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Escola de Psicologia

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Exploratory study of the women's HPA axis function during perinatal period: The impact of breastfeeding cessation and depression

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Despacho RT - 31 /2019 - Anexo 4

STATEMENT OF INTEGRITY

I hereby declare having conducted this academic work with integrity. I confirm that I have not used plagiarism or any form of undue use of information or falsification of results along the process leading to its elaboration.

I further declare that I have fully acknowledged the Code of Ethical Conduct of the University of Minho.

University of Minho, 19th of October 2021

Signature: Maria Inês Silva

Estudo exploratório do funcionamento do eixo HPA da mulher ao longo do período perinatal: o impacto da cessação da amamentação e da depressão

Resumo

Não obstante a maioria dos estudos seja consensual quanto ao impacto da amamentação no funcionamento do eixo HPA da mulher, o impacto da cessão da amamentação e da depressão no funcionamento do eixo HPA durante o período perinatal foi ainda pouco estudado. Este estudo teve como principal objetivo analisar o impacto da cessação da amamentação/amamentação exclusiva nas várias medidas de COR. Uma amostra de 30 mulheres primíparas com EPDS ≥ 9 no terceiro trimestre de gravidez reportaram o estado da amamentação, providenciaram amostras salivares de COR e responderam aos questionários que avaliam sintomas de depressão e ansiedade ao longo do período perinatal.

Os resultados revelam que a cessação da amamentação exclusiva tem impacto na diminuição da secreção global do COR durante o dia e que a cessão da amamentação exclusiva/amamentação tem impacto na redução do CAR. Efeitos de interação revelam que mulheres com mais sintomas de depressão na cessação da amamentação exclusiva têm uma maior diminuição na secreção global de COR durante o dia, após a cessação de amamentação exclusiva. Estes resultados ajudam a explicar o funcionamento do eixo HPA subjacente aos benefícios da amamentação e aos riscos da cessação da amamentação precoce, especialmente em mulheres com sintomas de depressão.

Palavras-chave: Amamentação; eixo HPA; cortisol; depressão; período perinatal.

Exploratory study of the women's HPA axis function during perinatal period: The impact of breastfeeding cessation and depression

Abstract

Although the majority of the studies are consensual about an association between breastfeeding and the function of HPA axis, data regarding breastfeeding and HPA axis function over the time, taking account depression are limited. This study main aimed to analyze the impact of exclusive breastfeeding and breastfeeding cessation on several measures of COR. A sample of 30 primiparous women with an EPDS ≥ 9 at the third pregnancy trimester, reported Breastfeeding status, provided salivary samples of COR and completed questionnaires that measure depressive and anxiety symptoms, over the perinatal period.

Results revealed exclusive breastfeeding cessation had an impact on the decrease of the overall secretion of COR during the day and both exclusive breastfeeding and breastfeeding cessation had an impact on the decrease of the CAR. The interaction effects between the impact of exclusive breastfeeding cessation and depressive symptoms on overall COR secretion during the day shows that women with more depressive symptoms at exclusive breastfeeding cessation have a greater decrease on the overall secretion of COR, after exclusive breastfeeding cessation. These results help to explain the functioning of the HPA axis underlying the benefits of breastfeeding and the risks of early breastfeeding cessation, especially in women with depressive symptoms.

Keywords: Breastfeeding cessation, HPA axis, cortisol, depression, perinatal period.

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Exploratory study of the women's HPA axis function during perinatal period: The impact of breastfeeding cessation and depression

Breastfeeding is associate to several benefits for the infant and for the mother (American Academy of Pediatrics, 2012; UNICEF & WHO, 2018). Breastfeeding promotes mother health, for example strengthen the immune system, and promotes mother mental health as well, by reducing stress and providing better sleep and well-being (Altemus et al., 1995; Groer et al., 2006; Henrichs et al., 2002; Walters et al., 2016). Breastfeeding is also a protective factor for depression during the postpartum period (Figueiredo et al., 2013; Hahn-Holbrook et al., 2013; Stuebe et al., 2013), and particularly in depressed pregnant women (Figueiredo et al., 2021). In opposite, early exclusive and non-exclusive breastfeeding cessation is associated with increased depression and anxiety symptoms during the postpartum period (Figueiredo et al., 2014; Fairlie etal., 2009; Stuebe et al., 2019; Ystrom, 2012).

Health welfares of breastfeeding women appears to be related with hormonal alterations regulated by the hypothalamic–pituitary–adrenal (HPA) axis. These alterations can be protective, especially for hormone-sensitive mothers, taking account hormone-gene-environment interactions (Gust et al., 2019). One of the central functions of the HPA axis is to regulate stress response through the synthesis, release and succeeding inhibition of three main hormones, corticotrophin releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and cortisol (COR) (Almanza-Sepulveda et al., 2020; Brunton & Russel, 2011). Suckling and breastfeeding is suggested to exert a short-term suppression of the HPA axis, with short-term attenuation of neuroendocrine stress responses, affecting CRH, ACTH and COR output; usually decreasing COR levels, particularly during stress situations (Altemus et al., 1995; Groer et al., 2006; Heinrichs et al., 2001, Mizuhata et al., 2020). Comparing to formula-feeding women, women who breastfeed may also be more protected from deleterious influences of stress on postpartum immunity (Ahn & Corwin, 2015).

It's well known that complex changes ocurr across pregnancy and the postpartum period in hormones regulated by the HPA axis, including COR (Brunton & Russell 2008; Groer et al., 2007; Noyola-Martínez et al., 2019). The HPA axis is activated during pregnancy, so the mother experiences a state of hypercortisolism, as shown by high levels of COR, CRH and ACTH (Conde & Figueiredo, 2014; Mastorakos & Ilias, 2003; Neves, 2020). COR increment in pregnancy is crucial for further breastfeeding, contributing for milk production while stimulates the secretory differentiation of breast cells into lactocytes (Brunton & Russel, 2008; Pang & Hartmann, 2007). During/after delivery and across the postpartum period, the HPA axis

abruptly recovers from its activated state during pregnancy and gradually returns to pre-pregnancy state (Duthie & Reynolds, 2012; Mastorakos & Ilias, 2003).

The relationship between HPA axis and women's mental health symptoms during perinatal period is not yet well understood. Suppressed HPA axis has been suggested as a factor in the etiology of postpartum affective disorders (Groer et al., 2007; Taylor et al., 2009). Susceptible women to HPA axis deregulation may be particularly vulnerable to develop stress-related disorders at this time (Brummelte & Galea, 2010). Literature has been showing HPA axis deregulation during the pre and the postpartum period in women with depression, including COR, CRH and ACTH secretion (Brunton & Russell 2008; Taylor et al., 2009; Brummelt & Galea, 2016). Many studies show an increase in women's COR levels in the presence of antenatal depressive symptoms, others can't find this association (for a revision see Illiadis et al., 2015). Some studies found high levels of COR in mothers with postpartum depression, while other studies point to lower levels of COR or no association at all (for a revision see Seth et al., 2016). Even though, postpartum depression is more often associated with hypocortisolemia, typically associated with chronic depressive states (Seth et al., 2016) and other health issues, like Addison's disease and immune problems (Manoharan et al., 2019; Sarapultsev et al., 2020). Over-all, results concerning the way HPA axis functions in the presence of pre and postpartum depression and anxiety symptoms are still inconclusive (for a revision see Almanza-Sepulveda et al., 2020).

Although the majority of the studies are consensual in regard an association between breastfeeding and HPA axis function, studies considering breastfeeding and HPA axis function over the time, taking account depression are limited. Namely, in most of the studies, the sample doesn't have a significant expression of clinical depressive symptoms, or significant associations between breastfeeding and depressive symptoms, therefore usually depressive symptoms don't enter in statistical models, making it difficult the assessment of its potential influence on the relationship between breastfeeding and HPA axis function.

In perinatal period, COR levels are frequently reported as an indicator of HPA axis function (Almanza-Sepulveda et al., 2020), COR levels can be measure by its overall secretion over a specific time period or by its median secretion levels in a specific moment (Pruessner, 2003). Preliminary work shows that depending on women's breastfeeding status, depressed women may have different COR levels outputs, breastfeeding women who were depressed had higher median secretion of evening COR, compared non-breastfeeding women who were depressed (Illiadis et al., 2015). Contrary, Bublitz et al. (2019) didn't found differences in

evening secretion of COR between women who were breastfeeding and bottle feeding, but depression symptoms didn't enter in the model.

Circadian changes of COR during the day are an important COR output to access the reactivity of HPA axis, particularly in perinatal period (Adam et al., 2017; Gust et al., 2019). The impact of breastfeeding on circadian changes COR during the day is not yet clarified. Tu et al. (2006) didn't found an impact of breastfeeding in the distribution of mothers into the three diurnal circadian COR groups, showing that breastfeeding didn't had an impact on circadian changes of COR during the day, in this study depressive symptoms were not included in the model. The stability of circadian COR during the day as shown to lower the risk of postpartum depression (Taylor et al., 2009), but is not known if breastfeeding has an impact on this relationship.

Cortisol awakening response (CAR) is a central variable to understand HPA axis reactivity and comprehend circadian changes of COR during the morning. The measure marks COR secretion increase over the first 30–45 min after morning awakening. CAR has been linked to a wide range of psychosocial, physical and mental health parameters (Stalder, 2016), especially in the perinatal period (Tu & Lupien, 2005; Dickens & Pawluski, 2018). Namely, Bublitz et al. (2019), as shown an influence of pregnancy CAR on further breastfeeding status, they found higher CAR over pregnancy among breastfeeding women compared to non-breastfeeding women. They also found that CAR mediate the negative association between socioeconomic status and breastfeeding, this result point that CAR may have an important role in the association between breastfeeding and distress in the perinatal period. Likewise, Ahn and Corwin (2015) found an impact of breastfeeding on CAR, predominantly breastfeeding women had higher CAR compared to those who bottle fed, through 6 months postpartum.

COR changes are associated with oxytocin, an important hormone related with breastfeeding in the postpartum period (Brunton & Russel, 2008; Henrichs, Neumann & Ehlert, 2002). In response to suckling, oxytocin is released into the circulatory system, taking part in multiple systems that affect HPA reactivity (Cox et al., 2015; Whitley et al., 2019). Depressive symptoms may influence the relationship between breastfeeding and oxytocin in mothers. During breastfeeding, women with depressive symptoms had lower oxytocin production than women without depressive symptoms (Whitley et al., 2019). Also, depressed women who stop breastfeeding at 8 weeks postpartum, exhibit low levels of oxytocin than non-depressed women who stopped breastfeeding (Lara-Cinisomo et al., 2017). Prior research also shows that breastfeeding may influence the association between oxytocin and COR in mothers with depressive symptoms. Among breastfeeding women without depressive symptoms, high levels

of oxytocin were associated with low COR levels, in contrast, among breastfeeding women with depressive symptoms, oxytocin was associated with high levels of COR (Cox et al., 2015). These results suggest that Oxytocin released during breastfeeding, in women with depressive symptoms, may increase COR response to stress, rather than blunting HPA activation as previously suggested by Henrichs et al., (2001, 2003).

To explore changes in HPA axis from pregnancy to postpartum, taking in account breastfeeding and significant depressive symptoms, can help explain the benefits of breastfeeding in the first year postpartum and the risks associated to early breastfeeding cessation, specifically in vulnerable pregnant women. From our knowledge, there are no study on breastfeeding cessation, depressive symptoms and COR. By studying what happens in COR measures, when women with significant depressive symptoms stop breastfeeding, could help explore the function of HPA axis and better understand the possible risks of early breastfeeding cessation in these women's health. In order to contribute towards the further understanding of HPA axis changes related to breastfeeding in women with mental health problems during pregnancy and postpartum, this exploratory investigation main aim to study the effect of breastfeeding cessation on salivary COR in a sample of primiparous women with clinically significant depressive symptoms in third timestre of preganancy, from third pregnancy trimester to 12 months postpartum. We specifically aim to study: (1) The impact of exclusive breastfeeding and breastfeeding cessation on the overall secretion of salivary COR during the day, on circadian changes of salivary COR during the day, and on CAR; (2) Interaction effects between exclusive breastfeeding/breastfeeding cessation and depressive symptoms on overall secretion of COR during the day, on circadian changes of COR during the day, and on CAR.

Method

Participants

Sample comprised 30 primiparous women. Most women were Caucasian (90%). More than a half were aged between 26-34 years old (63.3%), employed (53.3%), married or cohabiting with the partner (80%), and half of the women were between 9 and 12 years of schooling (53.3%). Most women had a term gestation (≥ 37 weeks; 93.3%) and more than a half had a vaginal delivery (56.7%) and half of the women (50%) had normal delivery with epidural.

All infants were born with normal birth weight (≥ 2500g) and the majority were born with normal birth length (≥ 48 cm; 83.3%) and were not resuscitated at birth (90%). More than a half of the infants were males (60%).

Table 1

Sample's socio-demographic characteristics

		n= 30 *n*	 %
Mother	Age		
	18-25	7	23.3
	26-34	19	63.3
	35-44	4	13.3
	Marital status		
	Married/cohabiting	24	80
	Single/divorced/widow	6	20
	Occupational status		
	Employed	16	53.3
	Unemployed/Household/student	13	43.3
	Years of schooling		
	< 12	14	46.6
	>12	16	53.3
Pregnancy and delivery	Time of gestation		
	< 37	2	6.7
	≥ 37	28	93.3
	Resuscitation at birth		
	No	27	10
	Yes	3	90
	Type of delivery 1		
	Vaginal	17	56.7
	Caesarean	13	43.3
	Type of delivery 2		
	Normal delivery with epidural	15	50
	Caesarean section with epidural	10	33.3
	Caesarean delivery with general anesthesia	3	10
	Vacuum cup delivery	2	6.7

Procedure

This study took part from a larger longitudinal study (Figueiredo, 2011) that received approval from the Ethical commissions of the institutions involved. Participants were recruited

during the third pregnancy trimester in two public hospitals in the North of Portugal. Exclusion criteria were not reading or writing Portuguese, being multiparous, having multiple gestations, having gestational complications and having pre-term infant or death-birth. The aims and the procedures of the study were explained, and those women who agreed to participate, signed an informed consent form and filled in the Edinburgh Postpartum Depression Scale (EPDS; Cox, et al., 1987).

Women with an EPDS ≥ 9 at the third pregnancy trimester were invited to participate in the study, answering the questionnaires and provide salivary samples on 2 consecutive days in 4 moments: upon awakening, 30 minutes later, and 3 and 12 hours after awakening. Salivary samples were collected in 4 moments during perinatal period: 3rd trimester of pregnancy, 2 weeks postpartum, and at 3 and 6 months postpartum.

From the 101 invited women with clinically significant depression symptoms, 4 of them were excluded because they were multiparous. From the 97 depressed primiparous women, 40 declined to participate and 57 (58.8%) agreed to participate. From the 57 women who were eligible to participate in this study, 32 (31.7%) returned the saliva sampling packs, 2 women didn't answer the questionnaires and didn't return the saliva sampling packs at the post-partum period and were excluded, so 30 (29.7%) primiparous women with clinically significant depressive symptoms were included in the data analysis (see Fig 1.). Non-significant differences were found on depression symptoms at the 3rd trimester of pregnancy between the participants who decline to participate, the participants who agreed to participate and did not return the saliva sampling packs and the participants who agreed to participate and returned the saliva sampling packs.

Repeated measures of Breastfeeding status were obtained at 2 weeks, and 3, 6 and 12 months postpartum. Of the 30 participant women, 28 (93.3%) returned complete data (considering the four salivary cortisol measurements during the day) at the third pregnancy trimester, 23 (76.7%) at 2 weeks, 19 (66.3%) at 3 months, 16 (53.3%) at 6 months postpartum, and 9 (30%) at 12 months. Repeated measures of depressive symptoms, anxiety symptoms and salivary cortisol were obtained at the third pregnancy trimester (M = 37.00 weeks of gestation; SD = 2.50), and at 2 weeks (M = 15.00 days; SD = 5.76), 3 months (M = 13.00 weeks; SD = 1.11), 6 months (M = 26.09 weeks; SD = 0.54) postpartum and 12 months (M =62.78; SD= 14.41).

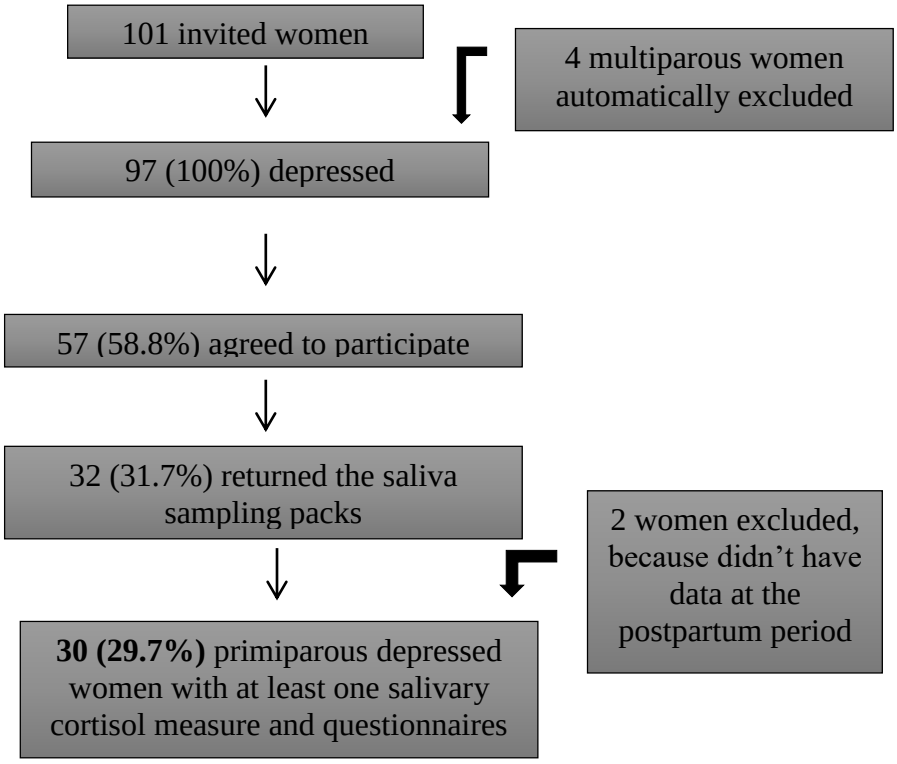

Fig. 1. Participants exclusion flow diagram.

Measures

Sociodemographic data. A Socio-demographic Questionnaire was used to collect women's sociodemographic data. The questionnaires included questions regarding women's demographic and obstetric information and infant's sex, weight, length, etc.

Breastfeeding. Breastfeeding was determined according to the Index of Breastfeeding Status (IBS; Labbok & Coffin, 1997; Labbok & Krasovec, 1990) through women's report of feeding practices at two weeks postpartum. In this study, for accessing breastfeeding, the IBS was modified to define feeding practices as breastfeeding (exclusive and partial breastfeeding) versus non-breastfeeding (exclusive artificial feeding). Breastfeeding included Labbock & Krasovec's all categories (full breastfeeding, partial breastfeeding and token). For accessing exclusive breastfeeding, feeding practices was defined as exclusive breastfeeding versus non-exclusive breastfeeding (partial and exclusive artificial feeding), as recommended by WHO, as the optimal infant feeding method (WHO, 2017). Exclusive breastfeeding included Labbock and Krasovec's level one (full breastfeeding: exclusive breastfeeding, no other liquid or solid is provided) and level two (almost exclusive breastfeeding: only vitamins, minerals or water are given in addition to breastfeeding). All other categories that involved any amount of artificial milk or solids (level three to seven) were defined as non-exclusive breastfeeding.

Salivary cortisol. A saliva collection pack with (1) 8 test tubes (Salivette®, Sarstedt, Germany); (2) a sheet with detailed instructions; and (3) a diary sheet for recording the date and time of saliva collection was provided to the participants at the hospital or sent by mail. Participants who agreed to perform cortisol assessments were asked to provide saliva samples on two consecutive days. Saliva samples were collected immediately upon awakening, 30 minutes later, and 3 and 12 hours after awakening. Participants were instructed not to brush their teeth nor eat or drink anything apart from water 30 minutes before sample collection. Women were also instructed to store the samples in a refrigerator for posterior collection by the researcher. Samples were centrifuged for 10 minutes at 2000 x g and stored at -80ºC until analysis.

Salivary cortisol samples were analyzed in duplicate using a commercial competitive enzyme immunoassay (ELISA; IBL, Germany) following manufacturer instructions. Mean values of duplicate determinations were considered for analysis. Intra- and inter-assay coefficients of variation were both below 10%.

Depressive symptoms. The EPDS (Cox et al., 1987) was used to assess depression symptoms. It is a self-report questionnaire comprising 10 items scored on a four-point Likert-type scale. Depressive symptoms are assessed within the previous seven days. The Portuguese version of the EPDS has shown good internal consistency for both pregnancy and postpartum (α = .82 and .88; Tendais et al., 2014). In the original sample the STAI-S also showed good internal consistency (Cronbach's α = 0.91). In the original sample the STAI-S also showed good internal consistency (Cronbach's α = 0.91). In the Portuguese version of the EPDS, an optimal cut-off of 9 was suggested to screen for depression during pregnancy. In the present sample, EPDS also showed good internal consistency (Cronbach's α = 0.84).

Anxiety symptoms. State-Trait Anxiety Inventory (STAI-S; Spielberger et al., 1983). The STAI was used to assess anxiety symptoms. It is a self-report questionnaire that comprises two subscales, the state anxiety subscale and the trait anxiety subscale. In this study we only used the state anxiety subscale. State anxiety symptoms are defined as symptoms felt at the moment. State anxiety is conceptualized as a transient emotional condition of the individual, characterized by subjectively experienced feelings of tension, together with a heightened activity of the autonomous nervous system. The state anxiety subscales have 20 items and scores ranges from 20 to 80. Higher scores on this subscale indicate higher anxiety symptoms. The optimal cut-off to consider clinically significant symptoms is 40 for pregnancy and 34 during the postpartum period (Tendais et al., 2014). In the Portuguese version of the STAI-S showed an excellent internal consistency for pregnancy and postpartum (Cronbach's α = 0.91

and 0.92, respectively) (Tendais et al., 2014). In the present sample the STAI-S also showed good internal consistency (Cronbach's α = 0.91).

Statistical analysis

Repeated measurements of salivary cortisol were obtained over time (2 consecutive days and 4 times during the day - immediately upon awakening, 30 minutes later, and 3 and 12 hours after awakening), several procedures were conducted to summarize cortisol data. Considering cortisol measurements at day 1 and 2, we opted firstly for day 1 measurements. When complete data (considering the 4 salivary cortisol measurements during the day) for day 1 was not available, salivary cortisol measurements were retrieved from day 2.

Following recommendations (Pruessner et al., 2003), the area under the curve (AUC) was used to summarize the information contained in the multivariate data obtained during the day. To assess the overall secretion during the day, the 'Area under the curve with the respect to ground' (AUC_G) was used. The 'Area under the curve with the respect to increase' (AUC_I) was used to estimate the circadian changes of cortisol (0, 30, 180, and 720 minutes after awakening). When results are negative, AUC_I is considered as "index of decrease", rather than an area (Stalder, 2016). To access the dynamic of the post-awakening cortisol response, AUC_I was used to estimate Cortisol Awakening Response (CAR), post-awakening cortisol concentrations (AUC_G) will be reported as additional information, as recommended in expert consensus guidelines (Stalder, 2016).

Two formulas were used to calculate the (AUC_G) and the (AUC_I), corresponding m to the measurement at each time point during the day and t to time to next measure (Pruessner et al., 2003).

Cortisol overall secretion during the day (*t1*=30 *t2*=150 *t3*=540*)*

$$\mathbf{AUC_G} = \frac{(m2+m1)*t1}{2} + \frac{(m3+m2)*t2}{2} + \frac{(m4+m3)*t3}{2}$$

Circadian changes of cortisol "Index of decrease" (t1=30 t2=150 t3=540)

AUC_I **= AUC$_G$ – [**m1* **(**t1+t2 + t3**)**

Cortisol Awakening Response (*t1=30*)

CAR= AUC_G – [m1* (*t*1)]

Cortisol post-awakening overall secretion (t1=30)

$$\mathbf{AUC_G} = \frac{(m2+m1)*t1}{2}$$

Growth curve models were estimated using multilevel modelling (e.g. Heck et al., 2010) to analyze the effect of exclusive breastfeeding cessation (1) on the overall secretion of Salivary COR during the day, (2) on circadian changes of salivary COR during the day, and (3) on CAR, adjusting depression. Anxiety symptoms were also analyzed and adjust for accessing its influence as a potential covariate. Exclusive breastfeeding cessation (baseline) was calculated for each participant and defined as the moment of each women ceased exclusive breastfeeding; and to analyze the effect of cessation of breastfeeding (1) on the overall secretion of COR during the day, (2) on circadian changes of COR during the day (AUCI), and (3) on CAR. Breastfeeding cessation (baseline) was calculated for each participant and defined as the moment of each women ceased breastfeeding.

The intercept corresponds to the outcome variables (each measure of salivary cortisol) at the baseline (moment they ceased exclusive breastfeeding or breastfeeding) and the slope represents the extent to which the outcome variables changed after the moment women ceased breastfeeding. Significant interactions were interpreted and graphed using one standard deviation above and below the grand mean of the predictor variables as high and low values. Interactions effects between breastfeeding cessation and depressive symptoms on salivary cortisol measures was not possible to access due to number of groups.

Statistical analyses were performed in a person-period dataset using SPSS version 27.0 (SPSS Inc., USA). Each participant had a record for each time point (third trimester of pregnancy, 2 weeks, 3 months, 6 months and 12 months), resulting on the observations before and after they ceased exclusive breastfeeding or breastfeeding, resulting in 150 observations. The effect size r (Rosenthal et al., 2000) was estimated for all significant effects and interpreted according to Cohen's guidelines (1988).

Results

Descriptive statistics regarding percentage were performed for the feeding method (exclusively breastfeeding, partial breastfeeding and exclusively artificial feeding) at each moment in postpartum period (see Table 2), M and SD were performed for the study variables (cortisol overall secretion during the day, circadian changes of salivary cortisol during the day and cortisol awakening response and depressive symptoms) at each assessment wave (see Table 3 and 4).

Table 2

Descriptive data of feeding method at each assessment wave

	Post-Partum							
	(2) 2 weeks		(3) 3 months		(4) 6 months		(5) 12 months	
Feeding method	n	**%**	n	**%**	n	**%**	n	**%**
Exclusive BF	19	63.3	18	60	6	30	0	0
Partial BF	6	27.7	3	10	9	20	5	16.7
Exclusive artificial feeding	2	6.7	7	23.3	7	23.3	12	40

Note. BF = Breastfeeding

Table 3

Descriptive data of the studied variables at each assessment wave

Variables	Pregnancy		Post-Partum							
	(1) Third trimester		(2) 2 weeks		(3) 3 months		(4) 6 months		(5) 12 months	
	n	*M (SD)*	*n*	*M (SD)*	*n*	*M (SD)*	*n*	*M (SD)*	*n*	*M (SD)*
Salivary cortisol overall secretion during the day (AUC_G)	28	247.95 (99.57)	23	99.78 (33.11)	19	87.49 (30.49)	15	105.48 (68.71)	9	98,24 57,42
Salivary cortisol circadian (AUC_I)	28	-115.78 (206.10)	23	-110.93 (150.27)	19	-71.56 (65.99)	15	-97.75 (78.69)	8	-85,15 (109,51)
CAR (AUC_I)	28	-347.11 (189.13)	23	-201,96 (145.27)	19	-151,56 (71.49)	15	-194.43 (114.41)	9	-0.21 (3,10)
Salivary cortisol post-awakening overall production (AUC_G)	28	16.61 (6.81)	23	8.75 (3.92)	19	7.49 (3.01)	15	8.8 (6.24)	9	7,46 (4,25)

Note. BF = Breastfeeding; AUC_G = Area Under the Curve respect to ground; AUC_I = "Index of decrease"; CAR = Cortisol Awakening Response

Table 4

Descriptive data of depressive symptoms at each assessment wave

	Pregnancy		Post-Partum							
	(1) Third trimester		(2) 2 weeks		(3) 3 months		(4) 6 months		(5) 12 months	
Variables	*N*	*M* (*SD*)	*n*	*M* (*SD*)	*n*	*M* (*SD*)	*n*	*M* (*SD*)	*n*	*M* (*SD*)
Depressive symptoms	30	12.00 (3.3)	27	7.81 (4.48)	28	5.39 (3.91)	22	6.95 (3.90)	12	5.83 (3.40)

Effects of exclusive breastfeeding cessation on salivary cortisol measures

Results revealed significant effects for exclusive breastfeeding cessation on the overall secretion of salivary COR during the day and CAR (see Table 5). Independently of the postpartum time, after the moment that women ceased exclusive breastfeeding they presented a decreased on the overall secretion of salivary COR during the day, β = -3.55, ρ = .005, effect size r = 0.75. Likewise, independently of postpartum time, after the moment that women ceased exclusive breastfeeding they presented a decreased on CAR, β = 4.81, ρ < .001, effect size r = 0.72.

No main effects for exclusive breastfeeding cessation were found on circadian changes of salivary COR, β = -.21, ρ= .836.

Effects of breastfeeding cessation on salivary cortisol measures

Results revealed significant effects for exclusive breastfeeding cessation on the CAR (see Table 6). Independently of the postpartum time, after the moment that women ceased breastfeeding they presented a decreased on the CAR, β = 3.55, ρ= .002, effect size r = .59.

No main effects for breastfeeding cessation were found on overall secretion of salivary COR, β = -1.1, ρ= .275 and on circadian changes of salivary COR, β = 1.47, ρ= .311.

Effects of depressive and anxiety symptoms on salivary cortisol measures

Results revealed significant effects for depressive symptoms on overall secretion of salivary COR during the day (see table 5), at the moment women ceased exclusive breastfeeding, more depressive symptoms were associated with higher overall secretion of salivary COR during the day, β = 2.31, ρ= .024, effect size r = **0,265.** Likewise results revealed significant effects for depression symptoms on overall secretion of salivary COR and CAR, at the moment women ceased breastfeeding (see table 6), more depressive symptoms were associated with higher overall secretion of salivary COR, β = 2.52, ρ= .014, effect size r = 0.27 and higher CAR, β = -2.75, ρ= .007, effect size r = 0.296.

No main effects for depressive symptoms were found on circadian changes of salivary COR (see table 5), at the moment women ceased exclusive breastfeeding, depressive symptoms were not associated with circadian changes of salivary COR, β = -.59 ρ= .155. Likewise, no main effects for depressive symptoms were found on circadian changes of salivary COR and on CAR (see table 6), at the moment women ceased

breastfeeding, depressive symptoms were not associated with circadian changes of salivary COR, β = -1.02, ρ = .558 and with CAR, β = , ρ = .165.

Likewise, results revealed no significant effects for anxiety symptoms on overall secretion on any salivary cortisol measure, at the moment women ceased exclusive breastfeeding, anxiety symptoms were not associated with overall secretion of COR, β = .32, ρ = .720 on circadian changes of salivary COR, β = -.78, ρ = .555 and on CAR, β = -1.05 , ρ = .140. Likewise, there was not found significant effects for anxiety symptoms on any salivary cortisol measure, at the moment women ceased breastfeeding, anxiety symptoms were not associated with overall secretion of COR at the moment women ceased breastfeeding, COR β = 1.22, ρ = .225, with circadian changes of salivary COR, β = -.82, ρ = .413 and with CAR, β = -1.94 ρ = .056.

Table 5

Effects of Cessation of exclusive breastfeeding and depression on salivary cortisol measures

Fixed effects	AUCg			AUCI			CAR (AUCi)		
	B	SE	95% CI	β	SE	95% CI	β	SE	95% CI
Intercept	4.87	17.79	[51.08, 121.10] ***	-2.69	28.32	[-132,61, -19.82]	-5.3	27.84	[-202,76, -92.07] ***
Time	-3.55	.64	[-3.71, -,84] *	-.21	.98	[-2,300, 1.89]	4.81	.83	[2.26, 5.69] ***
Depressive symptoms	2.31	2.04	[.64, 8.77] *	-.59	3.23	[-8,329, 4.53]	-1.40	3.21	[-10.867, 1.881]
Random effects									
Intercept + time	1.0	4.19	.61, 29.43	1.168	7.32	[1.6, 45.762049]	0.77	2378.51	[.25, 40, .21]
Residuals	4.67	1089.74	[3340.71, 7739.15]	5.27	2434.06	[8850.430, 18612.9808] ***	5.70	4.11	[9631.42, 19139.28] ***

*p < .05; ***p < .001

Time (0) corresponds to the moment of assessment wave the women ceased breastfeeding.

Table 6

Effects of breastfeeding cessation and depression symptoms on salivary cortisol measures

Fixed effects	AUCg			AUCI			CAR		
	β	SE	95% CI	B	SE	95% CI	β	SE	95% CI
Intercept	4.46	19.37	[47,77 124.86] ***	-1.55	30.86	[-109.51, 13.59]	-2.75	31.43	[-148.89, -23,64] *
Time	-1.1	.47	[-1.45, .417]	1.47	1.01	[-.61, 3.59]	3.55	.99	[1.48, 5.57] *
Depressive symptoms	2.52	2.18	[1.16, 9.840] *	-1.02	3.52	[-10.60, 3.42]	-2.75	3.60	[-17.07, -2.72] *
Random effects									
Intercept + time	.	.	.	5.31	2931.82	[2.096, 32.86]	1.31	4.99	[1.48, 29.14]
Residuals	6,364	1141,016	[5336.62, 9880.36] ***	1.42	5.826	[10773.81, 22528.67] ***	5.51	3166.82	[12227.13, 24904,27] ***

*p < .05; ***p < .001

Time (0) corresponds to the moment of assessment wave the women ceased breastfeeding.

Interactions effects between exclusive breastfeeding cessation and depressive symptoms on salivary cortisol measures.

Results revealed significant interaction effects between exclusive breastfeeding cessation and depressive symptoms on overall secretion of salivary COR (see table 7). Women with more depressive symptoms at the time they ceased exclusive breastfeeding, had a greater decreased on overall secretion of salivary COR, β=, ρ= .007, effect size r = 0.29 X after they ceased exclusive breastfeeding.

No main interaction effects between exclusive breastfeeding cessation and depressive symptoms (see table 7) were found on circadian changes of salivary COR, β= , ρ= .717 and on CAR, ρ= .685.

Table 7

Interaction Effects of exclusive breastfeeding cessation and depression on salivary cortisol measures

Fixed effects	AUCg			AUCI			CAR		
	β	SE	95% CI	β	SE	95% CI	β	SE	95% CI
Intercept	5.33	16.50	[54.67, 121.3] ***	-2.691	28.73	[-134.527, -20.0827] *	-5.28	27.96	[-203.05, -91.9] ***
Time X depressive symptoms	-2.75	.13	[-.60, -.097] *	.36	.21	[-.35, .50]	-.41	.18	[-.44, .3]
Random effects									
Intercept + time (Cessation of EBF)	1.02	9.48	[1.4, 66.34]	1.18	7.83	[1.77, 48.55]	.644	4.15	[.13, 56.1]
Residuals	3.55	1117.57	[2279.57, 6886.92] *	5.2	2437.37	[8691.87, 18474.13] *	5.65	2424.52	[9683.66, 19379.37] *

*p < .05; ***p < .001

Time (0) corresponds to the moment of assessment wave the women ceased breastfeeding.

Discussion

The main purpose of this study was to analyze the impact of both exclusive breastfeeding and breastfeeding cessation on overall secretion of COR during the day, on circadian changes of COR during the day and on CAR's changes, from the 3rd pregnancy trimester to 12 months postpartum, in a sample of primiparous women with significant depressive symptoms in the third trimester of pregnancy.

Descriptive results shows that longitudinal overall secretion of COR during the day corresponds in general to the normal fluctuations during perinatal period: highest levels of COR during pregnancy (Conde & Figueiredo, 2014; Mastorakos & Ilias, 2003; Neves, 2020), and decrease in COR levels across the postpartum period until 12 months postpartum (Duthie & Reynolds, 2012; Mastorakos & Ilias, 2003). Descriptive results regarding depressive symptoms, revealed a general decrease of the symptoms from third trimester of pregnancy pregnancy to 12 months postpartum.

Impact of exclusive breastfeeding/breastfeeding cessation on COR

Main results showed that exclusive breastfeeding cessation had an impact on the overall secretion of COR during the day. After the moment that women ceased exclusive breastfeeding, they presented a significant decreased on the overall secretion of COR during the day, pointing that before women ceased exclusive breastfeeding they had higher levels of overall secretion of COR during the day. This results can indicate that breastfeeding may exert a different response in HPA axis in women with antenatal depressive symptoms, it seems that breastfeeding in the presence of depressive symptoms, not necessarily exerts a suppression of the HPA axis, with short-term attenuation of neuroendocrine stress responses, decreasing COR levels (Altemus et al., 1995; Groer et al., 2006; Heinrichs et al., 2001, Mizuhata et al., 2020). In women with depressive symptoms in pregnancy, the impact of suckling and breastfeeding in HPA axis may happen otherwise, concerning different COR outputs, namely increasing overall secretion of COR, as suggested by prior research that showed that depressive women who were breastfeeding had higher median evening secretion of COR, compared to depressive women who weren't breastfeeding (Illiadis et al., 2015).

Likewise, main results shows that exclusive breastfeeding/breastfeeding cessation had an impact on women's CAR. Results shows that after ceasing exclusive breastfeeding or breastfeeding, women presented a significant decreased on the CAR, which may indicate that before women ceased exclusive breastfeeding, they had higher

levels of CAR consistent with Ahn and Corwin (2015) findings that revealed breastfeeding women had higher CAR through 6 months postpartum. It seems the impact of breastfeeding on CAR may be mainly significant in women with antenatal depressive symptoms, this reinforces the assumption that breastfeeding may exerts a different response in HPA axis with different CAR outputs in women experiencing distress, namely having significant depressive symptoms in pregnancy, which is congruent with prior results that showed CAR mediated the negative association between socioeconomic status and breastfeeding (Bublitz et al., 2019).

The results shows no impact of neither exclusive breastfeeding neither breastfeeding cessation on the circadian changes of COR during the day. These results are consistent with Tu et al. (2006) study, that didn't found an impact of breastfeeding in the distribution of mothers into the three diurnal circadian cortisol groups. Possibly breastfeeding has an impact on circadian changes of COR only in the morning (Ahn & Corwin, 2015) or evening (Illiadis et al., 2015), and not on the all diurnal pattern of COR.

Impact of depressive symptoms on COR

Results shows that depressive symptoms had also an impact on overall secretion of COR during the day and on CAR. In the moment women ceased either exclusive breastfeeding either breastfeeding, more depressive symptoms were associated with higher overall secretion of COR during the day and in the moment women ceased breastfeeding cessation, more depressive symptoms were associated with with higher CAR. This results also supports Stuebe et al., (2012) hypothesis that links shared neuroendocrine mechanisms in both failed breastfeeding and perinatal mood disorders, inadequate circulating COR associated with pregnancy depression may directly impact milk production at the level of the mammary differentiation and disruption of oxytocin and prolactin may reduce the attenuating effects of breastfeeding on the COR stress-response. Perhaps inadequate CAR in depressed women in pregnancy may influence early breastfeeding cessation and early breastfeeding cessation may deregulate the CAR, since pregnancy CAR as shown to influence breastfeeding status (Bublitz et al., 2019) and breastfeeding status has shown to impact the CAR (Ahn & Corwin, 2015).

We didn't found depressive symptoms effects on the circadian changes of COR during the day, in the moment they ceased exclusive breastfeeding, there was not found an association between depressive symptoms and circadian changes of salivary COR during the day. Possible depressive symptoms impacts circadian changes of COR only in

the morning, as shown by a deregulation on the CAR, and not on the on the all diurnal pattern.

Interaction effects of exclusive breastfeeding cessation and depressive symptoms on COR

Interaction effects between the impact of exclusive breastfeeding cessation and depressive symptoms, on the overall secretion of COR during the day, shows that women with more depressive symptoms at the time they ceased exclusive breastfeeding, had a greater decreased on the overall secretion of COR during the day, after they ceased exclusive breastfeeding. This is the first study that present this interaction, pointing that early exclusive cessation may have higher risks for health in pregnant with mental health problems, like depressive symptoms. This results are promising to help indorse the potential protective effect of breastfeeding, regulating HPA axis in women with depressive symptoms. Biologic mechanism underlying breastfeeding, may help regulate the levels of COR from pregnancy to the postpartum period by increasing levels of overall secretion of COR during the day and to increase the blunted CAR. Although is not yet clear the way HPA axis function in pregnancy and postpartum depression, this results corroborate the hypothesis that hypocortisolemia is associated with postpartum depression, breastfeeding can help regulate a blunted HPA axis in depressed women, particularly in women chronic depression or in hormone-sensitive mothers (Gust et al., 2019; Seth et al., 2016). This assumption is also congruent with prior research that suggests that Oxytocin released during breastfeeding, in women with depressive symptoms, may increase COR response to stress, rather than blunting HPA activation (Cox et al., 2015).

There was not found an interaction effect of exclusive breastfeeding cessation and depressive symptoms on circadian changes of COR during the day, thus we couldn't ratify that breastfeeding has an impact on the relationship between the stability of circadian COR during the day and postpartum depression (Taylor et al., 2009). Even though there was found an impact of exclusive breastfeeding cessation and depressive symptoms on CAR, interaction effects between exclusive breastfeeding cessation and depressive symptoms were not found on the CAR. Despite prior research suggests that pregnancy CAR may mediate the negative association between distress and breastfeeding (Bublitz et al., 2019), it's not clear if distress, like depressive symptoms, mediates the impact of breastfeeding on CAR.

Limitations and strengths

The main limitation was the the small sample size, that don't permit to generalize the results, despite the reduce number of participants, growth curve models permitted a total of 150 observations, using multilevel modelling. Also due to number of groups needed for statistical analyzes, it was not possible to earn interaction effects of breastfeeding cessation and depressive symptoms on COR variables.

Nevertheless the self-reported nature assessment, in this study EPDS and STAI presented good internal consistence and are a sensible and reliable measure for screening clinical significant symptoms and are frequently used in international studies. Participants collecting saliva samples, may not always have been followed precisely instructions, for example collecting saliva immediately upon awakening, which can be a limitation since delayed sampling after awakening may potential bring inaccuracy for CAR assessment, so results should be analyzed prudently.

The strengths of this study included the longitudinal nature with five assessment waves, allowing to analyze with accuracy the changes in depressive symptoms and breastfeeding over the perinatal period. Using growth models from pregnancy to postpartum to analyze the changes in COR, makes statistical analyses sensitive to individual differences and reduce the interference of the relationship between pregnancy COR and further breastfeeding.

Implications for clinical practice and research

Our results have important highlights for future research about the impact of exclusive breastfeeding and breastfeeding on HPA axis during perinatal period in women with depressive symptoms. Further research should continue to explore the function of HPA axis and better understand the risks of an early breastfeeding cessation and the protective effect of exclusive breastfeeding and breastfeeding in women with depressive symptoms, answering the questions: Breastfeeding regulate the HPA axis in depressed women, by increasing COR response to stress? Oxytocin released during breastfeeding, in depressed women may increase COR response to stress? Exclusive breastfeeding protects depressed pregnancy women from postpartum depression by regulating the HPA axis? Early breastfeeding cessation and perinatal depression shares the same neuro endocrine mechanisms?

The studies who access the relationship between breastfeeding, depressive symptoms and COR use a wide range of measures, especially in what concerns to COR measures, further research must take account expert recommendations by Pruessner et al. (2003) and Stalder, (2016), using the (AUCG) to assess the overall secretion during the day, the (AUCI) to estimate the circadian changes of cortisol and to access the dynamic of the post-awakening cortisol response, AUCI should be used to estimate the CAR. Use standard criteria and measurements for breastfeeding, depression and COR and accessed the differences across the time, regarding pregnancy and postpartum period, is main concern and directs future research.

This study help guide clinical practice, as it provides information about the risks of early breastfeeding cessation and potential protective effect of breastfeeding in women with significant depressive symptoms. In practice, it can improve screenings in pregnancy in order to promote further breastfeeding and health and prevent the risks of an early breastfeeding cessation in women significant depressive symptoms.

Conclusion

This is the first study that analyses the impact of both exclusive breastfeeding and breastfeeding cessation on salivary COR measures, taking account clinical significant depressive symptoms. The main findings suggests that both exclusive breastfeeding and breastfeeding cessation has an impact on COR in women with clinically significant depressive symptoms in pregnancy. Both exclusive breastfeeding and breastfeeding cessation seems to impact the decrease of the CAR and exclusive breastfeeding the decrease of overall secretion of COR during the day in these women. Also it's the first longitudinal study that revealed an interaction between exclusive breastfeeding cessation and depressive symptoms on overall secretion of COR during the day, it's necessary to continue to replicate this results. This findings are important for the literature about breastfeeding and depression, because it can can help explain the benefits of breastfeeding in the first year postpartum, and comprehend the risk factors regarding early breastfeeding cessation, specifically in vulnerable pregnant women, by help explaining the HPA axis function underlying.

Despite the well-known benefits of breastfeeding, global rates of breastfeeding haven't substantially increased in the past two decades, exclusive breastfeeding rates among children under 6 months are below 50 percent in most countries and in the poorest countries, late initiation and low rates of exclusive breastfeeding is less than 40 percent

(Walters et al., 2016). In Portugal, according to the Results of the Report of the National Food Survey and of Physical activity, 46 percent of the children were exclusive breastfed in a period of less than 4 months (Lopes et al., 2017). To understand the risk factors of early breastfeeding cessation and to promote exclusive breastfeeding for the first 6 months of life with continuation of breastfeeding for 1 year or longer, especially in women with depressive symptoms is a public health priority. Incorporate mental health screenings in pregnancy to provide care to women with mental health problems and provide sensitive care services meeting mother's specific needs, can help promote further breastfeeding and prevent the risks of an early breastfeeding cessation, improving mother and the children's health.

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Appendix

Ethical Commission Approval

Universidade do Minho

SECVS

Subcomissão de Ética para as Ciências da Vida e da Saúde

Identificação do documento: SECVS – 022/2014
Titulo do projeto: *Breastfeeding and postpartum depression*
Investigador(a) responsável: Dra. Bárbara Fernandes de Carvalho Figueiredo, da Escola de Psicologia, Universidade do Minho
Outros investigadores: Cláudia Alexandra Castro Dias, Escola de Psicologia, Universidade do Minho; Sónia Maria Pereira de Azevedo Brandão, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto; Ana Catarina Miranda Canário, Escola de Psicologia, Universidade do Minho; Rui Alexandre Nunes da Costa, Escola de Psicologia, Universidade do Minho; Cristina Isabel Nogueira Silva, Escola de Ciências da Saúde, Universidade do Minho; Diogo Jorge Pereira Vale Lamela da Silva, Escola de Educação, Instituto Politécnico de Viana do Castelo; Nadine Correia Santos, Escola de Ciências da Saúde, Universidade do Minho
Subunidade orgânica: Escola de Psicologia, Universidade do Minho
Outras Unidades: Serviço de Ginecologia e Obstetrícia, Hospital de Braga; Maternidade Júlio Dinis, Centro Hospitalar do Porto

PARECER

A Subcomissão de Ética para as Ciências da Vida e da Saúde (SECVS) analisou o processo relativo ao projeto intitulado *"Breastfeeding and postpartum depression"*.

Os documentos apresentados revelam que o projeto obedece aos requisitos exigidos para as boas práticas na experimentação com humanos, em conformidade com o Guião para submissão de processos a apreciar pela Subcomissão de Ética para as Ciências da Vida e da Saúde.

Face ao exposto, a SECVS nada tem a opor à realização do projeto.

Braga, 06 de maio de 2014.

A Presidente

(Maria Cecília de Lemos Pinto Estrela Leão)