Stress During Labor and Delivery Is Associated with Delayed Onset of Lactation among Urban Guatemalan Women[1,2,3]

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ABSTRACT A delayed onset of lactation (OL) is likely to have a negative influence on breast-feeding outcomes. Thus, it is important to identify risk factors for delayed OL. We examined prospectively the association between stress during labor and delivery and OL in a cohort of urban Guatemalan women. Healthy women ($n = 136$) were recruited during the first stage of labor in the labor unit of a social security hospital in Guatemala City. Women provided salivary samples for cortisol determinations 3.2 ± 3.2 h before birth and 1.7 ± 1.9 h and 17.1 ± 4.7 h after delivery. At the same time points women were asked to respond to a 17-item psychosocial stress and anxiety questionnaire. Women were called daily until OL occurred. Primiparous women had higher antepartum and early postpartum cortisol levels that were twice as high as those among multiparous women ($P < 0.05$). The parity differential in salivary cortisol concentrations vanished with time. General linear model multivariate analysis indicate that multiparous women ($n = 77$), irrespective of mode of delivery, had an earlier OL ($P < 0.05$) than primiparae who underwent emergency cesarean section deliveries ($n = 11$) (adjusted mean ± SEM, 2.5 ± 0.1 vs. 3.4 ± 0.3 d postpartum, respectively). OL took longer to occur among multiparous women with prenatal salivary cortisol levels above (vs. below) the 40th percentile [3 ± 0.2 ($n = 28$) vs. 2.4 ± 0.2 d postpartum ($n = 38$), respectively; $P = 0.02$]. Thus, stress during labor and/or delivery is likely to be a significant risk factor for delayed OL in urban Guatemala. J. Nutr. 132: 3055–3060, 2002.

KEY WORDS: • *breast-feeding* • *cortisol* • *lactogenesis stage II* • *onset of lactation* • *stress*

Women perceive the onset of lactation (OL[6]; i.e., initiation of copious milk production in the mammary gland) between a few hours and as many as 7 d after delivery. A delayed OL is likely to have major implications for infant nutrition because it may negatively affect breast-feeding behaviors. Secondary analyses of the 1991/1992 Epidemiology and Family Health Survey from Honduras showed that women with a delayed OL [i.e., >3 d postpartum (pp)] were twice as likely as their counterparts with an earlier OL to have had introduced non-human milk-based prelacteals (i.e., before OL). Among respondents with infants under 6 mo of age, use of these prelacteals was in turn associated with a fivefold increase in the risk of not being breast-fed or to not be exclusively breast-fed at the time of the survey (1). Pérez-Escamilla et al. (2) found in a longitudinal study in Hermosillo, Mexico, that the OL occurred later among those who were feeding formula to their babies by 1 wk pp compared with their fully breast-fed counterparts (3.3 vs. 2.9 d pp, respectively; $P \leq 0.05$). As in Honduras, the early use of formula was associated with an earlier termination of breast-feeding. In Hartford, CT, among women who were planning to breast-feed their children for at least 6 mo at the time of delivery, a delayed OL was significantly associated with a shorter duration of breast-feeding (3.4 mo vs. 11.7 mo, respectively; $P < 0.001$) (3).

The negative influence of a delayed OL on breast-feeding outcomes is perhaps explained by a higher likelihood of women becoming anxious about the adequacy of their milk supply followed by the introduction of infant formulas which in turn may lead to a further delay in OL (1–3). Different factors such as parity, birth weight, mode of delivery (4,5), infant feeding practices (2,4), insulin-dependent diabetes mellitus (6,7), maternal obesity (8,9) and maternal and/or infant stress (4,10) have been associated with a delayed OL.

The negative association between stress and milk production has been recognized for many years (11), but few studies have been performed in humans to attempt to understand the influence of stress during labor and delivery on OL. Chapman and Pérez-Escamilla (4) conducted a prospective study to identify the risk factors for delayed OL among U.S. women. Multivariate logistic regression analyses indicated that unscheduled cesarean section delivery and vaginal delivery with prolonged second stage of labor (i.e., "pushing" stage) were risk factors for delayed OL. Chen et al. (10) found an association among longer labor duration, maternal exhaustion, increased

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[6] Abbreviations used: GLM, general linear model; OL, onset of lactation; pp, postpartum.

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stress hormones, reduced breast-feeding frequency and delayed OL. To our knowledge, these relationships have not been tested in a developing country setting.

The objective of this project was to conduct a longitudinal study to examine the influence of stress during labor and delivery on OL among urban Guatemalan women.

MATERIALS AND METHODS

Study design

This study was approved by the Institutional Review Boards from the University of Connecticut, the Institute of Nutrition of Central America and Panama and the Instituto Guatemalteco de Seguridad Social. The study followed a longitudinal design and was conducted in Guatemala City between June and August 2000 in the labor and pp units of an Instituto Guatemalteco de Seguridad Social tertiary teaching hospital where ~12,000 babies are born per year. The hospital maternity ward serves mainly low-income employed women and housewives who live in periurban areas surrounding Guatemala City.

Subjects who participated in the study met the following criteria: *1*) telephone at home; 2) experiencing first stage of labor at time of recruitment; 3) term pregnancy (37–42 wk of gestation age by last menstrual period); 4) no medical or obstetric complications precluding breast-feeding, including being positive for the human immunodeficiency virus; 5) healthy newborn (born at term, birth weight >2500 g, 1-min Apgar score ≥7).

A total of 364 low-income pregnant women were contacted upon arrival to the low-risk labor unit. A screening questionnaire was administered to identify women who met the study selection criteria. Of the women who were contacted, 170 met these criteria and of these 16 did not complete the hospital follow-up because the baby was born after midnight or during the weekend. Eighteen women completed the hospital follow-up, but when personnel from the project tried to contact them, the telephone number was wrong or out of service or the mother decided to stay with other relatives. Thus, 136 women completed the hospital phase and the home telephone follow-up phase of the study (Fig. 1).

Salivary cortisol levels were measured: *1*) 3.2 ± 3.2 h before delivery (n = 137); 2) 1.7 ± 1.9 h after delivery of the placenta (n = 135); and 3) 17.1 ± 4.7 h pp (n = 153). A fourth salivary sample was obtained from 62 women at 29.3 ± 5.6 h pp; of these 27 had a vaginal delivery, 20 had a scheduled cesarean section delivery and 15 had an emergency cesarean section delivery. A fifth salivary sample was taken from four women at 81.9 ± 40 h pp; of these, two had vaginal deliveries and two had scheduled cesarean section deliveries.

Saliva samples were obtained using a Salivette, which consists of a plastic stopper, a tube insert, a centrifuge tube and a cotton cylinder. Women received instruction to hold the cotton cylinder under the tongue for 2–3 min and were encouraged to wet the cotton cylinder with saliva. The cotton cylinder was then placed in the tube insert and kept in a cooler with an ice pack for 4–6 h before being transported to the laboratory. Once in the laboratory, the tubes were refrigerated at −4°C for 10–12 h and centrifuged at room temperature for 15 min at 3000 rpm. Three salivary aliquots were separated in plastic vials and frozen at −20°C. Two sets of salivary samples were shipped from Guatemala to Storrs, CT, in a frozen state and then sent for cortisol determinations to Pennsylvania State University, University Park, PA, in a cooler with dry ice.

Maternal psychosocial stress was measured during labor and early and late pp with a 17-item questionnaire adapted from Hodnett and Simmons-Tropea (12) and Rini et al. (13) and pretested in this population. A modified shorter version was used during telephone follow-ups.

Medical charts were used to obtain information about type, date and time of delivery, type of anesthesia if any, vaginal tears or episiotomy, newborn's health status, gestational age at birth based on newborn's physical examination and date of last menstrual period, birth weight and Apgar scores 1 and 5 min after birth. A questionnaire was applied during the postnatal hospital stay to gather data on demographics, socioeconomic status, breast-feeding knowledge and infant feeding plans and intentions.

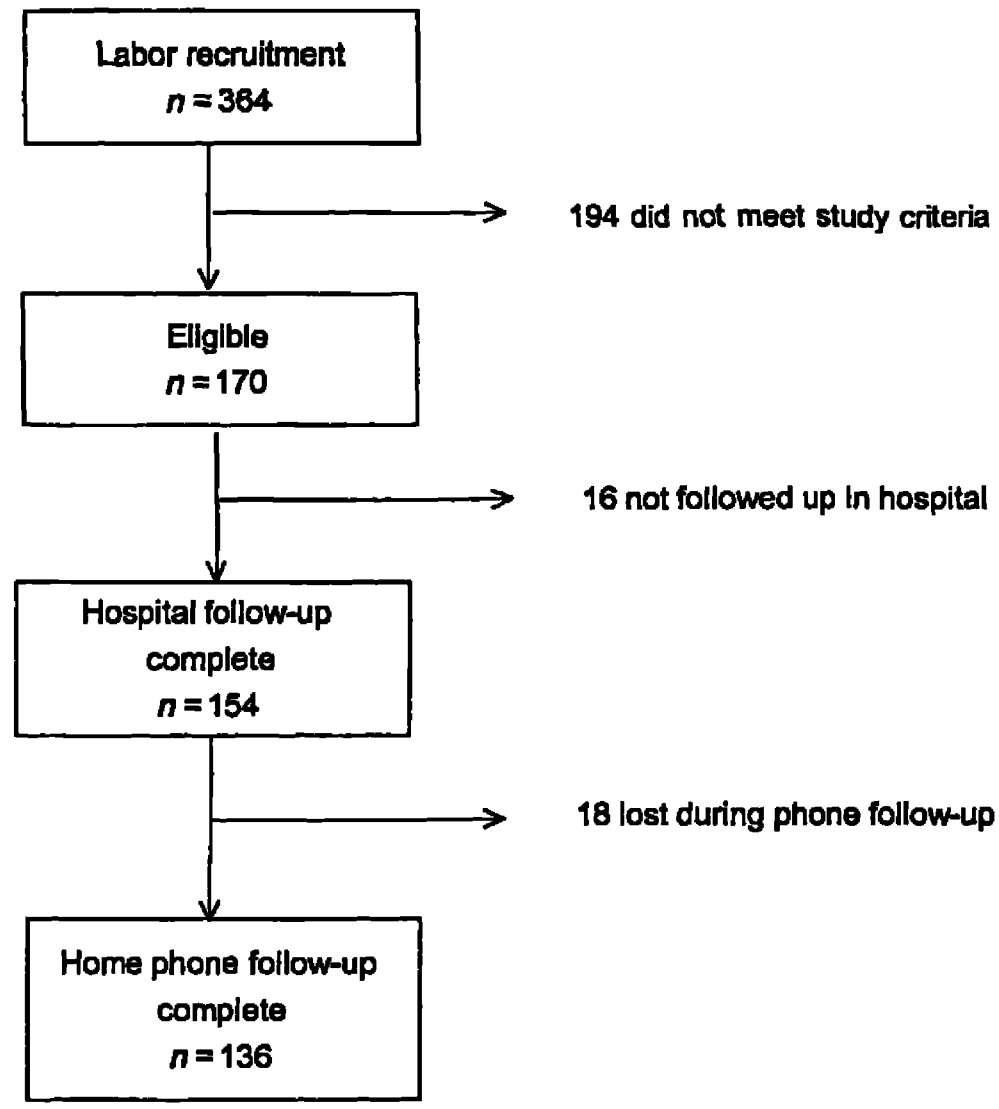

FIGURE 1 Study sample size and attrition. Women were recruited during the first stage of labor and followed up in the hospital at ~2 and 17 h after delivery. Those still in the hospital were also followed up 30 and 82 h after delivery. After hospital discharge, women were followed up in their homes via telephone until onset of lactation (OL) occurred.

Questions about OL symptoms were asked during the hospital stay and daily via telephone after hospital discharge until the mother reported OL following the methodology developed and validated by Chapman and Pérez-Escamilla (14,15). With this method, after the birth of the child subjects are interviewed at least once daily regarding breast symptoms (i.e., breast fullness, swelling, leakage) to evaluate clinical signs of lactogenesis. This symptom checklist is followed by the following question: "Has your milk come in yet?" If the response is positive, the subject is asked to explain how she knows that her milk has "come in" and the timing of OL is recorded in hours. Pérez-Escamilla and Chapman (15) have recently documented that the vast majority of women can identify when OL occurs and that they report similar symptoms of OL across cultures that are fully consistent with the physiology of lactogenesis stage II. Furthermore, conclusions reached from studies examining risk factors for delayed OL are remarkably similar when comparing maternal perception with more invasive procedures for measuring OL (i.e., infant test weighing, changes in milk composition).

Five community nutrition experts, of whom two are experts in breast-feeding research in Latin America, tested instruments for content validity. A pilot study was conducted before formal data collection with a convenience sample of 20 women contacted at different stages of labor and delivery to assess the clarity of the questionnaires and the need for further revision of them (i.e., face validity), data collection procedures and/or salivary sample collection techniques. The field coordinator and one of the study's research assistants were standardized in the administration of the screening and stress and anxiety forms. A second research assistant was trained on the application of the hospital and home phone follow-up forms. The standardization procedure involved applying the survey by either the field coordinator or an interviewer while both recorded simultaneously the responses provided by each woman. Results were compared between interviewers and field coordinator. A minimum level of 95% agree-

ment in responses was used before proceeding with formal data collection. Data collected during the pilot phase was not included in the final analyses.

Laboratory procedures

Salimetrics' HS-Cortisol Kits (Salimetrics, State College, PA) were used to measure salivary cortisol. This enzyme immunoassay uses 25 μL of saliva per test. The principle of this method is that cortisol standards and salivary cortisol compete with cortisol linked to horseradish peroxidase for rabbit antibody binding sites to cortisol. Bound cortisol peroxidase is measured through optical density read on a standard plate reader at 450 nm. The amount of cortisol peroxidase detected is inversely proportional to the amount of cortisol present (16). The assay has a range of sensitivity from 0.194 to 200 nmol/L. Method accuracy, determined by spike recovery, and linearity, determined by serial dilution, are 105 and 95%, respectively. Values from matched serum and saliva samples show the expected strong linear relationship ($r = 0.94$; $P < 0.0001$). All samples were tested in duplicate, and duplicate test values that varied by >7% were subject to repeat testing. The average intra- and interassay coefficients of variation were 7.99 and 8.89%, respectively. The mean of the duplicate tests was used in the statistical analyses.

Statistical analyses

The study field director (R. Grajeda) entered and cleaned the data using Epi-Info for Windows version 1.0 (17). All analyses were conducted with SPSS for Windows version 10.0.7 (18).

Analyses were based on the 136 women who completed both the hospital and phone follow-up phases of the study. Salivary cortisol levels were plotted by parity and against time before or after delivery. Mean cortisol concentrations were compared by parity at each time point using the Student *t* test. Sample size for first time of collection (i.e., ~3 h antepartum) was 112 because 11% of women could not wet the cotton cylinder due to dehydration and an additional 6.6% had unphysiologically high cortisol levels indicative of blood contamination, most likely as a result of biting the mouth walls during contractions. For the sample taken ~2 h pp (i.e., soon after the "pushing" effort) the corresponding figures were 12.5 and 16.9%, respectively, yielding a sample size of 96. As expected, dehydration or blood contamination was not a problem with the sample taken ~17 h pp. At this point only three women had to be excluded from the analytical sample ($n = 133$).

Bivariate analyses were conducted to examine the association between the independent variables and OL expressed both as a categorical (OL ≤3 d vs. >3 d pp) and as a continuous variable. In agreement with previous studies (15) the OL cut-off of 3 d pp was selected because it is likely that the level of concern of the mother and support individuals about the adequacy of the maternal milk supply for the infant will start increasing dramatically if the milk has not come in by this time (4). Chi-square analyses were conducted when OL was expressed categorically and Student's *t* test or analysis of variance when OL was continuous. Key independent variables examined were parity (primiparous vs. multiparous women), salivary cortisol levels at different time points (≤40th percentile vs. >40th percentile), type of delivery (vaginal, scheduled cesarean section, emergency cesarean section), second stage of labor duration (i.e., "pushing time"), infant gender, medications administered during labor and delivery, socioeconomic status, mother's age, cervical dilation upon recruitment and every item of the stress questionnaire. The 40th percentile of salivary cortisol was based on the distribution of this variable in the whole analytical sample and was selected based on *a*) exploratory analyses with this data set and *b*) the fact that using a lower cortisol cut-off would have left practically all primiparous women out of the analyses (because the overwhelming majority of primiparae were above the 35th percentile) (19).

Based on these exploratory bivariate analyses, two multivariate models were tested. The general linear model (GLM) was used to examine OL as a continuous variable by parity and delivery mode after adjusting for maternal age and cervical dilation upon recruitment (i.e., when the first salivary sample was taken). Multivariate logistic regression was used to test the same hypothesis using OL as a dichotomous variable (≤3 vs. >3 d pp). Likewise, multivariate logistic regression was used to test the association between salivary cortisol levels upon recruitment and OL after adjusting for maternal age and cervical dilation at the time when the first salivary sample was obtained. These two variables were controlled for in the multivariate analyses because they were the only two covariates significantly associated with OL in the bivariate analyses. Similar conclusions were reached with both GLM and logistic regression analyses; thus, only the former results are presented. Differences were considered to be significant when the two-sided probability value was ≤0.05.

RESULTS

Subjects

On average, women were in their middle 20s and had ~9 y of formal education, two pregnancies and 4 cm of cervical dilation upon recruitment. There were no differences in these variables between women who met the study criteria and those who left the study. However, women who dropped tended to have less education ($P = 0.10$) (Table 1).

Forty-three percent of the women were primiparous and, as expected, they were younger than their multiparous counterparts. There were no differences by parity associated with gestational age (measured by either last menstrual period or newborn's examination), cervical dilation upon recruitment or antepartum hospital stay. Primiparous women were more likely ($P < 0.01$) to have an emergency cesarean section delivery and less likely to have a vaginal delivery or a scheduled cesarean section than multiparae. Parity status was not associated with birth weight, "pushing time" during the second stage of labor or the newborn's gender (Table 2).

TABLE 1

Comparison of descriptive characteristics of study participants with nonparticipants from Guatemala City[1]

			Study participation				
	n	All	*n*	Yes	*n*	No	*P*-values[2]
Maternal age, *y*	169	25.8 ± 5.6	136	26.0 ± 5.8	33	25.1 ± 4.9	0.42
Maternal schooling, *y*	169	9.1 ± 3.8	136	9.3 ± 3.9	33	8.1 ± 3.5	0.10
Gestational age, *wk*	164	38.7 ± 1.7	133	38.8 ± 1.6	31	38.5 ± 1.8	0.41
Cervical dilation,[3] *cm*	170	4.1 ± 2.5	136	4.3 ± 2.5	34	3.6 ± 2.5	0.13
Pregnancies, *n*	170	2.1 ± 1.3	136	2.1 ± 1.3	34	2.3 ± 1.3	0.46

[1] Values are means ± SD.
[2] Student's *t* test probability value (participants vs. nonparticipants).
[3] Cervical dilation upon recruitment.

TABLE 2

Descriptive sociodemographic and biomedical characteristics of study participants from Guatemala City by parity[1]

	All $n = 136$	Primiparous $n = 58$	Multiparous $n = 78$	P[2]
Mother				
Age, *y*	26.0 ± 5.8	22.7 ± 3.9	28.3 ± 5.8	<0.01
Schooling, *y*	9.3 ± 3.9	10.0 ± 3.4	8.9 ± 4.2	0.10
Parity, *n*	2.1 ± 1.3	1.0 ± 0.0	2.9 ± 1.2	<0.01
Gestational age,[3] *wk*	38.7 ± 1.6	38.8 ± 1.5	38.7 ± 1.7	0.52
Cervical dilation,[4] *cm*	4.3 ± 2.5	4.7 ± 2.1	4.0 ± 2.7	0.12
Antepartum hospital stay, *h*	11.8 ± 6.7	12.4 ± 7.0	11.3 ± 6.4	0.31
Onset of lactation, *d pp*	2.7 ± 1.1	2.8 ± 1.1	2.6 ± 1.0	0.23
Newborn				
Gestational age,[5] *wk*	39.8 ± 1.0	39.8 ± 1.1	39.9 ± 1.1	0.52
Birth weight, *kg*	3.18 ± 4.23	3.14 ± 4.44	3.21 ± 4.34	0.37

	n	%	*n*	%	*n*	%	*P*-values[6]
Type of delivery							<0.01
Vaginal	102	75.0	47	81.0	55	70.5	
Schedule C-section	17	12.5	—	—	17	21.8	
Emergency C-section	17	12.5	11	19.0	6	7.7	
Pushing time[7]							0.20
≤15 *min*	75	75.0	31	68.9	44	80.0	
>15 *min*	25	25.0	14	31.1	11	20.0	
Gender							0.29
Female	68	50.0	32	55.2	36	46.2	
Male	68	50.0	26	44.8	42	53.8	

[1] Values are means ± SD.
[2] Student's *t* test probability value (primiparous vs. multiparous women).
[3] Gestational age based on date of last menstrual period.
[4] Cervical dilation upon recruitment.
[5] Gestational age based on newborn's physical examination.
[6] Chi-square test probability value.
[7] Corresponding to second stage of labor.

Upon recruitment during labor, primiparous women were more likely than their multiparous counterparts to report extreme pain [39.7 vs. 14.1%, respectively; $P < 0.001$ ($n = 136$)]. Primiparae were less likely to report not to be suffering [26.3 vs. 48.7%; $P = 0.027$ ($n = 135$)] and more likely to be very worried about their unborn children [22.4 vs. 5.1%; $P = 0.003$ ($n = 136$)] and to have families who were very worried about them [38.6 vs. 16.9%; $P = 0.013$ ($n = 134$)]. A lower percentage of primiparae felt that their unborn babies were very well [17.2 vs. 36.4%; $P = 0.050$ ($n = 135$)] and a lower proportion of them were able to think about anything other than the upcoming delivery [37.9 vs. 55.1%; $P = 0.047$ ($n = 136$)].

Cortisol levels during labor and delivery and pp

Salivary cortisol concentration increased from 27.8 ± 2.2 nmol/L in the first stage of labor to 64.1 ± 4.2 nmol/L soon after the delivery of the placenta, followed by a decline to 12.6 ± 1.3 nmol/L in the following 17 h. Primiparous women had higher antepartum and early postnatal cortisol levels that were almost twice as high as those among multiparous women ($P < 0.05$). The parity difference in salivary cortisol concentrations vanished with time (Fig. 2).

Onset of lactation

OL occurred at 2.7 ± 1.1 d pp (range 0.9–8.1 d pp); 27% of the women had a delayed OL (i.e., >3 d pp). Among primiparae, OL tended to occur ($P = 0.23$) 5.4 h later than among multiparae (2.8 ± 1.1 vs. 2.6 ± 1 d pp) (Table 2).

The symptoms most commonly associated with OL were breast fullness (99%), breast heaviness (97%), infant being full (92.6%), breast pain (91.9%), increase in breast temperature (89%), milk leakage (88.2%), milk in baby's mouth (86%), breast tingling (56.6%) and increase in body temperature (41.9%).

Stress and OL

Multivariate GLM analysis results found a significant association ($P = 0.04$) between parity/delivery mode and OL (Table 3). Multiparous women (irrespective of mode of delivery) had an earlier OL than primiparae who underwent emergency cesarean section deliveries. Likewise, primiparous women with vaginal deliveries tended to have ($P = 0.163$) an earlier OL than their counterparts who underwent an emergency cesarean section delivery (Fig. 3).

Among multiparous women, those with a prenatal salivary cortisol level above the 40th percentile (i.e., 16.55 nmol/L) had a more delayed OL than their counterparts with lower salivary cortisol levels (Table 3) [adjusted OL timing (mean ± SEM): 3 ± 0.2 d pp ($n = 28$) vs. 2.4 ± 0.2 d pp ($n = 38$), respectively; $P = 0.02$]. Among primiparae, cortisol levels were not associated with the timing of OL [2.8 ± 0.2 d pp ($n = 38$) vs. 3 ± 0.4 d pp ($n = 7$), respectively; $P = 0.73$].

In the multivariate analyses, maternal age was positively associated with OL but cervical dilation upon recruitment was no longer associated with OL (Table 3).

DISCUSSION

To our knowledge, this is the first published prospective study showing a positive association between maternal stress

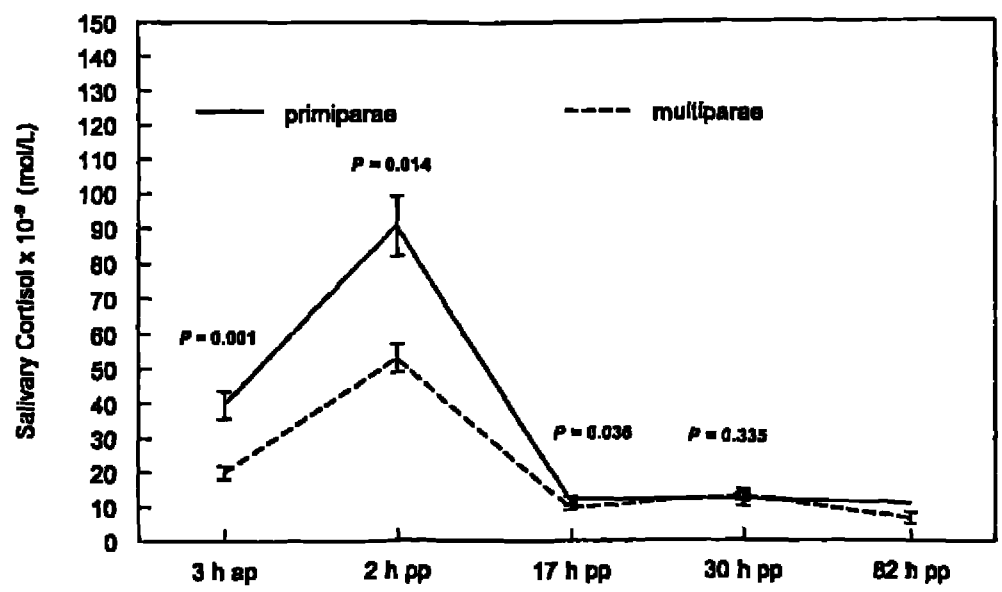

FIGURE 2 Salivary cortisol concentrations by parity, and antepartum (ap) and postpartum (pp) time among urban Guatemalan women. Sample sizes: *a*) 3 h ap ($n = 112$); *b*) 2 h pp ($n = 96$); *c*) 17 h pp ($n = 133$); *d*) 30 h pp ($n = 60$); *e*) 82 h pp ($n = 4$). Comparisons between primiparous and multiparous women based on Student's *t* test; *P*-values are shown. Error bars represent SEM. Data at 82 h pp are based on one primiparous woman and three multiparous women. Thus, at this time point no statistical comparisons were made.

TABLE 3

GLM multivariate regressions examining influence of stress on OL among urban Guatemalan women[1]

	All subjects (model 1)			Multiparae (model 2)		
	n	B ± SEM	*P*-values	*n*	B ± SEM	*P*-values
Parity-delivery type						
Primiparae-vaginal	47	−0.490 ± 0.350	0.163			
Multiparae	78	−0.866 ± 0.348	0.014			
Primiparae-emergency cesarean section	11					
Salivary cortisol						
<40th percentile				38	−0.573 ± 0.257	0.029
≥40th percentile				28		
Cervical dilation, *cm*	136	0.025 ± 0.037	0.495	66	0.052 ± 0.049	0.288
Maternal age, *y*	136	0.046 ± 0.018	0.010	66	0.054 ± 0.022	0.017
Model statistics						
F-value	2.7			4.9		
Total df	135			66		
P-value	0.032			0.004		

[1] Model 1 is based on whole analytical sample and excludes salivary cortisol concentration as an independent variable. Model 2 is based on multiparous women only and excludes the parity-delivery mode variable. The dependent variable in both models is the onset of lactation coded in days postpartum (pp). B: Regression coefficient; df: Degrees of freedom.

during labor and delivery and the timing of OL in a developing country population. This is also the first OL study to use salivary cortisol as a biological stress indicator. In agreement with studies conducted in the U.S. (4,10), results strongly suggest that stress during labor and delivery is a significant risk factor for a delayed OL. First, primiparous women who underwent emergency cesarean section deliveries were more likely than the rest of the sample to have a significantly delayed OL. A recent analytical overview indicates that previous studies (15) have identified mode of delivery (i.e., emergency cesarean section delivery, vaginal deliveries with prolonged second stage of labor) and primiparity as independent risk factors of delayed OL. In this study, it was the interaction between primiparity and a stressful delivery (i.e., emergency cesarean section delivery) that was associated with an increased risk of delayed OL.

In this study there were no subjects who had a vaginal delivery that pushed for >30 min. The active management of labor and delivery in the study hospital, which included widespread use of oxytocin and other labor inducers, perhaps explains this finding (19). Thus, the hypothesis that a prolonged second stage of labor is a risk factor for delayed OL among women undergoing vaginal deliveries (4) could not be tested with these data.

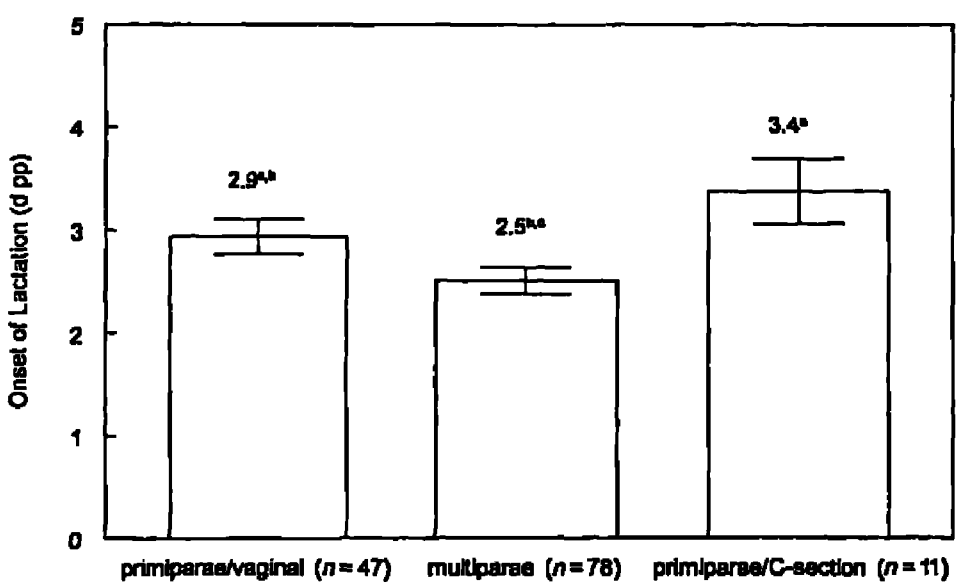

FIGURE 3 Onset of lactation (OL) by parity and type of delivery among urban Guatemalan women ($P = 0.04$). Shown is the general linear model (GLM) adjusted mean; error bars represent SEM. Means were adjusted for vaginal dilation upon recruitment and maternal age. Means without a common letter differ; $P < 0.05$.

Second, among multiparous women cortisol levels were positively associated with the timing of OL. Through this study it is not possible to establish whether high cortisol levels are causally linked with a delayed lactogenesis stage II among multiparae. Cortisol is required for lactogenesis to occur (20); thus, it is not known whether cortisol is simply a marker of other stress hormones or cortisol concentration beyond a threshold level impairs lactogenesis stage II. Indeed, it is possible that the relatively high cortisol levels among primiparous women, which were twice as high in the antepartum and early postnatal period compared with multiparae, explains why the association between this stress hormone and OL was not found in this subgroup. Further mechanistic research is clearly needed in this area.

OL in this population of low-income Guatemalan women occurred 2.7 ± 1.1 d pp and the incidence of delayed OL was 27%, both of which fall well within the range previously reported (15). OL symptoms reported by Guatemalan women, such as breast fullness, are in agreement with those reported in industrialized countries, confirming that women perceive OL using similar cues across cultures (15).

Salivary cortisol levels increased dramatically from the time of recruitment during the first stage of labor until the delivery of the placenta and then returned to baseline levels between 17 and 30 h pp. Cortisol levels were significantly and positively associated with cervical dilation upon recruitment and with several items of the psychosocial and anxiety scales (19). These observations indicate that salivary cortisol is a valid stress indicator during labor and delivery.

Study limitations

Subjects' access to a telephone was a key selection criterion in this study. Thus, the findings may have been biased toward a relatively more "privileged" group among the low-income population served by the social security system in Guatemala City. Thus, although no major differences in socioeconomic and demographic characteristics were observed between sub-

jects who participated and did not participate in the study, the results of this study can not be generalized to the whole low-income population served by public hospitals or neighborhood maternity clinics in Guatemala City.

The salivary cortisol technique is a useful approach to study stress and reproductive outcomes (including human lactation) prospectively. There were two limitations of the method used in this study: *1*) the inability of a small percentage of women to provide a saliva sample due to dehydration during labor and delivery and *2*) the likelihood of blood contamination of saliva samples, perhaps as a result of biting the internal surfaces of the mouth during labor and "pushing" contractions. By contrast, 17 h after delivery the overwhelming majority of women were able to provide adequate saliva samples. Thus, special precautions need to be taken when applying this technique during labor and delivery. Measures could include ensuring that women understand that they need to wet the cotton cylinder, providing subjects with neutral gum or other substances that facilitate saliva production but do not interfere with the assay and asking women to try to prevent biting their mouths' inner surfaces.

In conclusion, this study has important public health implications because women who experience psychosocial and/or birth-related biological stress during labor and delivery are likely to experience a delayed OL. Mechanistic research is needed to gain a better understanding of the neuroendocrinological mechanisms that may be responsible for the association between stress and impaired lactogenesis stage II. Results from this line of inquiry are likely to have profound infant feeding implications because preventing a delayed OL is likely to have a positive impact on breast-feeding outcomes (1–3).

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