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# Allopregnanolone and progesterone in estradiol treated severe postpartum depression and psychosis – Preliminary findings

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ABSTRACT

*Background:* Postpartum affective disorders may be associated with dysregulation of gonadal steroids. We investigated peripheral levels of allopregnanolone and progesterone in a combined group of women with postpartum onset of severe depression and/or psychosis who, as previously reported, responded with rapid symptom remission during sublingual estradiol treatment. The aim was to assess differences in allopregnanolone and progesterone between patients and healthy controls at baseline, and hormonal changes during estradiol treatment and symptom remission in patients.

*Methods:* Allopregnanolone and progesterone in serum were analyzed with radioimmunoassay before and four weeks after initiation of sublingual estradiol treatment in ten women with postpartum depression and four women with postpartum psychosis (ICD-10). Twenty-eight healthy postpartum controls were included for baseline comparison.

*Results:* Allopregnanolone declined significantly during estradiol treatment while there was a trend for lower baseline allopregnanolone levels in patients compared with healthy postpartum controls. The ratio between allopregnanolone and progesterone was significantly lower in patients compared with controls and it remained unchanged after clinical recovery.

*Limitations:* This study is a secondary analysis of two estradiol treatment studies based on availability of samples for the analysis of allopregnanolone. Healthy controls were assessed earlier after delivery. Data on potential confounders (somatic health, breastfeeding, other medication) were not available.

*Conclusions:* These preliminary findings suggest that clinical recovery of severe postpartum depression and psychosis during estradiol treatment does not seem to depend on increasing levels of allopregnanolone. Differences in progesterone metabolism may constitute a risk factor for severe postnatal affective dysregulation.

## 1. Introduction

The incidence of severe affective mental disorders is increased following childbirth (Munk-Olsen, Laursen, Pedersen, Mors, & Mortensen, 2006). Clinical and epidemiological studies suggest associations between disorders characterized by postnatal affective dysregulation. Women with postpartum onset major depression may have an increased risk for developing bipolar disorder (Sharma, 2018). Bipolar disorder increases the risk for postpartum psychosis which is distinguished by predominantly depressive, manic or mixed episodes (Chaudron & Pies, 2003). Preclinical and clinical studies, including estrogen treatment studies, suggest that perinatal hormonal fluctuations trigger affective dysregulation in vulnerable populations despite similar hormonal levels compared with healthy controls (Schiller, Meltzer-Brody, & Rubinow, 2015). Estrogen substitution in postpartum depression has been assessed to have only modest therapeutic value (Dennis, Ross, & Herxheimer, 2008) - studies have major limitations (Gentile, 2005) and include only one placebo-controlled randomized trial (Gregoire, Kumar, Everitt, Henderson, & Studd, 1996). Some authors have though interpreted the rapid and large estradiol treatment effects in some of these studies, including women with postpartum depression and psychosis, as support to the notion of a hormone sensitive subtype of perinatal affective disorders (Moses-Kolko, Berga, Kalro, Sit, & Wisner, 2009; Payne, Palmer, & Joffe, 2009; Schiller et al., 2015).

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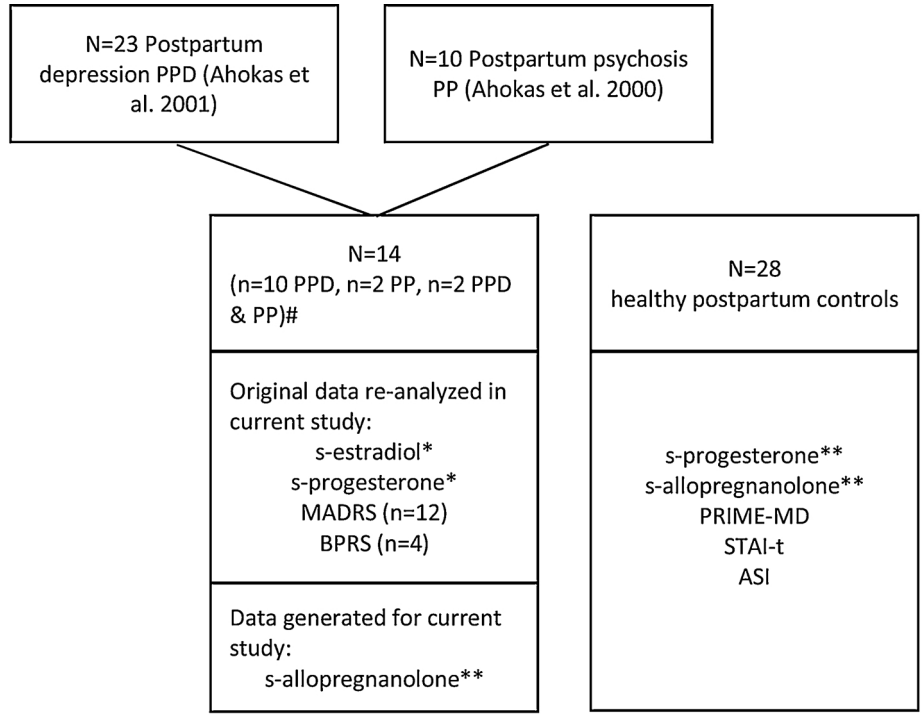


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# Among four women with postpartum psychosis two presented with psychotic depression. These were reported in both original studies but included only once in the current study
PPD – postpartum depression, PP – postpartum psychosis
MADRS – Montgomery Åsberg Depression Scale, BPRS- Brief Psychiatric Rating Scale
PRIME-MD – Primary Care Evaluation of Mental Disorders, STAI-t – Trait subscale of Spielberger State-Trait Anxiety Inventory, ASI – Anxiety Sensitivity-Index

**Fig. 1.** Chart depicting current study design based partially on data from prior studies.

Estrogen, progesterone and progesterone metabolites may be associated with postnatal affective dysregulation through effects on GABAergic neurotransmission (Schiller, Schmidt, & Rubinow, 2014). Altered GABA signaling and abnormal levels of the progesterone metabolite allopregnanolone have been found in depression and bipolar disorder (Carta, Bhat, & Preti, 2012). Some SSRIs, antipsychotics and lithium (Carta et al., 2012) have been found to increase allopregnanolone levels. Whether estradiol increases allopregnanolone levels without addition of progesterone seems unclear (Pluchino et al., 2009). Allopregnanolone administration seems to lead to rapid symptom reduction in postpartum depression (Meltzer-Brody et al., 2018).

Allopregnanolone has anti-depressive, anxiolytic, anticonvulsant, sedative/hypnotic and anti-nociceptive effects (Locci & Pinna, 2017). It acts primarily via allosteric modulation of the $GABA_A$-receptor (Paul & Purdy, 1992) and it also suppresses the hypothalamic-pituitary-adrenal (HPA) axis (Crowley & Girdler, 2014). $GABA_A$ subunit composition is associated with differential sensitivity to allopregnanolone levels and $GABA_A$-subunit-plasticity has been suggested as a homeostatic mechanism that maintains functioning at the network/circuit level during increasing pregnancy and decreasing postpartum allopregnanolone levels (MacKenzie & Maguire, 2014). Withdrawal from high allopregnanolone levels or failure to regulate subunit composition may trigger mood symptoms in vulnerable women (Schiller et al., 2014).

The evidence for abnormal peripheral allopregnanolone and progesterone levels in postpartum women with affective symptoms is though inconsistent (Schiller et al., 2015). Lower allopregnanolone levels have been reported in baby blues (Nappi et al., 2001) but not in postpartum depression (Deligiannidis et al., 2013; Pearson Murphy, Steinberg, Hu, & Allison, 2001). Postpartum depression was though associated with alterations in the allopregnanolone progesterone ratio (a biological relevant proxy for metabolism of progesterone to allopregnanolone) (Kimmel et al., 2016). Allopregnanolone levels were negatively associated with depressive symptoms in women with a history of postpartum depression (Schiller et al., 2014) that recurred after add-back of estradiol and progesterone following experimental hypogonadism (Bloch et al., 2000).

This study is based on a subsample of women with postpartum onset of severe depression and/or psychosis, who participated in two priorly published studies that found a rapid remission of psychiatric symptoms after high-dose sublingual estradiol treatment (Ahokas, Aito, & Rimon, 2000; Ahokas, Kaukoranta, Wahlbeck, & Aito, 2001). We combined women with postpartum depression and psychosis based on the suggested relationship with bipolar affective disorder (Di Florio & Meltzer-Brody, 2015) and the similar treatment responses to estradiol in the prior studies - conceptualizing one single hormone-sensitive subgroup of severe postpartum affective disorder (as suggested according to the Research Domain Criteria (RDoC) (Cuthbert & Insel, 2010)). We hypothesized that patients would have lower allopregnanolone levels than healthy controls and that increasing levels of estradiol due to estradiol treatment would affect allopregnanolone levels positively - pointing toward a potential mediation of the estradiol treatment effect by allopregnanolone.

The aim of this study was to compare allopregnanolone, progesterone and the allopregnanolone progesterone ratio in patients with postpartum onset of severe depression and psychosis with healthy postpartum controls. We also aimed to assess changes in these

neurosteroids in patients during estradiol treatment in relation to peripheral estradiol levels and depressive or psychotic symptoms.

## 2. Methods

### 2.1. Study participants and setting

The current study is based on a secondary analysis of remaining serum samples from two cohorts of women with postpartum onset affective disorder – fourteen patients with postpartum depression and/or postpartum psychosis - and twenty-eight healthy controls (Fig. 1). Consecutive patients were recruited at the duty unit/emergency outpatient department of the Department of Psychiatry at Helsinki City Hospital, Finland, with the aim to study estradiol treatment effects. The treatment effect data from originally twenty-three women with postpartum depression and ten women with postpartum psychosis have been published (Ahokas et al., 2000, 2001). Inclusion criteria for the study of postpartum depression were a diagnosis of major depression (ICD-10) with debut within 6 months postpartum according to a structured psychiatric interview by two psychiatrists, time since parturition less than 12 months, Montgomery-Åsberg-Depression-Scale (MADRS) $\geq 22$ and serum-estradiol concentration $\leq 200$ pmol/L (the level was hypothesized from clinical experience and all women fell below this limit). Exclusion criteria were a history of gynecologic, breast or thromboembolic disease (no woman was excluded), use of hormonal preparations (n = 2 excluded) and irregular use of study medication (n = 1 excluded). (Ahokas et al., 2001). Inclusion criteria for the study of postpartum psychosis was an ICD-10 diagnosis of postpartum psychosis. No women were excluded (Ahokas et al., 2000).

Baseline assessment of hemoglobin, red and white blood cells, sedimentation rate, C-reactive protein, thyroxin and thyroid stimulating hormone were in the normal range. No patient had resumed menstruation according to weekly oral enquiries during the study period. Three women were medicating with neuroleptic agents (risperidone (two women) and promazine) at study start, which was gradually tapered during the first week of estradiol treatment. Two women with postpartum depression started antidepressant medication (fluoxetine and venlafaxine) at treatment week three. None of the women with psychosis was breastfeeding. The following data were not available in this subsample but reported as followed in the original study populations: Thirteen women with postpartum depression were breastfeeding during the study period. Twelve women had not responded to psychotherapeutic treatment and eight women had not responded to treatment with antidepressants or antipsychotics. Three women with depression had made a recent suicide attempt. Two women were reported in both original studies as they fulfilled criteria for postpartum psychosis and depression but are reported only once in the current study.

Treatment consisted of sublingual 17beta-estradiol a) 1 mg x 3–8 for eight weeks (postpartum depression) or b) 1 mg x 3–6 for six weeks (postpartum psychosis) with goal serum-concentration of estradiol 400 pmol/L (level hypothesized as one-third from peak level during regular menses). Mean estradiol dose from treatment week 2 was 4.7 mg and 4.8 mg for women with psychosis or depression respectively.

The twenty-eight healthy postpartum controls were recruited during early pregnancy with follow-ups during late pregnancy and after parturition for a different and ongoing study conducted with the aim to study pregnancy and postpartum neurosteroid levels. In this study we used only data from the postpartum neurosteroid assessment. Twenty-seven of the healthy controls were breastfeeding.

The Ethical review committee at the Helsinki City Hospital approved the original study and patients gave written informed consent (Ahokas et al., 2000, 2001). Due to ethical reasons there was no placebo-treated group. For the purpose of investigating neuroendocrine variables not included in the original studies, ethical approval was granted by the Regional Ethical Review Board in Umeå, Sweden (Dnr: 2016/170-31). The ethical approval for study of the healthy controls was granted by the Regional Ethical Review Board in Umeå, Sweden (Dnr: 2011-146-31 M).

### 2.2. Psychological and psychiatric assessment

Standardized psychiatric diagnostic assessment in patients was performed according to ICD-10. Symptom severity was assessed at baseline and weekly with a) Montgomery-Åsberg-Depression-Scale (Montgomery & Asberg, 1979) (MADRS) in women with postpartum depression (10 items, score range 0–6 per item) and b) the 18-item Brief-Psychiatric-Rating-Scale (Overall & Gorham, 1962) (BPRS) (score range 0–6 per item) in women with postpartum psychosis.

Controls were assessed with the self-administered Swedish versions of the Primary Care Evaluation of Mental Disorders (PRIME-MD) (Spitzer et al., 1994) and the trait subscale of the State-Trait Anxiety Inventory (STAI; 20-item, 1–4 points per item) (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983) to exclude current mental disorders and anxiety proneness at inclusion and follow-ups, and the Anxiety Sensitivity Index for assessment of anxiety symptoms (ASI; 16-item, 0–4 points) (Reiss, Peterson, Gursky, & McNally, 1986) at inclusion.

### 2.3. Blood sampling procedure and assay

Fasting blood sampling in patients was performed between 7 a.m. and 9 a.m. before administration of the morning dose of estradiol at baseline and once weekly. Frozen samples were available for analysis of allopregnanolone at baseline and after 3–4 weeks of ongoing estradiol treatment in 4 patients with psychosis and 12 patients with depression.

In this study, blood samples provided by the controls at the postpartum follow-up were used for the assessment of allopregnanolone and progesterone.

Allopregnanolone in patients and controls was analyzed with identical methods (standard radioimmunoassay (RIA)) in the same laboratory at Umeå Neuroendocrine Centre (UNC), Sweden. The sensitivity of the assay is 25 pg, with an intra-assay coefficient of variation of 6.5% and an inter-assay coefficient of variation of 8.5%. The recovery of allopregnanolone was 83–85% and the results were compensated for recovery (described in detail by (Bixo, Andersson, Winblad, Purdy, & Bäckström, 1997)). Unpublished data on progesterone levels in patients were available in the original dataset after analysis at the laboratory in Helsinki, Finland. Progesterone serum levels in controls were measured with a sequential competitive immunoassay (Immulite®). All analyze kits were purchased from Diagnostic Products Corporation, Corporate Offices, Los Angeles, CA, USA.

### 2.4. Data analysis

Progesterone-values fell under the level of detection (0.8 nmol/L) in two patients at baseline and in 4 patients at follow-up. These were substituted by a constant (0.8/sqr2) (Hornung & Reed, 1990). In one case, where the value for progesterone was missing at the four-week follow-up, measurements from the 3-week and 6-week follow-up were used to impute the estimated 4-week value, assuming a linear relationship. The ratio between allopregnanolone and progesterone was calculated as an index for the metabolism of progesterone to allopregnanolone as a post-hoc analysis.

Initial analyses were carried out to evaluate skewness and kurtosis of the distributions (Shapiro—Wilks test). Neither the number of days between childbirth and symptom debut, days between childbirth and baseline assessment, progesterone, allopregnanolone and allopregnanolone/progesterone ratio levels at baseline nor BPRS at follow-up were normally distributed. One control was identified as a univariate outlier at baseline for allopregnanolone and one control was a univariate outlier for the ratio between allopregnanolone and pregnanolone at

baseline. According to analysis of Mahalanobis distance there were no multivariate outliers (Tabachnick, 2007). Exclusion of the univariate outliers did not affect the analyses and we consequently did not exclude them.

Non-parametric methods were used to assess relation- and inter-relationships between hormones and psychological variables, independent group differences between patients and controls at base-line (Mann-Whitney-U-test) and dependent group differences in hormonal and psychological variables between base-line and follow-up in patients (Wilcoxon-signed-rank-test). To adjust for differences in timing of assessment after birth in patients and controls, multiple linear regressions with the logarithm of allopregnanolone, progesterone and the ratio allopregnanolone/progesterone as dependent variables were performed.

The $p$ value was set at $< 0.05$.

The SPSS software, version 24.0.0.0, IBM Corp., USA was used for all statistical analyses.

## 3. Results

### 3.1. Baseline characteristics of the study group

Psychosis started mean 14 days (range 5–21 days) and depression mean 47 days (range 3–120 days) postpartum. Patients with psychosis were included mean 24 days (range 2–45 days) and patients with depression mean 70 days (range 7–120 days) after symptom start. Controls were assessed significantly earlier on mean postpartum day 6 (range 2–11 days). By follow-up (treatment day 24 (range 20–42 days) all patients had recovered clinically from depression (range MADRS 0–5) and psychosis (range BPRS 0–3) (Table 1). Controls did not fulfil criteria for any mental disorder according to PRIME-MD neither at inclusion during pregnancy nor at the pregnancy and postpartum follow-up. STAI at inclusion during pregnancy (mean 34.36, SD 5.92, n = 28) and postpartum follow-up (mean 31.7, SD 6.1, n = 27) was similar to the normal distribution for women (mean 34.79, SD 9.22 (Spielberger et al., 1983)). At inclusion, controls scored (mean 9.8, SD 5.0, n = 28) within the normal distribution of the Anxiety-Sensitivity-Index (mean 19.1, SD 9.11 (Reiss et al., 1986)).

### 3.2. Allopregnanolone, progesterone and allopregnanolone/progesterone ratio in patients and controls

In the unadjusted analyses patients had significantly lower levels of allopregnanolone and a significantly lower allopregnanolone progesterone ratio at baseline compared with controls (Table 2; Fig. 2).

We adjusted for time since childbirth, as allopregnanolone and progesterone correlated significantly negatively with time since childbirth in the bivariate analyses (Table 4). The difference in the ratio remained significant (p = 0.004) but the group difference in allopregnanolone was only trend significance (p = 0.091) (Table 2; Fig. 2).

### 3.3. Differences between clinical assessments and allopregnanolone, progesterone and estradiol in patients at baseline and follow up

A Wilcoxon-signed rank test indicated that allopregnanolone in patients at follow-up (Table 3) was significantly lower than at baseline (Z= -2.67, p = 0.008, n = 14). Progesterone levels decreased but the change was not significant (Z= -1.60, p = 0.109, n = 12) and also the allopregnanolone/progesterone ratio remained unchanged (Z= -0.63, p = 0.530, n = 12) during estradiol treatment.

As previously reported in the original groups (Ahokas et al., 2000, 2001), estradiol levels increased significantly (Z = 3.30, p = 0.001, n = 14) and depressive symptoms measured with MADRS decreased significantly (Z= -3.07, p = 0.002, n = 12). Psychotic symptoms measured with BPRS decreased from florid psychotic symptom levels with mean 77.5 points (SD 7.0) to mean 0.8 (SD 1.5) points but this change was not significant due to the small number of patients (Z= -1.83, p = 0.068, n = 4).

### 3.4. Correlations between clinical assessments and allopregnanolone, progesterone, allopregnanolone progesterone ratio and estradiol at baseline and follow up

Allopregnanolone was significantly positively correlated with progesterone in the whole group (Spearman's rho 0.39, p = 0.014) (Table 4) and in controls at baseline (rho = 0.57, p = 0.002). In patients, allopregnanolone correlated significantly with progesterone only at follow-up (rho 0.56, p = 0.045) but not at baseline (rho 0.45, p = 0.12) and there was a positive association between allopregnanolone baseline and follow-up levels (rho = 0.63, p = 0.017) (Table 5).

Estradiol did not show significant correlations with any of the neurosteroids neither at baseline nor at follow-up (Table 5).

There were no significant associations between symptom severity (MADRS or BPRS) and allopregnanolone, progesterone, estradiol or the ratio between allopregnanolone and progesterone neither at baseline nor at follow-up.

### 3.5. Change between clinical assessments and allopregnanolone, progesterone and estradiol from baseline to follow up

Change in depressive symptoms (measurement MADRS at follow-up minus measurement at baseline) was not associated with change in allopregnanolone, progesterone, estradiol or the allopregnanolone progesterone ratio. We did not assess for association with BPRS due to limited data in the bivariate correlations (n = 2–4). There was no significant correlation between time from birth to baseline (range 7–210

**Table 1**
Baseline characteristics of patients and controls.

| | Controls (n = 28) | | Patients (n = 14) | |
|---|---|---|---|---|
| | Mean (SD) | Median (range) | Mean (SD) | Median (range) |
| Age (years) | 25.5 (4.4) | 25.5 (18-34) | 27.5 (7.1) | 24.7 (18-42) |
| Start of symptoms after childbirth (days) | – | – | 37.6 (33.9) | 25.5 (3-120) |
| Symptom duration until treatment start (days) | – | – | 56.9 (40.4) | 52.5 (2-120) |
| Baseline (days after childbirth) * | 6.1 (3.0) | 5.0 (2-11) (n = 27) | 94.4 (56.8) | 87.5 (7-210) |
| Time baseline to follow-up (days) | – | – | 25.7 (6.9) | 22 (20-42) |
| MADRS (n = 12) | | | | |
| At baseline | – | – | 41.5 (2.5) | 41 (38-45) |
| At follow-up | – | – | 1.7 (1.7) | 1.5 (0-5) |
| BPRS (n = 4) | | | | |
| At baseline | – | – | 77.5 (7.0) | 78.5 (68-85) |
| At follow-up | – | – | 0.8 (1.5) | 0 (0-3) |

* significant difference between patients and controls (Mann-Whitney-U) (< 0.001).

**Table 2**
Unadjusted and adjusted differences of estradiol (nmol/L), allopregnanolone (nmol/L) and progesterone (nmol/L) in patients and controls.

| | Controls (n = 28) Mean (SD), median (IQR) | Patients (n = 14) Mean (SD), median (IQR) | p-value* (adjusted #) |
|---|---|---|---|
| Allopregnanolone | **3.59 (3.93), 2.08 (5.48) (n = 27)** | **0.58 (0.35), 0.48 (0.22) (n = 14)** | **p < 0.001** (p = 0.091) |
| Progesterone | 2.06 (2.20), 0.98 (2.07) (n = 27) | 1.64 (1.55), 1.20 (1.00) (n = 13) | p = 0.628 (p = 0.141) |
| Allo/Prog ratio | **2.29 (2.33), 1.44 (1.59) (n = 26)** | **0.45 (0.19), 0.42 (0.24) (n = 13)** | **p < 0.001 (p = 0.004)** |

Allo/Prog ratio = Allopregnanolone/Progesterone ratio.
* Unadjusted differences between patients and controls (Mann-Whitney-U). Significant differences are bolded.
\# Difference between patients and controls adjusted for childbirth to baseline (days) (Multiple linear regression). Significant differences are bolded.

days; Table 1) and time between baseline and follow-up (range 20–42 days; Table 1) and allopregnanolone and progesterone levels in patients (Table 5).

## 4. Discussion

The two major findings of this preliminary study were (a) that allopregnanolone declined during estradiol treatment and (b) that patients compared with healthy controls had a lower allopregnanolone progesterone ratio, which remained unchanged after clinical recovery.

We could not confirm our hypothesis that allopregnanolone would be lower in this group of hormone sensitive women with postpartum onset severe depression and psychosis compared with controls which is in line with other studies in postpartum depression (Deligiannidis et al., 2013; Pearson Murphy et al., 2001). Patients had lower levels of allopregnanolone at baseline but after controlling for timing of assessment this difference was only trend significant. Controls were assessed earlier than patients and allopregnanolone decreases continuously during the first weeks after birth (Gilbert Evans, Ross, Sellers, Purdy, & Romach, 2005). Baseline levels of allopregnanolone in patients and controls were comparable to those reported by (Pearson Murphy et al., 2001) employing the same method (RIA) at approximately the same time after parturition (Supplementary table S1).

Neither could we confirm our second hypothesis that allopregnanolone levels would increase during estradiol treatment in patients. We found a significant decrease in allopregnanolone levels during estradiol treatment. It is possible that a potential increase associated with estradiol treatment may have been overshadowed by the physiological postnatal decrease. But the majority of patients were assessed about three months after birth when allopregnanolone already has decreased (the allopregnanolone levels were similar to follicular phase levels of menstruating women (Hedstrom et al., 2015)), and there was no correlation between allopregnanolone and time. Thus, it seems unlikely that levels increased during estradiol treatment. It is possible that estradiol suppressed allopregnanolone and that levels increased after treatment was discontinued, but several studies suggest elevated allopregnanolone levels after estradiol administration (Pluchino et al., 2009). Decline in allopregnanolone during treatment co-occurred with symptom reduction, however, since there was no correlation between depression ratings and change in allopregnanolone, we cannot conclude that the decline is clearly associated with clinical recovery in these patients. Neither of the investigated steroids – allopregnanolone, progesterone, estradiol – was associated with psychiatric symptoms before or after mood stabilization but the narrow range of symptom levels at baseline and follow up may have affected these findings.

Thus, estrogen treatment does not appear to alleviate a deficiency in allopregnanolone, which may point against a suggested role of higher levels of allopregnanolone mediating treatment effects in postpartum affective disorders (Carta et al., 2012). Bimodal effects of both increased and decreased allopregnanolone levels have been associated with mood stabilization in premenstrual dysphoric disorder (Andreen et al., 2009) and with changes in GABA receptor sensitivity (Burke, Susser, & Hermann, 2019; MacKenzie & Maguire, 2014). As gonadal steroids and their metabolites can alter the composition of the $GABA_A$-receptor (MacKenzie & Maguire, 2014) it is possible that estradiol treatment or decreased levels of allopregnanolone may be associated with changes in $GABA_A$-receptor composition; affecting $GABA_A$ sensitivity, neurotransmission and mood stabilization. In a mouse model, failure to regulate subunit composition during pregnancy was associated with postpartum depressive behavior (Maguire & Mody, 2008).

Rather than being a treatment effect, decreased allopregnanolone may be a trait marker for disorder severity in women at risk of severe postpartum affective symptoms during stress or periods of hormonal change. Such changes may be associated with alterations in

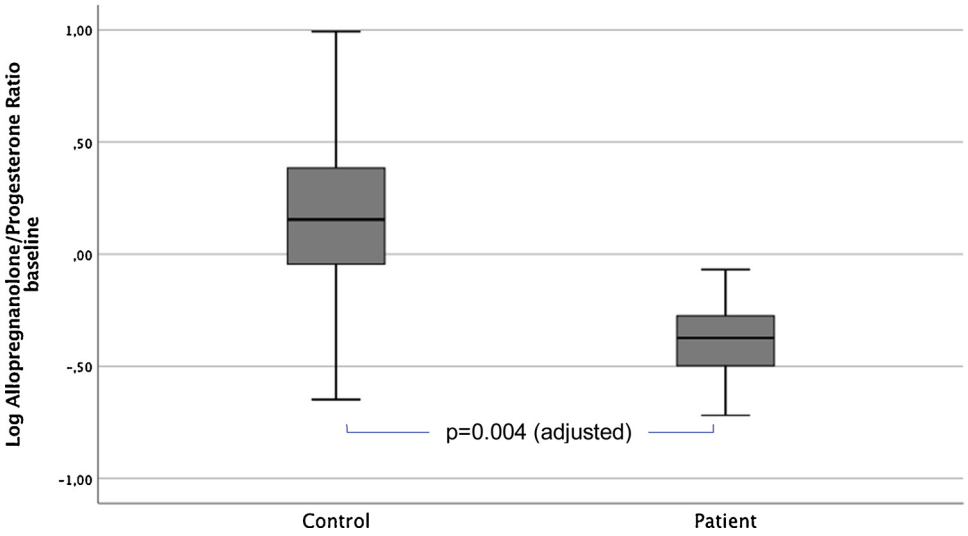


**Fig. 2.** Logarithm of allopregnanolone progesterone ratio in controls and patients at baseline .

**Table 3**
Differences of estradiol (nmol/L), allopregnanolone (nmol/L) and progesterone (nmol/L) levels in patients between baseline and follow-up.

| | Baseline<br>Mean (SD), median (IQR) | Follow-up<br>Mean (SD), median (IQR) | Difference<br>Test-statistic, p-value* |
|---|---|---|---|
| Allopregnanolone | **0.58 (0.35), 0.48 (0.22)** | **0.40 (0.11), 0.39 (0.13)** | **Z = -2.67, p = 0.008** |
| Progesterone | 1.64 (1.55), 1.20 (1.00) | 1.11 (0.50), 1.00 (0.98) | Z = -1.60, p = 0.109 |
| Allo/Prog ratio | 0.45 (0.19), 0.42 (0.24) | 0.40 (0.16), 0.34 (0.24) | Z = -0.63, p = 0.530 |
| Estradiol | **0.09 (0.04), 0.09 (0.05)** | **0.43 (0.18), 0.45 (0.27)** | **Z = 3.30, p = 0.001** |
| MADRS | **41.5 (2.5), 41.0 (5)** | **1.7 (1.7), 1.5 (3)** | **Z = -3.07, p = 0.002** |
| BPRS | 77.5 (7.0), 78.5 (13) | 0.8 (1.5), 0 (2) | Z = -1.83, p = 0.068 |

Allo/Prog ratio = Allopregnanolone/Progesterone ratio.
* Difference baseline to follow-up in patients (Wilcoxon-signed-rank). Significant differences are bolded.

**Table 4**
Correlations between allopregnanolone, progesterone, AP/P ratio, age, childbirth to baseline at baseline in patients and controls.

| | Allopregnanolone | Progesterone | AP/P ratio | Age | Birth to baseline |
|---|---|---|---|---|---|
| Allopregnanolone | 1.00 | | | | |
| Progesterone | **0.39** ** | 1.00 | | | |
| AP/P ratio | **0.71** *** | −0.31* | 1.00 | | |
| Age | 0.21 | 0.25 | 0.07 | 1.00 | |
| Birth to baseline | **−0.84** *** | **−0.39** ** | **−0.56** *** | −0.11 | 1.00 |

Spearman´s rho * < 0.1 ** < 0.05 *** < 0.01; significant differences bolded.
AP/P ratio = Allopregnanolone/progesterone ratio.

allopregnanolone biosynthesis (Locci & Pinna, 2017). Neurosteroids, including allopregnanolone, are involved in the homeostatic control of the HPA-axis (Crowley & Girdler, 2014). Failure to increase allopregnanolone may exacerbate HPA dysregulation to stress, which has potential pathophysiologic importance in postpartum affective disorders (Schiller et al., 2015). The risk for severe postpartum mental disorders is increased in women with prior affective episodes, including depressive, psychotic, suicidal and bipolar symptoms (Howard et al., 2014; Jones, Chandra, Dazzan, & Howard, 2014). But the majority of studies investigating allopregnanolone in perinatal populations have excluded either history of mental disorder (Nappi et al., 2001; Pearson Murphy et al., 2001), bipolar disorder or prior psychotic, suicidal or manic symptoms (Deligiannidis et al., 2013, 2016; Schiller et al., 2014). In studies including high-risk women, lower allopregnanolone was associated with depressive symptoms in late (Hellgren, Akerud, Skalkidou, Backstrom, & Sundstrom-Poromaa, 2014) but not in mid pregnancy (Hellgren, Comasco, Skalkidou, & Sundstrom-Poromaa, 2017). Women with prior uni- and bipolar disorder, at risk for development of postpartum depression, showed decreased mid but not late pregnancy allopregnanolone levels (Osborne et al., 2017) and change of allopregnanolone from mid to late pregnancy was associated with epigenetic variation in estrogen responsive genes (Osborne et al., 2016).

Progesterone is metabolized into allopregnanolone by 5-alpha-reductase and 3-alpha-hydroxysteroiddehyrogenase (3-alpha-HSD) that has been associated with depressive symptoms in pregnancy (Hellgren et al., 2017) and paranoid ideation in women with bipolar disorder (Johansson, Nikamo, Schalling, & Landen, 2012). As estradiol can modulate both enzymes (Pluchino et al., 2009), it may affect neurosteroid metabolism and balance. Patients in our study lacked correlation between allopregnanolone and progesterone at baseline, a finding in line with that reported in women with postpartum blues (Nappi et al., 2001). The correlation between allopregnanolone and progesterone became significant after clinical recovery, similar to the correlation in controls, which may point toward a more efficient metabolism or balance between allopregnanolone and progesterone.

Patients in our study had relatively higher levels of progesterone than allopregnanolone at baseline leading to a significantly declined ratio compared with controls in time adjusted analyses. Progesterone levels in patients were similar to levels reported in women with postpartum depression 2–9 weeks postpartum (Deligiannidis et al., 2013) and healthy controls at 4–6 weeks (Gilbert Evans et al., 2005) but lower than reported in patients at 3–9 weeks in (Deligiannidis et al., 2016) and higher than in healthy controls at 6–8 weeks (Pearson Murphy et al., 2001) (Supplementary Table S1). Progesterone levels in healthy controls were lower than levels reported during the first week (Deligiannidis et al., 2016; Nappi et al., 2001; Pearson Murphy et al., 2001) but levels vary widely within studies during the first days after

**Table 5**
Correlations between allopregnanolone, progesterone, estradiol, age, childbirth to baseline in patients at baseline and follow-up.

| | Allo bl | Allo fu | Prog bl | Prog fu | Estr bl | Estr fu | Age | Birth to bl | Bl to fu |
|---|---|---|---|---|---|---|---|---|---|
| Allo bl | 1.00 | **0.63**** | 0.45 | 0.28 | 0.07 | −0.10 | −0.17 | −0.26 | −0.12 |
| Allo fu | | 1.00 | −0.11 | **0.56**** | −0.20 | −0.24 | −0.24 | 0.07 | −0.10 |
| Prog bl | | | 1.00 | 0.32 | 0.11 | 0.39 | −0.12 | −0.53* | −0.25 |
| Prog fu | | | | 1.00 | 0.01 | 0.05 | −0.17 | 0.09 | −0.27 |
| Estr bl | | | | | 1.00 | −0.08 | **0.64**** | −0.02 | −0.17 |
| Estr fu | | | | | | 1.00 | −0.41 | **−0.58**** | 0.20 |
| Age | | | | | | | 1.00 | **0.54**** | −0.37 |
| Birth to bl | | | | | | | | 1.00 | −0.05 |
| Bl to fu | | | | | | | | | 1.00 |

bl = baseline, fu = follow-up.
Allo = Allopregnanolone, Prog = Progesterone, Estr = Estradiol.
* < 0.1.
** < 0.05; significant differences bolded.

birth and some of our controls, that were assessed after the first week, potentially decreased the summary measures that are more similar to values assessed at about two weeks (Supplementary Table S1). The ratio remained unchanged after symptom normalization. While neither Deligiannidis et al. (2016) nor Osborne et al. (2017) found differences in the allopregnanolone progesterone ratio between depressed and healthy women during pregnancy and the postpartum period, reported Kimmel et al. (2016) an association between the allopregnanolone progesterone ratio and the interaction between estradiol levels and methylation at the oxytocin receptor (that was predictive of postpartum depression) in perinatal at-risk women.

Even though the original studies were not placebo-controlled, treatment effects were likely associated with the increase in estradiol, as low levels were associated with relapse or decreased effectiveness as described in the original studies (Ahokas et al., 2000, 2001). As estradiol affects multiple mood regulating systems (e g HPA, immune and serotonin system) (Schiller et al., 2015), treatment effects are unlikely to affect only the neurosteroid system. Mood regulation in these patients is likely associated with complex changes in different neurotransmitters, neuroactive steroids and their receptors affecting different neurocircuitries.

This study has several limitations. The control group was assessed only once and significantly earlier after birth. Unfortunately, we lacked data on breastfeeding in patients with postpartum depression and were not able to match based on breastfeeding status. Allopregnanolone has been found to be higher in smokers (Duskova et al., 2012), hypertension (Luisi et al., 2000) and associated with higher pregnancy weight gain (Lundqvist, Sandstrom, & Backstrom, 2017) but lower in those with higher pregnancy BMI (Hellgren et al., 2017). We were not able to control for any of these variables. The blood samples for analysis of allopregnanolone in patients were obtained several years ago which may have affected the integrity of the samples. The distribution of levels in patients are though similar to levels at approximate postnatal timepoints in other studies (Supplementary Table S1). And neurosteroid levels seem to be stable at low temperatures according to a study in rats (Bixo, Backstrom, Cajander, & Winblad, 1986). Progesterone in patients and controls was assessed with similar methods at two different laboratories but levels approximate those reported also with other methods than RIA (Supplementary Table S1). Two women started antidepressants treatment week 3. Three women tapered neuroleptics during the first treatment week. We cannot exclude potential medication effects in these patients. The strengths of this study are that we could assess a group of women with severe mental disorders prospectively who had high ratings of both depressive and psychotic symptoms, including women with suicidal risk and who had not resumed menstruation. By combining women with severe affective symptoms that were sensitive for estradiol treatment effects and obtaining longitudinal data we were able to assess neurosteroids according to RDoC suggestions (Cuthbert & Insel, 2010). We did not correct for multiple comparisons according to reasoning from Rothman (1990), as the small sample size would likely have increased the type II error precluding potentially relevant findings, that might suggest further research of neurosteroids in severe postnatal affective disorders. Also, we only assessed peripheral levels. Neurosteroids cross the blood brain barrier easily and plasma concentrations similar to brain concentrations have been found in many, though not in all, studies (Paul & Purdy, 1992). Within the CNS, neuroactive steroid concentrations are region dependent (Bixo et al., 1997).

In this preliminary study, women with postpartum psychosis and depression showed a decrease of allopregnanolone during estradiol treatment. While neither allopregnanolone nor progesterone levels differed in comparison with healthy controls, change in neurosteroid balance was associated with estradiol treatment. These findings may support potential differences in neurosteroid metabolism as biomarkers in women at-risk for severe affective postpartum disorders. Future studies on potential changes in neurosteroid metabolism in high-risk affective perinatal populations seem warranted. Such studies, with careful control for the physiological postnatal hormonal changes and confounders, might be able to discern potential differences in progesterone metabolites and metabolizing enzymes. If substantiated, differences in allopregnanolone metabolism, might have implications for allopregnanolone treatment effects in severe populations.

## Financial disclosures

None of the contributing authors have any conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript.

## Ethical statement

Hereby, I, Marie Bendix, consciously assure that for the manuscript *Allopregnanolone and progesterone in estradiol treated severe postpartum depression and psychosis – preliminary findings* (Bendix M; Bixo M; Wihlbäck AC; Ahokas A; Jokinen J) the following is fulfilled:

1) This material has not been published in whole or in part elsewhere (except from being part of a PhD dissertation);
2) The manuscript is not currently being considered for publication in another journal;
3) All authors have been personally and actively involved in substantive work leading to the manuscript, and will hold themselves jointly and individually responsible for its content.

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## Contributors

AA designed the studies and collected the data in the original estradiol treatment studies. MBi and AW collected the data for the healthy controls. The current project was conceptualized by JJ, AA, MBi and MBe. MBi performed the measurements. MBe analyzed the data and drafted the paper. All authors reviewed the results, interpreted the data, and revised the paper. All authors revised and approved the final version of the manuscript.

## Declaration of Competing Interest

None.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.npbr.2019.10.003.

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