Advances in Neonatal Care

MATERNAL AND NEONATAL HAIR CORTISOL LEVELS AND PSYCHOLOGICAL STRESS AS PREDICTORS OF HUMAN MILK PRODUCTION

--Manuscript Draft--

Manuscript Number:	
Full Title:	MATERNAL AND NEONATAL HAIR CORTISOL LEVELS AND PSYCHOLOGICAL STRESS AS PREDICTORS OF HUMAN MILK PRODUCTION
Article Type:	Special Series: Human Milk Science
Keywords:	Psychological stress; Cortisol; human milk; Neonates; Pregnancy
Corresponding Author:	Maria Isabel Peralta-Ramirez, PhD SPAIN
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	
Corresponding Author's Secondary Institution:	
First Author:	Rafael A. Caparros-Gonzalez, RN, Bsc, PhD Psychology
First Author Secondary Information:	
Order of Authors:	Rafael A. Caparros-Gonzalez, RN, Bsc, PhD Psychology
	Borja Romero-Gonzalez, Msc
	Raquel Gonzalez-Perez, PhD
	Sandraluz Lara-Cinisomo, PhD
	Pablo L. Martin-Tortosa, RN
	Antonio Oliver-Roig, PhD
	Maria Isabel Peralta-Ramirez, PhD
Order of Authors Secondary Information:	
Manuscript Region of Origin:	SPAIN
Abstract:	Background: Several factors can influence the production of human milk. Purpose: To assess the influence of maternal psychological stress, maternal cortisol levels, and neonatal hair cortisol levels on the production of human milk. Methods: A prospective study was conducted at 2 public health centers in Andalusia, Spain. Participants were 60 pregnant women and their 50 neonates. Hair cortisol levels and psychological stress (pregnancy-specific stress [PDQ], perceived stress [PSS]), was evaluated during the third trimester and the postpartum period. Results: Higher PDQ scores during the third trimester were associated with later human milk production ($P < 0.05$). Higher PSS scores in the third trimester were associated with later human milk production ($P < 0.05$). Higher postpartum maternal hair cortisol levels were associated with a delayed secretory activation of human milk ($P < 0.05$). Implications for Research: Future studies should study the influence of psychological stress and cortisol levels on the variety of hormones involved in human milk production. Implications for Practice: Neonatal nurses and other health care providers are encouraged to should be familiar with the level of maternal prenatal stress and how much stress neonates were exposed to before birth.

M. Isabel Peralta-Ramirez
Mind, Brain, and Behavior Research Center
(CIMCYC)
Campus de Cartuja s/n, 18011
Universidad de Granada
Granada
Spain
Tel: +958244251
Email: mperalta@ugr.es

January 2019

Prof. Dr. Debra Brandon PhD, RN, CCNS, FAAN
Editor
Advances in Neonatal Care

Re: Advances in Neonatal Care paper submission

Dear Prof. Debra Brandon

Thank you for your email dated 04.December.2018, in which you said you would be happy to consider our new manuscript for the special series on Human Milk.

Enclosed you can find the manuscript entitled “MATERNAL AND NEONATAL HAIR CORTISOL LEVELS AND PSYCHOLOGICAL STRESS AS PREDICTORS OF HUMAN MILK PRODUCTION”. Human milk production is associated with a range of maternal variables. In our study, we aimed to assess the influence of maternal psychological stress, maternal cortisol levels, and neonatal hair cortisol levels on the production of human milk. We hypothesized that higher levels of maternal and neonate stress would be associated with a delayed production of human milk.

All of the authors have read and approved the paper and it has not been published previously nor is it being considered by any other peer-reviewed journal.

Please consider this manuscript for reviewing and publishing in ADVANCES IN NEONATAL CARE.

Yours sincerely,

M. Isabel Peralta-Ramirez
Mind, Brain, and Behavior Research Center (CIMCYC)
Campus de Cartuja s/n, 18011
Universidad de Granada
Granada
Spain
Tel: +958244251
Email: mperalta@ugr.es

MATERNAL AND NEONATAL HAIR CORTISOL LEVELS AND PSYCHOLOGICAL STRESS AS PREDICTORS OF HUMAN MILK PRODUCTION

Rafael A. Caparros-Gonzalez[a,d] RN, Bsc, PhD; Borja Romero-Gonzalez[b] Bsc, PhD Candidate; Raquel Gonzalez-Perez[c] PhD, Sandraluz Lara-Cinisomo[d] PhD, Pablo L. Martin-Tortosa RN[e] Antonio Oliver-Roig[f] RN, PhD, Maria Isabel Peralta-Ramirez[b] PhD

a. University of Jaen, Faculty of Health Sciences, Department of Nursing, Jaen, Spain

b. Mind, Brain, and Behavior Research Center (CIMCYC), University of Granada. Granada, Spain

c. Department of Pharmacology, CIBERehd, School of Pharmacy, Instituto de Investigación Biosanitaria ibs.University of Granada, Granada, Spain

d. University of Illinois, College of Applied Health Sciences, Department of Kinesiology and Community Health, USA

e. Gynecology and Obstetrics Department, Hospital Antequera, Antequera, Malaga, Spain

f. University of Alicante, Faculty of Health Sciences, Department of Nursing, Alicante, Spain

Category of the article: Special Series Human Milk Science

Institution where the work occurred

- Gongora Health Center, Granada, Spain

Funding Sources and Conflict of Interest Statement
This work was supported by the I+D Project 2PSI2015-63494-P" of the Spanish Ministry of Science and Innovation (url: http://www.idi.mineco.gob.es/portal/site/MICINN/menuitem.00d7c011ca2a3753222b7d1001432ea0/?vgnextoid=33881f4368aef110VgnVCM1000001034e20aRCRD); co-supported by funds/European Regional Development Fund (ERDF) ± a way to build Europe (MIPR).
None of the authors has biomedical financial interests or potential conflicts of interest.

List of Acronyms and Abbreviations used in the manuscript

CRH	Corticotropin-releasing hormone
CV	Coefficients of variance
NICU	Neonatal Intensive Care Unit
PDQ	Prenatal Distress Questionnaire
PSS	Perceived Stress Scale

Corresponding author:

M. Isabel Peralta-Ramirez
Mind, Brain, and Behavior Research Center (CIMCYC)
Campus de Cartuja s/n, 18011
Universidad de Granada
Granada
Spain
Tel: +958244251
Email: mperalta@ugr.es

MATERNAL AND NEONATAL HAIR CORTISOL LEVELS AND PSYCHOLOGICAL STRESS AS PREDICTORS OF HUMAN MILK PRODUCTION

Abstract

Background**:** Several factors can influence the production of human milk.

Purpose**:** To assess the influence of maternal psychological stress, maternal cortisol levels, and neonatal hair cortisol levels on the production of human milk.

Methods: A prospective study was conducted at 2 public health centers in Andalusia, Spain. Participants were 60 pregnant women and their 60 neonates. Hair cortisol levels and psychological stress (pregnancy-specific stress [PDQ], perceived stress [PSS]), was evaluated during the third trimester and the postpartum period.

Results: Higher PDQ scores during the third trimester were associated with later human milk production ($P < 0.05$). Higher PSS scores in the third trimester were associated with later human milk production ($P < 0.05$). Higher postpartum maternal hair cortisol levels were associated with a delayed secretory activation of human milk ($P < 0.05$).

Implications for Research: Future studies should study the influence of psychological stress and cortisol levels on the variety of hormones involved in human milk production.

Implications for Practice: Neonatal nurses and other health care providers are encouraged to should be familiar with the level of maternal prenatal stress and how much stress neonates were exposed to before birth.

Key Words: prenatal stress, cortisol, human milk, prospective study

BACKGROUND AND SIGNIFICANCE

The United Nations Children's Fund supports that breastfeeding and human milk are an ideal way to provide food in order to promote an adequate development of neonates.[1] The diverse range of bioactive and micronutrients present in human milk can ameliorate the neonates' immune system and protect them against infections in the short and long term.[2,3] Human milk is beneficial for all neonates, especially those requiring care in the Neonatal Intensive Care Unit (NICU).[4] Neonate mortality could be reduced if all neonates receive 6 months of exclusive human milk.[5] The World Health Organization has declared that every neonate should be fed human milk.[6]

The production of human milk is initiated by a two-stage process.[7,8,9] Stage I, secretory differentiation, takes place during midpregnancy.[9] At this time, the gland gets differentiated enough to produce a certain amount of milk components.[7,10] The secretion product, most commonly known as colostrum, can be obtained from the pregnant woman's breast and contains defensive substances such as inmunoglobulins.[11] Stage II, secretory activation, refers to the onset of an abundant quantity of human milk occurring after delivery, commonly known as milk coming in.[9] During secretory activation, human milk incorporates immunoglobulins, complex carbohydrates, and lactoferrin. When this occurs, women report swelling and fullness of the breasts, and leakage of milk.[7]

Previous studies have reported that there is an association between increasing maternal age, gestational diabetes, primiparity, C-section, and postpartum depression with a delayed onset of secretory activation.[12-16] Neonatal factors such as low birth weight and prematurity are associated with a later onset of secretory activation.[17,18] Figure 1 shows a summary of risk factors previously associated with a delayed onset of secretory activation.

(Please place Figure 1 about here)

An additional risk factor associated with a delay in the onset of secretory activation is maternal stress during pregnancy, resulting in pregnant women and their developing fetus being exposed to high levels of cortisol.[19-21] As part of the stress response, the hypothalamus produces corticotropin-releasing hormone (CRH). CRH promotes the release of cortisol so an organism can cope with a stressful situation.[22,23] The placenta produces high levels of placental CRH during pregnancy which stimulates the release of higher levels of maternal cortisol. What's more, during pregnancy, the pituitary gland doubles in size, boosting the release of maternal cortisol from the adrenal gland.[22] High cortisol levels during pregnancy can cross the placenta and reach the developing fetus. In fact, neonates' cortisol levels reflect chronic maternal stress during pregnancy.[21]

Cortisol levels of pregnant women have been measured using saliva, urine, blood, amniotic fluid, and human milk.[24-26] Although each method offers information regarding stress levels at the time samples are taken, these techniques are sometimes invasive and are affected by circadian rhythms and situational variables.[22,24] Alternatively, assessing hair cortisol levels is an innovative technique offering a retrospective chronic stress measure of the previous 3 months, is not affected by the time of the day, is not invasive, and is easy to preserve and transport.[27,28] Assessing cortisol levels from the past 3 months provides a deeper understanding of the influence chronic psychological stress can have on maternal and neonatal health.[28] Furthermore, neonatal hair cortisol levels provide information about fetal exposure to stress during the preceding 3 months of gestation while still in utero.[21]

A recent review found there is a paucity of studies available to fully understand the role of cortisol levels in mediating human milk secretion.[29] Although acute maternal and fetal stress during labor and delivery as well as the postpartum period have been associated with later secretion of human milk,[19] to our knowledge, no study has assessed the consequences of chronic maternal and neonatal stress during pregnancy and postpartum on the production of human milk. Thus, the potential associations between neonatal chronic stress and production of human milk has not yet been explored.[21]

The aim of this study was to assess the influence of maternal psychological stress, maternal cortisol levels, and neonatal hair cortisol levels on the production of human milk. We hypothesized that higher levels of maternal and neonate stress would be associated with a delayed production of human milk.

METHODS

Design

A prospective study was conducted to evaluate the association between prenatal and postnatal stress and the production of human milk in postpartum women. The independent variable was stress (maternal psychological stress, maternal hair cortisol levels, and neonatal hair cortisol levels). The dependent variable was secretory activation (onset of human milk after delivery).

Study Setting

Participants were recruited while attending an appointment with a community midwife at two public health centers in Andalusia, Spain (Figure 2). A sample of 81 women agreed to participate in the study. Eighteen pregnant women were excluded for not meeting inclusion criteria, declined to participate, or because of a language barrier. In addition, a pregnant woman decided to give birth in a different city, so she could not be evaluated after delivery and was therefore excluded from the study. Two pregnant women did not give consent to provide hair samples and were excluded. The final sample consisted of 60 pregnant women between 23 and 43 years of age (M = 32.97 years, SD = 4.04), and 60 neonates. All participants had a healthy and full-term newborn (M = 39.33 weeks, SD = 1.60). All babies at birth were stable and had an Apgar test of 9 to 10 at 5 minutes (M = 9.23, SD = 0.85).

The inclusion criteria for pregnant women were that they be 18 years of age or over, be fluent in the Spanish language, maintain their pregnancy in the Public Health System, and breastfeed their neonates for at least the first 72 hours after delivery. The inclusion criteria for neonates was that they be healthy, reach full-term and be delivered vaginally. Ten participants were excluded from the study because they had C-sections.

Exclusion criteria for pregnant women included presenting any pathology that could afect cortisol levels before or during pregnancy, Cushing’s disease, asthma, steroid medication, diabetes, and C-section. In summary, any condition that could affect cortisol levels. Exclusion criteria for neonates included tongue tie, cleft lip or cleft palate that could hinder the establishment of breastfeeding.

This study was approved by the Human Research Ethics Committee of the University of Granada (reference 881), the Research Committee on Biomedical Ethics, and the Research Ethics Committee of the health centers. The study protocol was implemented between February 2018 and June 2018 in accordance to the guidelines of the Declaration of Helsinki (AMM, 2008) and the Directive on good clinical practice

(Directive 2005/28/EC) of the European Union. Participation was voluntary and each participant signed an informed written consent document.

(Please place Figure 2 about here)

Measures

Biological measures

Hair cortisol levels were assessed through hair samples proximal to the scalp with a length no greater than 3 cm (assuming a growth rate of 1 cm/month, a 3 cm segment contains cortisol that has been deposited over approximately the last 3 months).

Samples consisted of approximately 150 strands of hair and were collected from the posterior vertex of the head.[30] The samples were wrapped in a piece of aluminum foil for protection and were stored in an envelope at room temperature.

Next, hair samples were sent for analysis to the Faculty of Pharmacy at the University of Granada. The hair samples were weighed and ground to a fine powder to break up the hair's protein matrix using a ball mill. Cortisol from the hair shaft was extracted into HPLC-grade methanol for incubation of the sample for 72 hours at room temperature in the dark with constant inversion using a rotator. After incubation, the supernatant was evaporated using a vacuum evaporator and the extract was reconstituted in 150 ul of phosphate buffered saline at a pH of 8.0. The reconstituted sample was immediately frozen at -20ºC for later analysis.[31-33]

Cortisol in hair samples were measured using a salivary ELISA cortisol kit with the reagent provided following the manufacturer's directions. Using a salivary ELISA cortisol kit is a validated method to evaluate hair cortisol levels. This procedure is highly positively correlated with liquid chromatograph-mass spectrometry (LC-

MS/MS).[33] The cortisol ELISA kit has a sensitivity of 1.0 ng/ml as reported by the manufacturer and the cross reactivity is as follows: prednisolone 13.6%, corticosterone 7.6%, deoxycosticosterone 7.2%, progesterone 7.2%, cortisone 6.2%, deoxycortisol 5.6%, pednisone 5.6%, and dexamethasone 1.6%. No cross-reaction was detected with dehydroepiandrosterone sulfate and tetrahydrocortisone.

The intra- and inter-assay variations were analyzed on internal quality assessment used for routine salivary cortisol measurement, using in duplicate on 8 consecutive assays. The intra-assay coefficients of variance (CV) were 2.7% at 10.7 ng/ml and 4.3% at 43.9 ng/ml. The inter-assay CVs were 4.4% and 6.3%, respectively. This protocol has been previously used with success in previous studies on pregnant women and neonates.[20,21,34]

Psychological assessment

Maternal perceived stress was assessed with the 14-item Spanish version of the PSS[35] to evaluate the perception of general stress during the previous month. The PSS has 14 items scores on a 5-point Likert scale (0 = never, 1 = almost never, 2 = once in a while, 3 = often, 4 = very often). The Cronbach's alpha reliability coefficient of the Spanish version is α = 0.81.[36] A community midwife administered the PSS during the third trimester and postpartum.

In order to measure pregnancy-specific stress, the Spanish version of the PDQ was used.[37,38] It is a 12-item questionnaire scored on a 5-point Likert scale from 0 (none at all) to 4 (extremely) to evaluate specific worries and concerns pregnant women have regarding medical problems, physical symptoms, body changes, labor, childbirth, relationships, and the infant's health. The Cronbach's alpha reliability coefficient of the Spanish version is α = .71.[38]

The community midwife was responsible for assessing the human milk production through secretory activation. For this purpose, the participants were assessed during postpartum using the Spanish version of the breastfeeding self-efficacy scale-short form. It consists of 14 items scored on a 5-point Likert scale ranging from 1 (not at all sure) to 5 (completely sure). The Cronbach's alpha reliability coefficient of the Spanish version is α = .91.[39] In this study, to determinate the moment of the onset of lactation, the item regarding maternal perception of the onset of lactation was used. Participants were asked "Has your milk come in yet?" A positive answer was followed by the next question: "When did your milk come in?" The answer to this question was then recorded in minutes. This procedure has been previously used with success in previous studies developed in USA and Spain (Europe).[39-42]

Sociodemographic and obstetrical data was collected using the Pregnancy Health Document,[43] the medical record of the health of every pregnant woman and her neonate.

Procedure

This study is part of a cohort study, called GESTASTRESS, designed to assess the association between maternal stress during pregnancy and detrimental consequences on maternal, fetal, and neonatal health.[44] Participants were recruited during the third trimester of pregnancy while attending a prenatal appointment with a community midwife. At this time, they were informed and given an informed consent document. Those agreeing to participate read and signed the informed consent. During the antenatal appointment, the community midwife distributed the psychological instruments, PDQ and PSS, which participants completed at home and submitted at their next appointment. The community midwife also took hair samples according to the established protocol.

After delivery, during the first postnatal appointment the community midwife assessed the onset of human milk using the protocol described earlier. The community midwife also administered the psychological questionnaire (PSS) and took hair samples from the mothers and their neonates. This assessment was performed during the first postpartum appointment with the community midwife.

Data Analysis

Due to a lack of normal distribution of hair cortisol levels, a logarithmic transformation (LN base e) was performed.[20,21] Descriptive statistical analysis was used to analyze sociodemographic and obstetrical data. Aiming to evaluate potential associations between the predictors (maternal and neonatal hair cortisol levels, PDQ, PSS), several bivariate Pearson correlations were carried out. A lack of high correlation (> 0.80) between the predictors and a value of the variance inflation factor (VIF) ≤ 10 indicates an absence of multicollinearity between predictors. This fact facilitates the assessment of the importance of each predictor in the statistical model.[45] Finally, to determine whether the levels of perceived stress, specific pregnancy stress, or hair cortisol levels predicted the moment of the onset of lactation, a hierarchical regression was performed. This approach is the best as it allows the researcher inputting known predictors first based on past work.[45] The statistical analyses were carried out using the statistical package SPSS version 20 (IBM, Armonk, NY) for Mac OSX 10.12.6.

RESULTS

Table 1 shows demographic and obstetric information regarding pregnant women and medical characteristics of the neonates participating in this study. The majority of pregnant women were European Spanish in their mid-30s, married and had attended university. All neonates in the present study were full-term with a mean birthweight of 3233.63 g (SD = 376.55), and a mean length of 50.28 cm (SD = 2.10).

The Pearson correlations between predictors were all < 0.80, and the VIFs were ≤ 10, indicating a lack of multicollinearity (see Table 2). Hence, maternal hair cortisol levels, neonatal hair cortisol levels, PDQs, and PSSs were included in the model as predictors in the model.

Hierarchical regression analyses revealed that the PDQ scores were significantly and positively associated with secretory activation of human milk (stage II) [R^2 = 0.23, F = 17.44, *P* = 0.01] ß = 4.17, *P* = 0.01. Higher PDQ scores were associated with a later human milk production. This association was maintained when maternal age, number of previous children, pain relief in labor, and pregnancy method, whether the pregnancy was spontaneous or assisted, were included in the model [R^2 = 0.25, F = 3.76, *P* = 0.01] ß = 0.48, *P* = 0.01. The PSS at the end of the third trimester also significantly and positively predicted a later secretory activation of human milk [R^2 = 0.62, F = 96.55, *P* = 0.01] ß = 0.79, *P* = 0.01, even when maternal age, number of previous children, pain relief in labor, and pregnancy method were included in the model [R^2 = 0.63, F = 18.92, *P* = 0.01] ß = 0.79, *P* = 0.01 (see Figure 3). Higher PSS scores at the third trimester were associated with a later human milk production. Postpartum PSS scores were not significantly associated with secretory activation of human milk.

Maternal hair cortisol levels during the postpartum period were significantly and positively associated with secretory activation of human milk [R^2 = 0.37, F = 35.38, *P* =

0.01] ß = 0.61, P = 0.01. Postpartum maternal hair cortisol levels were significantly associated with secretory activation of human milk even when maternal age, number of previous children, pain relief in labor, and pregnancy method were included in the model [R^2 = 0.40, F = 7.38, P = 0.01] ß = 0.62, P = 0.01 (see Figure 3). Prenatal maternal hair cortisol levels and neonatal hair cortisol levels did not predict secretory activation of human milk.

DISCUSSION

A longitudinal assessment was conducted to evaluate the effects of maternal psychological stress, maternal hair cortisol levels, and neonatal hair cortisol on the secretory activation of human milk. The findings showed that maternal psychological stress during the third trimester, and maternal hair cortisol levels during the postpartum period were predictive of secretory activation of human milk. The associations between maternal psychological stress and a delayed onset of lactation supports previous findings.[17,19] Still, our study is the first to show that maternal hair cortisol levels during the postpartum period can predict secretory activation of human milk. The associations found between maternal stress and delayed secretory activation are hypothesized to be due to detrimental effects of high levels of psychological stress and cortisol levels on the onset of prolactin which is the main hormone involved on the secretion of human milk.[46,47] Additionally, maternal stress may interfere with oxytocin levels which is the hormone involved in control of the milk ejection reflex.[19] As noted previously, cortisol is necessary for adequate lactogenesis. However, high levels of cortisol may impair lactogenesis stage II, secretory activation.[48] Thus, additional research that examines the individual and simultaneous role of these 3 vital hormones is necessary to clarify the etiology of delayed human milk production, particularly in the context of maternal stress.

Although other studies have found associations between pregnancy-specific stress (PDQ) and negative maternal and neonatal outcomes,[44,49] to our knowledge, our study is the first to study and show that high levels of the PDQ scores during the third trimester of pregnancy were associated with a delayed secretory activation of human milk. Unlike previous studies that have focused on postpartum reports of stress,[50,51] we found that assessing perceived stress (PSS) during the third trimester can indicate whether secretory activation of human milk will be delayed postpartum. Given that delayed secretion of human milk is associated with the discontinuation of breastfeeding,[52] excessive neonatal weight loss, and maternal obesity,[53] determining which women are at risk can reduce these negative consequences.

Our results come from a rigorous prospective study and follow the guidelines of a robust protocol.[44] Hair cortisol levels reflect chronic stress during the preceding 3 months.[28] Measuring maternal hair cortisol levels during the postpartum period provides information regarding stress levels a woman experiences during the third trimester.[54] Our study is the first to assess and find that high postpartum maternal hair cortisol levels were associated with delayed secretory activation (stage II).

Previous studies have reported that high levels of psychological stress, and blood and salivary cortisol levels at delivery had adverse effects on breastfeeding and the secretion of human milk.[55,56] However, these studies refer to an acute stress situation and require sometimes painful procedures (e.g. blood samples).[28]

While this study provides advances in knowledge regarding the role of maternal and neonatal stress on lactogenesis, it is not without limitations. For instance, stress levels were not assessed during the first and second trimester of pregnancy. Evaluating stress levels at these time-points (first and second trimester) can offer information regarding stress exposure preconception and in early pregnancy,[20] and how they are associated with human milk production.[56] Additionally, our study did not evaluate prolactin or oxytocin levels, which are directly involved in breastfeeding and the secretion of human milk.[46,47] Future studies should include these hormones to fully understand the mechanisms involved in delayed production of human milk.

In the present study, we could not find an association between neonatal hair cortisol levels and secretory activation of human milk. Maternal hair cortisol levels have only been associated with neonatal hair cortisol levels during the first trimester.[21] Because maternal hair cortisol levels were not assessed during the first trimester in this study, we hypothesized this lack of association was due to the absence of that information.

In conclusion, high levels of maternal psychological stress and cortisol levels during the third trimester of pregnancy are associated with a delayed production of human milk. Neonatal nurses and other health care providers should be aware of the noted associations between perinatal exposure to stress and delayed production of human milk to help women identify stress-reducing strategies.

Implications for Research

Our results show that prenatal stress is associated with delayed production of human milk. Maternal psychological stress and maternal cortisol levels appear to be predictors of secretory activation of human milk. However, continuing research should look at whether maternal stress during the first and/or second trimester may influence the production of human milk. Future studies should explore the influence of psychological stress and cortisol levels on the hormones involved in human milk production (e.g. prolactin and oxytocin).

Implications for Practice

The present study provides additional evidence that the production of human milk is associated with prenatal stress. Breastfeeding and the use of human milk in the NICU is beneficial and can reduce the mortality in neonates.[4,5] Neonatal nurses are encouraged to keep track of maternal stress and prenatal stress levels neonates were exposed to before birth. In this respect, neonatal nurses will better support those women willing and able to breastfeed their neonates while in the NICU.

REFERENCES

1. United Nations Children´s Fund. Breastfeeding: a mother´s gift, for every child. New York: UNICEF; 2018.
2. Murphy K, Curley D, O'Callaghan TF, et al. The composition of human milk and infant faecal microbiota over the first three months of life: a pilot study. *Sci Rep*. 2017;7:40597.
3. Hampel D, Dror DK, Allen LH. Micronutrients in human milk: analytical methods. *Adv Nutr*. 2018;9(suppl_1):313S-31S.
4. Bujold M, Feeley N, Axelin A, Cinquino C. Expressing Human Milk in the NICU. *Adv Neonatal Care*. 2018;18(1):38-48.
5. Spatz DL, Edwards TM. The use of human milk and breastfeeding in the neonatal intensive care unit: position statement 3065. *Adv Neonatal Care*. 2016;16(4):254.
6. World Health Organization, UNICEF. Planning guide for national implementation of the global strategy for infant and young child feeding. Geneva: WHO; 2007.

7. Neville MC, Morton J, Umemura S. Lactogenesis: the transition from pregnancy to lactation. *Pediatr Clin North Am.* 2001;48(1):35-52.

8. Hartmann PE. The lactating breast: an overview from down under. *Breastfeed Med*. 2007;2(1):3-9.

9. Pang WW, Hartmann PE. Initiation of human lactation: secretory differentiation and secretory activation. *J Mammary Gland Biol Neoplasia*. 2007;12(4):211-221.

10. Asztalos EV, Kiss A, da Silva OP, et al. Pregnancy gestation at delivery and breast milk production: a secondary analysis from the EMPOWER trial. *Matern Health Neonatol Perinatol*. 2018;4(1):21.

11. Rodríguez-Camejo C, Puyol A, Fazio L, et al. Antibody profile of colostrum and the effect of processing in human milk banks: Implications in immunoregulatory properties. *J Hum Lact.* 2018;34(1):137-147.

12. García-Fortea P, González-Mesa E, Blasco M, Cazorla O, Delgado-Ríos M, González-Valenzuela MJ. Oxytocin administered during labor and breast-feeding: A retrospective cohort study. *J Matern Fetal Neonatal Med.* 2014;27(15):1598-1603.

13. Lind JN, Perrine CG, Li R. The relationship between labor pain medication and delayed onset of lactation. *J Hum Lact*. 2014;30(2):167-173.

14. Prior E, Santhakumaran S, Gale C, Philipps LH, Modi N, Hyde MJ. Breastfeeding after cesarean delivery: A systematic review and meta-analysis of world literature. *Am J Clin Nutr.* 2012;95(5):1113-1135.

15. Brownell E, Howard CR, Lawrence RA, Dozier AM. Delayed onset lactogenesis II predicts the cessation of any or exclusive breastfeeding. *J Pediatr*. 2012;161(4):608-614.

16. Lara-Cinisomo S, McKenney K, Di Florio A, Meltzer-Brody S. Associations between postpartum depression, breastfeeding, and oxytocin levels in Latina mothers. *Breastfeed Med*. 2017;12(7):436-442.

17. Nommsen-Rivers LA. Does insulin explain the relationship between maternal obesity and poor lactation outcomes? An overview of the literature. *Adv Nutr*. 2016;7(2):407-414.

18. Nommsen-Rivers, LA, Dewey KG. Development and validation of the infant feeding intentions scale. *Matern Child Health J*. 2009;13(3):334-342.

19. Dewey KG. Maternal and fetal stress are associated with impaired lactogenesis in humans. *J Nutr*. 2001;131(11):3012S-5S.

20. Caparros-Gonzalez RA, Romero-Gonzalez B, Strivens-Vilchez H, Gonzalez-Perez R, Martinez-Augustin O, Peralta-Ramirez MI. Hair cortisol levels, psychological stress and psychopathological symptoms as predictors of postpartum depression. *PloS One*. 2017;12(8):e0182817.

21. Romero-Gonzalez B, Caparros-Gonzalez RA, Gonzalez-Perez R, Delgado-Puertas P, Peralta-Ramirez MI. Newborn infants' hair cortisol levels reflect chronic maternal stress during pregnancy. *PloS One.* 2018;13(7):e0200279.

22. Sandman CA, Glynn LM, Davis EP. Neurobehavioral consequences of fetal exposure to gestational stress. In: Reissland N, Kisilevsky B, eds. *Fetal Development*. Switzerland: Springer International Publishing; 2016:229-265.

23. Mastorakos G, Ilias I. Maternal and fetal hypothalamic-pituitary-adrenal axes during pregnancy and postpartum. *Ann N Y Acad Sci*. 2003;997:136-149.

24. Bergman K, Sarkar P, Glover V, O'Connor TG. Maternal prenatal cortisol and infant cognitive development: moderation by infant-mother attachment. *Biol Psychiatry*. 2010;67(11):1026-1032.

25. De Rezende MG, Garcia-Leal C, de Figueiredo FP, et al. Altered functioning of the HPA axis in depressed postpartum women. *J Affect Disord*. 2016;193:249-256

26. Grey KR, Davis EP, Sandman CA, Glynn LM. Human milk cortisol is associated with infant temperament. *Psychoneuroendocrinology*. 2013;38(7):1178-1185.

27. Mastorakos G, Ilias I. Maternal and fetal hypothalamic-pituitary-adrenal axes during pregnancy and postpartum. *Ann N Y Acad Sci*. 2003;997:136-149.

28. Wosu AC, Valdimarsdóttir U, Shields AE, Williams DR, Williams MA. Correlates of cortisol in human hair: implications for epidemiologic studies on health effects of chronic stress. *Ann Epidemiol.* 2013;23(12):797-811.

29. Fu M, Zhang L, Ahmed A, Plaut K, Haas DM, Szucs K, Casey TM. Does circadian disruption play a role in the metabolic–hormonal link to delayed lactogenesis II? *Front Nutr*. 2015;2:4.

30. Sauvé B, Koren G, Walsh G, Tokmakejian S, Van Uum SH. Measurement of cortisol in human hair as a biomarker of systemic exposure. *Clin Invest Med.* 2007;30(5):183-191.

31. Meyer J, Novak M, Hamel A, Rosenberg K. Extraction and analysis of cortisol from human and monkey hair. *J Vis Exp*. 2014;83:1-6.

32. Chen Z, Li J, Zhang J, et al. Simultaneous determination of hair cortisol, cortisone and DHEAS with liquid chromatography-electrospray ionization-tandem mass spectrometry in negative mode. *J Chromotgr B Analyt Technol Biomed Life Sci*. 2013;929:187-194.

33. Russell E, Kirschbaum C, Laudenslager ML, et al. Toward standardization of hair cortisol measurement: Results of the first international inter-laboratory round robin. *Ther Drug Monit.* 2015;37(1):71-75.

34. Garcia-Leon MA, Peralta-Ramirez MI, Arco-Garcia L, et al. Hair cortisol concentrations in a Spanish sample of healthy adults. *PloS one*. 2018;13(9):e0204807.

35. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. *J Health Soc Behav*. 1983;24(4):385-396.

36. Remor E. Psychometric properties of a European Spanish version of the Perceived Stress Scale (PSS). *Span J Psychol*. 2006;9(1):86-93.

37. Yali AM, Lobel M. Coping and distress in pregnancy: an investigation of medically high risk women. *J Psychosom Obstet Gynecol*. 1999;20(1):39-52.

38. Caparros-Gonzalez RA, Strivens H, Marinas-Lirola JC, et al. Internal consistency and convergent validity of the Spanish version of the Prenatal Distress Questionnaire. *J Reprod Infant Psychol*. 2015;33(3):E36.

39. Piñeiro-Albero RM, Ramos-Pichardo JD, Oliver-Roig A, et al. The Spanish version of the Prenatal Breast-feeding Self-efficacy Scale: Reliability and validity assessment. *Int J Nurse Stud.* 2013;50(10):1385-1390.

40. Chapman DJ, Perez-Escamilla R. Does delayed perception of the onset of lactation shorten breastfeeding duration? *J Hum Lact*. 1999;15(2):107-111.

41. Chapman DJ, Perez-Escamilla R. Maternal perception of the onset of lactation is a valid, public health indicator of lactogenesis stage II. *J Nutr*. 2000; 130(12):2972-2980.

42. Dimitraki M, Tsikouras P, Manac B, Gioka T, Koutlaki N, Zervoudis S, Galazios G. Evaluation of the effect of natural and emotional stress of labor on lactation and breastfeeding. Arch Gynecol Obstet. 2016;293(2):317-320.

43. Andalusian Ministry of Health. Pregnancy health document; 2010. Avaliable from: http://www.juntadeandalucia.es/salud/sites/csalud/galerias/documentos/c_3_c_1_vida_sana/embarazo_y_salud/ lactancia_materna/cartilla_embarazo.pdf Accessed January 22, 2019.

44. Caparros-Gonzalez RA, Garcia-Garcia I, Mariñas-Lirola JC, Peralta-Ramírez MI. [GESTASTRES cohort study protocol on the effects of stress during pregnancy by measuring the cortisol in women's and newborn's hair.] *Rev Esp Salud Publica*. 2018;92: pii: e201804027.

45. Field A. Discovering statistics using SPSS. Sage publications; 2009.

46. Benjamin Neelon SE, Stroo M, Mayhew M, Maselko J, Hoyo C. Correlation between maternal and infant cortisol varies by breastfeeding status. *Infant Behav Dev.* 2015;40:252-258.

47. Jonas W, Woodside B. Physiological mechanisms, behavioral and psychological factors influencing the transfer of milk from mothers to their young. *Horm Behav*. 2016;77:167-181.

48. Grajeda R, Pérez-Escamilla R. Stress during labor and delivery is associated with delayed onset of lactation among urban Guatemalan women. *J Nutr*. 2002;132(10):3055-3060.

49. Hasanjanzadeh P, Faramarzi M. Relationship between maternal general and specific-pregnancy stress, anxiety, and depression symptoms and pregnancy outcome. *J Clin Diagn Res*. 2017;11(4):VC04-VC07.

50. Shukri NH, Wells J, Mukhtar F, Lee MH, Fewtrell M. Study protocol: An investigation of mother-infant signalling during breastfeeding using a randomised trial to test the effectiveness of breastfeeding relaxation therapy on maternal psychological state, breast milk production and infant behaviour and growth. *Int Breastfeed J.* 2017;12(1):33.

51. Tan DJ, Lew JP, Jumhasan MB, Pang C, Sultana R, Sng BL. Investigating factors associated with success of breastfeeding in first-time mothers undergoing epidural analgesia: a prospective cohort study. *Int Breastfeed J.* 2018;13(1):42.

52. Fu M, Zhang L, Ahmed A, et al. Does circadian disruption play a role in the metabolic–hormonal link to delayed lactogenesis II? *Front Nutr*. 2015;2:4.

53. Preusting I, Brumley J, Odibo L, Spatz DL, Louis JM. Obesity as a predictor of delayed lactogenesis II. J Hum Lact. 2017;33(4):684-691.

54. Orta OR, Tworoger SS, Terry KL, et al. An evaluation of distal hair cortisol concentrations collected at delivery. *Stress*. 2018 Apr 3:1-1.

55. Thayer ZM, Agustin Bechayda S, Kuzawa CW. Circadian cortisol dynamics across reproductive stages and in relation to breastfeeding in the Philippines. Am J Hum Biol. 2018;30(4):e23115.

56. Orta OR, Tworoger SS, Terry KL, et al. Stress and hair cortisol concentrations from preconception to the third trimester. *Stress*. 2018;7:1-10.

Table and Figure Legends

Table 1: **Maternal Information and Neonatal Data.**

Table 2: **Pearson Correlations and Collinearity Statistic Between Predictors.**

Table 3: **Summary of Recommendations for Practice and Research**

Figure 1: **Risk Factors Previously Associated with Later Secretory Activation.**[12-18]

Figure 2: **Flow diagram of Pregnant Women and Neonates selection.**

Figure 3: **Scatterplot showing predictors of secretory activation of human milk.** (A-C). (A) PDQ scores at the third trimester. (B) PSS scores at the third trimester. (C) Maternal hair cortisol levels during the postpartum.

Summary of Recommendations for Practice and Research	
What we know:	• Human milk is the ideal way to feed neonates to promote an optimal development. • The production of human milk is initiated by a two-stage process: stage I (secretory differentiation) takes place during midpregnancy and stage II (secretory activation), occurring after delivery. • The production of human milk is associated with C-section, primiparity and psychological stress.
What needs to be studied:	• The effects of maternal cortisol levels during pregnancy and postpartum on the secretory activation of human milk. • Comparison the effects of neonatal cortisol levels on the secretory activation of human milk. • Additional prospective studies assessing the effects of maternal and neonatal stress at different time points on human milk production.
What we can do today:	• Provide neonatal nurses and health care providers a range of tools to assess prenatal stress. • Reduce high levels of stress during pregnancy. • Improve human milk production by controlling levels of maternal stress.

Table 1

TABLE 1. Maternal Information and Neonatal **Data.**			
			M (SD)/N(%)
Maternal Sociodemographic Information			
Age, years			33.47 (3.93)
Previous children			0.75 (0.72)
Previous miscarriages			0.45 (0.64)
Marital status	Single/divorced/widowed		12 (20%)
	Married/cohabitating		48 (80%)
Employment situation	Employed		51 (85.00%)
	Unemployed		9 (15.00%)
Level of education	High school		23 (38.30%)
	University or more		37 (61.70%)
Nationality	European Spanish		43 (71.70%)
	European (other than Spanish)		5 (8.30%)
	Maghrebi (Morocco)		9 (15.00%)
	Latin American		3 (5.00%)
	Maternal obstetrical information		
Pregnancy method	Spontaneous		51 (85.00%)
	Assisted reproductive technology		9 (15.00%)
Planned pregnancy	Yes		48 (80%)
	No		12 (20%)
Pain relief in labor	None		8 (13.3%)
	Epidural		44 (73.3%)
	Warm bath		8 (13.3%)
Neonatal Data			
Sex of the fetus	Female		29 (48.30%)
	Male		31 (51.70%)
Birth delivery	Vaginal (without instrument assistance)		50 (83.30%)
	Vaginal (with instrument assistance)		10 (16.70%)
Weeks of gestation[a]			39.33 (1.60)
Birthweight, g[a]			3233.63 (376.55)
Apgar test score (5 min)			9.23 (0.85)
Length, cm[a]			50.28 (2.10)

Head circumference, cm[a]			34.15 (1.59)
	Note: The values for scalar variables are displayed as means (standard deviations); the percentage of cases are displayed for dichotomic or categorical variables. [a]Neonatal information at time of birth		

TABLE 2. Pearson Correlations and Collinearity Statistic **Between Predictors**

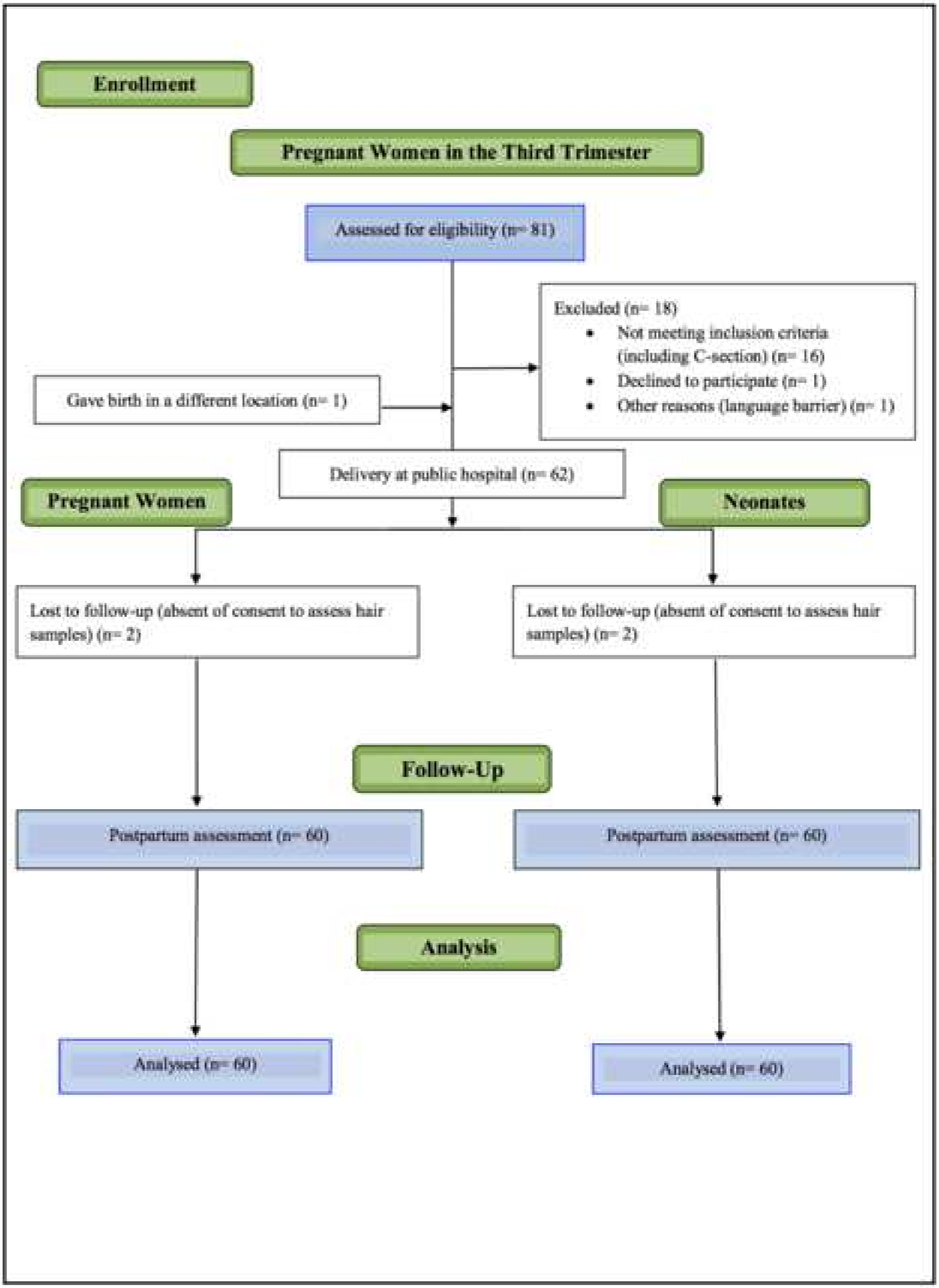
Time Frame		**3rd Trimester of Pregnancy**			**Postpartum**		**Collinearity Statistic**
	Predictors	Maternal cortisol	PDQ	PSS	Maternal cortisol	Neonatal cortisol	VIF
3rd Trimester of Pregnancy	Maternal cortisol	1					1.07
	PDQ	0.07	1				1.69
	PSS	0.07	0.31	1			1.96
Postpartum	Maternal cortisol	0.29	0.10	0.28	1		1.28
	Neonatal cortisol	0.01	0.01	0.17	0.29	1	1.05
	PSS	0.28	0.24	0.08	0.08	0.11	1.29

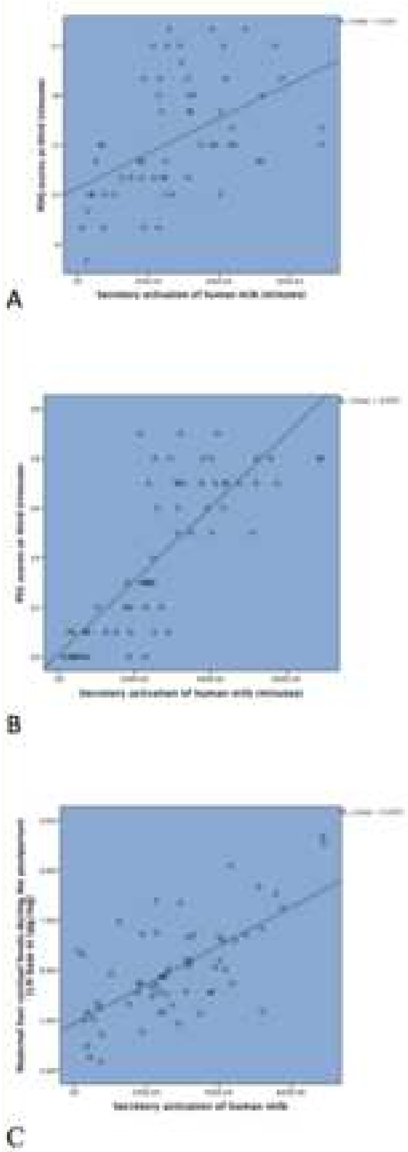
Note. Correlations between predictors were all non-significant: $p > 0.05$

Figure 1


Maternal factors
Age
Gestational diabetes
Primiparity
C-section
Obesity
Stress
Postpartum depression
SECRETORY ACTIVATION
Low birth weight
Prematurity
Neonatal factors

Figure 2 Click here to access/download;Figure;Figure 2.tiff





(A-C). Scatterplot showing predictors of secretory activation of human milk. (A) PDQ scores at the third trimester. (B) PSS scores at the third trimester. (C) Maternal hair cortisol levels during the postpartum.