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## Influence of prone positioning on premature newborn infant stress assessed by means of salivary cortisol measurement: pilot study

*Influência do posicionamento em prona sobre o estresse no recém-nascido prematuro avaliada pela dosagem de cortisol salivar: um estudo piloto*

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### ABSTRACT

**Objective:** This study sought to assess the influence of prone positioning on the stress of newborn premature infants through the measurement of the salivary cortisol concentration and the evaluation of physiological and behavioral responses before and after changes in body positioning.

**Methods:** Saliva samples were collected from newborn infants at two different times: the first (corresponding to the baseline) after a period of 40 minutes during which the infants were not subjected to any manipulation and were placed in the lateral or supine position, and the second 30 minutes after placement in the prone position. Variables including heart rate, respiratory rate, peripheral oxygen saturation, and the Brazelton sleep score were recorded before, during, and at the end of the period in the prone position.

**Results:** The sample comprised 16 newborn premature infants (56.3% male) with a gestational age between 26 and

36 weeks, postnatal age between 1 and 33 days, birth weight of 935 to 3,050g, and weight at the time of intervention of 870 to 2,890g. During the intervention, six participants breathed room air, while the remainder received oxygen therapy. The median salivary cortisol concentration was lower in the prone position compared to baseline (0.13 versus 0.20;  $p=0.003$ ), as was the median Brazelton sleep score ( $p=0.02$ ). The average respiratory rate was lower after the intervention ( $54.88\pm 7.15$  versus  $60\pm 7.59$ ;  $p=0.0004$ ). The remainder of the investigated variables did not exhibit significant variation.

**Conclusion:** Prone positioning significantly reduced the salivary cortisol level, respiratory rate, and Brazelton sleep score, suggesting a correlation between prone positioning and reduction of stress in preterm infants.

**Keywords:** Adrenal córtex/metabolismo; Hydrocortisone/analysis; Infant newborn/metabolism; Infant premature/metabolism; Prone position; Saliva/analysis; Stress, physiological; Intensive care units, neonatal

**Conflicts of interest:** None.

Submitted on November 20, 2013

Accepted on April 6, 2014

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DOI: 10.5935/0103-507X.20140025

### INTRODUCTION

The ability of preterm infants to perceive pain and stress is well established.<sup>(1-3)</sup> In particular, the actions of stressors may disorganize various systems in newborn preterm infants, including the autonomic, motor, and information systems, resulting in physiological and behavioral expressions of stress.<sup>(4)</sup> In regard to behavioral characteristics, preterm infants exhibit observable signs in response to stress.<sup>(5,6)</sup> With the aim of systematically assessing newborn infants' behavior, Als et al. formulated the Behavioral Assessment Scale (Brazelton

Neonatal Behavioral Assessment Scale - BNBAS) in 1973. This research tool was adapted to the physical and neurological responses and emotional behaviors of newborn infants while taking individual differences into account.<sup>(6)</sup> In regard to the physiological changes caused by stress, cortisol is one of the stress-related hormones commonly measured in newborn infants.<sup>(7)</sup> After the correlation between plasma and salivary cortisol levels was demonstrated,<sup>(8,9)</sup> subsequent studies on neonatal pain and stress were conducted using the noninvasive method of saliva collection for the measurement of cortisol concentration.<sup>(10-17)</sup>

As newborn infants exhibit physiological and behavioral signs of pain and stress that can be recognized by healthcare professionals, pharmacological and non-pharmacological resources may be employed in the neonatal intensive care unit (NICU) to manage such conditions.<sup>(4,12,13)</sup> In this regard, appropriate positioning is considered an important non-pharmacological intervention in premature newborns admitted to NICUs,<sup>(17-19)</sup> and according to some studies, the frequency of stress behaviors is lower when infants are placed in the prone position.<sup>(20,21)</sup>

The main objective of the present study was to establish whether prone positioning influences stress in premature newborn infants, as indicated by the salivary cortisol concentration. In addition, we also investigated the correlation between salivary cortisol concentration and physiological parameters, including heart rate (HR), respiratory rate (RR), and oxygen saturation (SatO<sub>2</sub>), and behavioral signs according to an adaptation of the BNBAS sleep score.

## METHODS

The present study was a pilot intervention study conducted at the NICU of the *Hospital Universitário do Oeste do Paraná* from August to September 2013. The study was approved by the research ethics committee of the *Universidade Estadual do Oeste do Paraná*, under protocol no. 322,268. All of the participants' parents or guardians signed an informed consent form.

The study population comprised newborn infants with a gestational age of 25 to 36 weeks who were clinically stable and exhibited normal physiological parameters before sample collection. The samples were collected at least one hour after the last feeding and 40 minutes after any manipulation of the infants. Premature infants in unstable hemodynamic conditions with grade III or IV intraventricular hemorrhage or subsequent leukomalacia, congenital defects of the nervous system, neurological

malformations or impairments, or kidney disease; using opiates, corticosteroids, or other drugs liable to interfere with the response to nociception; and requiring invasive procedures at the time of sample collection were excluded.

## Study protocol

The study design took into account the fact that the circadian cortisol cycle is not yet established in newborn infants.<sup>(10)</sup> Thus, each individual was considered as his or her own control, whereby the baseline and response measurements were paired. To minimize the presence of other potential stressors, sample collection was performed between 6:00 and 7:30 in the morning, a time when newborn infants are less subjected to manipulation and activities are minimal at the NICU, thus resulting in low levels of noise and exposure to light. The newborn infants were monitored by means of conventional pulse oximetry, digital skin thermometers, and observation of the respiratory pattern and behavioral responses by the principal investigator. The variables were recorded in an ad hoc form at two different times: before baseline sample collection (before oral hygiene) and after prone positioning of the infants.

Because excessive manipulation may be a source of bias in behavioral assessments, we chose to use the Brazelton sleep score, adapted to a study condition defined by the least possible manipulation of infants during intervention, although this score has known restrictions for use in assessments of premature infants.<sup>(6)</sup> The BNBAS aims to assess the behavior of neonates by observing responses in various systems in a given sequence, i.e., from the autonomic to the motor state and finally to the social interaction system.<sup>(6)</sup> According to the BNBAS, the state system describes the levels of consciousness, which range from quiet sleep to full crying, and measures the ability of infants to control their states and to process and respond to information from their caregiving environment.<sup>(6)</sup> Although there are restrictions in the use of the BNBAS in premature newborn infants, we decided to use the Brazelton sleep score because it is a purely observational scale and thus appropriate for the study condition, as excessive manipulation during the assessment of behavioral responses could represent a source of stress and introduce bias. Based on the original scale classification,<sup>(6)</sup> the participants were attributed scores according to the behavioral state of accommodation observed before and after the intervention as follows: state 1 - deep sleep, regular breathing, no movements; state 2 - light sleep, closed

eyes, occasional smooth movements; state 3 - drowsy, occasional opening of the eyes; state 4 - awake, eyes open, few bodily movements; state 5 - fully awake, continuous bodily movements; state 6 - crying.

One hour after the last feeding and immediately before the first saliva collection, the infant's oral cavity was gently but thoroughly cleansed using sterile cotton swabs and water, and all care was taken to avoid contamination of the samples by milk remnants. Next, 0.5 to 0.7mL of saliva was collected through careful aspiration of the floor of the mouth using a 10mL syringe coupled to a needleless flexible 18G intravenous catheter. Two saliva samples were collected. The first sample, corresponding to the baseline level, was collected after a period of at least 40 minutes during which the infants were subjected to no manipulation whatsoever before the oral cleansing. This sample was collected immediately after the oral cleansing, with no stabilization interval allowed between the cleansing procedure and saliva collection; this sample represented the pre-manipulation state, as the stress associated with such a procedure could have induced an increase in salivary cortisol secretion, the peak of which usually occurs after 20 to 40 minutes. After the baseline sample was collected, the newborn infants were placed in the prone position and inclined forward, with the head to one side and all four limbs in flexion. No restraints were removed or added beyond those already in use by the newborn infant at the time of the baseline sample collection.

Ten minutes were allowed for the infants to achieve stabilization in the prone position, and the second saliva sample was collected 20 minutes later. The samples were stored and transported to the *Laboratório de Análises Clínicas Alvaro* located in the city of Cascavel, Paraná state, Brazil, where the measurement of cortisol was performed.

The samples were processed using electrochemiluminescence (Roche Diagnostics Elecsys® 2010 Immunoassay System) and stored at 2 to 8 °C for 5 days and then discarded. The following parameters were recorded in an ad hoc form before the first sample collection, while the infants were placed in the prone position, and at the end of the procedure: HR, RR, SatO<sub>2</sub>, body temperature (T), and the Brazelton sleep score. Intercurrent events and other observations were also recorded in the abovementioned form.

### Statistical analysis

As this was a pilot study, the sample size was gradually increased to calculate the standard error and compensate for the 0.015-unit margin of error in the cortisol

measurement (value right below the precision level of the measurement) and 95% confidence interval relative to the sample mean. Parametric data were analyzed by means of the paired *t*-test, and non-parametric data were analyzed by means of the Wilcoxon test. The significance level was set at 5%. Although the paired *t*-test controls for variations that occur among newborn infants, to assess the variations that occur as a result of treatment only (i.e., before and after treatment), regression analysis was performed to assess the possible influence of the variables gestational and postnatal age. The statistical analyses were performed using the software Statistica 10® (Stat Soft, 2011). The clinical characteristics of the participants are described as frequencies, means, and standard deviations. The paired *t*-test and the Wilcoxon test were applied to matched pairs, and the significance level was set as  $p < 0.05$ .

### RESULTS

A total of 21 newborn infants were recruited. Of these, 16 were included in the analysis, and 5 were excluded because their saliva samples were too viscous for laboratory processing.

All 16 participants were preterm infants, with an average gestational age of 31.2 weeks, ranging from 26 to 36 weeks. The mean birth weight of the infants was 1,813.7g, ranging from 935 to 3,050g. On the day of the intervention, the mean body weight was 1,705g, ranging from 870 to 2,890g, and the mean age of the infants was 9.4 days old, ranging from 1 to 33 days. Nine participants were male, and nine participants had been delivered by cesarean section. The mean 5-minute Apgar score was 7.6, ranging from 4 to 10. Two participants were placed in oxygen hoods, eight were given oxygen masks, and six were not given oxygen therapy. Most participants (11) preferentially adopted the right lateral position; in addition, four adopted the left lateral position and one the supine position (Table 1).

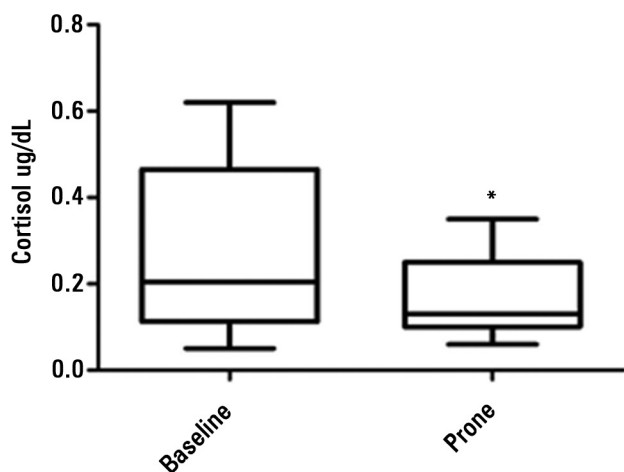
From the 42 collected saliva samples, 32 (76.19%) were appropriately processed and included for analysis. The salivary cortisol level after prone positioning decreased in 13 newborn infants (81.25%), increased in one (6.25%), and did not change in two (12.5%). The median salivary cortisol level after prone positioning was significantly lower compared to the baseline measurement (0.13 (0.1125-0.465) versus 0.20 (0.100-0.250);  $p = 0.003$ ) (Figure 1).

The infants' mean RR significantly decreased after the intervention ( $p = 0.0004$ ), from  $60 \pm 7.59$  breaths per minute

**Table 1 - Clinical data of premature newborn infants**

Variables	Results
Cesarean birth	9
Gestational age	31.3 (26-36)
Birth weight (g)	1,813.8 (935-3,050)
Weight on intervention day (g)	1,705 (870-2,890)
Five-minute Apgar score	7.6 (4-10)
Age (days)	9.4 (1-33)
Male gender	9
Oxygen therapy	
Hood	2
Mask	8
None	6
Baseline body position	
Right lateral	11
Left lateral	4
Supine	1

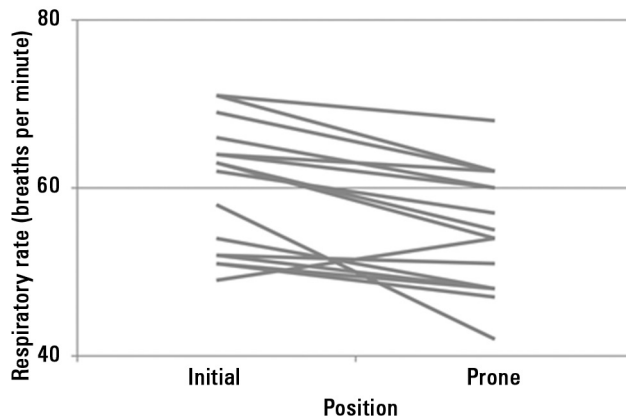
Values expressed as weeks and means.



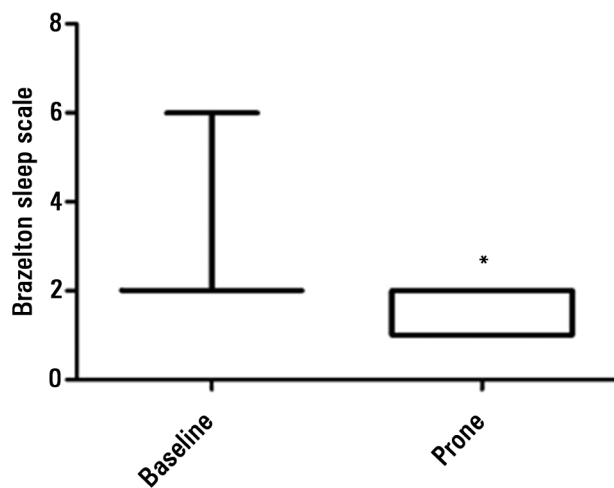
**Figure 1 - Variation in salivary cortisol concentration before and after prone positioning. \* p value=0.003/Wilcoxon test.**

at baseline to  $54.88 \pm 7.15$  after prone positioning (Figure 2). The mean HR was  $150.8 \pm 18.00$  (minimum/maximum: 119/186) at baseline versus  $146.5 \pm 17.12$  (114/175) after prone positioning, with no significant difference between measurements ( $p=0.17$ ). Neither the  $\text{SatO}_2$  ( $94.69 \pm 3\%$  versus  $95.56 \pm 2.22\%$ ;  $p=0.33$ ) nor T measurements ( $36.72$  versus  $36.73$ ;  $p=0.75$ ) exhibited significant differences before and after the intervention.

The Brazelton sleep score did not increase in any participant after prone positioning, decreased in seven (43.75%) and did not change in nine (56.25%) in comparison to the baseline score ( $p=0.02$ ) (Figure 3).



**Figure 2 - Comparison of the variation in respiratory rate before and after prone positioning of infants. p value=0.0004/paired t-test.**



**Figure 3 - Variation in Brazelton sleep score before and after prone positioning. \* p value=0.02/Wilcoxon test.**

## DISCUSSION

Technological advances in neonatal intensive care and research have contributed to an increase in the survival of newborn infants, including extremely premature ones.<sup>(22)</sup> At the same time, approximately 15% of surviving premature infants exhibit significant sequelae,<sup>(23)</sup> which may be related to the routine interventions applied by healthcare providers at NICUs that, while on the one hand provide life support, on the other cause pain, stress, and discomfort.<sup>(23,24)</sup> Thus, life support in premature infants should prioritize care measures centered on their development, among which body positioning is important.<sup>(5,6,18,19)</sup>

Although several studies have assessed the influence of positioning on premature newborn infant pain and stress,<sup>(15,20,21,25)</sup> the literature includes no reports on the



correlation between the salivary cortisol level as a marker of stress and prone positioning in premature infants placed in incubators. In addition, there are no reports on the use of the electrochemiluminescence method to measure the salivary cortisol level as a marker of stress following manipulation of premature newborn infants. The method most widely used for this purpose is the radioimmunoassay, which was not available at the time of the present study.

The *Hospital Universitário do Oeste do Paraná* is a tertiary care institution with 10 NICU beds. In 2012, the NICU admitted 257 newborn infants, of which almost all were referred from the hospital's own maternity unit. The NICU has not yet established a protocol for development-centered infant care, and prone positioning is only indicated when healthcare providers detect signs of respiratory distress in newborn infants.

The results of the present study revealed a significant reduction in the salivary cortisol level in 81.25% of the sample after prone positioning, corroborating previous studies that assessed the effect of this position on stress in premature newborn infants and found a reduction in the number of stress-indicating behaviors,<sup>(20,21,25)</sup> which may be correlated with lower cortisol levels. However, in contrast to previous studies<sup>(12,15)</sup> that found an influence of gestational and postnatal age on the salivary cortisol response, we did not find any correlation among such variables in our sample, including in infants who did not exhibit changes in the salivary cortisol level (12.5%) and in infants in which the cortisol levels increased (6.25%). Three of the largest reductions in the salivary cortisol levels found corresponded to infants with a gestational age over 32 weeks and a postnatal age of 1 day, which may suggest that the hormonal response is better in newborn infants with an increased gestational age, more stable condition, and shorter stay at the NICU.

In agreement with a previous study,<sup>(26)</sup> we found a reduction in RR following prone positioning, which lends support to previous reports describing improvements in the respiratory pattern and a reduction of RR following prone positioning.<sup>(27)</sup> However, our results are in contrast to those of others studies that detected an increase<sup>(28)</sup> or no change<sup>(29)</sup> in RR following prone positioning.

The results of the present study showed that the adapted Brazelton sleep score did not increase following prone positioning compared to baseline but did decrease in 44% of the sample. These findings provide further evidence of the positive effects of the prone position on sleep in premature newborn infants, which include fewer

arousals,<sup>(30-33)</sup> a longer length of quiet sleep,<sup>(30,31)</sup> and, consequently, lower energy expenditure.<sup>(29,30)</sup>

In the present study, the measurements of HR, T, and SatO<sub>2</sub> did not exhibit significant changes, in contrast to reports by other authors who found increases in HR,<sup>(28,29)</sup> a reduction in T,<sup>(29)</sup> and increases in SatO<sub>2</sub><sup>(27,30)</sup> with changes in body position. These differences may be partially explained by methodological differences in the procedures used for monitoring, such as the use of pulse oximetry instead of electrocardiography, and the short interval applied for posture maintenance.

Our body posture intervention was found to be safe, as no intercurrent events, such as a reduction in SatO<sub>2</sub> or apnea, occurred, and there was no need to either increase the oxygen supply or to interrupt the procedure. This finding is particularly important because stress is known to negatively influence the development of premature infants.<sup>(3-6,18,19,25)</sup> Thus, the results of the present study may motivate further research to reaffirm the adoption of prone positioning as an important positive stimulus for developing premature newborn infants.

Some methodological aspects of the present study may limit the interpretation of the results, such as the sample heterogeneity, which exhibited wide variations in the gestational and postnatal age and the respiratory function of the participants. Additionally, the small sample size did not allow stratification of the participants according to important groupings such as gestational and postnatal age, which may influence the regression analysis and point to the most vulnerable groups and determinant variables. In this sense, the length of time the infants were kept in the prone position may not have benefited infants with slower behavioral and physiological responses, i.e., the most premature infants who remain longer at the NICU and are consequently exposed to additional painful stimuli. In addition to the sample size, the use of the electrochemiluminescence method to measure salivary cortisol also characterized the present as a pilot study. However, the intra- and inter-assay coefficient of variation, as determined by future studies, will serve to establish the reproducibility of the electrochemiluminescence method in small-volume saliva samples, such as those used in the present study. An additional limitation may be the fact that the assessment of the Brazelton sleep score was observational, although it was performed by a single investigator. This assessment requires the utmost attention, and we cannot rule out the possibility that were the infants filmed at the time of the assessment and the videos subsequently reviewed by different blinded examiners, the scores given

may have been different. Finally, it should be noted that simple monitoring of the respiratory pattern and HR may not have been sensitive enough to detect discrete variations, and the lack of continuous recording represents a negative factor in the interpretation of those data.

## CONCLUSION

Our results indicate that prone positioning may significantly reduce the salivary cortisol level, respiratory rate, and Brazelton sleep score in stable premature

newborn infants admitted to a neonatal intensive care unit who are not stratified according to gestational or postnatal age. These findings suggest a possible correlation between the prone position and a reduction of stress in premature newborn infants. Although the measurements of temperature, heart rate, and peripheral oxygen saturation did not seem to be influenced by body position, future studies with improved methodology and larger samples may be able to detect a correlation between these variables and a population more sensitive to postural intervention.

## RESUMO

**Objetivo:** Avaliar a influência da postura em prona sobre o estresse no recém-nascido prematuro por meio da dosagem do cortisol salivar e da avaliação das respostas fisiológicas e comportamentais, antes e após o posicionamento.

**Métodos:** Foi realizada a coleta de saliva em cada recém-nascido em dois momentos: o primeiro (correspondente ao basal), sem manipulação prévia por 40 minutos, em decúbito lateral ou supino; e o segundo, 30 minutos após o posicionamento em prona. A frequência cardíaca e respiratória, saturação periférica de oxigênio e escala de sono de Brazelton foram registradas antes, durante e ao final do posicionamento em prona.

**Resultados:** Participaram do estudo 16 recém-nascidos prematuros (56,3% masculino) com idade gestacional de 26 a 36 semanas, com 1 a 33 dias de vida, e peso variando de 935 a 3.050g ao nascimento e de 870 a 2.890g no dia da intervenção.

Durante o posicionamento, seis recém-nascidos estavam em ar ambiente e os demais recebiam oxigênio suplementar. A mediana dos níveis de cortisol salivar foi menor durante o posicionamento em prona comparativamente ao basal (0,13 e 0,20;  $p=0,003$ ), assim como a do escore de sono de Brazelton ( $p=0,02$ ). A média da frequência respiratória foi menor após a intervenção ( $54,88\pm 7,15$  e  $60\pm 7,59$ ;  $p=0,0004$ ). As demais variáveis analisadas não apresentaram variação significativa.

**Conclusão:** O posicionamento em prona diminuiu significativamente os níveis de cortisol salivar, da frequência respiratória e do escore de sono de Brazelton, sugerindo a correlação entre essa postura e a diminuição do estresse nesses recém-nascidos.

**Descritores:** Córtex suprarrenal/metabolismo; Hidrocortisona/análise; Recém-nascido/metabolismo; Prematuro/metabolismo; Decúbito ventral; Saliva/análise; Estresse fisiológico; Unidades de terapia intensiva neonatal

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