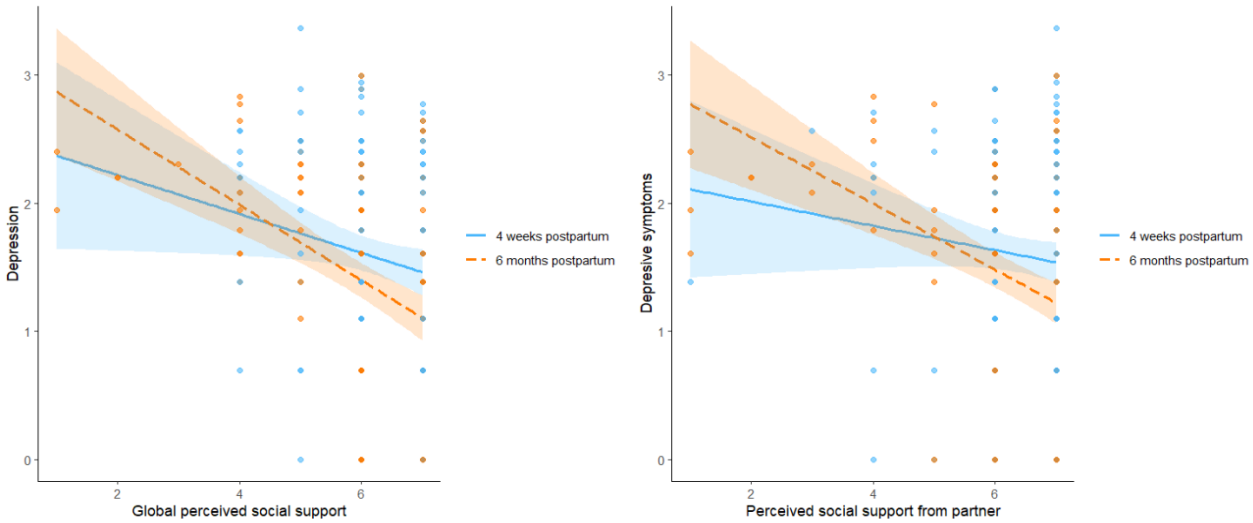
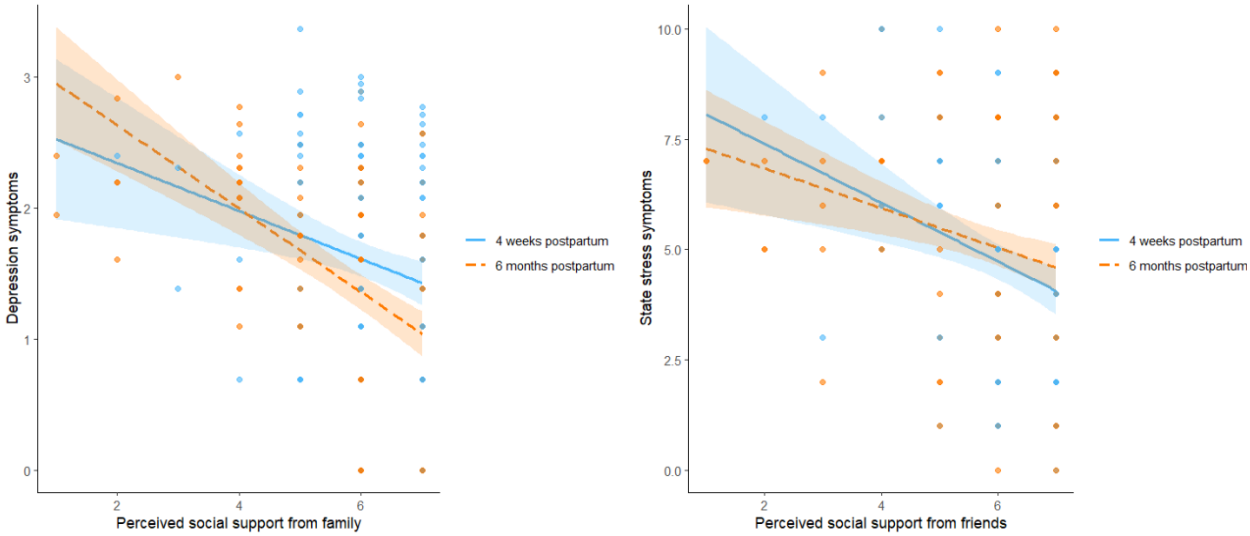


**Figure 14**

*The impact of perceived social support from family and friends on depression at postpartum*




When evaluating the relative strengths of the effects of different sources of social support on depression at 4 weeks postpartum, the results indicated that support from friends had a significant negative effect on depression ($\beta$= −0.26, *SE* = 0.08, *t*(160) = -3.34, *p*= 0.001), accounting for the largest proportion of variance explained (*lmg*= 0.077). In contrast, neither family support ($\beta$= −0.06, *SE* = 0.07, *t*(160)= −0.94, *p*= 0.35) nor partner support ($\beta$= 0.12, *SE* = 0.06, t(160)= 1.80, *p*= 0.07) significantly influenced depression. Variance inflation factor values ranged from 1.21 to 1.60, confirming no multicollinearity concerns (see Figure 15 and Supplementary Table 13).

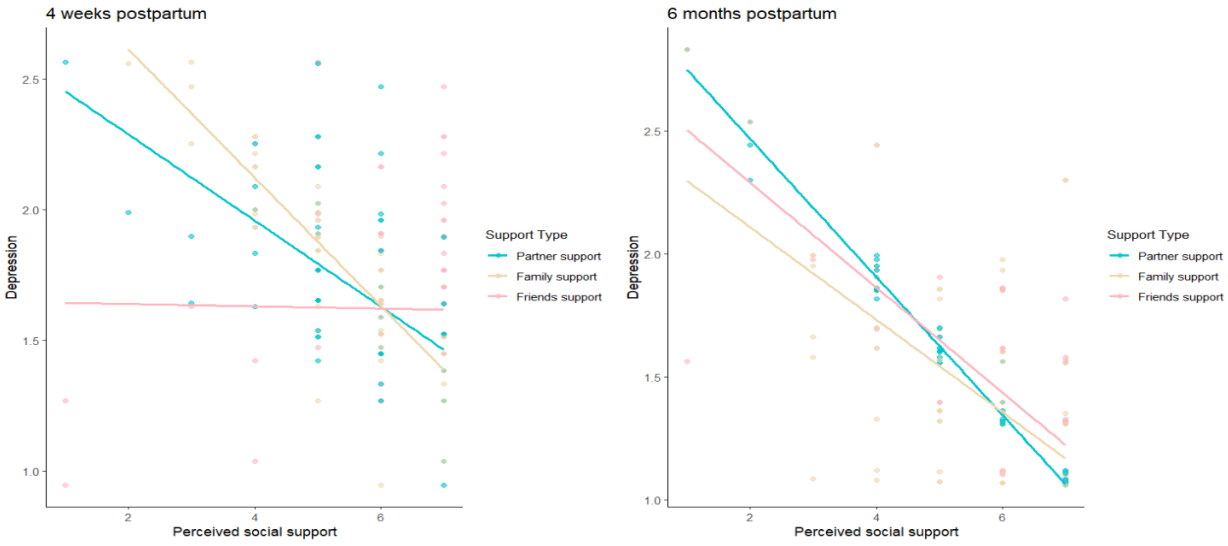
At 6 months postpartum, the results indicated that family support had a significant negative effect on depression ($\beta$= −0.25, *SE* = 0.08, *t*(118) = −3.13, *p*= 0.002), accounting for the largest proportion of variance explained (*lmg*= 0 .115). In contrast, neither partner support nor friends' support significantly influenced depression.

Variance inflation factor values ranged from 1.89 to 2.21, confirming no multicollinearity concerns (see Figure 15 Supplementary Table 13).

These results highlight the strong influence of friends' support in mitigating depression at early postpartum, while family support exerts a substantial buffering effect at mid-postpartum. Conversely, partner support showed no measurable impact on postpartum depression at either time point, indicating a potentially minimal mitigating role.

**Figure 15**

*The differential effects of support from partner, family and friends on depression over postpartum*




***General Well-being***

Well-being in gestational mothers was not influenced by social support over the postpartum period (see Supplementary Figures 4-5 and Supplementary Table 14).

### *Summary*

Social support from partners, family, and friends played a protective role in reducing stress and depressive symptoms during the postpartum period, particularly at 4 weeks postpartum. However, social support did not significantly influence maternal well-being.

At 4 weeks postpartum, support from family and friends emerged as the strongest and most consistent factor in mitigating both stress and depressive symptoms. Meanwhile, partner support showed a measurable effect on stress but not on depression. By 6 months postpartum, the dynamics shifted: the protective influence of friends' support on stress grew stronger, while partner support became a key mitigating factor for depression. In contrast, the effect of family support on depression persisted, though its influence on stress remained minimal.

When examining the differential effects of these sources of support over the postpartum period, a more nuanced picture emerged. Friends' support was the most significant predictor of both stress and depression at 4 weeks postpartum, highlighting its critical role in early maternal mental health. By mid-postpartum, family support had a substantial impact on reducing depressive symptoms. Overall, partner support showed no measurable effect on stress at either point, suggesting a more limited mitigating role.

### *Maternal Attachment*

Perceived social support from different sources enhanced global maternal attachment among gestational mothers over the postpartum period. At 4 weeks postpartum, global maternal attachment in gestational mothers was significantly enhanced by global perceived social support (*Est.* = 0.08, 95% CI [0.02, 0.14], *SE* =

0.03, *t* (196.69) = 2.67, *p* = .01), support from partner (*Est.* = 0.08, 95% CI [0.02, 0.14], *SE* = 0.03, *t* (214.87) = 2.78, *p* = .01), support from family (*Est.* = 0.11, 95% CI [0.06, 0.16], *SE* = 0.03, *t* (195.36) = 4.34, *p* < .001), and by support from friends (*Est.* = 0.07, 95% CI [0.02, 0.12], *SE* = 0.03, *t* (193.71) = 2.53, *p* = .01). However, the enhancing effects of social support diminished and were no longer significant by 6 months postpartum (see Figures 16-17 and Supplementary Table 15). Exceptionally, the positive effect of family support continued to influence global attachment by 6 months postpartum positively, although its effect was weaker (*Est.* = -0.07, 95% CI [-0.13, -0.01], *SE* = 0.03, *t* (140.88) = -2.38, *p* = .02), (see Figures 16-17 and Supplementary Table 15).

**Figure 16**

*The impact of global perceived social support and support from partners on global maternal attachment in gestational mothers at postpartum*

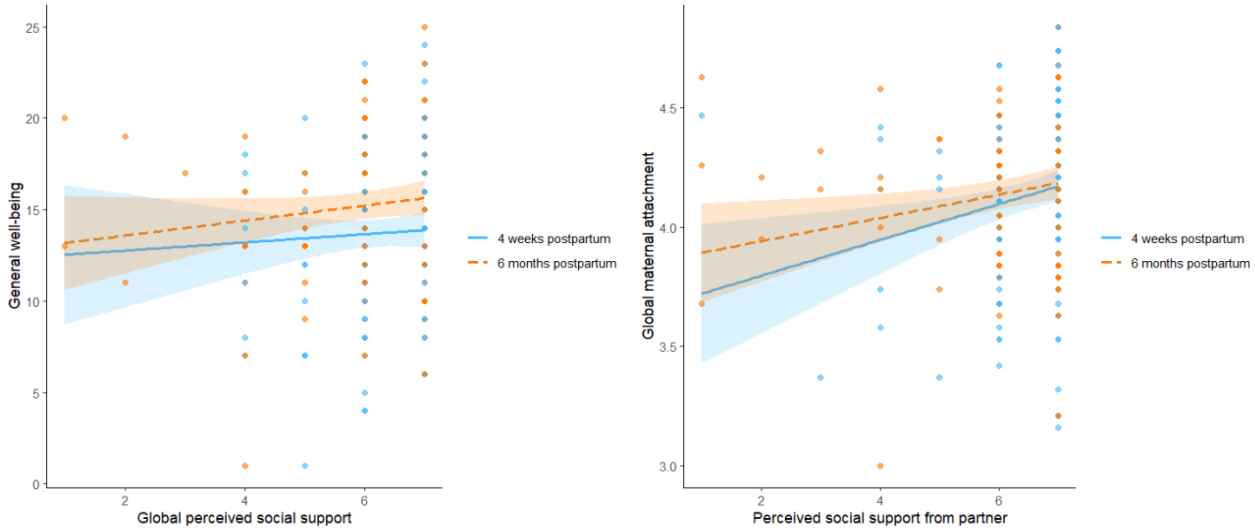
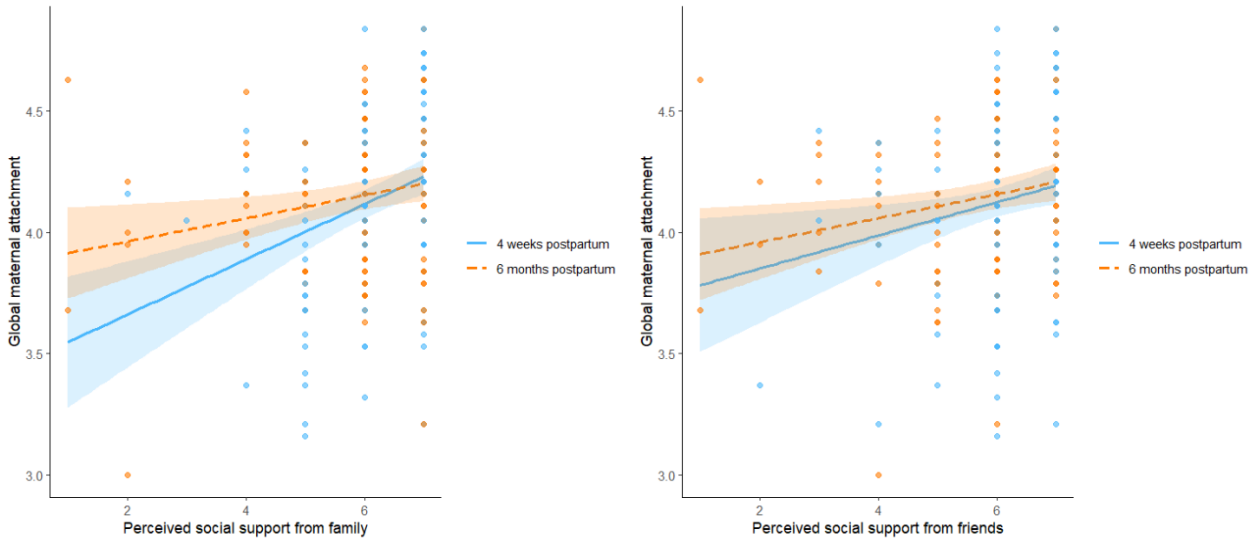


**Figure 17**

*The impact of perceived social support from family and friends on global maternal attachment in gestational mothers at postpartum*



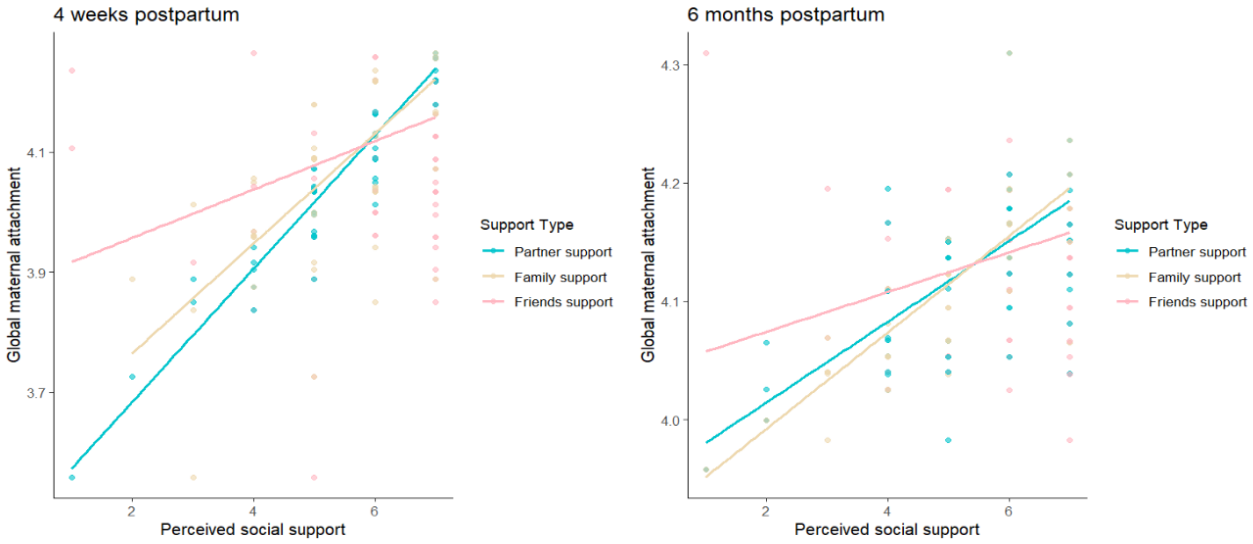

When evaluating the relative effects of social support from partners, family, and friends on global maternal attachment at 4 weeks postpartum, the results revealed a significant positive effect of family support on maternal attachment ($\beta$= 0.09, *SE*= 0.03, *t*(160)=2.76, *p*= 0.00), accounting for the largest proportion of variance explained (*lmg*= 0.06). In contrast, neither partner support nor friends' support significantly predicted maternal attachment. Variance inflation factor values ranged from 1.21 to 1.60, confirming no multicollinearity concerns (see Figure 17 and Supplementary Table 16). By contrast, at 6 months postpartum, none of the social support predictors had significant effects on depression. Variance inflation factor values ranged from 1.89 to 2.21, confirming no multicollinearity concerns (see Figure 18 and Supplementary Table 16).

These findings highlight the importance of family support in strengthening global maternal attachment at 4 weeks postpartum. In contrast, support from partners

and friends showed no measurable impact, and the influence of social support vanished at 6 months postpartum.

**Figure 18**

*The differential effects of support from partner, family and friends on maternal attachment over postpartum*




***Summary***

Global social support from global, support from partners, family, and friends enhanced maternal attachment during the postpartum period, though the effects varied by source over time. By 4 weeks postpartum, global perceived social support and support from partners, family, and friends significantly enhanced global maternal attachment. By 6 months postpartum, the positive effects of global, partner, and friend support on maternal attachment diminished, though family support continued to enhance global attachment. Overall, social support played a significant role in fostering maternal attachment early in the postpartum period, though its influence declined by 6 months postpartum.

Despite these results, a more nuanced picture emerged when exploring the differentiated effects of the distinct social support sources on maternal attachment over the postpartum period. Family support was the only and the most significant predictor of maternal attachment at 4 weeks postpartum, while all sorts of social support effects disappeared at 6 months postpartum.

## The Impact of Birth Experience on Affective Symptoms, Well-being and Maternal Attachment

### *Affective Symptoms and Well-being*

Global birth experience ameliorated affective symptoms and improved well-being over the postpartum period. At 4 weeks postpartum, global birth experience had a significant positive effect on stress (*Est.* = 7.93, 95% *CI* [2.55, 13.31], *SE* = 2.74, *t* = 2.90, *p* = 0.00), and depression (*Est.* = 0.92, 95% *CI* [0.43, 1.41], *SE* = 0.25, *t* = 3.67, *p* = 0.00), as well as a significant negative effect on well-being (*Est.* = -4.62, 95% *CI* [-7.16, -2.08], *SE* = 1.29, *t* = -3.57, *p* = 0.00), indicating that a more negative birth experience was associated with higher levels of stress, state stress, and depression, and lower well-being. By 6 months postpartum, these direct effects were no longer significant for stress (*Est.* = 2.96, 95% *CI* [-3.94, 9.86], *SE* = 3.49, *t* = 0.85, *p* = 0.40), depression (*Est.* = 0.17, 95% *CI* [-0.52, 0.86], *SE* = 0.35, *t* = 0.49, *p* = 0.63), or well-being (*Est.* = -1.21, 95% *CI* [-4.14, 1.72], *SE* = 1.48, *t* = -0.82, *p* = 0.41). The effects between birth experience and the 6-month postpartum session were also not significant for stress (*Est.* = -3.19, 95% *CI* [-8.24, 1.86], *SE* = 2.56, *t* = -1.25, *p* = 0.22), depression (*Est.* = -0.25, 95% *CI* [-0.77, 0.27], *SE* = 0.26, *t* = -0.97, *p* = 0.33), or well-being (*Est.* = 2.07, 95% *CI* [-0.09, 4.23], *SE* = 1.09, *t* = 1.91, *p* = 0.06), suggesting no significant changes in the effect of birth experience on these affective symptoms and well-being,

which remained stable over the postpartum time (see Figures 19-20 and Supplementary Table 17).

**Figure 19**

*The impact of global birth experience on stress and depression at postpartum*

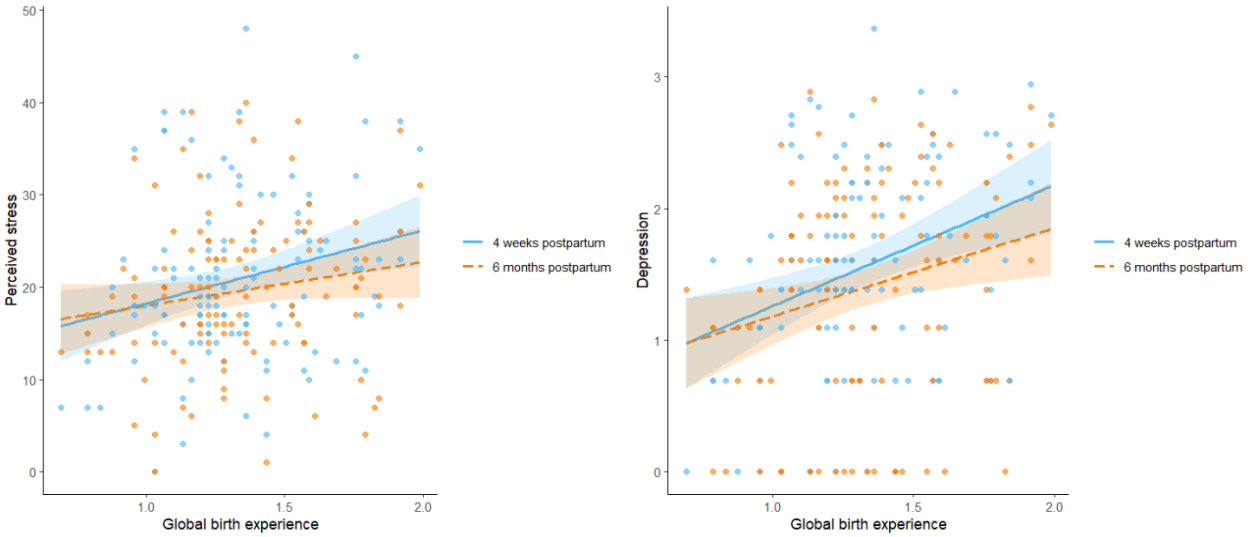
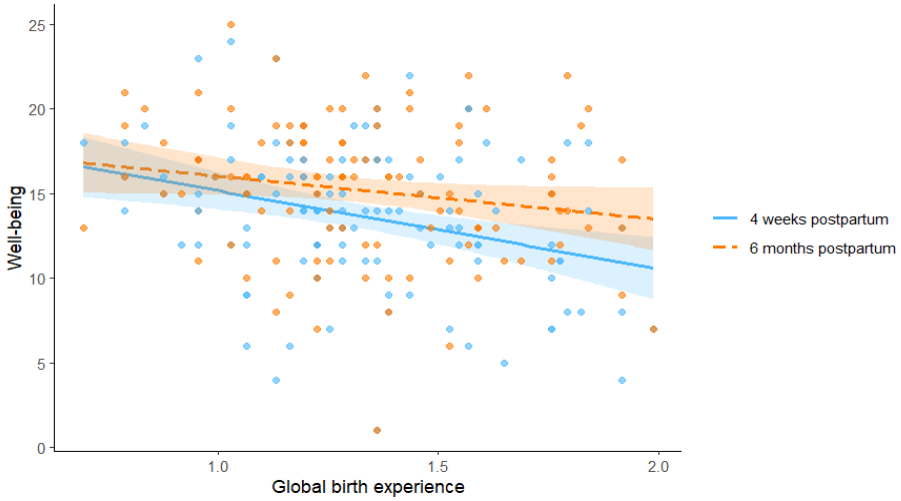



**Figure 20**

*The effect of global birth experience on well-being at postpartum*



### *Summary*

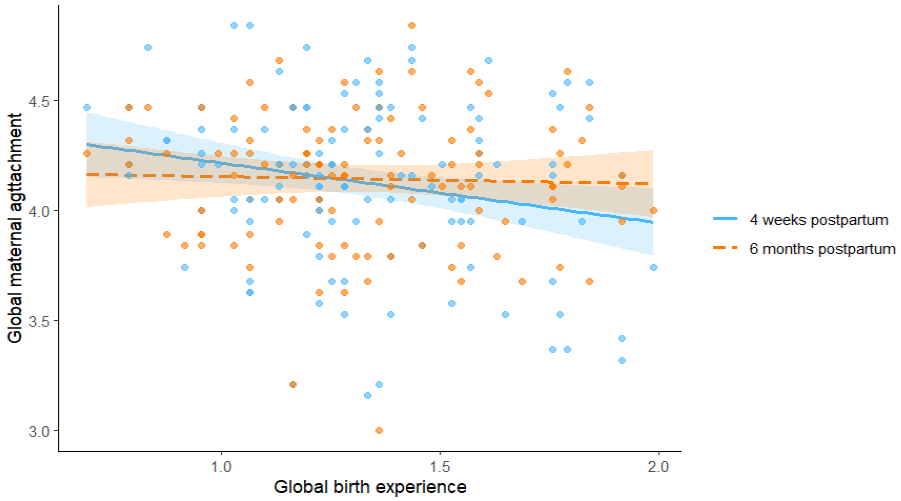
Gestational mothers who had a more negative birth experience exhibited significantly higher levels of stress and depressive symptoms, along with lower well-being at 4 weeks postpartum. By 6 months postpartum, the direct effects of birth experience on these affective symptoms and well-being were no longer statistically significant, yet the association remained relatively stable over time. This suggests that while the immediate postpartum period shows a strong link between birth experience and maternal mental health outcomes, this effect gradually diminishes but does not substantially change in direction or strength by 6 months postpartum.

### *Maternal Attachment*

Global birth experience significantly influenced maternal attachment over the postpartum period. At 4 weeks postpartum, global birth experience had a significant negative effect on global maternal attachment (*Est.* = -0.27, 95% CI [-0.49, -0.05], *SE* = 0.11, *t* = -2.52, *p* = .01), indicating that a more negative birth experience was associated with lower levels of attachment. Interaction effects between birth experience and the 6-month postpartum session were significant for global maternal attachment (*Est.* = 0.24, 95% CI [0.06, 0.42], *SE* = 0.09, *t* = 2.57, *p* = .01), indicating that the negative impact of a poor birth experience on maternal attachment lessened over time (see Figure 21 and Supplementary Table 18).

**Figure 21**

*The impact of global birth experience on maternal attachment*




***Summary***

Birth experience had a strong significant effect on maternal attachment at 4 weeks postpartum, while its impact vanished at 6 months postpartum.

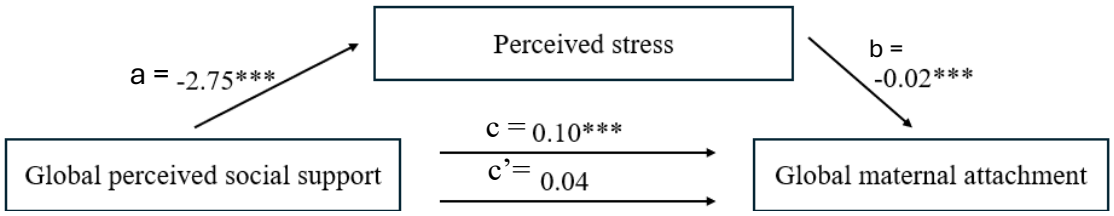
## The Mediation of Affective Symptoms over the Impact of Perceived Social Support on Global Maternal Attachment at Early Postpartum

### *Stress*

Stress mediated 55.47% (95% *CI* [0.25, 1.08], nominal $p < 0$, FDR-corrected $p < 0$) of the effect of global perceived social support (*Est.* = 0.10, 95% *CI* [0.04, 0.17], $p < 0$, FDR-corrected $p < 0$) on global maternal attachment at 4 weeks postpartum. The direct effect of global perceived social support was not statistically significant (*Est.* = 0.04, 95% *CI* [-0.004, 0.09], nominal $p = 0.092$, FDR-corrected $p = 0.09$), indicating that the relationship operates primarily through the mediated pathway (see Figure 22).

**Figure 22**

*Mediation of stress symptoms over the effect of global perceived social support on global maternal attachment at 4 weeks postpartum*

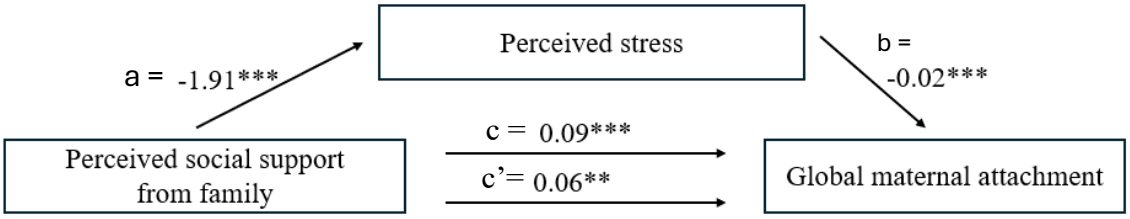



Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

Stress mediated 38.35% (95% *CI* [0.18, 0.70], nominal $p < 0.001$, FDR-corrected $p < 0.001$) of the effect of perceived social support from family (*Est.* = 0.10, 95% *CI* [0.05, 0.16], nominal $p < 0.001$, FDR-corrected $p < 0.001$) on global maternal attachment at 4 weeks postpartum (see Figure 23).

**Figure 23**

*Mediation of stress symptoms over the effect of perceived social support from family on global maternal attachment at 4 weeks postpartum*



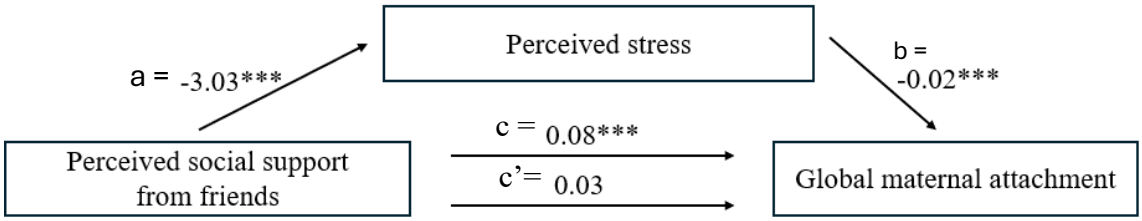

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

Stress mediated 71.02% (95% *CI* [0.42, 1.28], nominal $p < 0.001$, FDR-corrected $p < 0.001$) of the effect of perceived social support from friends (*Est.* = 0.09, 95% *CI* [0.04, 0.14], nominal $p < 0.001$, FDR-corrected $p < 0.001$) on global maternal attachment at 4 weeks postpartum. The direct effect of perceived social support from friends was not

statistically significant (*Est.* = 0.03, 95% *CI* [-0.02, 0.07], nominal *p* = 0.216, FDR-corrected *p* = 0.216), indicating that the relationship between social support from friends and maternal attachment quality operates primarily through its effect on stress (see Figure 24).

**Figure 24**

*Mediation of stress symptoms over the effect of perceived social support from friends on global maternal attachment at 4 weeks postpartum*



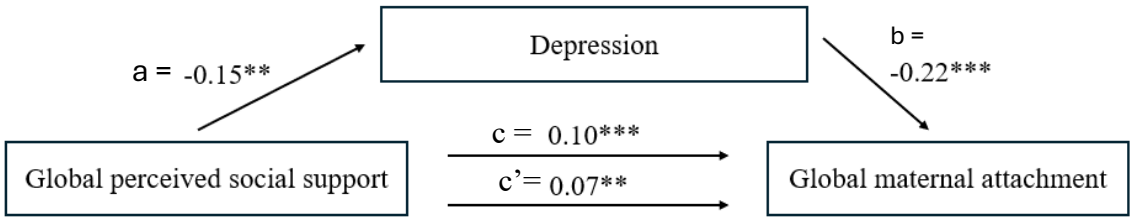

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

***Depression***

Depression mediated 33.68% (95% *CI* [0.06, 0.70], nominal *p* = 0.032, FDR-corrected *p* = 0.032) of the effect of global perceived social support (*Est.* = 0.10, 95% *CI* [0.04, 0.17], nominal *p* < 0.001, FDR-corrected *p* < 0.001) on global maternal attachment at 4 weeks postpartum (see Figure 25).

**Figure 25**

*Mediation of depressive symptoms over the effect of global perceived social support on global maternal attachment at 4 weeks postpartum*

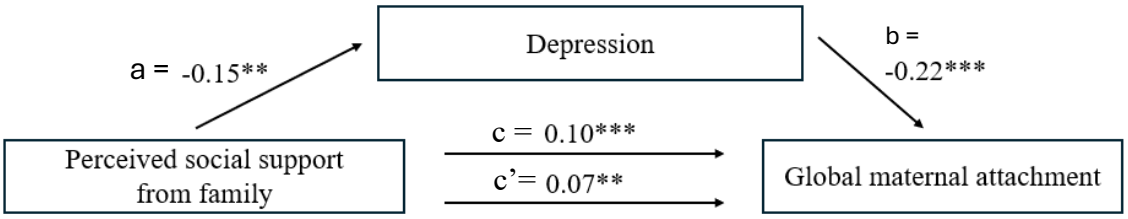



Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

Depression mediated 33.75% (95% CI [0.15, 0.61], nominal $p < 0.001$, FDR-corrected $p < 0.001$) of the effect of perceived social support from family (*Est.* = 0.10, 95% CI [0.05, 0.16], nominal $p < 0.001$, FDR-corrected $p < 0.001$) on global maternal attachment at 4 weeks postpartum (see Figure 26).

**Figure 26**

*Mediation of depressive symptoms over the effect of perceived social support from family on global maternal attachment at 4 weeks postpartum*



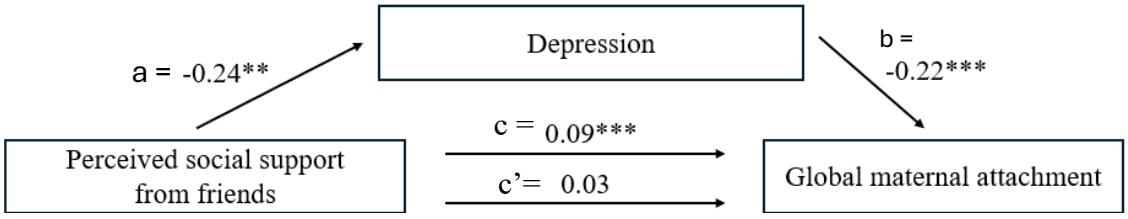

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

Depression mediated 60.68% (95% CI [0.35, 1.33], nominal $p < 0.001$, FDR-corrected $p < 0.001$) of the effect of perceived social support from friends (*Est.* = 0.09, 95% CI [0.03, 0.14], nominal $p < 0.001$, FDR-corrected $p < 0.001$) on global maternal attachment at 4 weeks postpartum. The direct effect of perceived social support from

friends was not statistically significant (*Est.* = 0.03, 95% CI [-0.01, 0.08], nominal $p$ = 0.13, FDR-corrected $p$ = 0.13), indicating that the relationship operates primarily through the mediated pathway of depression (see Figure 27).

**Figure 27**

*Mediation of depressive symptoms over the effect of perceived social support from friends on global maternal attachment at 4 weeks postpartum*




Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

***Summary***

The mediation analyses revealed that affective symptoms (stress and depression) and well-being significantly mediated the relationship between perceived social support and maternal attachment at 4 weeks postpartum. Across all sources of social support—global, family, and friends—mediated pathways accounted for a substantial proportion of the effect on global maternal attachment and attachment quality. In contrast, direct effects were often not statistically significant. This indicates that social support influences maternal attachment primarily by alleviating affective symptoms and enhancing well-being rather than directly affecting maternal attachment. However, perceived social support from the partner was not significantly mediated by any affective symptoms or well-being.

Support from friends consistently showed the strongest mediation through affective symptoms and well-being, particularly stress, depression, and well-being,

emphasising its critical role in maternal attachment formation. Global perceived social support had moderate mediation effects, stronger than family but weaker than friends, reflecting a broader but less targeted influence. Family support exhibited the least mediation.

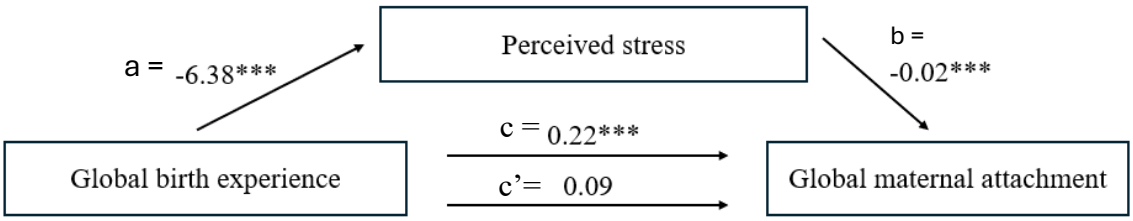
## The Mediation of Affective Symptoms over the Impact of Birth Experience on Maternal Attachment at Early Postpartum

### *Stress*

Stress mediated 58.88% (95% CI [0.33, 1.72], nominal $p < 0.001$, FDR-corrected $p < 0.001$) of the effect of global birth experience (*Est.* = 0.22, 95% CI [0.07, 0.37], nominal $p < 0.001$, FDR-corrected $p < 0.001$) on global maternal attachment at 4 weeks postpartum. The direct effect of global birth experience was not statistically significant (*Est.* = 0.09, 95% CI [-0.05, 0.21], nominal $p = 0.23$, FDR-corrected $p = 0.228$), indicating that the relationship operates primarily through the mediated pathway of stress (see Figure 28).

**Figure 28**

*Mediation of stress over the effect of global birth experience on global maternal attachment at 4 weeks postpartum*



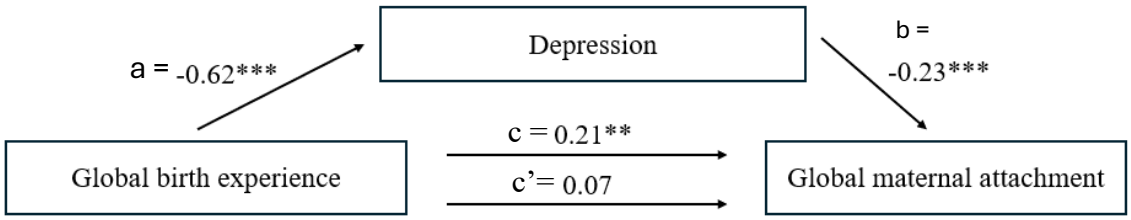

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

***Depression***

Depression mediated 65.94% (95% CI [0.35, 1.83], nominal $p < 0.001$, FDR-corrected $p = 0.011$) of the effect of global birth experience (*Est.* = 0.21, 95% CI [0.06, 0.35], nominal $p = 0.008$, FDR-corrected $p = 0.011$) on global maternal attachment at 4 weeks postpartum. The direct effect of global birth experience was not statistically significant (*Est.* = 0.07, 95% CI [-0.07, 0.20], nominal $p = 0.268$, FDR-corrected $p = 0.268$), indicating that the relationship primarily operates through the mediated pathway of depression (see Figure 29).

**Figure 29**

*Mediation of depression over the effect of global birth experience on global maternal attachment at 4 weeks postpartum*



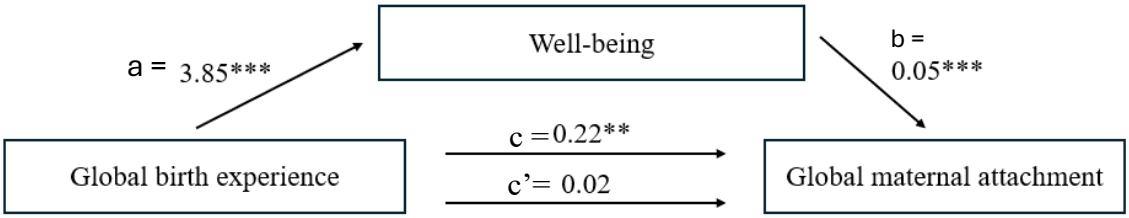

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

***Well-being***

Well-being mediated 92.03% (95% CI [0.59, 2.07], nominal $p < 0.001$, FDR-corrected $p = 0.005$) of the effect of global birth experience (*Est.* = 0.22, 95% CI [0.07, 0.37], nominal $p = 0.004$, FDR-corrected $p = 0.005$) on global maternal attachment at 4 weeks postpartum. The direct effect of global birth experience was not statistically significant (*Est.* = 0.02, 95% CI [-0.09, 0.13], nominal $p = 0.720$, FDR-corrected $p = 0.720$), indicating that the relationship primarily operates through the mediated pathway of well-being (see Figure 30).

**Figure 30**

*Mediation of well-being over the effect of global birth experience on global maternal attachment at 4 weeks postpartum*




Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

***Summary***

The mediation analyses revealed that affective symptoms (stress and depression) and well-being significantly mediated the relationship between global birth experience and maternal attachment at 4 weeks postpartum. The direct relationship between global birth experience and maternal attachment was not statistically significant. Instead, the influence of birth experience on maternal attachment was indirect, through its impact on mediating factors like affective symptoms and well-being. This highlights that birth experience alone does not directly enhance maternal attachment but does so by improving mental health and emotional well-being. Stress mediated over half of the relationship, while state stress accounted for a smaller but significant portion, around a quarter of the effect. Depression was the strongest mediator among the affective symptoms, explaining over 70% of the effect, while well-being emerged as the most significant mediator, accounting for over 85% of the relationship.

# Discussion

The present study explored the influence of a mother's mental health and well-being, in conjunction with social support and birth experience, on maternal attachment over pregnancy and the postpartum period in first-time gestational mothers. A strong and positive maternal attachment is vital to enhance the mother's emotional well-being and ensure the child's healthy emotional development. So, this underscores the relevance of exploring how the different psychological, social, and experiential-obstetric factors regulate the mother-to-infant bond. To address this, we investigated the dynamics and the interrelations between these factors to strengthen or weaken maternal attachment, focusing on the early postpartum period.

## Changes in Affective Symptoms, Well-being, Maternal Attachment and Social Support over Pregnancy and the Postpartum Period

### *Affective Symptoms and Well-being*

Our findings revealed substantial fluctuations in stress and depressive symptoms among first-time gestational mothers, spanning from preconception through pregnancy and into the early postpartum period. During pregnancy, these mothers demonstrated significant improvements in their stress and depressive symptoms, particularly in the second and third trimesters. However, in the early postpartum period, these mothers experienced a sharp worsening of their affective symptoms, which peaked around 4 weeks postpartum and remained elevated through 6 months postpartum.

Our findings provide further evidence for the proposed U-shaped trajectory of stress and depressive symptoms observed throughout pregnancy and into the postpartum period (Wang et al., 2023; Liou et al., 2014). However, Wang et al. (2023) reported a peak in depressive symptoms at three months postpartum, whereas our results indicate

an earlier exacerbation, beginning as early as the first month postpartum. This early emergence is consistent with findings by Zikic et al. (2024) and Iwata et al. (2016). Similarly, the early emergence of stress symptoms observed in our study aligns with the patterns reported by Liou, Wang, & Cheng (2014). Thus, our study not only supports the U-shaped trajectory of affective symptoms but also underscores the immediate postpartum period as a critical window for the onset of these symptoms in first-time mothers. In line with the observed trajectories in the affective symptoms, the gestational mothers' well-being improved over pregnancy, worsened very sharply in the early postpartum period and recovered slightly by mid-postpartum. While the improvement in well-being during pregnancy aligns with prior studies reporting gradual increases in life satisfaction and quality of life throughout this phase, our findings differ from earlier evidence suggesting that well-being significantly worsens immediately after childbirth, while it slightly improves and stabilises by mid-postpartum (e.g., Quick et al., 2023; Al Rehaili et al., 2023). These discrepancies may arise from differences in the aspects of well-being assessed by various instruments. Our study measured general well-being with an emphasis on emotional factors using the WHO-Wellbeing Index (WHO-5, 1998 version). By contrast, previous research employed tools like the Life Satisfaction Questionnaire (Fahrenberg et al., 2022) and the Quality-of-Life Questionnaire (WHOQOL Group, 1998), which consider broader dimensions of well-being, including psychological, physical, professional, and social life factors. Our study's focus on emotional health may explain the observed decline in well-being during the postpartum period, which mirrors the exacerbation of affective symptoms. In contrast, studies using instruments that evaluate well-being within the broader context of maternal quality of life have generally reported postpartum improvement. So, emotionally related well-being follows a distinct trajectory from the well-being related to motherhood.

Consistent with the decline in gestational mothers' well-being during the postpartum period, sleep quality reached its lowest point at 4 weeks postpartum. Sleep quality gradually worsened from mid-pregnancy, reaching its worst levels at 4 weeks postpartum, before showing modest improvement by 6 months postpartum. These findings go in line with previous studies that reported a steady decline in sleep quality as pregnancy progresses, with the most severe disruptions occurring during the final trimester and immediately after childbirth (e.g., Ojelere & Adeoye, 2024; Manconi et al., 2024; Umeno et al., 2020). In contrast with earlier research that examined the trajectory of sleep quality up to the immediate postpartum period, our findings describe a broader depiction of sleep quality until mid-postpartum. On top of that, our findings reveal a significant gradual restoration of sleep quality by 6 months postpartum.

Moreover, our findings highlight parallel trajectories between the recovery of sleep quality and the trajectory of general well-being throughout the postpartum period.

As a complementary analysis, we compared affective symptoms and well-being between mothers who conceived naturally and those who used assisted reproduction methods. The results revealed that the natural conception group exhibited higher perceived stress at 4 weeks postpartum. However, no significant differences were found between the two groups for the other affective symptoms or well-being variables. We controlled for the covariate of time searching for pregnancy, which was not influential. Given the isolated nature of this finding, the elevated perceived stress levels in the natural conception group may be considered anecdotal.

This study highlights the U-shaped trajectory of affective symptoms, which aligns closely with the patterns observed in well-being and sleep quality. During pregnancy, mothers experience an amelioration of their affective symptoms and an

improvement in their well-being and sleep quality. However, in the early postpartum period, mothers present a sharp exacerbation of their affective symptoms together with a worsening of their well-being and sleep quality.

By mid-postpartum, affective symptoms begin to decrease along with an improvement in subside in well-being and sleep quality. These findings suggest that well-being and sleep quality are interrelated with affective symptoms; these factors influence each other.

***Maternal Attachment***

Gestational mothers demonstrated a significant and gradual strengthening in their global maternal attachment. Their maternal attachment showed a notable increase beginning at 34-36 weeks of pregnancy, with further strengthening at 4 weeks postpartum and remaining high at 6 months postpartum. In contrast, non-gestational mothers exhibited minimal changes in their maternal attachment. Gestational mothers exhibited more consistent and pronounced maternal attachment developments than non-gestational mothers.

The minimal changes in the maternal attachment of non-gestational mothers during pregnancy and the postpartum period, compared to the gradual formation of attachment in gestational mothers, suggests that the biological adaptations associated with pregnancy may have a more substantial impact on the development of attachment during gestation, rather than the social transition to motherhood. Thus, social factors related to becoming a mother may exert a stronger and delayed influence on the formation of maternal attachment later in the postpartum period as the mothers' role of non-gestational mothers becomes more relevant.

Our findings align with previous evidence stating that maternal-foetal attachment develops in the first trimester of pregnancy and steadily increases through the second and third trimesters (e.g. Rossen et al., 2017). Additionally, our results support the idea that early formation of maternal-foetal attachment predicts a stronger mother-infant bond in the postpartum period (e.g. Petri et al., 2018). Considering the scarcity of evidence describing the evolution of maternal attachment over the postpartum period, the present study has broadened the depiction of the maternal attachment trajectory over the extended postpartum period. Specifically, we delineated whether maternal postnatal attachment keeps growing, weakens or stabilises over time.

As a complementary analysis, we compared the longitudinal trajectories of maternal attachment between mothers who conceived naturally and mothers who used an assisted reproduction method. The results indicated that the mothers from the natural conception group had slightly lower maternal attachment than the assisted reproduction group. Nevertheless, in the postpartum period, there were no significant differences between the groups. The stronger maternal attachment observed among the mothers from the assisted reproduction group may be that these others are more enthusiastic about their pregnancy due to the difficulties faced during the period while they were looking for pregnancy.

### *Social Support*

Perceived social support among gestational mothers declined by 6 months postpartum, whereas non-gestational mothers generally exhibited stable or slightly heightened social support over the postpartum period. Most research has explored social support employing cross-sectional associations with affective and maternal attachment variables (e.g., Liu et al., 2020). In contrast, the present study explored the longitudinal

course of perceived social support from early to mid-postpartum, revealing that the quality and intensity of the mothers' social support is more intense during the early weeks after birth and decreases from early to mid-postpartum.

Thus, social support may be particularly relevant in the immediate postpartum period, when mothers may require more emotional support because they are still adapting to their new role as mothers. However, as the situation stabilises and mothers adapt to their new role, their need for external emotional support lessens.

## The Impact of Social Support on Affective Symptoms, Well-being and Maternal Attachment over the Postpartum Period

### *Affective Symptoms and Well-being*

Our findings demonstrate that high levels of perceived social support significantly buffer the emergence of postpartum affective symptoms in gestational mothers. However, these positive effects did not apply to mothers' well-being. Global perceived social support, as well as support from family and friends, showed a consistent negative association with postpartum stress and depressive symptoms at early postpartum. In contrast, partner support had a delayed effect only on the mothers' depressive symptoms by six months postpartum. Strikingly, when comparing the differential effect of the distinct social supports, friend support became the only substantial influential factor of stress and depression at early postpartum. Meanwhile, family support became a strong predictor for depression at 6 months postpartum. Conversely, partner support had no effects on either stress or depression.

These results underscore the relevance of social support—particularly from family and friends—in promoting mothers' emotional well-being during the early postpartum period. The crucial role of family support aligns partially with prior research

highlighting the robust impact of family support in mitigating postpartum depression and stress (Liu et al., 2020) and the beneficial role of social support in enhancing well-being during early postpartum (Al Rehaili et al., 2023). In contrast with previous research, our findings reveal that friends' support is the most influential social support factor and that family support has a delayed substantial influence on mothers' emotional well-being, compared to the weak influence of partner support. A possible explanation is that mothers may perceive partner support primarily as instrumental help, so it has a minimal impact on their emotional well-being. In contrast, family support and particularly friend support are seen as more emotionally driven, directly enhancing mothers' emotional well-being.

This study offers a more nuanced understanding of how the impact of social support on mothers' affective symptoms evolves from early to mid-postpartum. Specifically, we highlight how important might be for mothers to count on friend support during the early postpartum weeks when they are still adapting to their new roles. Because of that, they may seek emotional support, especially from friends, to broaden their social support network beyond their most intimate support from partners and family. In contrast, as mothers might take partner support for granted, it may have a minimal impact on them. Moreover, family support becomes more relevant later in the postpartum period when mothers emotionally adapt to their new roles and may need extra instrumental support from close relatives when their partners' support is insufficient.

### *Maternal Attachment*

Similar to the findings on affective symptoms, social support from partners, family, and friends positively influenced maternal attachment at early postpartum. By mid-postpartum, however, these effects diminished or disappeared. Startlingly, when comparing the differential effect of the distinct social supports, family support became the only substantial influential factor of maternal attachment at early postpartum. Meanwhile, partner and friend support had no strong effects on maternal attachment. These findings partially align with previous research indicating that family support plays a significant role in shaping postpartum bonding (MacMillan et al., 2021; Wu et al., 2024). Our results partially align with previous studies indicating that partner support has long-lasting effects on maternal attachment over the postpartum period (Martin & Brock, 2023). Nevertheless, our study reveals that partner support weakens later in the postpartum period and its effect is masked by a more substantial impact of family support on maternal postnatal attachment. This finding suggests that mothers perceive partner support as instrumental help, while family support may be seen as emotional support.

## The Impact of Birth Experience on Affective Symptoms, Well-being and Maternal Attachment in Early Postpartum

### *Affective Symptoms and Well-being*

Our findings revealed that gestational mothers who experienced a more negative birth reported elevated levels of stress and depression and reduced general well-being in the early postpartum period. In contrast, those with positive birth experiences reported fewer affective symptoms and better well-being. By 6 months postpartum, the influential power of birth experience diminished, although the direction of its effect

remained stable. These findings align closely with previous studies indicating that a negative birth experience exacerbates postpartum anxiety and depressive symptoms. In contrast, a positive birth experience mitigates affective symptoms at postpartum (e.g., Froeliger et al., 2023). However, the diminishing effects we observed do not support evidence suggesting that the impact of birth experience persists over the postpartum period, extending up to 8 months postpartum (e.g., Bell et al., 2016). This discrepancy may be because the lasting effects of birth experience were observed on anxiety symptoms, whereas we explored depression and stress symptoms. Overall, our findings present the birth experience as a powerful protective factor against postpartum affective symptoms. Additionally, this study contributes to the growing body of evidence on the impact of birth experience on postpartum stress symptoms and overall well-being.

### *Maternal Attachment*

Our results indicate that a mother's birth experience significantly affects her maternal attachment during the early postpartum period, whereas this effect weakens by mid-postpartum. Specifically, mothers who had more negative birth experiences reported weaker maternal attachment a few weeks after birth. This finding goes in line with previous research emphasising the importance of birth experiences in forming maternal postnatal attachment (Döblin et al., 2023).

Notably, our study adds to the growing evidence that a mother's subjective experience of childbirth can be as influential, or even more, on maternal attachment than objective factors such as the mode of delivery (Junge-Hoffmeister et al., 2022). This perspective challenges the broader research stating that objective birth factors are stronger predictors of maternal bonding (e.g., Moniri et al., 2023). Therefore, we highlight the significance of a mother's subjective perception of her birth experience in

the development of maternal postnatal attachment, which remains an understudied aspect of childbirth.

**The Mediation of Affective Symptoms and Well-being over the Effect of Social Support on Maternal Attachment at Early Postpartum**

Our mediation models revealed that stress and depressive symptoms significantly mediated the effect of perceived social support on maternal attachment at 4 weeks postpartum. These findings indicate that social support influences maternal attachment indirectly by alleviating affective symptoms rather than exerting a direct effect on attachment. Among the sources of social support, support from friends demonstrated the strongest mediation, global support had a moderate impact, and family support exhibited the weakest mediation. These results underscore the indirect pathway of social support in improving maternal mental health to foster stronger maternal attachment.

Our findings align with previous research describing the indirect role of social support in shaping maternal attachment through its regulation of mothers' affect (e.g., MacMillan et al., 2021; Martin & Brock, 2023). However, we present a distinct mechanism through which social support indirectly influences maternal postnatal attachment. While prior studies proposed an interaction model in which social support moderates the relationship between depressive symptoms and maternal attachment—acting as a buffer to reduce the negative impact of depressive symptoms, mainly via partner and family support—we propose a mediation model. In our mediation model, social support indirectly strengthens maternal attachment by first alleviating affective symptoms rather than directly moderating the effects of depressive symptoms on maternal attachment.

While previous studies have highlighted family and partner support as the most substantial contributors to reducing postpartum affective symptoms and fostering maternal attachment (e.g., Martin & Brock, 2023), our findings identify friends' support as the most influential factor. In contrast, family support exerted a weaker effect, and partner support was discarded from the mediation analyses since it did not demonstrate a main contribution to explaining maternal postnatal attachment among the different social support sources. Again, a possible explanation for either the weak or the non-existent mediating effect of family and partner support is that mothers may perceive partner and family support as instrumental help and take it for granted, especially for partner support. For these reasons, mothers may be less emotionally moved by the support from their partners or family. In contrast, the mothers' expectations of emotional support from their friends probably explain the strong mediation effect of friend support.

This study uncovers a new mechanism through which social support indirectly impacts maternal attachment by regulating emotional well-being.

**The Mediation of Affective Symptoms and Well-being over the Effect of Birth Experience on Maternal Attachment at Early Postpartum**

The mediation analyses revealed that birth experience influences maternal postnatal attachment through a pathway similar to social support. Affective symptoms (perceived stress and depression) and well-being significantly mediated the relationship between global birth experience and maternal attachment in the first postpartum month. The mothers' emotional well-being mediating effect indicates that birth experience impacts attachment indirectly by improving mothers' emotional well-being. Among the

mediators, well-being was the strongest, followed closely by depression, while perceived stress mediated about half of the relationship.

These findings partially align with and expand on previous evidence suggesting that birth experience influences maternal attachment indirectly through its impact on postpartum depression, as proposed by Eitenmüller et al. (2022). Nevertheless, while the Eitenmüller et al. (2002) study presented a moderated effect in which birth experience buffered the effect of the mothers' depressive symptoms on their maternal attachment, we propose a mediation effect from affective symptoms and well-being over the impact of birth experience on postanal attachment. The mediating role of the emotional well-being factors underscores the critical role of maternal mental health in shaping the mother-to-infant bond, particularly during the early postpartum period.

Overall, the present studies emphasise the predictive power of the subjective birth experience over the maternal postnatal attachment in the early postpartum period through its direct effect on mothers' emotional well-being.

**Limitations and Future Research Directions**

The present study has several limitations that future research should consider. One limitation is the use of the WHO-Well-being Index (*WHO-5*, 1998 version; Topp et al., 2015) to assess maternal well-being is another limitation of the current study, as it measures general quality of life but does not capture aspects specific to the postpartum maternal experience. Using this general scale may explain why our results showed a weaker association between social support and maternal well-being. More targeted tools, such as the Satisfaction with Life Scale (Larsen et al., 1985, cited in Quick et al., 2023) and the Flourishing Scale (Diener et al., 2010, cited in Quick et al., 2023), may be more appropriate for assessing well-being and life satisfaction in mothers, as they directly address aspects relevant to the maternal context. Future studies should

incorporate such scales to precisely evaluate maternal-specific well-being and identify factors that enhance life satisfaction during the postpartum period.

The maternal postnatal attachment scale also showed limited sensitivity, exhibiting ceiling effects with peak scores reached by the third trimester and maintained from early to mid-postpartum. These ceiling effects likely mask the natural progression and strengthening of maternal attachment that typically continues in the postpartum period. Future research could benefit from integrating self-report attachment scales with observational and experimental methods, such as behavioural observations of mother-infant interactions, to better capture maternal attachment changes over time and mitigate ceiling effects.

Another limitation is the narrow time window in which we explored the longitudinal trajectories of affective symptoms, well-being, maternal attachment and social support until six months postpartum. This short study period limited the follow-up of any other changes in these variables over later postpartum stages. Extending the follow-up exploration to one year postpartum might better delineate the evolution of the dynamics and interrelations between affective symptoms, social support, and maternal attachment.

Finally, the small sample of non-gestational mothers as a comparison group limited our capabilities to distinguish between the effects of biological changes related to pregnancy and the social experience of transitioning to motherhood on the mothers' emotional well-being and maternal attachment. A larger, balanced comparison group of non-gestational mothers would provide a clearer picture of the unique contributions of biological and social factors in shaping maternal attachment and emotional well-being postpartum.

## Conclusions

This study makes several important contributions to understanding the trajectories and the complex interplay between affective symptoms, well-being, social support, and birth experience in shaping maternal attachment during the early postpartum period for first-time gestational mothers. First, this study reinforces the U-shaped trajectory of affective symptoms over pregnancy and postpartum, paralleling the orchestrated trajectories of well-being and sleep quality. Meanwhile, our findings illustrate how maternal attachment gradually strengthens during pregnancy and stabilises over postpartum. Additionally, our results reveal how the mothers' perception of the social support received diminishes over the postpartum time.

Our results also underscore the detrimental effects of affective symptoms on maternal attachment, particularly in the first month postpartum. At the same time, the present study expands prior evidence about the positive and strong influence of general well-being on maternal attachment, an influential factor that has been underexplored.

Furthermore, we underscore the criticality of the influence of social support and subjective birth experience on regulating mothers' emotional well-being and the development of maternal attachment in the very early postpartum period. Regarding social support, our findings reveal the stronger effect of family support over partner support on mothers' emotional well-being and maternal attachment. Of note is that we introduce friends' support as a novel and the most powerful influential factor.

Most importantly, the present study illustrates the indirect pathways through which social support and birth experience influence maternal attachment by regulating the mothers' emotional well-being in the early postpartum period. The depiction of the indirect pathways of social support and birth experience on maternal attachment has

relevant implications for future clinical interventions. Future interventions should focus on prompting and strengthening social support from friends and family and ensuring a positive perception of the birth experience. This intervention approach would help mitigate postpartum affective symptoms and promote a healthy maternal postnatal attachment.

# Study 2

## The Cumulative Production and Conjugation of Steroid Hormones during Pregnancy Predict Postpartum Depressive Mood

# Introduction

## Hormonal Trajectories over Pregnancy and the Postpartum Period

Pregnancy entails significant fluctuation in the steroid levels to maintain pregnancy and to prepare the woman's body for childbirth. These hormonal fluctuations begin early in pregnancy and continue until labour, followed by a rapid decline at immediately after childbirth.

Progesterone and 17α-hydroxyprogesterone steadily increase from early gestation, peaking just before labour. These hormones are crucial for maintaining the endometrium and preventing contractions, thereby supporting the development of the foetus (Liang et al., 2020; Glynn et al., 2016; Schock et al., 2016). Similarly, oestradiol and estrone levels rise consistently from early pregnancy, increasing nearly 9 to 10 times by delivery. This increase enhances uterine blood flow and promotes foetal growth, with oestradiol following a quadratic trajectory and peaking near the end of pregnancy (Liang et al., 2020; Glynn et al., 2016; Schock et al., 2016). Likewise, prolactin levels, crucial for preparing the woman's body for lactation, increase significantly during pregnancy (Schock et al., 2016).

In contrast to the steady rise of progesterone and oestradiol, estriol-16-glucuronide and estrone 3-sulfate show a more rapid increase, particularly before week 24. These hormones, primarily produced by the placenta, support later-stage pregnancy by modulating various metabolic processes critical for foetal development and labour preparation (Liang et al., 2020). Other steroid metabolites, such as tetrahydrodeoxycorticosterone (THDOC), also increase rapidly before week 24 (Liang et al., 2020).

Unlike the accentuated and rapidly increasing trajectories of progesterone and oestradiol, dehydroepiandrosterone (DHEA) and testosterone follow distinct courses. Testosterone rises gradually and slowly throughout pregnancy and remains relatively stable (Marceau et al., 2021). Despite testosterone's weaker trajectory, it still influences foetal development (Schock et al., 2016). By contrast, DHEA decreases significantly in the first trimester, suggesting a less direct influence on pregnancy maintenance (Marceau et al., 2021).

Following childbirth, most of these hormones—including oestradiol, progesterone, estriol-16-glucuronide, prolactin and THDOC—rapidly decline to baseline levels due to the removal of the placenta, which indicates the end of pregnancy and its hormonal demands, while testosterone and DHEA remain relatively stable (Liang et al., 2020; Glynn et al., 2016).

Overall, the trajectories of steroid hormones throughout pregnancy involve a steady rise in some hormones, such as progesterone, oestradiol, and estrone, alongside rapid surges in others like estriol-16-glucuronide and THDOC. At the same time, DHEA and testosterone show slower and more stable trajectories. These fluctuations are critical in supporting foetal development and preparing the woman's body for childbirth. After delivery, these hormone levels sharply drop, except for DHEA and testosterone, which remain stable (Liang et al., 2020; Glynn et al., 2016).

Despite advancements in understanding hormonal trajectories during pregnancy and childbirth, research remains scarce examining the dynamic and longitudinal changes in steroid metabolite levels from pregnancy into the early postpartum period. Existing studies suggest that steroid levels follow a linear, ascending trajectory throughout pregnancy, with a sharp decline at childbirth. Nevertheless, future research

should explore the fluctuation levels of the steroids in more detail and expand this longitudinal examination from early pregnancy all the way through and up to at least a few weeks after childbirth to determine whether the steroid levels remain very low or if they stabilise in the few days following childbirth. An expanded longitudinal exploration of the steroid trajectories would provide a more comprehensive picture of how steroid fluctuations across pregnancy and until the early postpartum period.

## The Influence of the Steroid Hormones' Deviations over Pregnancy and the Postpartum on the Emergence of Depressive Symptoms and on the Maternal Attachment Development at Postpartum

### *Depressive Symptoms*

Steroid hormones and their metabolites exert a substantial impact on women's emotional well-being over pregnancy and the postpartum period, particularly on postpartum depressive symptoms. Steroid fluctuations during pregnancy and the postpartum period are associated with the onset and the course of postpartum depressive symptoms in mothers.

Hormonal deviations during pregnancy may already affect women's mental health. Deviations in the allopregnanolone concentrations, a neuroactive metabolite of progesterone, appear to trigger depressive symptoms. Higher levels of allopregnanolone in mid-pregnancy (around 17 weeks) lead to a heightened risk of persistent depressive symptoms over pregnancy that continue into the postpartum period (Björvang et al., 2024). Each additional increase in allopregnanolone levels raises the odds of persistent depression by 7% (Björvang et al., 2024). Accordingly, lower allopregnanolone levels during the second trimester (around 19 weeks) significantly reduces the risk of postpartum depression by 63% (Osborne et al., 2017). However, conflicting evidence

suggests that lower levels of allopregnanolone in late pregnancy (37–40 weeks) are associated with more severe depressive symptoms (Hellgren et al., 2014). These inconsistencies suggest that allopregnanolone may influence depressive symptoms differently across gestational periods. Elevated progesterone metabolites, such as 5α-dihydroprogesterone (5α-DHP), during pregnancy are also associated with pregnancy-related depression (Pearson Murphy et al., 2001).

In the postpartum period, steroid levels continue to influence mothers' mental health. Progesterone has a substantial impact on postpartum mental health. During the early postpartum period, a sharper decline in progesterone levels is observed among mothers with postpartum depression, indicating that this abrupt hormonal drop may trigger depression, especially in first-time mothers (Kikuchi et al., 2021). While studies during pregnancy do not consistently find a direct link between progesterone levels and postpartum depression, the sudden decline in progesterone after childbirth appears to be a potent trigger of mood destabilisation. Women with a history of postpartum depression are more sensitive to these hormonal drops, predisposing them to depressive episodes right after childbirth (Bloch et al., 2000). In one study, 62.5% of women with a history of postpartum depression developed mood symptoms following hormonal withdrawal, compared to no mood changes in women without such a history (Bloch et al., 2000).

Testosterone is another steroid linked to postpartum depression. Elevated levels of testosterone four weeks before labour predict the onset of postpartum depression (Aswathi et al., 2015; Parízek et al., 2014). Further, elevated testosterone levels immediately after childbirth (within 24–28 hours) and at 6 weeks postpartum are also associated with higher risks of postpartum depression (Aswathi et al., 2015; Parízek et al., 2014).

Apart from steroid hormones, prolactin as a peptide hormone also impacts mothers' mental health. Women who experience a higher descending of their prolactin levels within the first 7 days postpartum are more likely to experience depressive symptoms. Prolactin levels are also lower in women who develop depression later (6–10 weeks postpartum), with breastfeeding—known to increase prolactin levels during and after a breastfeeding session—associated with reduced postpartum depressive symptoms (Abou-Saleh et al., 1998).

Despite the converging evidence about a relationship between steroid hormones and pregnancy-related depression, there are time constraints that have limited the exploration of this association. Most studies have focused on examining the effect of steroid fluctuations during late pregnancy or at immediate postpartum, limiting the understanding of how prolonged longitudinal steroidal deviations may affect postpartum depressive symptoms. This time constraint underscores the need for an exploration of the effects of the steroids' trajectories for a more extended period, ideally from preconception to postpartum. Besides, there is an urge for more comprehensive investigations examining steroid levels across pregnancy and into early postpartum to determine the underlying mechanisms that influence the emergence of postpartum depressive symptoms. So, this comprehensive approach may help to resolve the conflicting findings about the mechanism by which allopregnanolone triggers depressive symptoms.

### *Maternal Attachment*

Steroid hormones, particularly oestradiol and progesterone, substantially influence the formation and strengthening of maternal attachment across different pregnancy and postpartum stages. Research indicates that mothers who exhibit higher

quality of maternal postnatal attachment often show distinct hormonal trajectories during pregnancy. In the early study by Fleming et al. (1997), the authors observed that hormonal concentrations at specific stages of pregnancy did not appear to be directly or independently associated with postpartum maternal attachment. Instead, the overall longitudinal trajectories of steroid hormones throughout the pregnancy influenced maternal postnatal attachment (Fleming et al., 1997). A lower ratio of oestradiol-to-progesterone and a smaller decline of this ratio from early pregnancy to 4 days postpartum were linked to stronger maternal attachment at five and seven months postpartum (Fleming et al., 1997). From the effect of this ratio, oestradiol appeared to be the main influential factor, as its levels were inversely correlated with maternal postnatal attachment (Fleming et al., 1997).

Similarly, a recent study by Glynn et al. (2016) found that a slower rate in the oestradiol-to-progesterone ratio is associated with stronger postnatal maternal attachment. However, unlike the findings of Fleming et al. (1997), Glynn et al. reported that the influence of this slower rate in this ratio occurs over a much narrower gestational window, specifically from 20 to 34 weeks of pregnancy, and notably ends before childbirth. Additionally, Glynn et al. observed that the effects of steroid trajectories on maternal postnatal attachment extend up to 12 months postpartum. Furthermore, like the earlier study, Glynn et al. identified that a slower acceleration rate of the oestradiol levels from gestational week 28 up to the end of pregnancy was associated with greater maternal sensitivity at 1 year postpartum. However, in contrast with the previous study, they also found an association with a slower rise in progesterone from week 36 (Glynn et al., 2016).

Research on the effects of steroid trajectories during pregnancy and into childbirth on maternal postnatal attachment remains very limited. Moreover, the few

existing studies present conflicting findings regarding the specific gestational windows during which the rates of steroid concentration changes impact postpartum maternal attachment. There is also disagreement about the relative influence of oestradiol compared to progesterone on maternal attachment. The scarce research depicting the longitudinal trajectories of steroid hormones over pregnancy and until the early postpartum period in human mothers and the divergent findings call for further research exploring the longitudinal trajectories of the steroids and their impact on maternal postnatal attachment. Additionally, the narrow focus of previous research on oestradiol and progesterone indicates a strong need to explore the impacts of other key steroids in the development of maternal attachment.

## Justification of the Study

Fluctuations in steroid hormones throughout the perinatal period play a critical role in regulating maternal mood, with rapid declines following childbirth that are associated with postpartum depressive symptoms (Etyemez et al., 2023; Kikuchi et al., 2021). Additionally, changes in steroid hormone levels during the later stages of pregnancy and immediately after childbirth—particularly in progesterone and oestradiol—appear to influence maternal postnatal attachment. However, this evidence is limited (Glynn et al., 2016).

While substantial evidence supports the relationship between steroid hormone levels and maternal mental health, the association between steroid fluctuations and maternal postnatal attachment is less consistent. Moreover, most existing research has concentrated on the effects of hormonal changes within a narrow time frame, typically from late pregnancy to the immediate postpartum period, often neglecting broader hormonal trajectories in the perinatal period that may influence postpartum maternal mental health and attachment. There is a strong need for a more comprehensive and longitudinal approach, from preconception throughout the pregnancy to the early postpartum period, to solve the inconsistent previous findings and the narrow postpartum time frame explored.

This study adopts a novel approach to address the limitations of recent research. It examines the impact of cumulative and longitudinal changes in steroid fluctuations over an extended period—from preconception through pregnancy and into early postpartum—on postpartum depressive symptoms and maternal attachment. We anticipate that this innovative perspective will expand the current body of evidence and help resolve existing inconsistencies in the literature.

## Objectives

The present study examines the role of steroid metabolites in shaping postpartum mental health and well-being, as well as their impact on maternal attachment. Specifically, the study aims to examine how the cumulative production and conjugation of steroid metabolites from preconception to 4 weeks postpartum correlate with mothers' postpartum depressive symptoms, general well-being, and maternal postnatal attachment in first-time gestational mothers. Specifically, the study aims to:

*Objective 1*: Explore the fluctuations in the steroid metabolites' levels over pregnancy until 4 weeks postpartum.

*Objective 2*: Examine the association between cumulative changes in steroid metabolites' concentrations from preconception through pregnancy to 4 weeks postpartum and postpartum depressive symptoms, general well-being, and maternal attachment.

## Methods of Study 2

### Participants

In the present study, we analysed data from a subset of participants drawn from the larger sample of Study 1. Specifically, this study focused on 135 nulliparous Spanish women aged 25 to 45 who initially planned to conceive within six months of the study's start. These participants successfully became pregnant and completed all experimental sessions, from pre-conception to 4 weeks postpartum. However, 15 participants were excluded from the analyses due to missing metabolomic data: 2 were missing data at one point, 12 at two-time points, and one at three-time points—consequently, the final analysed sample comprised 120 first-time gestational mothers (see Table 1).

**Table 1**

*Demographic and obstetric data of the participants*

| | N= 120 |
|---|---|
| **Age, years at baseline** | 34.5 ± 3.9 |
| **BMI, kg/m$^2$ at baseline** | 24.3 ± 3.9 |
| **Sex of the baby, male** | 66 (55%) |
| **Assisted reproduction method** | 60 (50%) |
| **Delivery** | |
| **Vaginal** | 78 (65%) |
| **Planned C-section** | 18 (15%) |
| **Unplanned C-section** | 24 (20%) |
| **Perceived Social Support Scale score (IQR)** | 6.0 (1.0) |
| **Birth Experience Questionnaire – score (IQR)** | 2.6 (1.5) |
| **Baseline** | 10.83 ± 17.5 |
| **18-22 pregnancy weeks** | 18.07 ± 0.99 |
| **34-36 pregnancy weeks** | 33.93 ± 3.08 |
| **4 weeks postpartum** | 37.99 ± 6.9 |

| | |
|---|---|
| **Perceived social support scale** | 6.0 ± 1.0 |
| **Birth Experience Questionnaire** | 2.6 ± 1.5 |

Values are mean ± SD, median (IQR) or N (%). Abbreviations: BMI, body mass index IQR, interquartile range.

This research was approved by the Ethics Committee at the Hospital del Mar Research Institute (Ref: 2017/7450/I), the Hospital Clínic de Barcelona (Ref: HCB/2018/0357), and the Hospital Universitari Quirón Dexeus (Ref: 7/2/2017), following the Declaration of Helsinki guidelines. All participants signed a consent form before participating in the study.

***Instruments***

We employed the same tests, scales and questionnaires as in Study 1 to gather demographic and obstetric data, as well as to assess postpartum depression symptoms, general well-being and maternal postnatal attachment: *General Demographic & Obstetric Questionnaire*, *Mini International Neuropsychiatric Interview* (Sheehan et al., 2000), *Edinburg postnatal depression scale* (Cox, Holden, & Sagovsky, 1987), *Well-Being Index* (*WHO-5*, 1998 version; Topp et al., 2015), *Maternal Postnatal Attachment Scale* (Navarro-Aresti et al., 2016), *Perceived Social Support Scale* (Gregory et al., 1988), and *Birth Experience Questionnaire* (Saxbe, Taline Horton, & Bryna Tsai, 2018).

**Procedure**

We selected the major urinary glucuronides and sulphate conjugates of the steroid hormones: oestradiol, estriol, pregnenolone, progesterone, dehydroepiandrosterone, and testosterone. The targeted panel of urinary phase II metabolites that were measured throughout the pregnancy (see Supplementary Table 1).

The reagents and materials used for steroid analysis are described in supplementary information. To obtain information about the production of each steroid hormone, we selected the main conjugated forms for each steroid metabolite, i.e. glucuronide- and sulphate-conjugated metabolites (see Supplementary Table 1). Thereby, we could evaluate not only the effect of the production of steroid hormones but also the role of conjugation on the selected neuropsychological outcomes. Urinary steroid conjugates were assessed by a targeted LC-MS/MS approach adjusted using the methods reported for their determination in urine (Zimet et al., 1988; McLeod et al., 2017). Briefly, 15 µL of internal standards mix (see supplementary materials for composition) and 1 mL of 4% aqueous phosphoric acid was added to 1 mL urine sample.

A solid phase extraction was preconditioned with 2 mL 100% methanol and 2 mL 2% FA in water Oasis HLB 3cc cartridges (Waters Associates, Milford, MA, USA). After washing with 2 mL of 2% formic acid in water, steroids were eluted in two stages: the first elution was performed with 1 mL of 2% FA in methanol, followed with 1 mL of methanol; the second one was performed with 1 mL of 5% ammonia in methanol followed with 1 mL of methanol. Both elutes from the same sample were dried separately under $N_2$ at 40 ºC, reconstituted in 1 mL of methanol, and combined, and the mix was evaporated under $N_2$ at 40 ºC. The residue was reconstituted with 100 µL of water acetonitrile (9:1), and 5 µL was injected into the UHPLC-MS/MS system.

The UHPLC-MS/MS system consisted of an Acquity UPLC Class I chromatographic system equipped with an Acquity UPLC CSH C18 column (2.1 × 100 mm i.d., 1.7 µm) and coupled to a triple quadrupole (XEVO TQ-S micro) mass spectrometer equipped with an orthogonal Z-spray-electrospray ionisation source (all from Waters Associates). Details from gradient and MS/MS conditions can be seen in Supplementary information.

Targeted metabolites were monitored and quantified using selective reaction monitoring (SRM) mode. The specific transitions and optimum collision energy are summarised in Supplementary Table 1 for each detected steroid phase II metabolite.

Samples were processed in several batches along with corresponding calibration curves (prepared in steroid-stripped urine as described in supplementary materials) and a set of quality control samples injected at least twice per batch analysis. MassLynx V4.1 and TargetLynx XS software (Waters) were used for data management, instrument manipulation, and quantification of urinary metabolites by UHPLC-MS/MS analysis.

Concentrations of urinary steroids were normalised to concentrations of excreted creatinine and expressed in ng/mg of creatinine.

**Statistical Analysis**

We first conducted a preliminary analysis using mixed-effects linear regression models to explore changes in steroid metabolite levels from pre-conception through pregnancy and into the postpartum period. In these models, the pre-conception stage was set as the baseline for comparison, allowing us to examine how steroid metabolite levels fluctuated across different gestational and postpartum stages. This approach enabled us to account for individual variability and the repeated measures nature of the data, ensuring that our analysis captured both within-subject changes and the influence of specific gestational time points on metabolite levels. By incorporating fixed effects for each time point and random effects to address individual differences, we were able to control for potential confounding factors and identify key trends in metabolite fluctuations.

We employed simple effects analyses to gain deeper insights into these trends, which detailed within-subject comparisons. This allowed us to assess changes in steroid

metabolite levels across all gestational and postpartum stages while accounting for individual baseline differences. These analyses highlighted significant changes, particularly during critical periods such as 34-36 weeks of pregnancy and at 4 weeks postpartum, where notable shifts in metabolite levels were observed. These findings provided a more precise understanding of how steroid metabolites evolve throughout pregnancy and early postpartum.

In exploring the main study objective, we calculated the Area Under the Curve (AUC) from pre-conception (T1) to 4 weeks postpartum (T4), (AUCT1-T4) for each steroid metabolite, separately for glucuronide and sulphate forms. AUC is a summary metric commonly used to quantify changes in a variable across time, capturing the magnitude and duration of its fluctuations. In this case, the AUC provides a single value summarising how these levels evolve, considering their concentration and the time interval between measurements. The AUC allowed us to evaluate the impact of cumulative steroid production and conjugation during pregnancy on postpartum outcomes, including depression symptoms, general well-being, and maternal postnatal attachment scores. For statistical analysis, non-detectable values were converted to half of the lowest concentration detected across all samples, and creatinine-adjusted concentrations were log10-transformed, following standard procedures in the field.

Additionally, we examined the ratios of glucuronide to sulphate forms of steroid metabolites, given their significant differences in bioavailability, bioactivity, biostability, and function. These ratios provide critical insights into the physiological relevance of these metabolites. Detailed information regarding these analyses can be found in the supplementary materials (see Supplementary Table 2).

The associations between AUCT1-T4 and postpartum outcomes (depression, general well-being, and maternal postnatal attachment) were analysed using generalised linear models. After assessing the distribution of response variables, we selected the Poisson family model for depression and the Gaussian family model for general well-being and maternal postnatal attachment. The models were adjusted for key covariates, including maternal age, body mass index, conception method, perceived social support, birth experience, and baby's sex. The Benjamini–Hochberg procedure (Benjamini and Hochberg, 1995) was applied to control the false discovery rate (FDR), with an FDR-corrected p-value below 0.05 considered statistically significant.

# Results

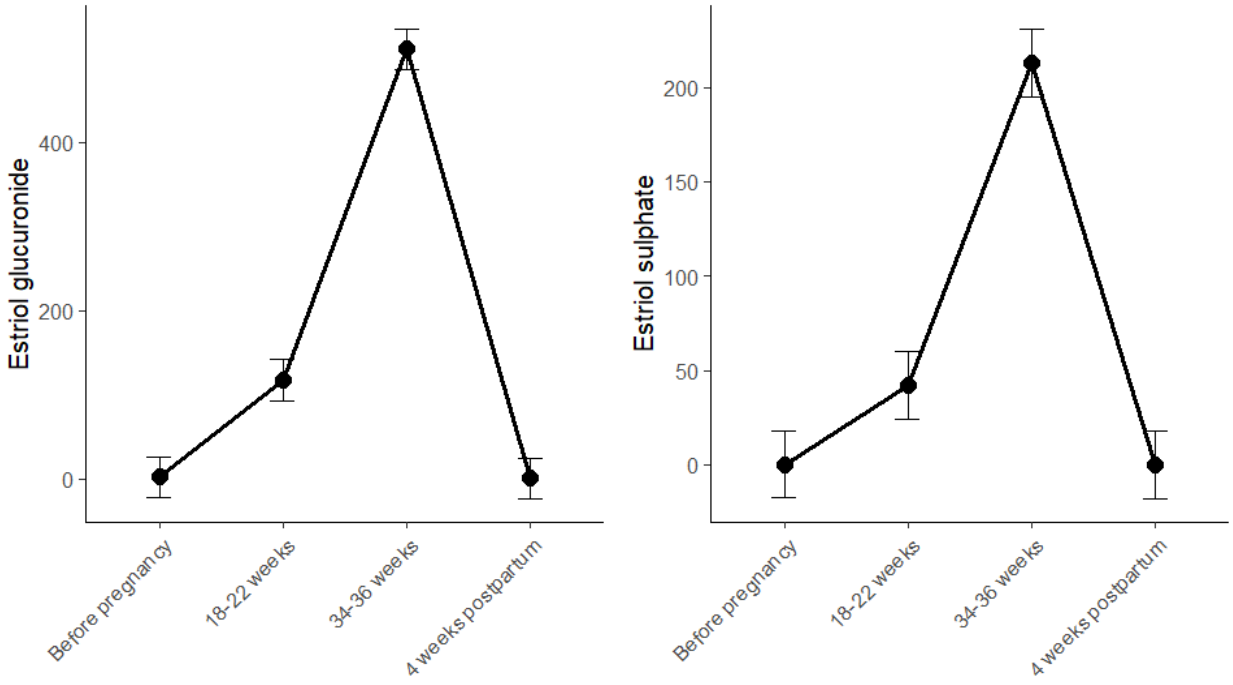
## Changes in the Steroid Metabolites Levels from Pre-Conception to Early Postpartum

### *Oestrogens*

Estriol glucuronide and estriol sulphate levels showed a marked increase at 18-22 weeks of pregnancy compared to pre-conception (*Est.* = 93.07, 95% *CI* [69.4, 120.5], *SE* = 17.51, *t* (464.41) = 5.31, $p < .00$, $\beta std$=0.85; *Est.* = 34.09, *SE* = 10.86, *t* (464.34) = 3.14, $p = 0.00$, $\beta std$=0.43, respectively). These increases became even more pronounced at 34-36 weeks of pregnancy, where estriol glucuronide and sulphate levels rose significantly higher than pre-conception (*Est.* = 411.68, 95% *CI* [388.0, 439.1], *SE* = 17.52, *t* (465.88) = 23.50, $p < .00$, $\beta std$= 3.75; *Est.* = 172.26, *SE* = 10.86, *t* (465.81) = 15.86, $p < 0.00$, $\beta std$=2.20, respectively). Conversely, at 4 weeks postpartum, estriol glucuronide and sulphate levels showed a marked decline, returning to levels comparable to pre-conception, with no significant differences observed (*Est.* = -1.33, 95% *CI* [-25.1, 26.2], *SE* = 17.54, *t* (465.07) = -0.08, $p = 0.94$; *Est.* = -0.12, *SE* = 10.88, *t* (464.96) = -0.01, $p = 0.99$), (see Figure 1).

**Figure 1**

*Changes in estriol glucuronide and estriol sulphate from pre-conception to early postpartum*



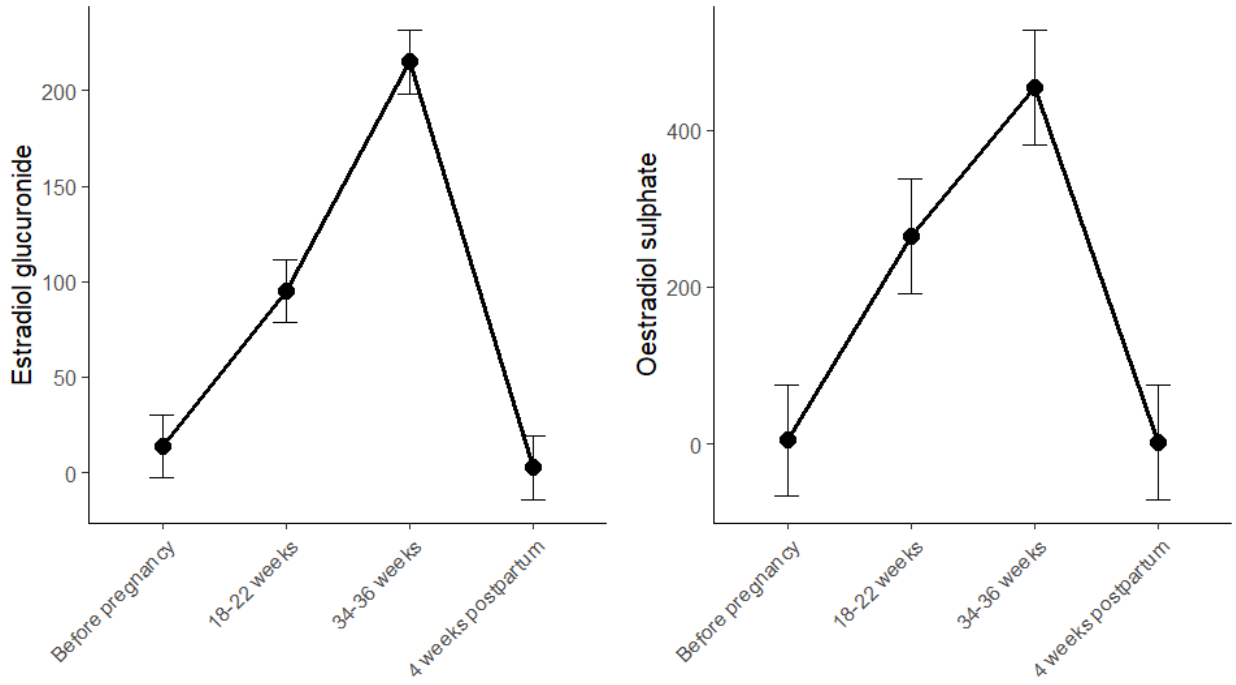

The simple effects analyses highlighted that estriol glucuronide and estriol sulphate levels significantly increased at 34-36 weeks of pregnancy compared to 18-22 weeks ($M_{diff.}$ = 318.61, $SE$ = 17.7, $t$ (457) = 17.957, $p$ < .00; $M_{diff.}$ = -138.17, $SE$ = 11.0, $t$ (457) = -12.56, $p$ < 0.00, respectively). Conversely, the levels of estriol glucuronide and sulphate significantly decreased at 4 weeks postpartum compared to 34-36 weeks of pregnancy ($M_{diff.}$ = -413.01, $SE$ = 17.8, $t$ (458) = -23.237, $p$ < .00, $M_{diff.}$ = 172.38, $SE$ = 11.0, $t$ (457) = 15.64, $p$ < 0.00), (see Supplementary Table 1).

These findings suggest that estriol glucuronide and sulphate levels rise notably during pregnancy, particularly in the third trimester, before dropping back near preconception levels postpartum.

Oestradiol glucuronide and sulphate levels significantly increased at 18-22 weeks of pregnancy (*Est.* = 65.25, *SE* = 9.93, *t* (464.06) = 6.57, *p* < .001, *βstd*=0.93; *Est.* = 212.46, *SE* = 39.89, *t* (464.05) = 5.33, *p* < .001, *βstd*=0.69, respectively) and at 34-36 weeks of pregnancy (*Est.* = 162.38, *SE* = 9.94, *t* (465.51) = 16.34, *p* < .001, *βstd* =2.31; *Est.* = 365.60, *SE* = 39.91, *t* (465.50) = 9.16, *p* < .001, *βstd*=1.19, respectively) compared to the preconception. Conversely, at 4 weeks postpartum, the oestradiol glucuronide and sulphate levels did not significantly differ from the pre-conception, as the decline was sharp enough that it almost returned to the pre-conception levels (*Est.* = -9.69, *SE* = 9.95, *t* (464.58) = -0.97, *p* = .33, *Est.* = -1.08, *SE* = 39.97, *t* (464.57) = -0.03, *p* = .98, respectively), (see Figure 2).

**Figure 2**

*Changes in oestradiol glucuronide and oestradiol sulphate from pore-conception to early pregnancy*



The simple effects analysis depicted that oestradiol glucuronide and oestradiol sulphate levels significantly increased at 34-36 weeks of pregnancy compared to 18-22 pregnancy weeks ($M_{diff.}$ =−120.2, *SE*=11.1, *t*(369) =−10.793, *p*<.0001; ($M_{diff.}$=−189.86, *SE*=48.4, *t*(369)=−3.927, *p*=.0006, respectively). By contrast, the oestradiol glucuronide and sulphate levels significantly dropped at 4 weeks postpartum compared to 34-36 pregnancy weeks ($M_{diff.}$=212.7, *SE*=11.2, *t*(370)= 19.063, *p*<.0001; ($M_{diff.}$=453.25, *SE*=48.5, *t*(369)=9.352, *p*<.0001, respectively) and to 18-22 pregnancy weeks ($M_{diff.}$= 92.6, SE=11.2, *t*(368)= 8.299, *p*<.0001; $M_{diff.}$=263.39,S*E*=48.4, *t*(368)=5.438, *p*<.0001, respectively), (see Supplementary Table 3).

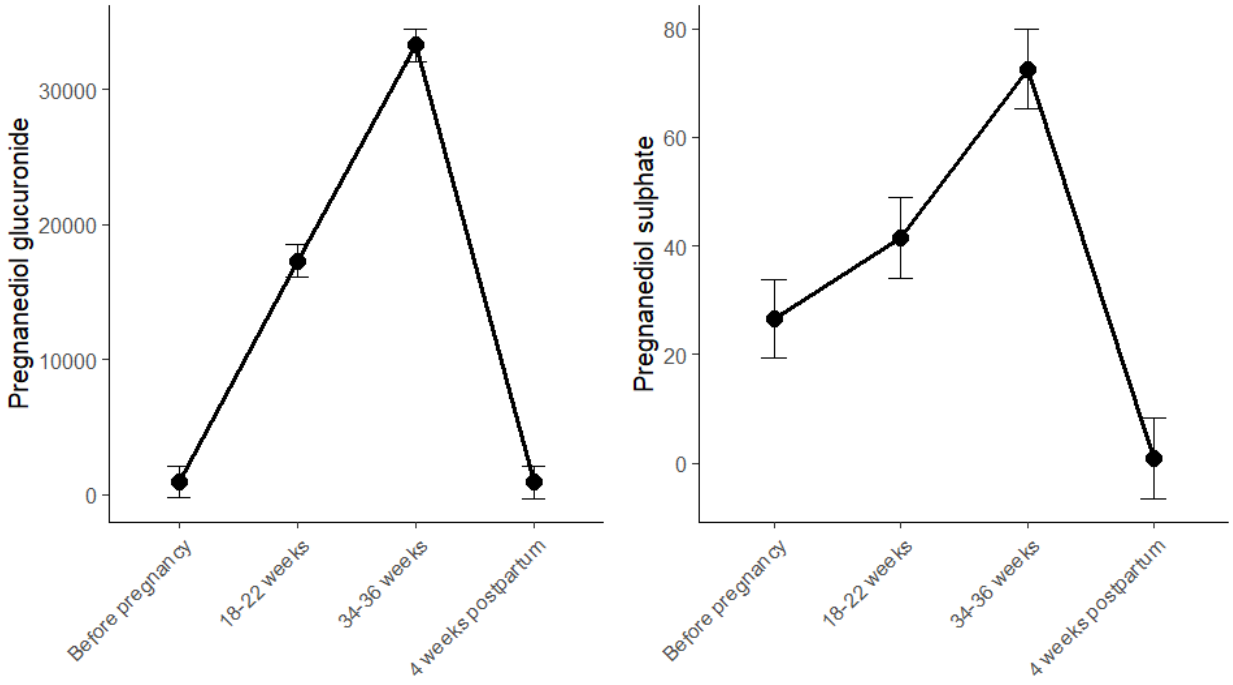
These findings suggest that oestradiol glucuronide and sulphate levels rise notably during pregnancy, particularly in the third trimester, before dropping back near preconception levels postpartum.

***Progestogens***

Pregnanediol glucuronide and pregnanediol sulphate levels significantly increased at 18-22 weeks of pregnancy (*Est.* = 13457.03, *SE* = 969.87, *t* (464.23) = 13.88, *p* < .001, *βstd*=2.43; *Est*. =14.87, *SE*= 4.85, *t*(377.09)=3.07, *p*=.002, *βstd*= 0.39, respectively) and at 34-36 weeks of pregnancy (*Est.* = 26391.27, *SE* = 970.25, *t* (465.68) = 27.20, *p* < .001, *βstd*=4.81; *Est.*= 46.00, *SE*=4.85, *t*(378.54)=9.48, *p*<.001, *βstd*= 1.19, respectively) compared to the preconception. Conversely, the pregnanediol glucuronide and sulphate levels at 4 weeks postpartum did not significantly differ from the pre-conception, as the decline was sharp enough that it almost returned to the pre-conception levels (*Est.* = 205.71, *SE* = 971.70, *t* (464.79) = 0.21, *p* = .83; *Est.*= −25.66, *SE*=4.86, *t*(377.63)= −5.28, *p*<.001, *βstd*= −0.67, respectively), (see Figure 3).

**Figure 3**

*Changes in pregnanediol glucuronide and pregnanediol sulphate from pre-conception to early postpartum*



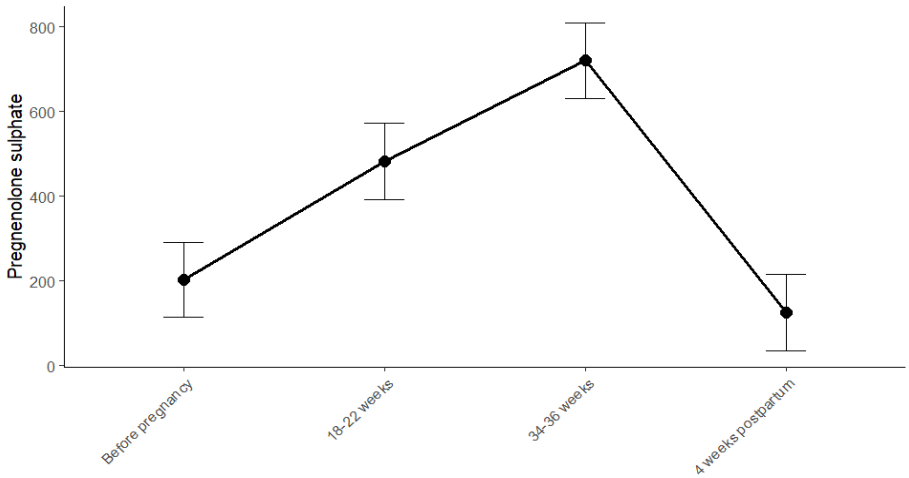

The simple effects analysis depicted significant increases in the pregnanediol glucuronide and sulphate levels at 34-36 pregnancy weeks compared to 18-22 pregnancy weeks ($M_{diff}$=−-16010.4, $SE$=857, $t$(370) =−18.677, $p$<.0001; $M_{diff}$=−31.1, $SE$=4.92, $t$(369) =−6.326, $p$<.0001). Conversely, the pregnanediol glucuronide and sulphate levels were significantly lower at 4 weeks postpartum than 34-36 pregnancy weeks ($M_{diff.}$ =32392.1, $SE$=859, $t$(371)= 37.707, $p$<.0001; $M_{diff}$=40.5, $SE$=4.93, $t$(368)= 8.222, $p$<.0001), and 18-22 pregnancy weeks ($M_{diff.}$ =16381.7, $SE$=859, $t$(370)= 19.073, $p$<.0001; $M_{diff}$=71.7, $SE$=4.93, $t$(369)=14.529, $p$<.0001), (see Supplementary Table 3).

These results indicate a pronounced increase in pregnanediol glucuronide and sulphate levels during pregnancy, particularly towards the later stages, followed by a significant decrease postpartum, almost returning to pre-conception levels.

Pregnenolone sulphate levels significantly increased at 18-22 weeks of pregnancy (*Est.*= 279.73, *SE*=41.84, *t*(372.39)=6.69, *p*<.001, *βstd*= 0.85) and at 34-36 weeks of pregnancy (*Est.* = 517.60, *SE*=41.91, *t*(373.46)=12.35, *p*<.001, *βstd*=1.57) compared to the preconception. Conversely, at 4 weeks postpartum, the pregnenolone sulphate levels did not significantly differ from the pre-conception, as the decline was sharp enough that it almost returned to the pre-conception levels (*Est.* = −78.09, *SE*=41.95, *t*(372.54) = −1.86, *p*=.06, *βstd*= −0.24), (see Figure 4).

**Figure 4**

*Changes in pregnenolone sulphate from pre-conception to early pregnancy*




The simple effects analysis depicted significant increases in the pregnenolone sulphate levels at 34-36 pregnancy weeks compared to 18-22 pregnancy weeks ($M_{diff.}$= −237.9, *SE*=42.1, *t*(365)= −5.646, *p* <.0001). Conversely, the pregnenolone sulphate levels were significantly lower at 4 weeks postpartum than 34-36 pregnancy weeks

($M_{diff}$= 595.7, *SE*= 42.2, *t*(365)= 14.101, *p* <.0001), and 18-22 pregnancy weeks ($M_{diff.}$= 357.8, *SE*=42.2, *t*(364)= 8.485, *p* <.0001), (see Supplementary Table 3).

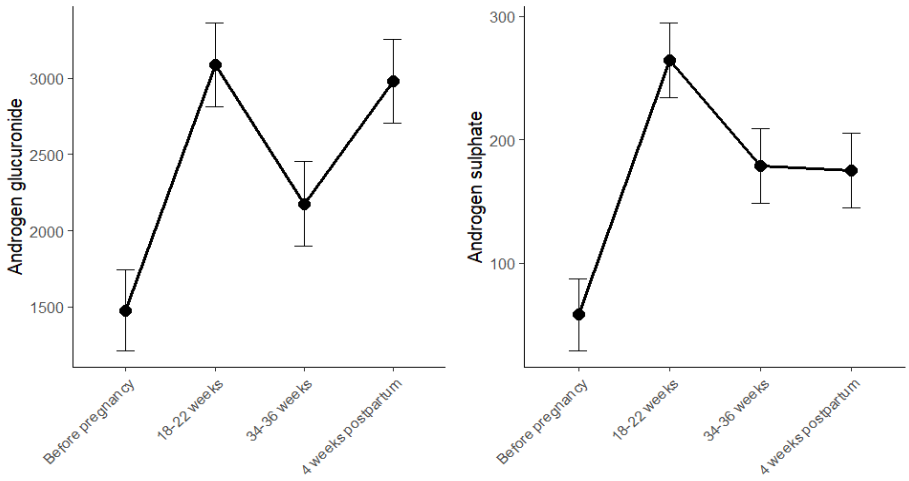
These results indicate a pronounced increase in pregnenolone sulphate levels during pregnancy, particularly towards the later stages, followed by a significant decrease at early postpartum, almost returning to pre-conception levels.

***Androgens***

Androgen glucuronide and androgen sulphate levels significantly increased at 18-22 weeks of pregnancy (*Est.* = 1759.59, *SE* = 161.25, *t* (462.40) = 10.91, *p* < .001, *βstd*= 1.22; *Est.*= 206.34, *SE*= 17.53, *t*(375.71)= 11.77, p< .001, *βstd*= 1.49, respectively) and remained significantly high at 34-36 weeks of pregnancy (*Est.* = 1146.00, *SE* = 161.40, *t* (463.71) = 7.10, *p* < .001, *βstd*= 0.53; *Est.* = 120.57, *SE*= 17.55, *t*(377.05)= 6.87, *p*< .001, *βstd*= 0.87) compared to the preconception levels. At 4 weeks postpartum, the androgen glucuronide and sulphate levels were still significantly higher compared to pre-conception (*Est.* = 1635.10, *SE* = 161.58, *t* (462.69) = 10.12, *p* < .001, *βstd*=1.14; *Est.*= 117.03, *SE*= 17.57, *t*(376.03)= 6.66, *p*<.001, *βstd*=0.84), (see Figure 5).

**Figure 5**

*Changes in androgen glucuronide and androgen sulphate from pre-conception to early postpartum*



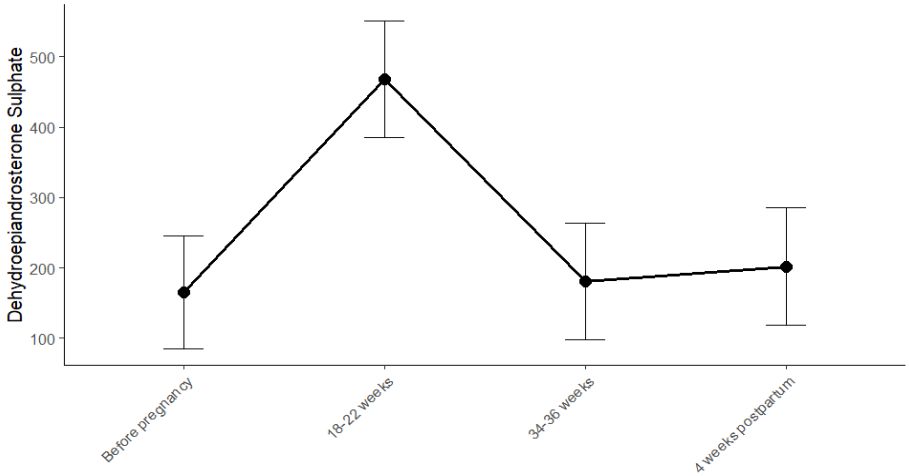

The simple effects analysis revealed that, at 34-36 pregnancy weeks, androgen glucuronide and sulphate stabilised their levels and were significantly lower compared to 18-22 pregnancy weeks ($M_{Diff.}$= 914, *SE*=168, *t*(368)= 5.427, *p*<.0001; $M_{diff.}$= 85.77, *SE*= 17.7, *t*(367)= 4.839, *p*<.0001). At 4 weeks postpartum, the androgen sulphate levels were significantly lower than at 34-36 pregnancy weeks ($M_{diff.}$= -1505 SE=167 t(377)= -9.022, *p* <.0001), while the androgen glucuronide levels were significantly lower than at 18-22 pregnancy weeks ($M_{diff.}$= 89.31, SE= 17.7, *t*(366)= 5.032, *p*<.0001). Despite the stability of the androgen glucuronide and sulphate levels at 34-36 pregnancy weeks and 4 weeks postpartum, they were still significantly higher than at pre-conception, as reported previously (see Supplementary Table 3).

These findings indicate that androgen glucuronide and sulphate levels rise sharply, peaking at 18-22 weeks. Subsequently, these levels decline moderately at 34-36 weeks and 4 weeks postpartum, where they stabilise.

Dehydroepiandrosterone sulphate levels significantly increased at 18-22 weeks of pregnancy (*Est.* = 303.35, *SE* = 45.23, *t* (374.84) = 6.71, $p < .001$, $\beta std = 0.85$) compared to pre-conception. However, these levels at 34-36 weeks of pregnancy (*Est.* = 15.27, *SE* = 45.28, *t* (376.12) = 0.34, $p = .74$) and at 4 weeks postpartum (*Est.* = 36.66, *SE* = 45.34, *t* (375.09) = 0.81, $p = .42$) did not significantly differ from the pre-conception. These non-significant contrasts suggest that the dehydroepiandrosterone sulphate levels returned close to preconception levels by 34-36 weeks of pregnancy and remained stable at 4 weeks postpartum (see Figure 6).

**Figure 6**

*Changes in dehydroepiandrosterone sulphate from pre-conception to early postpartum*




The simple effects analysis revealed that dehydroepiandrosterone sulphate levels are significantly lower at 34-36 weeks ($M_{diff.}$=288.1, *SE*=45.7, *t*(366)=6.308, $p<.0001$) and at 4 weeks postpartum ($M_{diff.}$=266.7, *SE*=45.7, *t*(365)=5.832, $p<.0001$) compared to 18-22 weeks (Supplementary Table 3).

These results indicate dehydroepiandrosterone sulphate levels rise significantly during early to mid-pregnancy but decline as the pregnancy progresses and remain relatively stable at early postpartum, returning close to pre-conception levels.

***Summary***

The results of the study revealed significant changes in the levels of various steroid metabolites from preconception through pregnancy and the postpartum period in first-time gestational mothers. Estriol glucuronide and sulphate, oestradiol glucuronide and sulphate, pregnanediol glucuronide and sulphate, and pregnenolone sulphate increased markedly during pregnancy, peaking at 34-36 weeks before sharply declining at early postpartum, returning to preconception levels. Pregnenolone sulphate levels increased significantly during pregnancy, especially towards the later stages, but did not fully return to preconception levels postpartum. Androgen glucuronide, androgen sulphate, and dehydroepiandrosterone sulphate levels rise sharply, peaking at 18-22 weeks. Subsequently, these levels decline moderately at 34-36 weeks and 4 weeks postpartum, where they stabilise.

**Association of the Cumulative Production of Steroid Metabolites with Postpartum Depression Symptoms, Well-being and Maternal Attachment**

Postpartum depressive symptoms exhibited the most pronounced association with the AUCs of most metabolites, as shown in Figure 7. Interestingly, sulphated metabolites were inversely related to postpartum depressive symptoms, whereas glucuronide metabolites showed a positive association (*Residual Deviance* = 341.2, *degrees of freedom* = 108; *Null Deviance* = 425.3, *degrees of freedom* = 115). Specifically, sulphate forms of progestogens (pregnanediol) and androgens were negatively associated with depression ($\beta$ = -0.30, 95% *CI* [−0.44, −0.16], *SE* = 0.07, *Z*

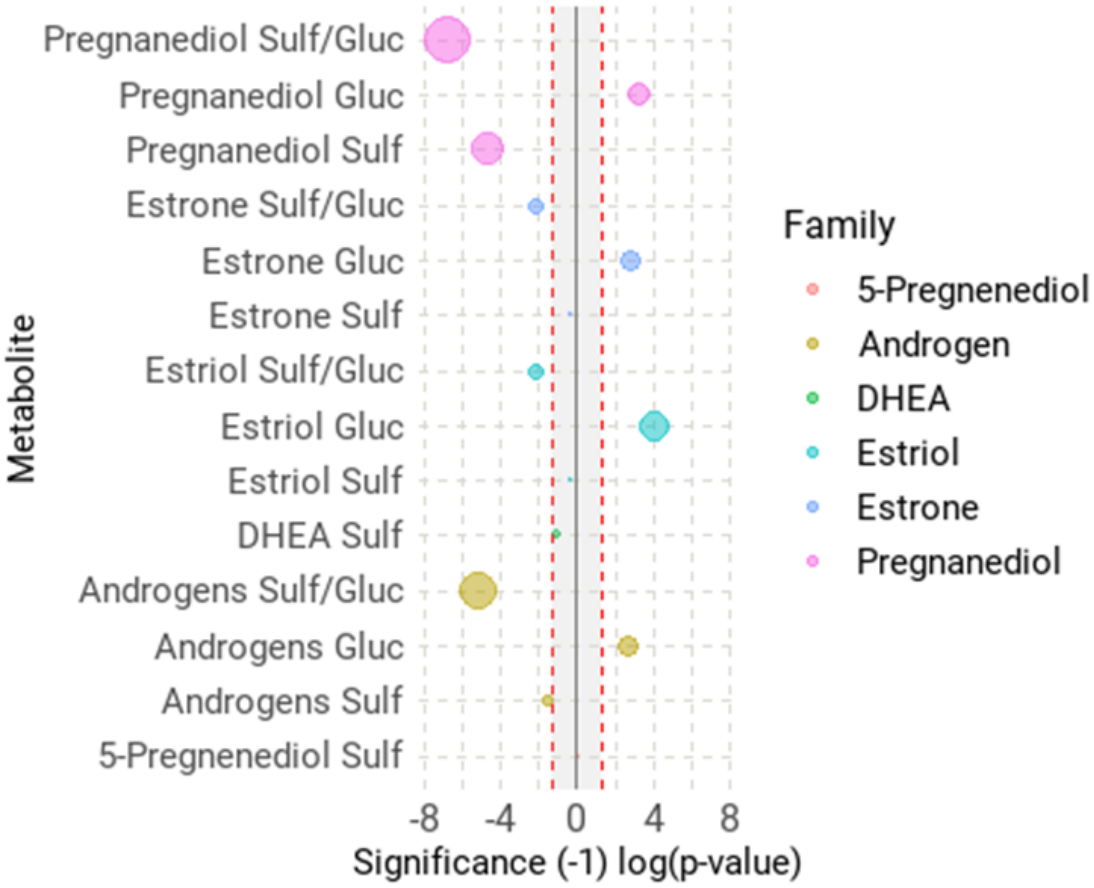
(108) = -4.26, FDR-corrected $p < 0.00$; and $\beta$ = -0.12, 95% *CI* [-0.23, -0.01, *SE* = 0.05, *Z* (108) = -2.21, FDR-corrected $p = 0.03$, respectively). In contrast, glucuronide forms of oestrogens, including estriol and estrone, were positively associated with depression ($\beta = 0.39$, 95% *CI* [0.19, 0.59], *SE* = 0.10, *Z* (108) = 3.92, FDR-corrected $p < 0.001$; and $\beta = 0.20$, 95% *CI* [0.07, 0.32], *SE* = 0.06, *Z* (108) = 3.14, FDR-corrected, $p = 0.00$, respectively). Accordingly, the ratios of sulphate to glucuronide for testosterone, estriol, oestradiol and progesterone exhibited negative associations with postpartum depression ($\beta$ = -0.27, 95% *CI* [-0.39, -0.15], *SE* = 0.06, *Z* (108) = -4.48, FDR-corrected $p < 0.001$; $\beta$ = -0.13, 95% *CI* [-0.23, -0.03], *SE* = 0.04, *Z* (108) = -2.71, FDR-corrected, $p = 0.01$; $\beta$ = -0.10, 95% *CI* [-0.18, -0.03], *SE* = 0.03, *Z* (108) = -2.73, FDR-corrected, $p = 0.01$; and $\beta$ = -0.33, 95% *CI* [-0.46, -0.21], *SE* = 0.06, *Z* (108) = -5.24, FDR-corrected, $p <$ 0.00; respectively). The dehydroepiandrosterone and pregnenolone were the only ones that did not show any of the aforementioned associations (See Figure 7). Results from the associations of depressive symptoms, Well-being, and maternal attachment with the accumulation of the steroids assessed are detailed in **Supplementary Table 4**.

Sulphated metabolites, including those generated from testosterone, progesterone, and estriol, showed a positive association with well-being, although only at a trend level ($\beta = 0.12$, 95% *CI* [0.00, 0.23], *SE* = 0.05, *t* (109) = 2.05, nominal $p =$ 0.042, FDR-corrected, $p < 0.09$ for testosterone; $\beta = 0.17$, 95% *CI* [0.03, 0.31], *SE* = 0.07, *t* (109) = 2.45, nominal $p = 0.01$, FDR-corrected $p = 0.05$ for progesterone; and $\beta$ = 0.08, 95% *CI* [−0.01, 0.17], *SE* = 0.04, *t* (109) = 1.69, nominal $p = 0.09$, FDR-corrected, $p = 0.17$ for estriol). Sulphate to glucuronide ratios were positively associated with wellbeing at a trend level ($\beta$= 0.162, 95% *CI* [0.03, 0.28], *SE* = 0.06, *t* (109) = 2.48, nominal $p = 0.01$, FDR-corrected, $p = 0.05$ for testosterone; $\beta$= 0.17, 95% *CI* [0.04,0.29], *SE* = 0.065, *t* (109) = 2.60, nominal $p = 0.01$, FDR-corrected $p = 0.05$ for

progesterone; and β= 0.13, 95% *CI* [0.03,0.23], *SE* = 0.05, *t* (109) = 2.55, nominal, *p* = 0.01, FDR-corrected *p* = 0.05 for estriol). Finally, there were no significant associations between steroid metabolites and global maternal attachment scores (See Figure 7 and Supplementary Table 4).

**Figure 7**

*The association between the steroid metabolites' AUC with depression symptoms, well-being and maternal attachment at early postpartum*




Associations of phase II steroids with postpartum depression. For consistency, values on the x-axes and circle sizes were calculated by rounding slopes (+1 or -1) multiplied by the negative logarithm of p-values from adjusted generalised linear models. Red dashed lines indicate the boundaries for a log-adjusted p-value of < |2.99| (equivalent to a p-value < 0.05)—abbreviations: DHEA, dehydroepiandrosterone, gluc, glucuronide; sulf, sulfate.

***Summary***

Postpartum depressive symptoms demonstrated the strongest associations with the AUCs of most steroid metabolites. Sulphated metabolites were inversely associated with postpartum depression, particularly progestogens (pregnanediol) and androgens, while glucuronide metabolites, including oestrogens such as estriol and estrone, were positively associated with depressive symptoms. Ratios of sulphate to glucuronide metabolites for testosterone, estriol, oestradiol, and progesterone also exhibited negative associations with postpartum depression, indicating a potential protective role of sulphated forms. However, dehydroepiandrosterone and pregnenolone showed no significant associations with depression.

Regarding well-being, sulphated metabolites from testosterone, progesterone, and estriol showed positive associations, albeit at trend levels. Similarly, sulphate-to-glucuronide ratios for testosterone, progesterone, and estriol displayed positive trend-level associations with well-being. No significant relationships were observed regarding maternal attachment between steroid metabolites and global maternal attachment scores.

## Discussion

In this study, we first examined the trajectories of steroid metabolite concentrations from preconception through pregnancy and into early postpartum. Our findings align with previous research, which states a dramatic gradual rise in steroid levels over pregnancy, reaching peak concentrations in the third trimester and sharply decreasing immediately after childbirth (Glynn et al., 2016; Shock et al., 2016; Liang et al., 2020). Specifically, we observed substantial gradual increases from mid-pregnancy up to 34–36 weeks in estriol glucuronide and sulphate, in oestradiol glucuronide and sulphate, and pregnanediol glucuronide and sulphate, followed by a rapid decline at one month postpartum, approaching preconception levels. Meanwhile, androgen glucuronide levels rose significantly in mid-pregnancy and remained elevated at one month postpartum. Besides, pregnenolone sulphate levels showed significant increases in late pregnancy but did not fully return to preconception levels after birth. Finally, dehydroepiandrosterone sulphate (DHEA-S) increased in mid-pregnancy, stabilised near baseline by 34–36 weeks, and remained steady in the first month postpartum.

With the steroid metabolites' trajectories depicted, we then investigated the associations between cumulative steroid production and conjugation—from preconception through pregnancy to one month postpartum—and mothers' postpartum depressive symptoms, general well-being, and maternal postnatal attachment. We found strong correlations between postpartum depressive symptoms and cumulative changes in the steroid levels (pregnanediol, androgens, estrone, and estriol) at one month postpartum. Notably, higher levels of sulphated steroids were associated with fewer postpartum depressive symptoms, while elevated glucuronide steroid levels correlated with increased depressive symptomatology. Postpartum well-being showed a potential association with the cumulative production of steroid metabolites (testosterone,

pregnanediol, and estriol), though this link did not survive multiple comparison adjustments. There was no significant relationship between cumulative steroid production and maternal postnatal attachment.

These findings extend prior research showing associations between postpartum depressive symptoms and various oestrogens, progestogens, and androgens (Kikuchi et al., 2021; Aswati et al., 2015; Nappi et al., 2001). Unlike prior studies that focused on linear and longitudinal changes in the steroids' concentrations in specific pregnancy stages or during brief postpartum periods (Hellgren et al., 2015; Osborne et al., 2017; Björväng et al., 2024), this study examined the cumulative steroid fluctuations from pre-pregnancy through postpartum. Our results underscore that sulphated versus glucuronide steroids had distinct associations with depressive mood and well-being at one month postpartum. Higher levels of sulphated steroids were associated with fewer postpartum depressive symptoms, while glucuronide steroids were associated with exacerbated postpartum depressive symptoms. Additionally, a higher sulphate-to-glucuronide ratio was associated with reduced postpartum depressive symptoms and showed a tendency to improve postpartum well-being.

Animal studies suggest that steroid sulphates may convert into neuroactive steroids in the brain through a desulfation process, potentially modulating mood by acting as neuroactive agents (Corpéchot et al., 1981). Sulphated steroids in the brain may arise from either endogenous synthesis or uptake from the bloodstream, which could suggest that a higher proportion of sulphated steroids may help maintain neuroactive steroid availability, contributing to mood regulation and protection against postpartum depression. Prolonged elevated neuroactive steroid levels during pregnancy, followed by a sharp and abrupt decline at childbirth, which results in reductions of GABA concentrations, a mood-regulator neurotransmitter, may, in turn, lead to the

emergence of affective symptoms. Conversely, the sulphate-to-glucuronide steroid ratio during pregnancy could act as a neuroactive reservoir, offering a protective effect against the manifestation of postpartum depressive symptoms.

Our results further suggest that the cumulative effects of steroid sulphate-to-glucuronide ratios may positively influence maternal mental well-being. However, this association was not statistically significant after conducting multiple comparisons. This limited finding may reflect using a general well-being scale rather than a specific instrument to assess maternal well-being. Additionally, we did not observe statistically significant associations between cumulative steroid levels and postpartum maternal attachment. The weakness of these findings may be due to the ceiling effects of the maternal attachment scale, which might make it difficult to detect the expected continuous attachment growth during the postpartum period.

**Study Limitations and Future Research Directions**

Several limitations in this study may account for some of the non-significant or weak results. First, using urine samples to measure steroid metabolites rather than systemic fluids (e.g., plasma or serum) may have limited the measurement accuracy, as systemic fluids directly reflect steroids' biological activity within target sites. Although urine sampling is a non-invasive instrument and allows the analysis of a very high number of samples, it only detects metabolites that are somewhat ready for excretion and does not identify active circulating forms. Future studies should complement the urine samples with blood sampling to obtain a more accurate measure of bioactive steroid levels.

Another area for improvement is our sample size, which may have constrained our results' statistical power and robustness. Additionally, limiting our follow-up to one

month postpartum has restricted the exploration of longer-term trends in steroid changes and their associations with depressive symptoms, well-being, and maternal attachment over the postpartum period. Future studies should consider gathering larger samples and extending the follow-up postpartum period to capture these dynamics more comprehensively.

The weak association between pregnancy-related steroids and maternal well-being may stem from the general nature of the WHO-Well-being Index (*WHO-5*, 1998 version; Topp et al., 2015), which may lack sensitivity to well-being related to motherhood. So, future studies should employ scales such as the Satisfaction with Life Scale (Larsen et al., 1985, cited in Quick et al., 2023) or the Flourishing Scale (Diener et al., 2010, cited in Quick et al., 2023), which precisely measure life satisfaction during motherhood, to obtain more significant results.

Finally, the absence of significant associations between steroids and maternal attachment may be due to limitations within the attachment scale itself, which may have a ceiling effect, as maternal attachment appeared to peak by the third trimester and remained high at postpartum. Future research should integrate self-reports with observational and experimental measures to capture a more accurate and comprehensive view of maternal attachment development at postpartum.

## Conclusions

This study identifies a potentially novel mechanism by which steroid hormones may influence maternal mental health at postpartum. Our findings suggest that a higher sulphate-to-glucuronide ratio during pregnancy could protect mothers from suffering postpartum depressive symptoms through the increase in the availability of neuroactive steroids in the brain. This insight could have significant clinical implications, providing a potential basis for clinical interventions aimed at mitigating postpartum depressive mood through steroid modulation.

## General Conclusion

In conclusion, both studies provide a better understanding of how social, experiential, and hormonal factors impact mothers' affect and maternal attachment during the postpartum period. On top of that, these studies identify new pathways through which these factors influence maternal affective symptoms and attachment. The first study demonstrates that social support and the birth experience affect maternal postnatal attachment indirectly by influencing mothers' affective symptoms. The second study uncovers an impact of the cumulative levels of steroids during pregnancy on postpartum depressive symptoms, suggesting that a higher sulphate-to-glucuronide ratio during pregnancy may help protect new mothers from experiencing postpartum depression.

These novel insights about the underlying mechanisms and factors influencing mothers' mental health and maternal attachment could help design future clinical interventions. These interventions may prioritize strengthening support from friends and family, ensuring positive birth experiences, and developing specific hormonal treatments for mood regulation. Ultimately, this novel intervention approach may help mitigate the exacerbation of postpartum affective symptoms, promote positive maternal attachment and support healthy infant emotional development.

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## Study 1: Supplementary Materials

**Table S1**

*Changes in affective symptoms, well-being and maternal attachment over pregnancy and postpartum in gestational mothers*

| | Before Pregnancy | 18-22 Weeks | 34-36 Weeks | 4 Weeks Postpartum | 6 Months Postpartum |
|---|---|---|---|---|---|
| **Stress Symptoms** | *M* = 17.0, *SD* = 0.738, 95% CI [15.6, 18.5] | *M* = 15.6, *SD* = 0.740, 95% CI [14.2, 17.1] | *M* = 17.5, *SD* = 0.742, 95% CI [16.0, 19.0] | *M* = 21.0, *SD* = 0.738, 95% CI [19.5, 22.4] | *M* = 19.6, *SD* = 0.738, 95% CI [18.2, 21.1] |
| **Depression Symptoms** | *M* = 1.61, *SD* = 0.07, 95% CI [1.48, 1.75] | *M* = 1.31, *SD* = 0.07, 95% CI [1.17, 1.45] | *M* = 1.33, *SD* = 0.07, 95% CI [1.19, 1.46] | *M* = 1.57, *SD* = 0.07, 95% CI [1.44, 1.71] | *M* = 1.43, *SD* = 0.07, 95% CI [1.30, 1.57] |
| **General Wellbeing** | *M* = 15.7, *SD* = 0.35, 95% CI [15.0, 16.4] | *M* = 16.4, *SD* = 0.35, 95% CI [15.7, 17.1] | *M* = 15.8, *SD* = 0.35, 95% CI [15.1, 16.4] | *M* = 13.7, *SD* = 0.35, 95% CI [13.0, 14.4] | *M* = 15.1, *SD* = 0.35, 95% CI [14.5, 15.8] |
| **Sleep Quality** | | | | | |
| **Global Maternal Attachment** | - | *M* = 4.01, *SD* = 0.03, 95% CI [3.95, 4.06] | *M* = 4.17, *SD* = 0.03, 95% CI [4.11, 4.23] | *M* = 4.14, *SD* = 0.03, 95% CI [4.08, 4.19] | *M* = 4.16, *SD* = 0.03, 95% CI [4.10, 4.21] |
| **Global Perceived Social Support** | - | - | - | *M* = 6.14, *SD* = 0.09, 95% CI [5.96, 6.31] | *M* = 5.86, *SD* = 0.09, 95% CI [5.68, 6.03] |
| **Perceived Social Support from Partner** | - | - | - | *M* = 6.41, *SD* = 0.10, 95% CI [6.22, 6.59] | *M* = 6.15, *SD* = 0.09, 95% CI [5.97, 6.33] |
| **Perceived Social Support from Family** | - | - | - | *M* = 6.11, *SD* = 0.10, 95% CI [5.90, 6.31] | *M* = 5.76, *SD* = 0.10, 95% CI [5.56, 5.97] |

| | | | | | |
|---|---|---|---|---|---|
| **Perceived Social Support from Friends** | - | - | - | *M* = 6.07, *SD* = 0.10, 95% CI [5.87, 6.26] | *M* = 5.71, *SD* = 0.10, 95% CI [5.51, 5.90] |

**Table S2**

*Changes in affective symptoms, well-being and maternal attachment over pregnancy and postpartum period in non-gestational mothers*

| | **Before Pregnancy** | **18-22 Weeks** | **34-36 Weeks** | **4 Weeks Postpartum** | **6 Months Postpartum** |
|---|---|---|---|---|---|
| **Stress Symptoms** | *M* = 16.2, *SD* = 1.727, 95% CI [12.8, 19.6] | *M* = 18.0, *SD* = 1.727, 95% CI [14.6, 21.4] | *M* = 19.1, *SD* = 1.727, 95% CI [15.7, 22.5] | *M* = 17.4, *SD* = 1.727, 95% CI [14.0, 20.8] | *M* = 17.8, *SD* = 1.727, 95% CI [14.4, 21.2] |
| **State Stress Symptoms** | *M* = 5.48, *SD* = 0.47, 95% CI [4.54, 6.42] | *M* = 5.83, *SD* = 0.47, 95% CI [4.54, 6.42] | *M* = 6.26, *SD* = 0.47, 95% CI [5.32, 7.20] | *M* = 3.83, *SD* = 0.47, 95% CI [2.88, 4.77] | *M* = 5.04, *SD* = 0.47, 95% CI [4.10, 5.98] |
| **Depression Symptoms** | *M* = 5.48, *SD* = 0.47, 95% CI [4.54, 6.42] | *M* = 5.83, *SD* = 0.47, 95% CI [4.88, 6.77] | *M* = 6.26, *SD* = 0.47, 95% CI [5.32, 7.20] | *M* = 3.83, *SD* = 0.47, 95% CI [2.88, 4.77] | *M* = 5.04, *SD* = 0.47, 95% CI [4.10, 5.98] |
| **General Well-being** | *M* = 16.7, *SD* = 0.82, 95% CI [15.1, 18.3] | *M* = 16.7, *SD* = 0.82, 95% CI [15.1, 18.3] | *M* = 15.2, *SD* = 0.82, 95% CI [13.6, 16.8] | *M* = 14.8, *SD* = 0.82, 95% CI [13.2, 16.4] | *M* = 15.8, *SD* = 0.82, 95% CI [14.2, 17.4] |
| **Global Maternal Attachment** | - | *M* = 4.14, *SD* = 0.07, 95% CI [4.01, 4.27] | *M* = 4.29, *SD* = 0.07, 95% CI [4.16, 4.42] | *M* = 4.26, *SD* = 0.07, 95% CI [4.12, 4.39] | *M* = 4.17, *SD* = 0.07, 95% CI [4.04, 4.30] |
| **Global Perceived Social Support** | - | - | - | *M* = 6.00, *SD* = 0.21, 95% CI [5.59, 6.41] | *M* = 6.17, *SD* = 0.21, 95% CI [5.76, 6.58] |
| **Perceived Social** | - | - | - | *M* = 6.22, *SD* = 0.25, 95% | *M* = 6.88, *SD* = 0.23, 95% |

| | | | | | |
|---|---|---|---|---|---|
| **Support from Partner** | | | | CI [5.73, 6.70] | CI [6.42, 7.33] |
| **Perceived Social Support from Family** | - | - | - | *M* = 5.70, *SD* = 0.24, 95% CI [5.22, 6.17] | *M* = 5.57, *SD* = 0.24, 95% CI [5.09, 6.04] |
| **Perceived Social Support from Friends** | - | - | - | *M* = 6.13, *SD* = 0.23, 95% CI [5.68, 6.58] | *M* = 6.13, *SD* = 0.23, 95% CI [5.68, 6.58] |

**Table S3**

*Changes in affective symptoms and well-being over pregnancy and postpartum period in nulliparous women*

| | **Before Pregnancy** | **18-22 Weeks** | **34-36 Weeks** | **4 Weeks Postpartum** | **6 Months Postpartum** |
|---|---|---|---|---|---|
| **Stress Symptoms** | *M* = 17.0, *SD* = 1.442, 95% CI [14.1, 19.8] | *M* = 17.7, *SD* = 1.442, 95% CI [14.9, 20.6] | *M* = 17.2, *SD* = 1.456, 95% CI [14.3, 20.0] | *M* = 18.8, *SD* = 1.442, 95% CI [15.9, 21.6] | *M* = 17.8, *SD* = 1.456, 95% CI [15.0, 20.7] |
| **State Stress Symptoms** | *M* = 5.21, *SD* = 0.40, 95% CI [4.43, 6.00] | *M* = 5.21, *SD* = 0.40, 95% CI [4.43, 6.00] | *M* = 5.63, *SD* = 0.40, 95% CI [4.84, 6.43] | *M* = 5.06, *SD* = 0.40, 95% CI [4.27, 5.85] | *M* = 4.94, *SD* = 0.40, 95% CI [4.15, 5.74] |
| **Depression Symptoms** | *M* = 5.21, *SD* = 0.40, 95% CI [4.43, 6.00] | *M* = 5.21, *SD* = 0.40, 95% CI [4.43, 6.00] | *M* = 5.63, *SD* = 0.40, 95% CI [4.84, 6.43] | *M* = 5.06, *SD* = 0.40, 95% CI [4.27, 5.85] | *M* = 4.94, *SD* = 0.40, 95% CI [4.15, 5.74] |

| | | | | | |
|---|---|---|---|---|---|
| **General Well-being** | *M* = 15.6, *SD* = 0.68, 95% CI [14.2, 16.9] | *M* = 15.7, *SD* = 0.68, 95% CI [14.3, 17.0] | *M* = 16.4, *SD* = 0.69, 95% CI [15.0, 17.7] | *M* = 16.4, *SD* = 0.68, 95% CI [15.1, 17.8] | *M* = 16.5, *SD* = 0.69, 95% CI [15.1, 17.8] |

**Table S4**

*Stress between natural conception and assisted reproduction groups over pregnancy and postpartum*

| | **Natural Conception** | **Assisted Reproduction** |
|---|---|---|
| **Before pregnancy** | $M_{diff.}$ = 17.56, *SE* = 1.05, *df* = 331.51, *CI* = [15.50, 19.63] | $M_{diff.}$ = 16.59, *SE* = 1.04, *df* = 331.51, *CI* = [14.54, 18.64] |
| **18-22 weeks** | $M_{diff.}$ = 16.55, *SE* = 1.05, *df* = 331.51, *CI* = [14.48, 18.61] | $M_{diff.}$ = 14.78, *SE* = 1.04, *df* = 331.51, *CI* = [12.73, 16.83] |
| **34-36 weeks** | $M_{diff.}$ = 17.55, *SE* = 1.06, *df* = 336.05, *CI* = [15.47, 19.63] | $M_{diff.}$ = 17.46, *SE* = 1.04, *df* = 331.51, *CI* = [15.41, 19.51] |
| **4 weeks postpartum** | $M_{diff.}$ = 22.61, *SE* = 1.05, *df* = 331.51, *CI* = [20.55, 24.68]) | $M_{diff.}$ = 19.44, *SE* = 1.04, *df* = 331.51, *CI* = [17.40, 21.49] |
| **6 months postpartum** | $M_{diff.}$ = 19.91, *SE* = 1.05, *df* = 331.51, *CI* = [17.85, 21.99] | $M_{diff.}$ = 19.32, *SE* = 1.04, *df* = 331.51, *CI* = [17.27, 21.37] |

**Table S5**

*Depression between natural conception and assisted reproduction groups over pregnancy and postpartum*

| | **Natural Conception** | **Assisted Reproduction** |
|---|---|---|
| **Before pregnancy** | $M_{diff.}$ = 1.69, *SE* = 0.0984, *df* = 321, *CI* = [1.50, 1.88] | $M_{diff.}$ = 1.55, *SE* = 0.0976, *df* = 321, *CI* = [1.35, 1.74] |
| **18-22 weeks** | $M_{diff.}$ = 1.40, *SE* = 0.0984, *df* = 321, *CI* = [1.21, 1.59] | $M_{diff.}$ = 1.22, *SE* = 0.0976, *df* = 321, *CI* = [1.03, 1.41] |
| **34-36 weeks** | $M_{diff.}$ = 1.34, *SE* = 0.0989, *df* = 326, *CI* = [1.14, 1.53] | $M_{diff.}$ = 1.32, *SE* = 0.0976, *df* = 321, *CI* = [1.13, 1.51] |
| **4 weeks postpartum** | $M_{diff.}$ = 1.63, *SE* = 0.0984, *df* = 321, *CI* = [1.44, 1.82] | $M_{diff.}$ = 1.52, *SE* = 0.0981, *df* = 326, *CI* = [1.33, 1.72] |
| **6 months postpartum** | $M_{diff.}$ = 1.41, *SE* = 0.0989, *df* = 326, *CI* = [1.22, 1.60]) | $M_{diff.}$ = 1.44, *SE* = 0.0976, *df* = 321, *CI* = [1.25, 1.64] |

**Figure S1**

*Depression between natural conception and assisted reproduction groups over pregnancy and postpartum*

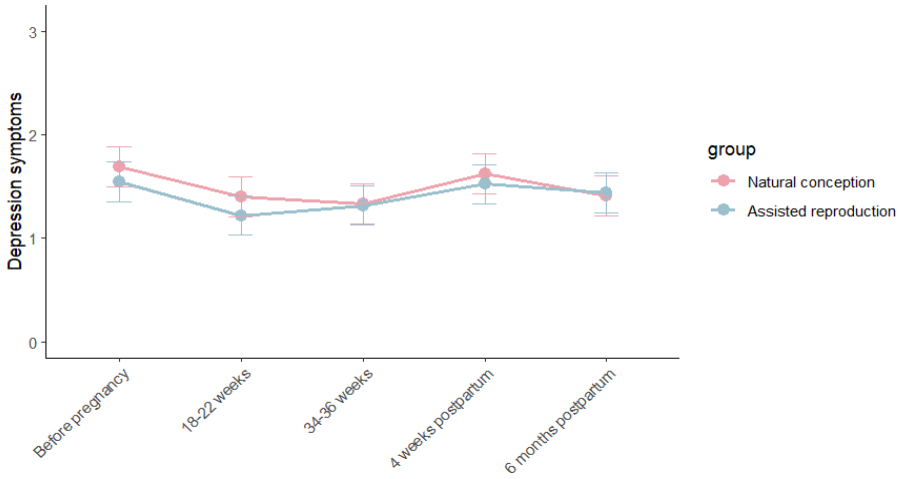


**Table 6**

*Well-being between natural conception and assisted reproduction groups over pregnancy and postpartum*

| | Natural Conception | Assisted Reproduction |
|---|---|---|
| **Before pregnancy** | $M_{diff.}$ = 15.5, *SE* = 0.501, *df* = 321, *CI* = [14.5, 16.5] | $M_{diff.}$ = 15.9, *SE* = 0.497, *df* = 321, *CI* = [14.9, 16.9] |
| **18-22 weeks** | $M_{diff.}$ = 15.9, *SE* = 0.501, *df* = 321, *CI* = [14.9, 16.8] | $M_{diff.}$ = 16.9, *SE* = 0.497, *df* = 321, *CI* = [15.9, 17.8] |
| **34-36 weeks** | $M_{diff.}$ = 15.8, *SE* = 0.504, *df* = 325, *CI* = [14.8, 16.8] | $M_{diff.}$ = 15.7, *SE* = 0.497, *df* = 321, *CI* = [14.7, 16.6] |
| **4 weeks postpartum** | $M_{diff.}$ = 13.5, *SE* = 0.501, *df* = 321, *CI* = [12.6, 14.5] | $M_{diff.}$ = 13.8, *SE* = 0.497, *df* = 321, *CI* = [12.8, 14.8] |
| **6 months postpartum** | $M_{diff.}$ = 15.1, *SE* = 0.501, *df* = 321, *CI* = [14.1, 16.1] | $M_{diff.}$ = 15.2, *SE* = 0.497, *df* = 321, *CI* = [14.2, 16.2] |

**Figure S2**

*Well-being between natural conception and assisted reproduction groups over pregnancy and postpartum*

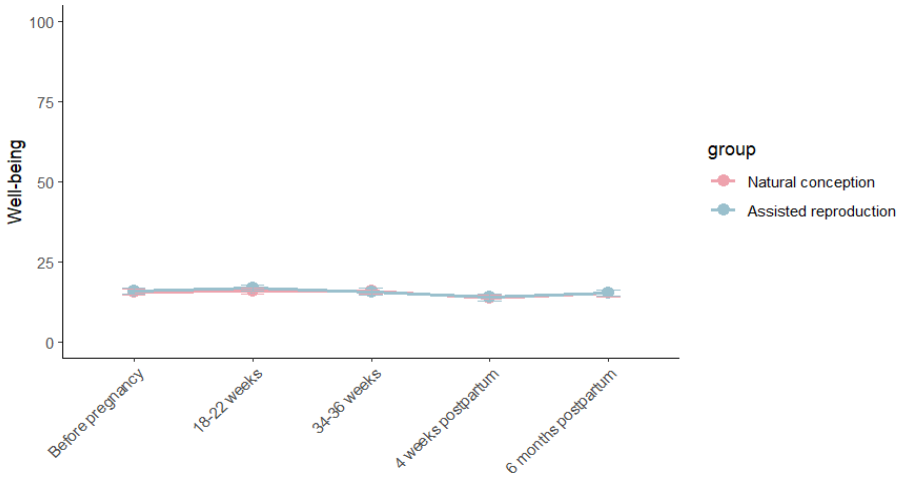


**Table S7**

*Sleep quality between natural conception and assisted reproduction groups over pregnancy and postpartum*

| | Natural Conception | Assisted Reproduction |
|---|---|---|
| **Before pregnancy** | $M_{diff.}$ = 1.59, $SE$ = 0.0577, $df$ = 451, $CI$ = [1.47, 1.70] | $M_{diff.}$ = 1.60, $SE$ = 0.0565, $df$ = 441, $CI$ = [1.48, 1.71] |
| **18-22 weeks** | $M_{diff.}$ = 1.80, $SE$ = 0.0573, $df$ = 446, $CI$ = [1.69, 1.91] | $M_{diff.}$ = 1.78, $SE$ = 0.0565, $df$ = 441, $CI$ = [1.67, 1.90] |
| **34-36 weeks** | $M_{diff.}$ = 2.08, $SE$ = 0.0577, $df$ = 451, $CI$ = [1.97, 2.20] | $M_{diff.}$ = 2.01, $SE$ = 0.0568, $df$ = 446, $CI$ = [1.90, 2.12] |
| **4 weeks postpartum** | $M_{diff.}$ = 2.40, $SE$ = 0.0569, $df$ = 441, $CI$ = [2.29, 2.52] | $M_{diff.}$ = 2.37, $SE$ = 0.0568, $df$ = 446, $CI$ = [2.25, 2.48] |
| **6 months postpartum** | $M_{diff.}$ = 2.10, $SE$ = 0.0569, $df$ = 441, $CI$ = [1.99, 2.21] | $M_{diff.}$ = 2.17, $SE$ = 0.0565, $df$ = 441, $CI$ = [2.05, 2.28] |

**Figure S3**

*Sleep quality between natural conception and assisted reproduction groups over pregnancy and postpartum*

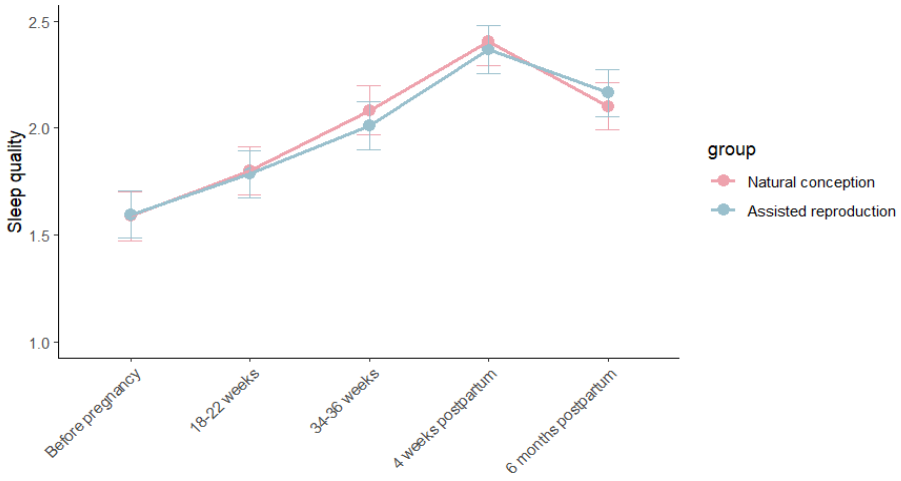


**Table S8**

*Global maternal attachment between natural conception and assisted reproduction groups over pregnancy and postpartum*

| Session | Natural Conception | Assisted Reproduction |
|---|---|---|
| **18-22 weeks** | $M_{diff.}$ = 3.95, $SE$ = 0.0411, $df$ = 299, $CI$ = [3.87, 4.03] | $M_{diff.}$ = 4.06, $SE$ = 0.0408, $df$ = 299, $CI$ = [3.98, 4.15] |
| **34-36 weeks** | $M_{diff.}$ = 4.11, $SE$ = 0.0413, $df$ = 303, $CI$ = [4.03, 4.19] | $M_{diff.}$ = 4.23, $SE$ = 0.0408, $df$ = 299, $CI$ = [4.15, 4.31] |
| **4 weeks postpartum** | $M_{diff.}$ = 4.09, $SE$ = 0.0411, $df$ = 299, $CI$ = [4.01, 4.17] | $M_{diff.}$ = 4.19, $SE$ = 0.0408, $df$ = 299, $CI$ = [4.11, 4.27] |
| **6 months postpartum** | $M_{diff.}$ = 4.12, $SE$ = 0.0411, $df$ = 299, $CI$ = [4.03, 4.20] | $M_{diff.}$ = 4.20, $SE$ = 0.0408, $df$ = 299, $CI$ = [4.12, 4.28] |

**Table S9**

*The impact of affective symptoms on maternal attachment at postpartum*

| | 4 weeks postpartum | 6 months postpartum | Interaction with 6 months postpartum |
|---|---|---|---|
| **Perceived stress** | *Est.* = -0.02, 95% *CI* [-0.03, -0.02], *SE* = 0.00, *t*(221.09) = -7.84, *p* < .001 | *Est.* = -0.15, 95% *CI* [-0.29, -0.01], *SE* = 0.07, *t*(136.05) = -2.12, *p* = .04 | *Est.* = 0.01, 95% *CI* [0.00, 0.01], *SE* = 0.00, *t*(139.82) = 2.22, *p* = .03 |
| **Depression** | *Est.* = -0.21, 95% *CI* [-0.27, -0.15], *SE* = 0.03, *t*(203.64) = -6.86, *p* < .001 | *Est.* = -0.21, 95% *CI* [-0.33, -0.09], *SE* = 0.06, *t*(133.04) = -3.62, *p* < .001 | *Est.* = 0.14, 95% *CI* [0.07, 0.21], *SE* = 0.04, *t*(137.06) = 3.94, *p* < .001 |
| **Well-being** | *Est.* = 0.05, 95% *CI* [0.04, 0.06], *SE* = 0.01, *t*(230.53) = 9.74, *p* < .001 | *Est.* = 0.13, 95% *CI* [-0.06, 0.32], *SE* = 0.10, *t*(134.42) = 1.35, *p* = .18 | *Est.* = -0.01, 95% *CI* [-0.02, 0.00], *SE* = 0.01, *t*(134.13) = -1.98, *p* = .05 |
| **Sleep quality** | *Est.* = -0.30, 95% *CI* [-0.46, -0.14], *SE* = 0.08, *t*(182.68) = -3.56, *p* < .001 | *Est.* = -0.41, 95% *CI* [-0.83, 0.01], *SE* = 0.21, *t*(150.83) = -1.92, *p* = .06 | *Est.* = 0.16, 95% *CI* [-0.02, 0.34], *SE* = 0.09, *t*(149.76) = 1.78, *p* = .08 |

**Table S10**

*The impact of perceived social support on stress at postpartum*

| | 4 weeks postpartum | 6 months postpartum | Interaction:6 months postpartum |
|---|---|---|---|
| **Global perceived social support** | *Est.* = -3.04, 95% *CI* [-4.64, -1.44], *SE* = 0.81, *t*(204.27) = -3.74, *p* < .001 | *Est.* = -8.10, 95% *CI* [-18.54, 2.34], *SE* = 5.29, *t*(147.72) = -1.53, *p* = .13 | *Est.* = 1.05, 95% *CI* [-0.65, 2.75], *SE* = 0.86, *t*(147.07) = 1.22, *p* = .22 |
| **Perceived social support from partner** | *Est.* = -1.50, 95% *CI* [-2.94, -0.06], *SE* = 0.73, *t*(222.95) = -2.07, *p* = .04 | *Est.* = -0.19, 95% *CI* [-9.47, 9.09], *SE* = 4.70, *t*(139.18) = -0.04, *p* = .97 | *Est.* = -0.22, 95% *CI* [-1.65, 1.21], *SE* = 0.73, *t*(137.91) = -0.29, *p* = .77 |
| **Perceived social support from family** | *Est.* = -2.59, 95% *CI* [-3.97, -1.21], *SE* = 0.70, *t*(204.55) = -3.68, *p* < .001 | *Est.* = -4.12, 95% *CI* [-13.22, 5.00], *SE* = 4.62, *t*(146.45) = -0.89, *p* = .37 | *Est.* = 0.38, 95% *CI* [-1.12, 1.88], *SE* = 0.76, *t*(145.80) = 0.50, *p* = .62 |
| **Perceived social support from friends** | *Est.* = -3.48, 95% *CI* [-4.88, -2.08], *SE* = 0.71, *t*(204.57) = -4.92, *p* < .001 | *Est.* = -12.05, 95% *CI* [-21.27, -2.83], *SE* = 4.66, *t*(149.85) = -2.59, *p* = .01 | *Est.* = 1.70, 95% *CI* [0.17, 3.23], *SE* = 0.77, *t*(149.25) = 2.21, *p* = .03 |

**Table S11**

*The differential effects of support from partner, family and friends on stress over postpartum*

| | 4 weeks postpartum | 6 months postpartum |
|---|---|---|
| **Perceived social support from partner** | $\beta$= 0.43, 95% *CI* [-0.98, 1.85], *SE* = 0.72, *t*(161) = 0.61, *p* = .55, *lmg* = 0.004, *VIF* = 1.21 | $\beta$ = 0.07, 95% *CI* [-1.53, 1.68], *SE* = 0.81, *t*(119) = 0.09, *p* = .93, *lmg* = 0.019, *VIF* = 2.21 |
| **Perceived social support from family** | $\beta$ = -0.50, 95% *CI* [-2.03, 1.03], *SE* = 0.77, *t*(161) = -0.64, *p* = .52, *lmg* = 0.025, *VIF* = 1.48 | $\beta$ = -1.59, 95% *CI* [-3.19, 0.01], *SE* = 0.81, *t*(119) = -1.97, *p* = .051, *lmg* = 0.055, *VIF* = 2.12 |
| **Perceived social support from friends** | $\beta$ = -3.01, 95% *CI* [-4.70, -1.32], *SE* = 0.86, *t*(161) = -3.51, *p* < .001, *lmg* = 0.090, *VIF* = 1.60 | $\beta$ = -0.70, 95% *CI* [-2.20, 0.81], *SE* = 0.76, *t*(119) = -0.92, *p* = .36, *lmg* = 0.029, *VIF* = 1.89 |

**Table S12**

*The impact of perceived social support on depression at postpartum*

| | 4 weeks postpartum | 6 months postpartum | Interaction:6 months postpartum |
|---|---|---|---|
| **Global perceived social support** | *Est.* = -0.15, 95% *CI* [-0.31, 0.00], *SE* = 0.08, *t*(203.51) = -1.98, *p* = .05 | *Est.* = 0.73, 95% *CI* [-0.24, 1.70], *SE* = 0.49, *t*(146.92) = 1.49, *p* = .14 | *Est.* = -0.16, 95% *CI* [-0.32, 0.01], *SE* = 0.08, *t*(146.23) = -1.93, *p* = .06 |
| **Perceived social support from partner** | *Est.* = -0.09, 95% *CI* [-0.22, 0.04], *SE* = 0.07, *t*(222.11) = -1.40, *p* = .16 | *Est.* = 0.92, 95% *CI* [0.06, 1.78], *SE* = 0.43, *t*(138.18) = 2.13, *p* = .04 | *Est.* = -0.18, 95% *CI* [-0.32, -0.04], *SE* = 0.07, *t*(137.03) = -2.63, *p* = .01 |
| **Perceived social support from family** | *Est.* = -0.19, 95% *CI* [-0.31, -0.06], *SE* = 0.06, *t*(206.33) = -2.90, *p* < .001 | *Est.* = 0.60, 95% *CI* [-0.23, 1.43], *SE* = 0.42, *t*(146.08) = 1.42, *p* = .16 | *Est.* = -0.14, 95% *CI* [-0.28, -0.00], *SE* = 0.07, *t*(145.05) = -2.01, *p* = .05 |
| **Perceived social support from friends** | *Est.* = -0.23, 95% *CI* [-0.37, -0.09], *SE* = 0.07, *t*(207.79) = -3.35, *p* < .001 | *Est.* = -0.24, 95% *CI* [-1.15, 0.67], *SE* = 0.46, *t*(151.71) = -0.54, *p* = .59 | *Est.* = 0.00, 95% *CI* [-0.15, 0.15], *SE* = 0.08, *t*(151.16) = 0.06, *p* = .95 |

**Table S13**

*The differential effects of support from partner, family and friends on depression over postpartum*

| | 4 weeks postpartum | 6 months postpartum |
|---|---|---|
| **Perceived social support from partner** | $\beta$ = 0.12, *SE* = 0.08, 95% *CI* [0.01, 0.23], *t*(161) = 1.50, *p* = .14, *lmg* = 0.009, *VIF* = 1.21 | $\beta$ = -0.04, *SE* = 0.10, 95% *CI* [-0.24, 0.16], *t*(118) = -0.41, *p* = .68, *lmg* = 0.045, *VIF* = 2.21 |
| **Perceived social support from family** | $\beta$ = -0.06, *SE* = 0.07, 95% *CI* [-0.12, 0.00], *t*(161) = -2.14, *p* = .03, *lmg* = 0.026, *VIF* = 1.48 | $\beta$ = -0.25, *SE* = 0.12, 95% *CI* [-0.49, -0.01], *t*(118) = -2.08, *p* = .04, *lmg* = 0.115, *VIF* = 2.12 |
| **Perceived social support from friends** | $\beta$ = -0.26, *SE* = 0.09, 95% *CI* [-0.38, -0.14], *t*(161) = -3.02, *p* = .003, *lmg* = 0.077, *VIF* = 1.60 | $\beta$ = -0.01, *SE* = 0.09, 95% *CI* [-0.19, 0.17], *t*(118) = -0.07, *p* = .94, *lmg* = 0.030, *VIF* = 1.89 |

**Table S14**

*The impact of perceived social support on well-being at postpartum*

| | 4 weeks postpartum | 6 months postpartum | Interaction:6 months postpartum |
|---|---|---|---|
| **Global perceived social support** | *Est.* = 0.22, 95% *CI* [-0.51, 0.95], *SE* = 0.37, *t*(191.26) = 0.61, *p* = .55 | *Est.* = 0.45, 95% *CI* [-4.18, 5.08], *SE* = 2.35, *t*(140.90) = 0.19, *p* = .85 | *Est.* = 0.19, 95% *CI* [-0.56, 0.94], *SE* = 0.38, *t*(140.23) = 0.48, *p* = .63 |
| **Perceived social support from partner** | *Est.* = 0.28, 95% *CI* [-0.38, 0.94], *SE* = 0.33, *t*(214.06) = 0.86, *p* = .39 | *Est.* = 0.37, 95% *CI* [-3.66, 4.40], *SE* = 2.04, *t*(135.30) = 0.18, *p* = .86 | *Est.* = 0.20, 95% *CI* [-0.43, 0.83], *SE* = 0.32, *t*(133.97) = 0.62, *p* = .54 |
| **Perceived social support from family** | *Est.* = 0.52, 95% *CI* [-0.11, 1.15], *SE* = 0.32, *t*(193.51) = 1.60, *p* = .11 | *Est.* = 0.82, 95% *CI* [-3.24, 4.88], *SE* = 2.06, *t*(140.70) = 0.40, *p* = .69 | *Est.* = 0.14, 95% *CI* [-0.53, 0.81], *SE* = 0.34, *t*(140.01) = 0.42, *p* = .67 |
| **Perceived social support from friends** | *Est.* = 0.42, 95% *CI* [-0.24, 1.08], *SE* = 0.33, *t*(190.31) = 1.28, *p* = .20 | *Est.* = 2.12, 95% *CI* [-2.02, 6.26], *SE* = 2.10, *t*(141.91) = 1.01, *p* = .31 | *Est.* = -0.09, 95% *CI* [-0.78, 0.60], *SE* = 0.35, *t*(141.24) = -0.25, *p* = .81 |

**Figure S4**

*The impact of perceived social support global and from partner on well-being at postpartum*

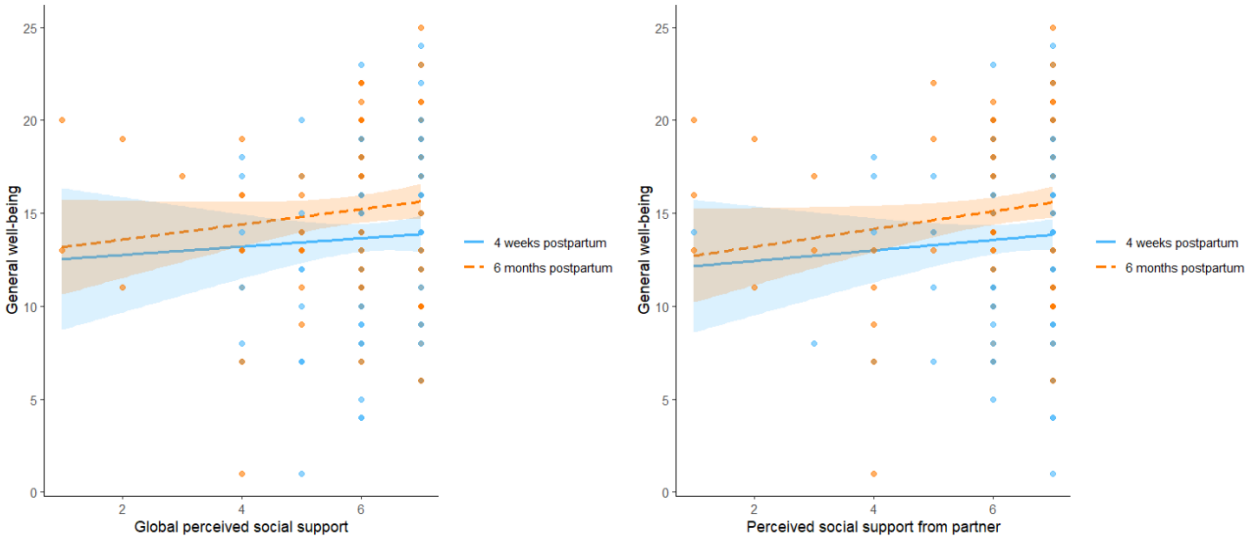


**Figure S5**

*The impact of perceived social support from family and friends on well-well-being at postpartum*

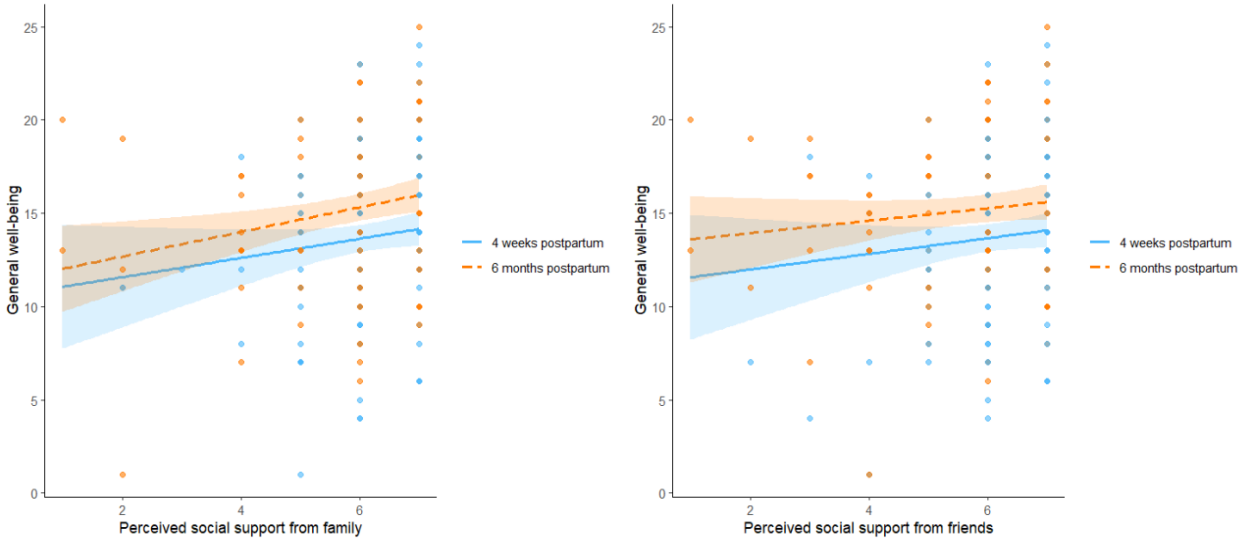

**Table S15**

*The impact of perceived social support on maternal attachment at postpartum*

| | 4 weeks postpartum | 6 months postpartum | Interaction:6 months postpartum |
|---|---|---|---|
| **Global perceived social support** | *Est.* = 0.08, 95% *CI* [0.02, 0.14], *SE* = 0.03, *t*(196.69) = 2.67, *p* = .01 | *Est.* = 0.30, 95% *CI* [-0.09, 0.69], *SE* = 0.20, *t*(143.53) = 1.52, *p* = .13 | *Est.* = -0.04, 95% *CI* [-0.10, 0.02], *SE* = 0.03, *t*(142.86) = -1.39, *p* = .17 |
| **Perceived social support from partner** | *Est.* = 0.08, 95% *CI* [0.02, 0.14], *SE* = 0.03, *t*(214.87) = 2.78, *p* = .01 | *Est.* = 0.20, 95% *CI* [-0.14, 0.54], *SE* = 0.17, *t*(135.59) = 1.17, *p* = .24 | *Est.* = -0.03, 95% *CI* [-0.09, 0.03], *SE* = 0.03, *t*(134.26) = -1.00, *p* = .32 |
| **Perceived social support from family** | *Est.* = 0.11, 95% *CI* [0.06, 0.16], *SE* = 0.03, *t*(195.36) = 4.34, *p* < .001 | *Est.* = 0.43, 95% *CI* [0.09, 0.77], *SE* = 0.17, *t*(141.57) = 2.57, *p* = .01 | *Est.* = -0.07, 95% *CI* [-0.13, -0.01], *SE* = 0.03, *t*(140.88) = -2.38, *p* = .02 |
| **Perceived social support from friends** | *Est.* = 0.07, 95% *CI* [0.02, 0.12], *SE* = 0.03, *t*(193.71) = 2.53, *p* = .01 | *Est.* = 0.15, 95% *CI* [-0.19, 0.49], *SE* = 0.17, *t*(143.61) = 0.84, *p* = .40 | *Est.* = -0.02, 95% *CI* [-0.08, 0.04], *SE* = 0.03, *t*(142.95) = -0.66, *p* = .51 |

**Table S16**

*The differential effects of support from partner, family and friends on maternal attachment over postpartum*

| | 4 weeks postpartum | 6 months postpartum |
|---|---|---|
| **Perceived social support from partner** | $\beta$ = -0.00, *SE* = 0.01, 95% *CI* [-0.02, 0.01], *t*(160) = -0.33, *p* = .74, *lmg* = 0.004, *VIF* = 1.21 | $\beta$ = -0.03, *SE* = 0.01, 95% *CI* [-0.05, -0.01], *t*(119) = -2.08, *p* = .04, *lmg* = 0.005, *VIF* = 2.21 |
| **Perceived social support from family** | $\beta$ = 0.09, *SE* = 0.02, 95% *CI* [0.05, 0.13], *t*(160) = 4.50, *p* < .001, *lmg* = 0.066, *VIF* = 1.48 | $\beta$ = 0.03, *SE* = 0.01, 95% *CI* [0.01, 0.05], *t*(119) = 2.12, *p* = .03, *lmg* = 0.013, *VIF* = 2.12 |
| **Perceived social support from friends** | $\beta$ = 0.04, *SE* = 0.01, 95% *CI* [0.02, 0.06], *t*(160) = 3.00, *p* = .003, *lmg* = 0.029, *VIF* = 1.60 | $\beta$ = 0.04, *SE* = 0.01, 95% *CI* [0.02, 0.06], *t*(119) = 4.08, *p* < .001, *lmg* = 0.024, *VIF* = 1.89 |

**Table S17**

*The impact of birth experience on affective symptoms and well-being*

| | 4 weeks postpartum | 6 months postpartum | Interaction: 6 months postpartum |
|---|---|---|---|
| **Perceived stress** | *Est.* = 7.93, 95% *CI* [2.53, 13.33], *SE* = 2.74, *t*(176.36) = 2.90, *p* < .01 | *Est.* = 2.96, 95% *CI* [-3.92, 9.84], *SE* = 3.49, *t*(116.00) = 0.85, *p* = .40 | *Est.* = -3.19, 95% *CI* [-8.27, 1.89], *SE* = 2.56, *t*(116.00) = -1.25, *p* = .22 |
| **Depression** | *Est.* = 0.92, 95% *CI* [0.42, 1.42], *SE* = 0.25, *t*(188.70) = 3.67, *p* < .001 | *Est.* = 0.17, 95% *CI* [-0.52, 0.86], *SE* = 0.35, *t*(115.08) = 0.49, *p* = .63 | *Est.* = -0.25, 95% *CI* [-0.76, 0.26], *SE* = 0.26, *t*(115.07) = -0.97, *p* = .33 |
| **Well-being** | *Est.* = -4.62, 95% *CI* [-7.15, -2.09], *SE* = 1.29, *t*(163.51) = -3.57, *p* < .001 | *Est.* = -1.21, 95% *CI* [-4.14, 1.72], *SE* = 1.48, *t*(116.00) = -0.82, *p* = .41 | *Est.* = 2.07, 95% *CI* [-0.09, 4.23], *SE* = 1.09, *t*(116.00) = 1.91, *p* = .06 |

**Table S18**

*The impact of birth experience on maternal attachment*

| | **4 weeks postpartum** | **6 months postpartum** | **Interaction: 6 months postpartum** |
|---|---|---|---|
| **Global maternal attachment** | *Est.* = -0.27, 95% *CI* [-0.48, -0.06], *SE* = 0.11, *t*(166.51) = -2.52, *p* = .01 | *Est.* = -0.30, 95% *CI* [-0.56, -0.04], *SE* = 0.13, *t*(116.00) = -2.37, *p* = .02 | *Est.* = 0.24, 95% *CI* [0.05, 0.43], *SE* = 0.09, *t*(116.00) = 2.57, *p* = .01 |

## Study 2: Supplementary Materials

**Table S1**

*Selected phase II steroid metabolites and the MS characteristics used in their determination*

| Hormone | Metabolite | Precursor Ion (m/z) | Product Ion (m/z) | Collision Energy (eV) |
|---|---|---|---|---|
| **Estradiol** | Estrone sulfate | 349 | 269 | 30 |
| | Estrone glucuronide | 445 | 269 | 30 |
| **Estriol** | Estriol 3-sulfate | 367 | 287 | 35 |
| | Estriol 3-glucuronide | 463 | 287 | 30 |
| | Estriol 16α-glucuronide | 463 | 287 | 30 |
| **Pregnenolone** | Pregn-5-ene-3β,20S-diol 3β-sulfate | 397 | 97 | 30 |
| | Pregn-5-ene-3β,20S-diol 20α-sulfate | 397 | 97 | 30 |
| **Progesterone** | 5α-Pregnane-3β,20α-diol 20α-sulfate | 399 | 97 | 40 |
| | 5α-Pregnane-3α,20α-diol 20α-sulfate | 399 | 97 | 40 |
| | 5β-Pregnane-3α,20α-diol 3α-glucuronide | 495 | 75 | 30 |
| **Dehydroepiandrosterone** | Androst-5-en-3β-ol-17-one 3β-sulfate | 367 | 97 | 30 |
| **Testosterone** | Androsterone sulfate | 369 | 97 | 35 |
| | Etiocholanolone sulfate | 369 | 97 | 35 |
| | Androsterone glucuronide | 465 | 75 | 30 |
| | Etiocholanolone glucuronide | 465 | 75 | 30 |

| | | | | |
|---|---|---|---|---|
| **Internal Standard** | Androsterone-d4 glucuronide | 469 | 75 | 30 |
| | Epitestosterone-d4 glucuronide | 467 | 75 | 35 |
| | Estradiol-d4 3-sulfate | 354 | 274 | 30 |
| | 3-S{18O}3-Epiandrosterone-sulfate | 375 | 103 | 35 |
| | Epitestosterone-d3 sulfate | 370 | 98 | 35 |

**Reagents and Materials**

Aqueous ammonia solution (25%), methanol, acetonitrile and formic acid (FA) (LC-MS grade) were from Merck (Darmstadt, Germany). Sigma-Aldrich (Louis, MO, USA) and VWR Prolabs Chemicals (Leuven, Belgium) provided phosphoric acid and ammonium formate. Ultrapure water was obtained using a Milli-Q purification system (Millipore Ibérica, Barcelona, Spain).

References to phase II steroid compounds' standards suppliers are given along with MS characteristics of metabolites in Table 5.

**Preparation of stripped urine for calibration curves**

The urine pool from different subjects was passed through an Oasis HLB 6 cc cartridge (Waters), which had previously been conditioned with 3 mL of methanol and equilibrated with 3 mL of water. The unretained portion of urine was collected after loading, checked for the absence of residual steroid metabolites, and labelled as steroid-stripped urine.

**LC conditions**

Chromatographic separation of phase II steroid urinary metabolites was performed at a flow rate of 400 uL/min at 30ºC on an (all from Waters Associates) using a fixed to Acquity UPLC CSH C18 VanGuard Pre-Column (2.1 × 5 mm i.d., 1.7 μm) (all from Waters Associates). Acetonitrile: water (9:1, v/v) and water, both with ammonium formate (25 mM), were used as organic and aqueous mobile phase solvents, respectively. The gradient elution program was applied, where the percentage of organic solvent was linearly changed as follows: 0 min, 10%; 0.5 min, 10%; 15 min, 47%; 15.5 min, 100%; 18,5 min, 100%; 19 min, 10%; 20 min, 10%. In total, the chromatographic method lasted 20 min.

**MS/MS conditions**

MS/MS analysis was carried out on nitrogen used as drying and nebulising gas. The desolvation gas flow was 1200 L/h, and the cone gas flow was 50 L/h. A cone voltage of 20 V and a capillary voltage of 2 kV were used in negative ionisation mode. The nitrogen desolvation temperature was 600 °C, and the source temperature was 150°C.

**Quantification strategy**

The response of compounds was calculated as the ratio between the analyte's and internal standard's areas. Concentrations of endogenous steroids were calculated using slopes of compound-specific calibration curves built in steroid-stripped urine. Metabolites for which quantitative standards were not available were relatively quantified using calibration curves of available standards of their isomers or structurally resemble steroid metabolites, assuming equal response for representative mass spectrometric transitions.

**Table S2**

*Formulae applied for the evaluation of the production of the different conjugation*

| Hormone | Metabolite conjugation | Formula |
|---|---|---|
| **Pregnenolone** | 5-Pregnenediol Sulf | (pregn-5-ene-3β,20S-diol 3β-sulfate + pregn-5-ene-3β,20S-diol 20α -sulfate) |
| **Testosterone** | Androgens Sulf | (androsterone sulfate + etiocholanolone sulfate) |
| | Androgens Gluc | (androsterone glucuronide + etiocholanolone glucuronide) |
| | Androgens Sulf/Gluc | (androsterone sulfate + etiocholanolone sulfate)/ (androsterone glucuronide + etiocholanolone glucuronide) |
| **DHEA** | DHEA Sulf | androst-5-en-3β-ol-17-one 3β-sulfate |
| **Estriol** | Estriol Sulf | estriol 3-sulfate |
| | Estriol Gluc | (estriol 3-glucuronide + estriol 16α-glucuronide ) |
| | Estriol Sulf/Gluc | estriol 3-sulfate / (estriol 3-glucuronide + estriol 16α-glucuronide ) |
| **Oestradiol** | Estrone Sulf | estrone sulfate |
| | Estrone Gluc | estrone glucuronide |
| | Estrone Sulf/Gluc | estrone sulfate / estrone glucuronide |
| **Progesterone** | Pregnanediol Sulf | (5α-pregnane-3β,20α-diol 20α -sulfate + 5α-pregnane-3α,20α-diol 20α -sulfate) |
| | Pregnanediol Gluc | 5β-pregnane-3α,20α-diol 3α-glucuronide |
| | Pregnanediol Sulf/Gluc | (5α-pregnane-3β,20α-diol 20α -sulfate + 5α-pregnane-3α,20α-diol 20α -sulfate) / 5β-pregnane-3α,20α-diol 3α-glucuronide |

**Table S3**

*Means and Standard Deviations of Steroid Metabolites across all Time Points for Gestational Mothers*

| Steroid metabolites | Before pregnancy | 18-22 weeks | 34-36 weeks | 4 weeks postpartum |
|---|---|---|---|---|
| **Estriol glucuronide** | *M* = 1.88, *SE* = 12.6, 95% *CI* [-22.9, 26.7] | *M* = 94.95, *SE* = 13.0, 95% *CI* [69.4, 120.5] | *M* = 413.56, *SE* = 13.0, 95% *CI* [388.0, 439.1] | *M* = 0.55, *SE* = 13.0, 95% *CI* [-25.1, 26.2] |
| **Estriol sulphate** | *M* = 0.18, *SE* = 7.95, 95% *CI* [-15.4, 15.8] | *M* = 34.27, *SE* = 8.18, 95% *CI* [18.2, 50.3] | *M* = 172.44, *SE* = 8.18, 95% *CI* [156.4, 188.5] | *M* = 0.06, *SE* = 8.21, 95% *CI* [-16.1, 16.2] |
| **Oestradiol glucuronide** | *M* = 0.18, *SE* = 7.95, 95% *CI* [-15.4, 15.8] | *M* = 34.27, *SE* = 8.18, 95% *CI* [18.2, 50.3] | *M* = 172.44, *SE* = 8.18, 95% *CI* [156.4, 188.5] | *M* = 0.06, *SE* = 8.21, 95% *CI* [-16.1, 16.2] |
| **Oestradiol sulphate** | *M* = 4.85, *SE* = 30.4, 95% *CI* [-54.9, 64.7] | *M* = 217.31, *SE* = 31.3, 95% *CI* [155.8, 278.8] | *M* = 370.46, *SE* = 31.3, 95% *CI* [309.0, 432.0] | *M* = 3.78, *SE* = 31.4, 95% *CI* [-57.9, 65.5] |

| | | | | |
|---|---|---|---|---|
| **Pregnanediol glucuronide** | *M* = 992, *SE* = 724, 95% *CI* [-430, 2413] | : *M* = 14449, *SE* = 745, 95% *CI* [12986, 15911] | *M* = 27383, *SE* = 745, 95% *CI* [25920, 28845] | *M* = 1197, *SE* = 747, 95% *CI* [-270, 2664] |
| **Pregnanediol sulphate** | *M* = 26.14, *SE* = 3.24, 95% *CI* [19.78, 32.5] | *M* = 34.93, *SE* = 3.33, 95% *CI* [28.39, 41.5] | *M* = 59.94, *SE* = 3.33, 95% *CI* [53.40, 66.5] | *M* = 1.54, *SE* = 3.34, 95% *CI* [-5.02, 8.1] |
| **Androgen glucuronide** | *M* = 1615, *SE* = 142, 95% *CI* [1337, 1893] | *M* = 3374, *SE* = 145, 95% *CI* [3089, 3660] | *M* = 2761, *SE* = 145, 95% *CI* [2476, 3046] | *M* = 3250, *SE* = 146, 95% *CI* [2964, 3536] |
| **Androgen sulphate** | *M* = 58, *SE* = 14.1, 95% *CI* [30.2, 85.7] | *M* = 271, *SE* = 14.5, 95% *CI* [242.3, 299.3] | *M* = 210, *SE* = 14.5, 95% *CI* [182.0, 238.9] | *M* = 193, *SE* = 14.5, 95% *CI* [164.9, 222.0] |
| **Pregnenolone sulphate** | *M* = 194, *SE* = 37.1, 95% *CI* [120.9, 267] | *M* = 423, *SE* = 37.9, 95% *CI* [348.9, 498] | *M* = 618, *SE* = 37.9, 95% *CI* [544.0, 693] | *M* = 134, *SE* = 38.0, 95% *CI* [59.8, 209] |
| **Dehydroepiandrosterone Sulphate** | *M* = 165, *SE* = 40.9, 95% *CI* [84.8, 246] | *M* = 469, *SE* = 42.1, 95% *CI* [385.7, 551] | *M* = 180, *SE* = 42.1, 95% *CI* [97.7, 263] | *M* = 202, *SE* = 42.2, 95% *CI* [118.8, 285] |

*Note: M = Mean, SE = standard error, CI = confidence interval.*

**Table S4**

*The Association of the accumulated phase II steroids associated with depressive symptoms, well-being and maternal attachment at 4 weeks postpartum*

| | | Depression | | | | | Well-Being | | | | | Maternal Postnatal Attachment | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hormone | Metabolite conjugation [a] | Slope | Z-value (108) | SE | P-value | FDR | Slope | T-value (109) | SE | P-value | FDR | Slope | T-value (109) | SE | P-value | FDR |
| Pregnenolone | 5-Pregnanediol Sulf | 0.002 | 0.020 | 0.081 | 0.984 | 0.984 | -0.02 | -0.244 | 0.083 | 0.808 | 0.876 | 0.101 | 1.655 | 0.061 | 0.101 | 0.348 |
| Testosterone | Androgens Sulf | -0.124 | -2.215 | 0.056 | 0.027 | 0.038 | 0.121 | 2.057 | 0.059 | 0.042 | 0.136 | 0.049 | 1.111 | 0.044 | 0.269 | 0.536 |
| | Androgens Gluc | 0.298 | 3.074 | 0.097 | 0.002 | 0.004 | -0.022 | -0.223 | 0.100 | 0.824 | 0.876 | -0.015 | -0.199 | 0.074 | 0.842 | 0.872 |
| | Androgens Sulf/Gluc | -0.277 | -4.484 | 0.062 | <0.001 | <0.001 | 0.162 | 2.481 | 0.065 | 0.015 | 0.107 | 0.068 | 1.386 | 0.049 | 0.169 | 0.445 |
| DHEA | DHEA Sulf | -0.044 | -1.666 | 0.026 | 0.096 | 0.122 | 0.047 | 1.661 | 0.029 | 0.100 | 0.219 | 0.038 | 1.779 | 0.021 | 0.078 | 0.348 |
| Estriol | Estriol Sulf | -0.033 | -0.706 | 0.047 | 0.480 | 0.56 | 0.083 | 1.698 | 0.049 | 0.092 | 0.219 | 0.036 | 0.985 | 0.036 | 0.327 | 0.554 |
| | Estriol Gluc | 0.399 | 3.927 | 0.102 | <0.001 | <0.001 | -0.151 | -1.416 | 0.107 | 0.160 | 0.289 | -0.101 | -1.279 | 0.079 | 0.204 | 0.455 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Estriol Sulf/Gluc | -0.134 | -2.711 | 0.049 | 0.007 | 0.01 | 0.131 | 2.556 | 0.051 | 0.012 | 0.107 | 0.066 | 1.695 | 0.039 | 0.093 | 0.348 |
| Oestradiol | Estrone Sulf | -0.021 | -0.63 | 0.033 | 0.529 | 0.57 | 0.048 | 1.441 | 0.034 | 0.153 | 0.289 | 0.026 | 1.033 | 0.025 | 0.304 | 0.551 |
| | Estrone Gluc | 0.202 | 3.148 | 0.064 | 0.002 | 0.004 | -0.038 | -0.567 | 0.066 | 0.572 | 0.790 | -0.041 | -0.843 | 0.049 | 0.401 | 0.554 |
| | Estrone Sulf/Gluc | -0.107 | -2.739 | 0.039 | 0.006 | 0.01 | 0.087 | 2.143 | 0.041 | 0.034 | 0.136 | 0.055 | 1.808 | 0.030 | 0.073 | 0.348 |
| Progesterone | Pregnanediol Sulf | -0.305 | -4.263 | 0.072 | <0.001 | <0.001 | 0.174 | 2.453 | 0.071 | 0.016 | 0.107 | 0.128 | 2.441 | 0.053 | 0.016 | 0.157 |
| | Pregnanediol Gluc | 0.508 | 3.439 | 0.148 | 0.001 | 0.001 | -0.117 | -0.756 | 0.155 | 0.452 | 0.655 | -0.085 | -0.743 | 0.114 | 0.459 | 0.555 |
| | Pregnanediol Sulf/Gluc | -0.336 | -5.241 | 0.064 | <0.001 | <0.001 | 0.170 | 2.609 | 0.065 | 0.010 | 0.107 | 0.125 | 2.590 | 0.048 | 0.011 | 0.157 |

[a]The sum of sulfated metabolites of androgens, 5-pregnanediol and pregnanediol, and the sum of glucuronidated metabolites of androgens, estriol and pregnanediol are detailed in Table 2. --Distribution of response variables was investigated before selecting the model's families. Slopes, Z-value (Poisson family model for depression symptoms), T-value (Gaussian family model for well-being and maternal attachment), and P-values from generalised linear models. Models were adjusted for the mother's age, body mass index, conception method, the perceived social support scale, the birth experiences questionnaire, and the baby's sex. Abbreviations: DHEA, Dehydroepiandrosterone; FDR, false, discovery rate-adjusted p-value; gluc, glucuronide; SE, Standard error; sulf, sulfate; and Sulf/Gluc, sulfate-to-glucuronide ratios.

**Table S5**

*Commercial source for standards*

| Standard | Source |
|---|---|
| estrone sulfate | Merk (Sigma-Aldrich, Burlington, MA, USA) |
| estrone glucuronide | *Steraloids (Newport, RI, USA)* |
| estriol 3-sulfate | kindly provided by Prof. C. Shackelton from UCSF Benioff Children's Hospital Oakland Research Institute |
| estriol 3-glucuronide | kindly provided by Prof. C. Shackelton from UCSF Benioff Children's Hospital Oakland Research Institute |
| estriol 16α-glucuronide | kindly provided by Prof. C. Shackelton from UCSF Benioff Children's Hospital Oakland Research Institute |
| pregn-5-ene-3β,20S-diol 3β-sulfate | qualitatively synthesized *in-house* at small scale based on earlier described methodology [McLeod MD, 2017] |
| pregn-5-ene-3β,20S-diol 20α - sulfate | qualitatively synthesized *in-house* at small scale based on earlier described methodology [McLeod MD, 2017] |
| 5α-pregnane-3β,20α-diol 3β,20α - bisulfate | quantitatively synthesised on customer demand, according to published methodology [McLeod MD, 2017] |
| 5α-pregnane-3α,20α-diol 20α - sulfate | qualitatively synthesised *in-house* at a small scale based on earlier described methodology [McLeod MD, 2017] |
| 5β-pregnane-3α,20α-diol 3α-glucuronide | *Steraloids (Newport, RI, USA)* |
| androst-5-en-3β-ol-17-one 3β-sulfate | Merk (Sigma-Aldrich, Burlington, MA, USA) |

| | |
|---|---|
| androsterone sulfate | *Australian National Measurement Institute (Pymble, Australia)* |
| etiocholanolone sulfate | *Australian National Measurement Institute (Pymble, Australia)* |
| androsterone glucuronide | *Australian National Measurement Institute (Pymble, Australia)* |
| etiocholanolone glucuronide | *Australian National Measurement Institute (Pymble, Australia)* |
| androsterone-d4 glucuronide | *Australian National Measurement Institute (Pymble, Australia)* |
| epitestosterone-d4 glucuronide | *Australian National Measurement Institute (Pymble, Australia)* |
| estradiol-d4 3-sulfate | TRC-Canada (Toronto Research Chemicals, Toronto, Canada) |
| 3-S{$^{18}$O}$_3$-epiandrosterone-sulfate | quantitatively synthesised on customer demand, according to published methodology [McLeod MD, 2017] |
| epitestosterone-d3 sulfate | *Australian National Measurement Institute (Pymble, Australia)* |