# The Impact of Holding on Stress and Bonding in Mother-Infant Pairs during Therapeutic Hypothermia

Research Protocol | IRB Number: 1183063

**Introduction:** The inability to hold an infant being treated with therapeutic hypothermia in the neonatal intensive care unit has been subjectively reported by ours and other research groups as a significant source of stress for parents[1-7]. We aim to assess the impact of holding on endocrinological markers of stress and bonding. Specifically, we plan to collect salivary cortisol and oxytocin levels from infants undergoing therapeutic hypothermia and their mothers prior to and immediately after a 30-minute holding period. We anticipate elevated oxytocin and decreased cortisol levels as quantitative markers of increased bonding and stress reduction in the mother-infant dyad after holding.

**Background:** Therapeutic hypothermia is a standard of care intervention that has been consistently shown to increase disability-free survival in term infants with neonatal encephalopathy[8-13]. Infants treated with therapeutic hypothermia often have central lines, are on continuous electroencephalogram (EEG) monitoring, and are intubated. Due to concerns for dislodging equipment and potential for warming the infant, parents historically have not been permitted to hold their infants during therapeutic hypothermia. While therapeutic hypothermia has important neurological benefits to the newborn, prior research conducted with nurses and parents indicates that the experience is psychologically traumatic[1,2,4,5,14]. A parent's inability to hold their infant during therapeutic hypothermia is often cited as a major impediment to parent-infant bonding[2].

For prematurely born infants, not treated with therapeutic hypothermia, skin-to-skin care has emerged as a therapeutic intervention that substantially improves bonding between mothers and infants[15]. It has also been shown to have an analgesic effect on infants[16] and cortisol lowering effects[17] on mothers and infants. Likewise, oxytocin, a hormone produced by the pituitary gland and associated with maternal-infant bonding and stress reduction, has been shown to increase in mothers while they hold their infants[18]. Previous research demonstrates correlation between salivary and serum oxytocin levels[19]. Skin-to-skin care has led to reduced salivary cortisol levels and increased salivary oxytocin levels in 28 premature infants and their parents.[20]

Holding a newborn is a critical moment, described as the moment in which the new parent feels like a parent for the first time[2]. Holding a baby is a necessary precursor to feeding a baby which is a fundamental maternal instinct. Barriers to holding are perceived as barriers to feeding which can disrupt the maternal bond with an infant. In prematurely born infants, the inability to feed a baby is associated with a higher level of maternal stress[21]. High levels of stress matter because maternal depression and anxiety frequently occur in the NICU[22] and research on very preterm infants has shown alterations in language development and higher anxiety traits in children of mothers who expressed higher levels of stress[23].

Increased bonding and reduced stress may lead to improved neurodevelopmental outcomes for infants being treated with therapeutic hypothermia and lower rates of psychological trauma for mothers. We anticipate the sampling of salivary cortisol and oxytocin will quantitatively demonstrate an endocrinological biomarker for stress reduction and increased bonding for mother-infant dyads who are being treated with therapeutic hypothermia. The results of this study will support and promote protocolized holding interventions for infants during therapeutic hypothermia, in a similar vein as skin-to-skin care for the prematurely born patient population.

**Preliminary Data:** A mixed methods study completed by our team demonstrated feasibility of holding medically stable infants for 30 minutes during therapeutic hypothermia (manuscript under review at Acta Paediatrica). There were no reported adverse events during holding including dislodging of central lines or EEG

leads or early termination of holding due to vital sign or other infant instability. A thin foam barrier was placed between the mother and infant to prevent unintentional rewarming. The mean infant temperature prior to holding was 33.4ºC and at completion of holding the mean temperature was 33.5ºC (p=0.18). There was no significant bradycardia (heart rate less than 80 bpm), hypotension (mean arterial pressure less than 40 mmHg) or oxygen desaturation (less than 93%) (Figure 1). Mothers and nurses responded to survey questions with overwhelming enthusiasm for the perceived benefits of holding. Nurses reported that holding during hypothermia seemed safe. Additionally, mothers responded to our survey with reports of lower stress levels and improved ability to bond with their baby after holding.

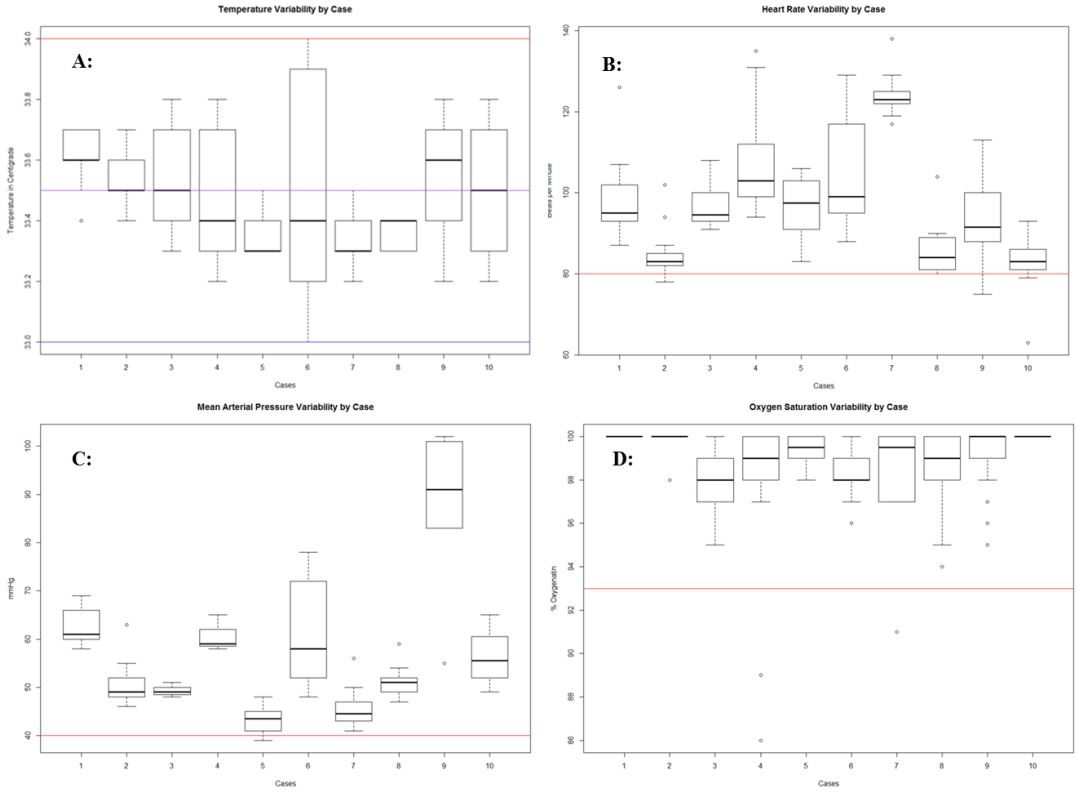



**Figure 1:** A: Temperature by case; B: Heart rate by case; C: Mean arterial pressure by case; D: Oxygen saturation by case; bold horizontal lines are the mean for the 30 minute period of holding for each case.

## Hypothesis

We hypothesize that measurable increases in salivary oxytocin levels, coinciding with the reported qualitative increased levels of bonding, will be observed after the holding period. We anticipate the reported stress reduction after holding to be quantified by measurable decreases in salivary cortisol levels. We hypothesize these hormone changes will be present in both the mother and the infant when compared to samples taken without the holding intervention.

**Primary outcome:** To compare the change in the level of oxytocin in maternal saliva between two conditions; one assessing the difference between oxytocin levels before and immediately after a 30-minute period of holding during therapeutic hypothermia compared to the second condition in which pre and post-levels of salivary oxytocin surrounding a 30-minute period of "not holding" are obtained.

**Secondary outcomes:**

1. To compare the change in the level of cortisol in maternal saliva between the same two conditions

2. To compare the change in the level of oxytocin and cortisol in infant saliva between the same two conditions

3. Comparison between the holding and "not holding" conditions of infant vital signs including temperature, heart rate, mean arterial blood pressure and oxygen saturation collected 30 minutes prior to holding, every 2 minutes during, and 45 minutes after holding.

4. Subjective maternal reports of the experience of holding the infant during hypothermia and responses to questions about stress level and bonding.

5. Subjective nurse report about the experience of supporting holding.

**Significance**

The subjective evidence of psychological stress for mothers in the neonatal intensive care unit (NICU) has been well-reported in prior research. This is exceptionally challenging for mothers of infants undergoing therapeutic hypothermia, who historically have not been permitted to hold their infants due to concerns for dislodging equipment and potential infant re-warming. Our previous research has demonstrated feasibility of holding infants during therapeutic hypothermia. This study aims to elucidate quantitative data to support holding as a potential standard intervention during therapeutic hypothermia. We aim to measure the proposed benefits of holding by using endocrinological biomarkers. Future research may use these biomarkers to noninvasively (saliva, not blood) assess the impact of stress and bonding on neurodevelopmental outcomes in infants treated with hypothermia, as well as a myriad of other clinically stressful scenarios in the NICU.

**Methods:**

Study Design: This is a single center, prospective randomized controlled crossover mixed methods study of the effect of a 30-minute holding intervention on salivary levels of cortisol and oxytocin in mothers and infants treated with therapeutic hypothermia.

Power Calculation: The power calculation was performed using the data from the Vittner paper[20] for our primary outcome, the maternal oxytocin response to holding. The maternal oxytocin level was chosen as the primary outcome for several reasons. First, we are confident in the ability to obtain salivary samples from an adult and second because several of the mothers in the pilot study reported symptoms during or after holding that would be consistent with increased oxytocin levels such as uterine contractions and/or increased breast milk production.

A sample size of 15 from a finite population of infants treated with hypothermia annually (N=40), will have 80% power to detect a difference in means of 113 pg/ml, (a first condition $\mu$=275 pg/ml and a second condition $\mu$=162 pg/ml) assuming a conservative estimate for standard deviation of 175 pg/ml using a paired t-test with a 0.05 two-sided level of significance. Of the 40 babies treated with therapeutic hypothermia each year, 25-33% are expected to meet exclusion criteria.

Location: A convenience sample of infants undergoing therapeutic hypothermia and their mothers will be recruited from the neonatal intensive care unit at Maine Medical Center.

Inclusion/exclusion criteria: Inclusion criteria will be; gestational age at birth of 35 weeks or greater, absence of clinical or electrographic seizures during the first 24 hours of therapeutic hypothermia, and designation as "clinically stable" by the attending neonatologist with the infant on room air, nasal cannula, continuous positive

airway pressure or intubated on conventional ventilator. Exclusion criteria will be use of inhaled nitric oxide for persistent pulmonary hypertension of the newborn, high frequency oscillator ventilation, presence of electrographic seizures, use of vasopressors or paralytic agents, presence of chest tubes, wound vacuums, or drains, and in utero opiate exposure.

Informed consent: Dr. Alexa Craig is the neonatal neurologist involved in the clinical care of all infants at Maine Medical Center treated with therapeutic hypothermia. Within this patient population, Dr. Craig will identify potential patients to recruit for the study. Dr. Craig will describe the research study to mothers of infants who meet the inclusion criteria. Therapeutic hypothermia treatment takes 72 hours to complete (three days). Drs. Craig, Fox or Utley will wait 24 hours into therapeutic hypothermia to determine if the infant meets all inclusion criteria and does not meet any exclusion criteria. At this time, Drs. Craig, Fox, Deerwester, or Utley will obtain a single signed informed consent from the mother on behalf of herself and on behalf of the infant. After randomization, a calendar of events will be created with mothers, NICU nurses and research personnel for the holding plan on day two and day three of hypothermia.

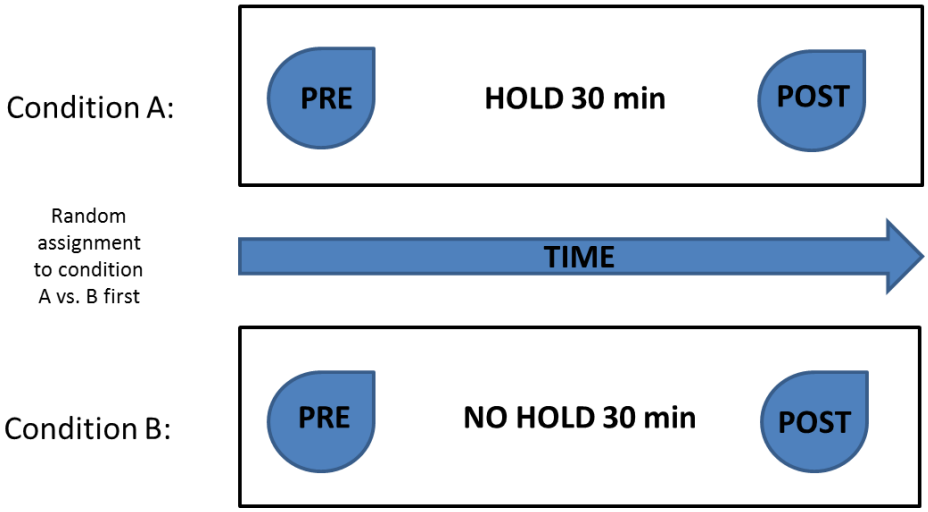
Assisting mothers to hold their infants is within the scope of practice and routine clinical duties for NICU nurses, including babies born prematurely (up to 23 weeks) and intubated babies. A discussion will take place between the NICU nurse and research personnel prior to enrolling a patient into this study. Any concerns or discomfort can be addressed, and no nurse will be obliged to participate in the study. Should a nurse decline to assist with holding a patient in this study, another nurse volunteer may step in. The patient's nurse will be expected to continue to care for the patient in all non-study related aspects. Nurses will go through an informed consent process before completing the questionnaire. There is no penalty for declining to complete the questionnaire.

Randomization: Infants and mothers will be randomly assigned to the holding condition occurring on the second versus the third day of hypothermia. A random number generator will be used with those numbers being translated into a list of sequentially numbered opaque envelopes that will be opened by the principal investigator (PI) in order at the time of randomization following the informed consent process (Figure 1).

Data Collection and Management:

After obtaining consent and randomization, a plan will be made with the mother and a discussion will take place regarding the timing of events. Mothers will be assisted with holding their infants for a single 30-minute period using the holding protocol developed for the feasibility study (Appendix A). Holding will occur between 9:00 am and 11:00am, to account for the diurnal variation of cortisol, on day two or day three of hypothermia treatment. Transitioning of the infant to the mother's arm will require the assistance of the NICU nurse caring for the baby and one other nurse. For condition A, four samples of saliva will be obtained; two from the mother (pre and post holding) and two from the infant (pre and post holding). The first sample will be collected prior to holding, and the second sample immediately at the end of holding before the infant is placed back in the bed (condition A). For condition B, four additional samples of saliva will be obtained; two from the mother (pre and post "not holding") and two from the infant (pre and post "not holding").

Figure 1: New Study Design. All infants exposed to both conditions but in a randomized order




All of the infants at Maine Medical Center treated with therapeutic hypothermia are recorded in the Maine Medical Center Neonatal Encephalopathy REDCap database (IRB NET976245-1). We will access the database for demographic and descriptive characteristics for the participants of this this study. Medical history will also be obtained from this database, including mothers' obstetric history and infants' medical history. This will be entered into a different REDCap database specific for this study.

A ten-question survey will be given to Mothers after holding. An informed consent form with be obtained and a de-identified 15-question survey will be given to nurses immediately after assisting with holding. Infant data and maternal surveys will be stored in a binder in a locked office in the neonatal intensive care unit or at 55 Spring Street, Scarborough, ME. The data will periodically be manually entered into the HIPPA compliant REDCap by co-investigators.

Safety: Infant vital signs will be continuously monitored during holding and "not holding" and recorded at the following intervals; 30 minutes prior to, every two minutes during, and 45 minutes after holding to monitor infant safety. Early termination of holding will occur if there is an adverse event as defined in the feasibility study. This includes a change in infant temperature greater than 0.5°C above or below 33.5°C. This cutoff is used based on historical data at our institution in which the infant core temperature during therapeutic hypothermia normally varies by up to 0.5°C above or below 33.5°C. Accidental dislodgement of central lines or disruption of EEG leads during holding will also be considered adverse events. Early termination of holding will occur if there is clinical instability of infant heart rate, blood pressure or oxygen saturation present for greater than 2 recorded measurements; infant bradycardia defined as heart rate less than 80 beats per minute[11], infant hypotension with mean arterial pressure (MAP) less than 40 mmHg[11], oxygen saturation of less than 93%[24], or evidence of clinical instability as determined by the NICU nurse and/or attending neonatologist.

Laboratory Studies**:** Saliva will be collected from mothers by passive drool method, the gold standard when collecting oral fluid for biological testing. Infant saliva will be obtained using a 1 mL syringe and suctioning saliva from the infants mouth until 0.1mL of volume is obtained. Research indicates that oxytocin increases in anticipation of breast-feeding and begins to normalize after 30 minutes[25]. Therefore, mothers will be asked to pump prior to the first saliva collection, which will allow prior anticipatory oxytocin increases to normalize. The collection will take place within the 30-minute window after pumping, prior to the rebound increase in oxytocin level. The mother will be asked not to eat, drink, smoke, or exercise for one hour before the study

session - all factors that confound salivary cortisol levels. Infants receiving therapeutic hypothermia are not fed by mouth so this issue will not impact infant subjects. The half-life of oxytocin has been shown to be 10-15 minutes[18]. The half-life for cortisol has been shown to be one hour[26]. After collection, the saliva will be mixed with RNAprotect® Saliva Reagent with cOmplete™ protease inhibitor, to stabilize the sample. It will then be frozen immediately at -20°C, transferred to Dr. Jill Maron's lab, and stored at -80°C, a temperature which has been shown to stabilize cortisol and oxytocin samples.[20,27,28] An enzyme-linked immunosorbent assay (ELISA) will be used to analyze cortisol and oxytocin concentrations in saliva[28] at Dr. Jill Maron's lab at Tufts in Boston. A premade salivary cortisol ELISA from ENZO Life Sciences (ADI-901-071) and a premade oxytocin ELISA from ENZO (ADI-901-153A) will be used. Samples will be run in triplicate. The cortisol and oxytocin levels will be tested in one batch at the completion of the study.

The sensitivity of the cortisol assay is 56.72 pg/ml. The intra- and interassay coefficients of variation are 6.6% and 7.8% respectively. Crossreactivities for the cortisol ELISA include: Prednisolone (122.35%), Corticosterone (27.68%), 11-deoxycortisol (4.0%), Progesterone (3.64%), Prednisone (0.85%), Testosterone (0.12%) and less than <0.10% androstenedione, cortisone and estradiol. The sensitivity of oxytocin assay is 15 pg/ml, without correcting for concentration. The intra- and interassay coefficients of variation are 10.2% and 16.5% respectively for our anticipated oxytocin concentration (100-300 pg/ml[11]). The oxytocin ELISA is reported to cross react with the following substances: mesotocin (7%), Arg-vasotocin (7.5%), Ser ,Ile -Oxytocin (<0.2%), TRH (<0.2%), Somatostatin (<0.2%), Met-Enkephalin (<0.2%), VIP (<0.2%), Lys -Vasopressin (<0.2%), Arg -Vasopressin (<0.2%), α−ANP (<0.2%), Growth Hormone (<0.2%), Tocinoic acid (<0.2%), and Melanostatin (<0.2%).

Data Analysis: Descriptive characteristics of infants and their mothers will be presented by percentages for categorical variables and by means and standard deviation (or non-parametric equivalent) for continuous variables. Salivary cortisol and oxytocin concentrations will be determined by comparing the optical density of the ELISA result to a control curve. A paired t-test will be used to assess for significant differences in hormone levels pre and post-holding. Responses to surveys will be described using proportions of "agree", "strongly agree", "disagree" and "strongly disagree."

**Potential Problems:** Our previous research demonstrated feasibility of holding clinically stable infants for 30 minutes during therapeutic hypothermia. Based on extensive experience of allowing mothers to hold critically ill premature infants, our NICU nurses developed and implemented a safe technique to transition hypothermic infants from the bed to their mother's arms without adverse events. The thermal barrier successfully prevented rewarming of the infant.

For this subsequent study on salivary biomarkers, we are confident in the ability to transition infants safely to holding, but acknowledge that problems could occur with saliva collection or storage, since these are the new components of the protocol. There is also the potential for difficulty in coordinating the timing of maternal pumping of breast milk and the collection of the first maternal "pre-holding" saliva specimen. We intend to address this with education and written instructions, as well as assistance from nursing staff. There may also be potential issues that arise around the availability and proper storage of dry ice. We will work with the Maine Medical Center patient laboratory to develop a specimen handling and storage protocol. The specimens, once collected, will be hand delivered to the lab for storage.

**Appendix A: Updated Safety Protocol**

REASON FOR RESEARCH:
Holding in normothermic infants has been shown to reduce stress hormones, decrease crying and irritability, regulate heart rate and breathing patterns, enhance mother-infant bonding, and provides more restful sleep patterns. This research project is aimed at understanding whether or not this holds true for hypothermic babies and their mothers.

OUTCOME MEASURE:
Maternal and Infant saliva will be collected before and after holding. Saliva will be tested for cortisol and oxytocin levels. We hypothesize that there will be a decrease in cortisol (marker of stress) and increase in oxytocin (marker of bonding) after the 30-minute holding period, when compared to samples obtained 30 minutes apart without holding taking place.

ELIGIBILITY CRITERIA:
- Infant with gestational age ≥35 weeks treated with therapeutic hypothermia
- Infant without seizures in the first 24 hours on EEG
- Infants who are clinically stable either intubated, on bubble CPAP, nasal cannula or who have no respiratory support. This criterion MUST be confirmed by the attending neonatologist.
- Infants with all types of vascular access are eligible.
    - Use appropriate caution and attention to peripheral arterial lines and central lines (PICC, UAC, UVC, tunneled central catheters)
- Informed consent for research study signed by mother who is at MMC.

EXCLUSION CRITERIA:
- High frequency oscillator ventilation
- Use of inhaled nitric oxide
- Persistent pulmonary hypertension of the newborn
- Presence of seizures on EEG
- Use of vasopressors or paralytic agents
- Presence of chest tubes, wound vacuum or drains
- Neonatal abstinence syndrome

GENERAL INSTRUCTIONS:
- Holding will occur exclusively as part of the research study.
- May ONLY be performed by an RN in conjunction with the research team
- Instruct the mothers to plan to stay in the NICU for a least one to two hours
- Only the mother can hold the infant during the research project.
- Instruct mother to take care of her own personal needs (i.e. bathroom) before holding. The mother will be asked not to eat, drink, smoke, or exercise for one hour before the study session. The mother will be asked to pump breastmilk within 30 minutes prior to holding.
- Encourage mothers to discuss their concerns regarding holding with their infant's nurse.

EQUIPMENT:
1. Comfortable chair/recliner
2. Research personnel will be present to assist the RN with transition of infant from Giraffe stand to mother's arms, monitor vital signs, and handle saliva specimens.
3. Pillows for support

4. Insulating barrier
5. Four saliva specimen collection kits for infant
6. Four saliva specimen collection kits for Mother

PROCEDURE FOR HOLDING INTERVENTION:

1. Assemble equipment
2. Obtain saliva for Mother and Infant per kit collection protocol.
3. Obtain initial set of vital signs (heart rate, respiratory rate, oxygen saturation, blood pressure and temperature). Record complete set of vitals 15 minutes into the 30-minute holding session. Record another complete set of vitals after holding session is over. Research team to record temperature and oxygen saturation every 2 minutes during holding. Temperature will be recorded from the esophageal probe.
4. Position reclining chair next to Giraffe stand.
5. Have mother sit in recliner and place thermal layer over her chest and abdomen
6. Transfer infant to mothers by placing hands under the cooling blanket in order to lift blanket and infant together
7. Support infant's head and lines immediately after transfer
8. Assess need for increased oxygen by monitoring the oxygen saturation
9. Offer pillows as necessary for additional support.
10. If the infant shows signs/symptoms of not tolerating holding (i.e. drop in oxygen saturation below 90% or if temperature increases greater than 1°C) STOP HOLDING and return infant to Giraffe stand. Continue to monitor cardiorespiratory status of the infant for 30 minutes following holding.
11. At the end of holding, prior to transferring infant back to Giraffe stand, obtain second saliva samples from mother and infant.

PROCEDURE FOR NO-HOLDING INTERVENTION:

1. Assemble equipment
2. Obtain saliva for Mother and Infant per kit collection protocol.
3. Obtain initial set of vital signs (heart rate, respiratory rate, oxygen saturation, blood pressure and temperature)
4. After 30 minutes, obtain second saliva samples from mother and infant.

DOCUMENTATION:

1. RN will document in EPIC the three sets of vital signs taken before, during and after holding session (HR, RR, BP, T, O2)
2. RN will document in EPIC note the length of holding time upon completion of session and any complications from holding

**References**

1. Craig A, James C, Bainter J, Lucas FL, Evans S, Glazer J. Survey of Neonatal Intensive Care Unit Nurse Attitudes Toward Therapeutic Hypothermia Treatment. *Adv Neonatal Care.* 2017;17(2):123-130.
2. Heringhaus A, Blom MD, Wigert H. Becoming a parent to a child with birth asphyxia-From a traumatic delivery to living with the experience at home. *Int J Qual Stud Health Well-being.* 2013;8:1-13.
3. Lemmon ME, Donohue PK, Parkinson C, Northington FJ, Boss RD. Communication Challenges in Neonatal Encephalopathy. *Pediatrics.* 2016;138(3).

4. Nassef SK, Blennow M, Jirwe M. Experiences of parents whose newborns undergo hypothermia treatment following perinatal asphyxia. *Journal of obstetric, gynecologic, and neonatal nursing : JOGNN / NAACOG.* 2013;42(1):38-47.
5. Thyagarajan B, Baral V, Gunda R, Hart D, Leppard L, Vollmer B. Parental perceptions of hypothermia treatment for neonatal hypoxic-ischaemic encephalopathy. *J Matern Fetal Neonatal Med.* 2017:1-7.
6. Craig AK, Gerwin R, Bainter J, Evans S, James C. Exploring Parent Experience of Communication About Therapeutic Hypothermia in the Neonatal Intensive Care Unit. *Adv Neonatal Care.* 2018;18(2):136-143.
7. Craig AK, Gerwin R, Bainter J, Evans S, James C. Exploring parent expectations of neonatal therapeutic hypothermia. *J Perinatol.* 2018.
8. Azzopardi D, Brocklehurst P, Edwards D, et al. The TOBY Study. Whole body hypothermia for the treatment of perinatal asphyxial encephalopathy: a randomised controlled trial. *BMC Pediatr.* 2008;8:17.
9. Azzopardi D, Robertson NJ, Cowan FM, Rutherford MA, Rampling M, Edwards AD. Pilot study of treatment with whole body hypothermia for neonatal encephalopathy. *Pediatrics.* 2000;106(4):684-694.
10. Gluckman PD, Wyatt JS, Azzopardi D, et al. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. *Lancet.* 2005;365(9460):663-670.
11. Jacobs SE, Berg M, Hunt R, Tarnow-Mordi WO, Inder TE, Davis PG. Cooling for newborns with hypoxic ischaemic encephalopathy. *Cochrane Database Syst Rev.* 2013;1:CD003311.
12. Shankaran S, Laptook AR, Ehrenkranz RA, et al. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med.* 2005;353(15):1574-1584.
13. Shankaran S, Pappas A, Laptook AR, et al. Outcomes of safety and effectiveness in a multicenter randomized, controlled trial of whole-body hypothermia for neonatal hypoxic-ischemic encephalopathy. *Pediatrics.* 2008;122(4):e791-798.
14. Lemmon ME, Donohue PK, Parkinson C, Northington FJ, Boss RD. Parent Experience of Neonatal Encephalopathy. *J Child Neurol.* 2017;32(3):286-292.
15. Tessier R, Cristo M, Velez S, et al. Kangaroo mother care and the bonding hypothesis. *Pediatrics.* 1998;102(2):e17.
16. Gray L, Watt L, Blass EM. Skin-to-skin contact is analgesic in healthy newborns. *Pediatrics.* 2000;105(1):e14.
17. Gitau R GX, Bond, C, et al. Acute effects of maternal skin-to-skin contact and massage on saliva cortisol in preterm babies. *Journal of Reproductive and Infant Psychology.* 2002;20(6):83-88.
18. Cong X, Ludington-Hoe SM, Hussain N, et al. Parental oxytocin responses during skin-to-skin contact in pre-term infants. *Early Hum Dev.* 2015;91(7):401-406.
19. Feldman R, Gordon I, Zagoory-Sharon O. Maternal and paternal plasma, salivary, and urinary oxytocin and parent-infant synchrony: considering stress and affiliation components of human bonding. *Dev Sci.* 2011;14(4):752-761.
20. Vittner D, McGrath J, Robinson J, et al. Increase in Oxytocin From Skin-to-Skin Contact Enhances Development of Parent-Infant Relationship. *Biol Res Nurs.* 2018;20(1):54-62.
21. Chourasia N, Surianarayanan P, Adhisivam B, Vishnu Bhat B. NICU admissions and maternal stress levels. *Indian J Pediatr.* 2013;80(5):380-384.
22. Segre LS, McCabe JE, Chuffo-Siewert R, O'Hara MW. Depression and anxiety symptoms in mothers of newborns hospitalized on the neonatal intensive care unit. *Nurs Res.* 2014;63(5):320-332.
23. Woodward LJ, Bora S, Clark CA, et al. Very preterm birth: maternal experiences of the neonatal intensive care environment. *J Perinatol.* 2014;34(7):555-561.
24. Poets CF. When do infants need additional inspired oxygen? A review of the current literature. *Pediatr Pulmonol.* 1998;26(6):424-428.
25. White-Traut R, Watanabe K, Pournajafi-Nazarloo H, Schwertz D, Bell A, Carter CS. Detection of salivary oxytocin levels in lactating women. *Dev Psychobiol.* 2009;51(4):367-373.
26. Mitchell A CJ, Yates C, et al. Challenges , Guidelines and Systematic Review of Salivary Cortisol Research in Preterm Infants. *e-journal of Neonatology Research.* 2012;2(1):44-51.

27. Neu M, Goldstein M, Gao D, Laudenslager ML. Salivary cortisol in preterm infants: Validation of a simple method for collecting saliva for cortisol determination. *Early Hum Dev.* 2007;83(1):47-54.
28. Grewen KM, Davenport RE, Light KC. An investigation of plasma and salivary oxytocin responses in breast- and formula-feeding mothers of infants. *Psychophysiology.* 2010;47(4):625-632.