Hormonal effects of estrogen and progesterone in postpartum depression

Roxana Mihaela Barbu, Cristina-Maria Gavrilescu, Elena Cojocaru, Răducu Ionuț Popescu, Daniela Ababei, Walther Bild

ABSTRACT
Throughout life, women make a sequential transition through states modulated by the relative levels of sex steroid hormones that include pre-puberty, menarche, menstruation, pregnancy, breastfeeding and menopause. In addition, exogenous sex hormones can further alter normal hormonal states, so the risk of major depression in multiparous women is twice as high as in nulliparous women and is particularly high during the years after menopause. These "reproductive depressions" involve episodes of depression that occur specifically during the premenstrual, postpartum and perimenopause phases in women. There is substantial evidence that estrogen and progesterone vary considerably throughout a woman's life and contribute to changes in brain structure and function. These findings are consistent with functional data indicating an important role for estrogen and progesterone in mediating emotional processing. The aim of the study was to explore the reciprocal relationships between sex steroid hormones, estrogen and progesterone throughout physiological and pathophysiological conditions in nulliparous females compared to multiparous females and to identify the cause of depression in multiparous females. Material and method: 20 Swiss mice, nulliparous females and 15 Swiss mice, multipair females with three pregnancies in number,
were used, for which the hormonal dosing was done. Single variance analysis (ANOVA) was used. Results: in the group of nulliparous female mice, an increase of the average values of estrogen was observed, compared to the group of multiparous female mice. Progesterone levels were not significantly different in the two groups of mice. Conclusions: estrogen values changed significantly between the parameters of nulliparous values compared to multiparous female mice, and progesterone values did not change in the two groups of mice studied, suggesting that the increase in estrogen values in multiparous females might cause postpartum depression.

KEYWORDS:
Postpartum depression, estrogen, progesterone.

INTRODUCTION
Based on the fact that studies using animal models have provided important perspectives on the pathogenesis, mechanisms and new therapeutic approaches of human diseases, in this study we provide an overview of existing evidence for the physiological, behavioral, cellular and molecular actions of estrogen and progesterone in the context of neurotransmission control in the central nervous system circuits that regulate mood and motivation and discuss the pathology that leads to mental disorders, such as depression in nulliparous and multiparous women.

Postpartum depression is a common complication of childbirth, affecting about 15% of multiparous women (1). A hormonal aetiology has long been hypothesized due to sudden and substantial fluctuations in steroid hormone concentrations associated with pregnancy and the immediate postpartum period. There is also convincing evidence that estrogen, progesterone and related compounds are important in the activity of the central nervous system at physiological concentrations (2). Estrogen and progesterone are steroid hormones derived from enzymatic changes in cholesterol. In women, the production of estrogen and progesterone takes place primarily in the ovary, but also in the adrenal gland and in other locations, such as adipose tissue. Gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) modulate the synthesis and secretion of ovarian sex hormone. FSH and LH are thus released from the pituitary gland under the control of the central nervous system, mediated predominantly by the hypothalamus, which in turn is widely connected with other areas of the central nervous system (3, 4).

Different stages of women's lifespan can be characterized depending on the relative levels of sex steroid hormones. Thus, estrogen and progesterone levels are low throughout childhood, but increase dramatically at the onset of puberty under the influence of pulsatile gonadotropin release from the pituitary gland (5). This process usually leads to the appearance of a regular menstrual cycle, divided into two phases: the follicular phase when serum estrogen levels are high and progesterone low and the luteal phase, in which the progesterone level is high compared to estrogen levels. The late luteal phase is associated with a spectrum of premenstrual symptoms, including headache, bloating, cramps, breast tenderness, weight changes, irritability, decreased concentration, depression and anxiety. The two major biologically active estrogens in non-pregnant women are estrone and estradiol, while pregnant women also produce significant amounts of estriol (6, 7)
During pregnancy, both estrogen and progesterone increase steadily in the three trimesters and then return to baseline after birth. During the postpartum phase, estrogen levels are relatively low, which has been implicated in the pathophysiology of postpartum depression (8). The high level of prolactin present during breast-feeding suppresses the release of gonadotropin-releasing hormone, estrogen and progesterone synthesis and ovulation. (9). Substantial evidence from several sources supports the role of estradiol, the physiological form of estrogen, which is involved in affective regulation, so that estrogen modulates "beneficially" the systems involved in the pathophysiology of depression; regulates the synthesis, metabolism and concentration of classical neurotransmitter receptors involved in depression (serotonin, dopamine and norepinephrine); regulates the activity of the basal and stimulated hypothalamic-pituitary-adrenal axis; acts like antidepressants in stimulating the brain's derived neurotrophic factor; is neuroprotective in a variety of models; improves mitochondrial respiratory efficiency and prevents the formation of oxygen free radicals that are thought to adversely affect the energy of mitochondria in depression; finally, at several levels, it prevents or counteracts the proinflammatory processes described as contributing to depression (10, 11).

In this context, the aim of the presentation was to compare the values of hormonal doses for estrogen and progesterone in nulliparous and multiparous female mice, and to identify the etiology of depression in multiparous, starting from the idea that fluctuating levels of the two reproductive hormones trigger a psychiatric affective pathology.

MATERIAL AND METHOD
Experimental animals used Swiss mice, adult females, of which 20 nulliparous and 15 multiparous weighing 18-20 g, were used for all experiments. The animals were housed in individual cages at a temperature of 21 ± 2°C, nictemeral cycle of 12-12 hours, with food and water at their discretion. All animals were kept in similar environmental and feeding conditions.

Blood collection and testing
In the morning, between 8 and 9 am, Swiss mice were weighed, anesthetized with inhaled isoflurane, and blood samples were collected by taking blood from the left ventricle directly into the vacutainer, approximately 1.5 - 2 ml / mouse, then placed immediately on ice. The serum was isolated by centrifugation at 2500xg for 15 min. The serum was aliquoted and frozen at -20 °C until analysis. Serum estradiol and progesterone were analysed by ELISA (mouse kit Estradiol, cat. RTC009R, BioVendor, Czech Republic; progesterone kit for mice, cat. RTC006R, BioVendor, Czech Republic).

The wells were coated with anti-estradiol antibody and anti-progesterone antibody. An unknown amount of hormone present in the sample competes with a hormone-radish peroxidase (HRP) conjugate for binding to the coated antibody. Each serum sample was analyzed in duplicate with each of the four ELISA kits according to the manufacturer's instructions. The absorbance at each test was read at a wavelength of 450 nm with a plate reader (State Fax 303 Plus, Awarness Technology Inc, USA). Based on the standard curves on each plate, the hormonal concentration of each hormone was determined for each mouse in the two groups studied.
Biochemical estimates

Estrogen dosing in female Swiss mice was performed by the competitive ELISA method, using immobilized antigen. Estradiol is one of the main components of natural estrogens and is the main estrogen secreted during the menstrual cycle. The estradiol calibration range is between 0.312 - 20ng/mL, and the limit of detection of estradiol in the body is equal to 0.157 ng/mL.

Progesterone was dosed in female Swiss mice using the sandwich ELISA method, using the HRP-labeled antibody. Progesterone is a female steroid hormone with a variety of physiological effects. In the follicular phase of the menstrual cycle, progesterone is produced at low levels. The progesterone calibration range is between 0.312 - 20ng/mL, and the limit of detection of estradiol in the body is equal to 0.173 ng/mL.

Data Analysis.

We used single variance analysis (ANOVA). Results are expressed as mean ± SEM. F values for which P <0.05 were considered statistically significant.

RESULTS

In this study we demonstrated the hypothesis that fluctuations in reproductive hormone levels during pregnancy and the postpartum period trigger postpartum depression in some women, depending on the pathological effects and values of sex steroid hormones such as estrogen and progesterone, using experimental animals - Swiss mice, nulliparous females and multipairs weighing between 18g - 25g. We analyzed two batches of animals, thus, a batch of 20 nulliparous female mice and the second batch of 15 multiparous female mice. For the two groups, a comparison was made between the values of estrogen and progesterone concentrations for each female nulliparous and multiparous mouse.

Fig. 1 Mean values in nulliparous and multiparous female mice for the levels of estradiol. The values are mean ± S.E.M. (n=20 in nulliparous group and n=15 in multiparous),**p<0.001 vs. nulliparous group.

Figure 1 represents a difference in the analysis of the average values of the group of nulliparous female mice compared to the multiparous ones, in the analysis of estrogen. Thus, in the group of nulliparous female mice, an increase of the average values is observed, compared to the group of multiparous female mice.
Figure 2 shows no significant difference in the analysis of mean values in the group of nulliparous female mice compared to multiparous female mice in the analysis of progesterone. Thus, progesterone levels are not significantly different in the two groups of mice.

Regarding the group differences between the group of nulliparous mice and multiparous mice, we noticed that in multiparous female mice the average estrogen values have a significant increase compared to the nulliparous female, but at the same time, in the progesterone analysis there is no difference in the analysis mean values in the group of nulliparous female mice compared to multiparous female mice. Our results are consistent with the observed epidemiological relationship between the effects of estrogen and progesterone and the type of female mice used, nulliparous and multiparous. However, based on the analysis performed, it was observed that the multiparous female mouse does not suffer pathological changes during the menstrual cycle compared to the nulliparous female mouse, but subsequently may suffer from postpartum depression.

**DISCUSSION**

The present study investigated the relationship between the effects of estrogen and progesterone on non-reproductive depression, compared to reproductive depression, using nulliparous and multiparous female mice, in which the two hormones were dosed, thus identifying the hormonal differences found in the two groups of study.

Despite decades of research to identify the causes of postpartum depression (PPD) and to develop effective methods of screening, prevention and treatment, PPD remains common, affecting between 7 and 20% of women after childbirth. PPD is one of the most important public health problems we can address, it only affects women at an extremely vulnerable time, but it also has harmful effects on children and families. Many have speculated that PPD is caused by the rapid change in the reproductive hormones estradiol and progesterone before and immediately after birth (12, 13).

Although a number of studies in human and non-human animals suggest that changes in reproductive hormone levels contribute to PPD (13), several studies have failed to detect
an association between hormone concentrations and PPD symptoms (14). For example, cross-sectional studies in humans examining differences between groups in ovarian hormone levels and depressive symptoms in the postpartum period have failed to demonstrate the association between absolute concentrations of estrogen, progesterone, and PPD (15). In contrast, studies treating PPD with estradiol have successfully reduced depressive symptoms (16), and animal studies have shown that withdrawal of estradiol and progesterone causes depression-like behavior (17).

Despite all the research that aimed to identify the causes of postpartum depression, PPD remains common and the causes are poorly understood. Many have attributed the appearance of depression to the rapid perinatal change of reproductive hormones. Although a number of studies in human and non-human animals support the role of reproductive hormones in postpartum depression, several studies have failed to detect an association between hormone concentrations and this postpartum depression. There are three main lines of investigation that addressed the role of reproductive hormones in PPD: animal studies, correlated studies of postpartum hormone levels and mood symptoms, and hormone manipulation studies. Reproductive hormones influence virtually any biological system involved in PPD, and a subgroup of women appear to be particularly sensitive to the effects of perinatal changes in hormone levels. The peripartum period is characterized by a rapid and significant physiological change in plasma levels of endocrine hormones, peptides and neuroactive steroids (18). Several lines of research actively examine the potential roles of estrogen, progesterone, cortisol, corticotropin-releasing hormone, adrenocorticotropic hormone, oxytocin, prolactin, testosterone and thyroid function in postpartum depression (19).

Untreated postpartum depression can have substantial adverse effects on the well-being of the mother and child with lasting consequences. PPD with onset during pregnancy is associated with an increased risk of maternal substance abuse, preeclampsia, premature birth and low birth weight. PPD can affect a woman's ability to take care of herself and the baby, having a negative impact on the child's cognitive, behavioral and emotional development (20). Thus, there is evidence that reproductive hormones directly influence the biological systems and neural circuits involved in depression, suggesting that the hormonal instability inherent in the perinatal period could contribute to mood disorder in PPD.

CONCLUSIONS
This study shows that, depending on the period in which the female was, before or after pregnancy, in the two groups of nulliparous and multiparous female mice, the hormonal concentrations for estrogen and progesterone have very different values from physiological to pathological. We demonstrated a significant increase between the parameters of estrogen values in the two groups of mice studied, but no significance for progesterone values. Thus, we have a significant increase between the parameters of reproductive hormone fluctuations - estrogen, and for progesterone no significant significance was found between the group of nulliparous and multiparous female mice, which triggers the affective hormonal disorder in some women in the postpartum period. During the postpartum phase, estrogen levels are relatively low, which has been implicated in the pathophysiology of postpartum depression. The study noticed that changes in multiple biological
systems, such as the immune system, hypothalamic-pituitary-adrenal cortex and lactogenic hormones, contribute to the pathophysiology of postpartum depression.

In conclusion, we suggest that significant changes in estrogen levels in multiparous women can sometimes trigger postpartum depression, and subsequently, can initiate menopause, with detrimental effects on female fertility.

ACKNOWLEDGEMENTS AND DISCLOSURES

The authors declare that they have no potential conflicts of interest to disclose.

REFERENCES

1. Gavin NI, Gaynes BN, Lohr KN, Meltzer-Brody S, Gartlehner G, Swinson T. Perinatal depression: a systematic review of prevalence and incidence. Obstet Gynecol. 2005; 106(5 Pt 1):1071–83.
2. Suda S, Segi-Nishida E, Newton SS, Duman RS. A postpartum model in rat: behavioral and gene expression changes induced by ovarian steroid deprivation. Biol Psychiatry. 2008; 64(18471802):311–319.
3. Ahokas A, Kaukoranta J, Wahlbeck K, Aito M. Estrogen deficiency in severe postpartum depression: successful treatment with sublingual physiologic 17beta-estradiol: a preliminary study. J Clin Psychiatry. 2001; 62(5):332–6.
4. Schiller CE, O’Hara MW, Rubinow DR, Johnson AK. Estradiol modulates anhedonia and behavioral despair in rats and negative affect in a subgroup of women at high risk for postpartum depression. Physiol Behav. 2013; 119:137–144.
5. Bernstein IH, Rush AJ, Yonkers K, et al. Symptom features of postpartum depression: are they distinct? Depress Anxiety. 2008; 25(1):20–26.
6. Wisner KL, Sit DY, McShea MC, et al. Onset timing, thoughts of self-harm, and diagnoses in postpartum women with screen-positive depression findings. JAMA Psychiatry. 2013; 70(5):490–498.
7. Finocchi C, Ferrari M. Female reproductive steroids and neuronal excitability. Neurol Sci Off J Ital Neurol Soc Ital Soc Clin Neurophysiol. 2011; 32 (Suppl 1):31–35.
8. Rubinow DR, Girdler SS. Hormones, heart disease, and health: individualized medicine versus throwing the baby out with the bathwater. Depress Anxiety, 2011; 28(4):282–296.
9. Santin AP, Furlanetto TW. Role of Estrogen in Thyroid Function and Growth Regulation. J Thyroid Res. 2011; 2011:e875125.
10. Berent D, Zboralski K, Orzechowska A, Galecki P. Thyroid hormones association with depression severity and clinical outcome in patients with major depressive disorder. Mol Biol Rep. 2014; 41(4):2419–2425.
11. Krause D, Jobst A, Kirchberg F, et al. Prenatal immunologic predictors of postpartum depressive symptoms: a prospective study for potential diagnostic markers. Eur Arch Psychiatry Clin Neurosci. 2014:1–10.
12. Blackmore ER, Groth SW, (Din) Chen D-G, Gilchrist MA, O’Connor TG, Moynihan JA. Depressive symptoms and proinflammatory cytokines across the perinatal period in African American women. J Psychosom Obstet Gynecol. 2013; 35(1):8–15.
13. Dr. Crystal Edler Schiller, Ph.D., Dr. Samantha Meltzer-Brody, M.D., M.P.H., and Dr. David R. Rubinow, M.D., The Role of Reproductive Hormones in Postpartum Depression, CNS Spectr. 2015; 20(1): 48–59.
14. Tarantino LM, Sullivan PF, Meltzer-Brody S. Using animal models to disentangle the role of genetic, epigenetic, and environmental influences on behavioral outcomes associated with maternal anxiety and depression. Front Psychiatry Front Res Found. 2011; 2:44.
15. Moses-Kolko EL, Fraser D, Wisner KL, et al. Rapid habituation of ventral striatal response to reward receipt in postpartum depression. Biol Psychiatry. 2011; 70(4):395–9.
16. Maguire J, Ferando I, Simonsen C, Mody I. Excitability Changes Related to GABAA Receptor Plasticity during Pregnancy. J Neurosci. 2009; 29(30):9592–9601.
17. Schiller CE, Schmidt PJ, Rubinow DR. Allopregnanolone as a mediator of affective switching in reproductive mood disorders. Psychopharmacology (Berl). 2014:1–11.
18. Djebaili M, Guo Q, Pettus EH, Hoffman SW, Stein DG. The neurosteroids progesterone and allopregnanolone reduce cell death, gliosis, and functional deficits after traumatic brain injury in rats. J Neurotrauma. 2005; 22(1):106–118.
19. Michael A, Jenaway A, Paykel ES, Herbert J. Altered salivary dehydroepiandrosterone levels in major depression in adults. Biol Psychiatry. 2000; 48(10):989–995.
Correspondence:

Cristina-Maria Gavrilescu,
Lecturer, MD, PhD, „Grigore T. Popa” University of Medicine and Pharmacy, Iaşi, Ist Medical Department, Iaşi, Romania. cristina.gavrilescu@umfiasi.ro

Submission: 28 July 2020
Acceptance: 8 Sep. 2020