Basal Salivary Oxytocin and Skin to Skin Contact among Lactating Mothers of Premature Infants

Jessica Marie Gordon

University of South Florida, jboricua1920@gmail.com

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Basal Salivary Oxytocin and Skin to Skin Contact among Lactating Mothers of Premature Infants

by

Jessica M. Gordon

A dissertation submitted in fulfillment of the requirements for the degree of Doctor of Philosophy Department of Nursing College of Nursing University of South Florida

Co-Major Professor: Maureen W. Groer, PhD, RN, FAAN Co-Major Professor: Denise Maguire, PhD, RN, CNL Melissa M. Shelton, PhD, RN, CNE Lance Wyble, MD, MPH

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ABSTRACT

This dissertation research explored mechanisms of human milk (HM) expulsion by describing the effects of skin to skin contact (SSC) on maternal basal oxytocin (OT) secretion among 20 premature mothers with hospitalized premature infants. This one-group, repeated measures design consisted of: 1) daily SSC with covariant data via self-report diary and 2) maternal salivary OT with and without SSC at 4 time points were collected over a 7 day time frame. Results indicate that mean levels of basal OT increase over time (M 234 pg/ml, SD 108 pg/ml time point 1; M 257 pg/ml, SD 125 pg/ml time point 3). Through multilevel model data analysis basal OT was found to have a meaningful amount of dependence on SSC frequency (t(16) = 6.389, p = < 0.001) and SSC duration (t(17) = 6.867, p = < 0.001) with coefficient estimates that indicate that basal OT exposed to 75-85 minutes of SSC per day are 92 pg/ml higher. These findings provide preliminary data that suggest that lactating mothers with premature infants sustain positive effects of SSC that increase basal OT secretion over time.
CHAPTER ONE:

INTRODUCTION

Introduction to the Problem

Infant mortality among the 3.6 million infant deaths reported by the World Health Organization (Robert et al., 2010) occurred within the first 28 days of life with nearly 50% within the first 24 hours relative to prematurity, infections and asphyxia. In the United States, medical advancements in neonatal resuscitation have improved survival rates of infants at birth. However, prematurity remains one of the top three leading causes of death annually (Mathew & MacDorman, 2011). Of the surviving premature infants, many are at high risk for comorbidities, such as necrotizing enterocolitis (NEC), sepsis, and impaired neurocognitive development (Gartner et al., 2005). Immediate postnatal care with an emphasis on neonatal warmth and early initiation of breastfeeding are effective interventions that reduce infant mortality and prevent co-morbidities of prematurity (Robert et al., 2010; Ganapathy, Hay, & Kim, 2012; Meier, Engstrom, Patel, Jegier, & Bruns, 2010). Compared to
conventional incubator care, skin to skin contact (SSC) equally stabilizes neonatal warmth and thermoregulation (Bystrova et al., 2003). However, SSC is more effective in positive breastfeeding outcomes (Aghdas, Talat, & Sepideh, 2013; Hurst, Valentine, Renfro, Burns, & Ferlic, 1997).

**Statement of the Problem**

Despite the evidence that supports the benefits of SSC and breastfeeding, SSC is seldom practiced in western cultures (Moore, Anderson, Bergman & Dowswell, 2012). In addition, breastfeeding rates for postpartum mothers of premature infants are statistically lower than women who birth healthy term infants. Mothers of premature infants who successfully initiate breastfeeding are less likely to sustain breastfeeding until neonatal discharge because of low human milk volume (Spatz, 2004; Zanardo et al., 2011).

Mechanisms that impact human milk volume are complex, with bio-behavioral underpinnings. Immediately after delivery, mothers with premature infants are often physically challenged by pregnancy complications, trauma associated with preterm delivery, and mentally challenged by barriers in privacy, overall concern for their infants’ health and emotional distress secondary to grief, loss or separation (Sisk, Quandt, Parson, & Tucker, 2010; Shah, Clements & Poehlmann, 2011). These
Compounding circumstances often induce additional psychological stressors that manifest maternal feelings of fatigue, anxiety, stress or even depression (Obeidat, Bond, & Callister, 2009; Morelius, Theodorsson, & Nelson, 2005; Flacking, 2007; Sisk et al., 2010; Shah et al., 2011). Physiologically, such bio-behavioral stressors negatively affect the physiology of human milk synthesis by inhibiting the secretion of lactogenic hormones necessary to stimulate lactogenesis (i.e. human milk synthesis) (Buhimschi, 2004; Dewey, 2001; Bruckmaier & Wellnitz, 2008; Ueda, Yokoyama, Irahara & Aono, 1994; Newton, M. & Newton, N. R., 1948).

During lactogenesis, oxytocin is an important hormone responsible for galactokinesis, a process in which human milk is ejected from the mammary gland (Buhimschi, 2004). A poor ejection reflex due to insufficient oxytocin secretion perpetuates a cycle that negatively affects the amount of human milk expelled from the mammary glands. This in turn leads to lactation failure due to inadequate human milk volume (Buhimschi, 2004).

An increase in oxytocin secretion can be triggered in response to non-noxious stimuli such as touch or massage among animal subjects (Uvnas-Moberg, 1996). Similarly, tactile stimulation via infant holding has been shown to enhance oxytocin secretion among lactating mothers of full term human
infants (Ueda et al., 1994). Additionally, tactile stimulation via direct SSC has been shown to positively influence human milk volume among mothers with preterm infants (Hurst, 2007; Hill et al., 2009). Conceivably, SSC can be an effective method that enhances both oxytocin secretion and human milk volume. However, the true clinical significance of the relationship between SSC and basal oxytocin levels on human milk volume have yet to be determined.

**Purpose of the Study**

The purpose of this research is to explore mechanisms of galactokinesis by analyzing the effects of SSC on maternal oxytocin basal salivary levels among lactating mothers with hospitalized premature infants. Findings from this research are expected to inform the long term research goal of a program of research on biobehavioral mechanisms of SSC on human lactation and maternal psychological health.

**Specific Aims**

The aims of this study are:

Aim 1: Explore the relationship between basal salivary oxytocin levels and amount of skin to skin contact (SSC) among lactating mothers with hospitalized premature infants.

Research Questions:
1a. Does maternal basal salivary oxytocin differ across 7 days in mothers before SSC?
1b. Does SSC frequency affect basal salivary oxytocin levels?
1c. Does SSC duration affect basal salivary oxytocin levels?

**Definition of Relevant Terms**

**Skin to Skin Contact (SSC)** occurs when a naked neonate is placed prone on his/her mother’s bare chest (Moore et al., 2012). The use of early SSC immediate post birth has been known to reduce infant mortality (Lawn, Mwansa-Kambafwile, Horta, Barros, & Cousens, 2010). Beyond the first hour of birth, intermittent and continuous SSC among premature infants regulates neonatal oxygen saturation, heart rate, breathing, glucose and assists transitioning neonates to breastfeed (Bergman, Linley, & Fawcus, 2004; Nyqvist et al., 2010).

**Lactogenesis** is a two stage process of human milk synthesis (Hurst, 2007). Stage I represents the onset of human milk synthesis initiated during the second trimester of pregnancy. Once the placenta is expelled after birth, progesterone levels decline rapidly and prolactin is secreted (Buhimschi, 2004; Nedkova & Tanechv, 1995). Within four days postpartum,
increasing prolactin levels then trigger the beginning of copious milk synthesis known as lactogenesis II (Hurst, 2007).

**Oxytocin** is a nine amino acid neuropeptide secreted from the posterior pituitary gland responsible for uterine contractility at birth and human milk ejection during lactation (Gabor, 2012). More recent studies among the social sciences have linked oxytocin as an important biomarker that facilitates parental affiliation and stress modulation within animal and human subjects (Feldman, Gordon, & Zagoory-Sharon, 2011. Synchronous maternal-infant behaviors found in these studies implicates that mothers who provide more affectionate tactile contact have an increase in oxytocin release following maternal-child interaction.

**Galactokinesis** is the process in which human milk is ejected from the mammary gland (Buhimschi, 2004). During breastfeeding, nerve impulses generated from areolar stimulation via massage and suckling activate the central nervous system to elicit the pulsatile release of oxytocin from the posterior pituitary (Matthiesen, Ransjö-Arvidson, Nissen, & Uvnäs-Moberg, 2001; Ueda, Yokoyama, Irahara, & Aono, 1994). Excreted oxytocin is then carried through the blood stream to the mammary gland, where oxytocin finally attaches to epithelial cell receptors located on milk-secreting alveoli in the milk ducts. This causes the milk duct cells to contract, resulting in expulsion of milk from the mammary gland (Hurst, 2007).
Assumptions

Mothers with premature infants in the neonatal intensive care unit experience stressors that trigger episodes of depression, anxiety (Carvalho, Linhares, Padovani, & Martinez, 2009; Padovani, Linhares, Pinto, Duarte, & Martinez, 2008; Ukpong, Fatoye, Oseni, & Adewuya, 2003) and elevated cortisol levels. Over time, the recurrent practice of SSC intervention has been shown to reduce maternal cortisol levels by 32% (Morelius et al., 2005). This evidence suggests that SSC can be considered a behavior that down regulates psychological stress responses. However, due to the complexity of such a phenomenon, inclusion of stress as a dependent variable is beyond the scope of this research and will not be measured. Nonetheless based on the SSC research discussed earlier by Morelius et al. (2005) it is assumed that post-partum mothers of premature infants are experiencing a high stress state prior to SSC intervention.

Significance

This study has a high level of significance for nursing science because it explores a new biomarker that will aid in describing the relationship between SSC and secretion of oxytocin lactogenic hormone. Secretion of oxytocin has been known to elicit maternal “feel good” responses that improve
maternal mood (Samuel et al., 2015). A new understanding of the relationship of SSC and basal oxytocin level is expected to emerge that adds to the understanding of the physiology of galactokinesis or human milk expulsion. These finding may indirectly explain how to sustain exclusive human milk feedings for premature infants. Immunologic benefits of breast milk, consumed by the premature infant via exclusive human milk feedings offers a significant contribution to neonatal health care outcomes by reducing the incidences of neonatal complications associated with premature delivery, such as sepsis, NEC, and death.

References


Flacking, R., Ewald, U., & Starrin, B. (2007). "I wanted to do a good job": Experiences of 'becoming a mother' and breastfeeding in mothers of very preterm infants after discharge from a neonatal unit. Social Science and Medicine, 64(12), 2405-2416. doi: 10.1016/j.socscimed.2007.03.008


CHAPTER TWO:
LITERATURE REVIEW

Objective

The objective of this chapter is to review and critique scientific evidence that supports current knowledge of skin to skin contact, oxytocin and human milk volume. First, an introduction to the topic with reference to breastfeeding rates among premature infants will provide data that supports the significance of the problem. Next, linkages between maternal-infant factors that contribute to insufficient human milk volume will be summarized based on an evidence based schematic. Additional biobehavioral factors that influence insufficient human milk volume will be further discussed to emphasize the compounding effects of the maternal-infant relationship on human milk synthesis. Physiologic effects of compounding factors among lactating women will be further analyzed to understand maternal stress responses that may inhibit lactogenesis and galactokinesis. Mediating effects of skin to skin contact (SSC) will be presented as a means to deregulate maternal stress responses in order to influence oxytocin and galactokinesis.
Concluding remarks will provide a summary that identifies gaps in the literature requiring further research and present a logic model that aims to fill the gaps by exploring the relationship between basal salivary oxytocin levels and SSC.

Literature considered for this review was extracted from PubMed, CINAHL and Web of Science databases utilizing a combination of keywords with “skin to skin contact”, “kangaroo care”, “breastfeeding”, “human milk volume”, “prematurity” and “oxytocin”. Three-hundred and ten abstracts were retrieved and reviewed for content. After excluding systematic reviews, print duplications, irrelevant literature and animal model designs, 31 studies were included for review.

**Human Milk Feedings and Premature Infants**

The American Academy of Pediatrics (2012) recommends exclusive breastfeeding for the first six month of life as the gold standard of nutrition for all newborns, including premature infants. Nutritional benefits of exclusive human milk feedings support optimal growth and development, while immunologic components reduce risks of necrotizing enterocolitis, sepsis and death (Gartner et al., 2011; Patel et al., 2013). Statistically, breastfeeding initiation rates for postpartum mothers of premature infants are significantly lower than women who birth healthy term infants and fewer than 50% of these mothers sustain
breastfeeding until neonatal discharge due to insufficient human milk volume (less than 500 milliliters per 24 hours 7-10 days post-partum) (Hill & Aldag, 2005; Hill, Aldag, & Chatterton, 1999; Spatz, 2004).

Insufficient human milk volume was identified as one of the leading causes for breastfeeding cessation by the Centers for Disease Control and Prevention (Li, Fein, Chen, & Grummer-Strawn, 2008) among 1,323 lactating mothers of full-term or late preterm infants weighing at least 5 pounds at birth. Self-report questionnaires completed by participants queried whether or not mothers had stopped breastfeeding or pumping for her infant. Those who answered yes were then asked to rate the importance of 32 reasons for breastfeeding cessation on a 4-point Likert scale ranging from not important to very important. The top seven factors that accounted for 54% of the motives for breastfeeding cessation included: (1) lactation issues related to latch-on or nipple/breast problems, (2) psychosocial reasons related to breastfeeding attitudes and social support, (3) nutritional concerns about milk supply, (4) lifestyle issues related to diet, smoking, and autonomy, (5) medical reasons related to the mother's or infant's illness, (6) milk pumping concerns related to mothers willingness or accessibility to express breast milk, and (7) self-weaning issues related to biting or losing interest. Regardless of the weaning age, the most consistent
reason for breastfeeding cessation of the top seven factors among 43-55% of all participants included perceptions of insufficient milk supply.

**Insufficient Human Milk Volume Schematic**

Dewey (2001) links perceptions of insufficient milk supply to biologic and behavioral stressors that influence the physiology of human milk synthesis in a schematic that identifies two causative pathways that influence insufficient human milk volume related to the birthing process. The first pathway originates with mode of delivery (e.g. vaginal vs. cesarean birth), while the second originates with medications necessary for birth (e.g. epidurals). Conceptually, adverse birthing outcomes that influence the mode of delivery also influence medications necessary for birth, likewise, medications that are not tolerated by the fetus during the labor influence the mode of delivery. Secondary stressors that are precipitated by mode of delivery and medications include duration/difficulty of labor and infant suck reflex. In response to these stressors, human milk production is compromised because of a delay in breastfeeding onset or nipple stimulation. Effects of a suppressed suckle-induced hormone response lead to insufficient human milk volume until mechanisms of human milk synthesis
(lactogenesis) and human milk ejection (galactokinesis) are restored.

In addition to these pathways, Dewey (2001) further describes how the reciprocal nature of maternal-infant breastfeeding relationship inversely influences human milk volume. For example, early gestational age impacts the infant’s suckling behavior at the breast. Ineffective suckling of the breast can trigger a delayed onset of milk synthesis which can inversely cause the mother to be stressed resulting in insufficient human milk volume. These compounding effects can negatively affect the physiology of lactogenesis and galactokinesis (Table 1).

McNeilly, Robinson, Houston, and Howie (1983) measured physiologic effects of infant suckling behavior in a small sample. One group included five women in early stages of lactogenesis and the second group included five women in later stages of lactogenesis. Among all women, oxytocin was found to be released in a pulsatile manner during suckling with higher concentrations three to 10 minutes before suckling began. Stimuli that triggered the release of oxytocin were in response to the baby crying (n=5), the baby becoming restless in expectation of the feed (n=3) and as the mother prepared for the feeding (n=2). Stimulation of the nipple during suckling was the only stimuli that solicited prolactin release. These results
indicate milk ejection reflex, with release of oxytocin, occurs in most women before the tactile stimulus of suckling with a second release of oxytocin in response to the suckling stimuli itself. Implications of the study indicate that lack of suckling stimulation or ineffective suckling behavior can delay the release of lactogenic hormone and result in a poor milk ejection reflex. Results also implicate that breastfeeding mothers should be protected from stress during suckling and immediately before nipple stimulation, when a conditioned release of oxytocin is expected to occur to prevent the inhibition of oxytocin secretion (McNeilly et al., 1983).

Additional Factors that Influence Human Milk Synthesis & Volume

Multiple maternal-infant factors influence human milk synthesis (Table 2). Biologic factors that adversely affect the physiology of human synthesis and ejection reflex include: parity, mode of delivery, body mass index, comorbidities, smoking, medications, breast anatomy, stress, anxiety, infant gestational age and weight (Fuertes, Santos, Beeghly, & Tronick, 2006; Gizzo et al., 2012; Handlin et al., 2012; Hill et al., 1999). Behavioral factors that indirectly impact human milk synthesis include: maternal self-efficacy and motivation to breastfeed, nursing frequency, method of nipple stimulation, use of supplements or pacifiers, social support and infant

Psychosocial constructs and social determinants also highly impact breastfeeding duration in addition to maternal perception of human milk volume (Li, Fein, Chen, & Grummer-Strawn, 2008). Hispanics and mothers with household incomes significantly lower than the federal poverty level are most likely to stop breastfeeding prematurely. Other social determinants include maternal demographics like women of younger age, unmarried, primiparous, lower education, enrollment in government women infant and children nutrition programs (WIC) programs, and a resident of the Midwest or the South (Li, Fein, Chen, & Grummer-Strawn, 2008).

**Physiologic Effects of Biobehavioral Factors that Influence Human Milk Synthesis & Volume**

As mentioned in Dewey’s (2001) schematic, compounding effects of biobehavioral factors related to the maternal-infant breastfeeding relationship induce psychologic stress responses. Zanardo et al. (2011) addressed psychological distress on lactation performance in mothers with late preterm infants. This study enrolled 42 mothers with singleton infants born 34-36 weeks gestation. After consent, participants completed a self-report survey identifying select socio-demographics, feeding
preference, psychosocial care, pre and post pregnancy. Each participant was interviewed for 30 minutes 3-4 days postpartum to complete three validated questionnaires: (1) State-Trait Anxiety Inventory (STAI), (2) the Edinburgh Postnatal Depression Scale (EPDS) and (3) the Psychological Stress Measure. Data analysis revealed that mothers who delivered late preterm infants had significantly lower breastfeeding rates had significantly higher levels of depression, stress, and anxiety compared to mothers of term infants. While this data helped identify that psychological stressors induced by physiologic (pain) or emotional (anxiety) measures negatively affected breastfeeding, the inclusion of lactogenic biomarkers limits the knowledge gained about the relationship of psychologic stressors on lactogenic hormone secretion, human milk synthesis and perhaps human milk volume.

Animal model research designs were the first to identify that hypothalamic-pituitary axis (HPA) reactivity to physical and emotional stressors were lessened during lactation (Neumann et al., 1998). Tu, Lupien and Walker (2006) tested this hypothesis among human subjects by investigating HPA responsiveness related to feeding methods and psychological stressors among human subjects. Sixty-six women enrolled were in between 5-20 weeks postpartum. Participants were asked to participate in two stress exposure sessions. One was the
standard Trier Social Stress Test and the other an emotional video evoking threats to children. Participants that were breastfeeding ended up with reduced responsiveness to the Trier Social Stress Test and child-related stressors. Tu and Lupien (2006) hypothesized that such findings may indicate that changes in neural mechanisms that modulate the HPA axis during pregnancy and lactation desensitize stress circuits, thus reducing the overall stress induced cortisol secretion.

Groer and Davis (2006) concur with the desensitization and down-regulated HPA stress response phenomenon, but perceived the phenomenon as a protective measure in lactating women. This was evidenced by their cross sectional study that compared mothers exclusively breastfeeding verses formula feeding. Among formula feeding mothers, biomarkers (serum levels of interferon-gamma and interleukin-10) were decreased in addition to elevated perceived stress, mood and negative life experience.

Given that lactogenic hormone secretion (oxytocin) depends on the activation of the HPA, it is possible that desensitization may negatively affect lactogenic hormone secretion from the posterior pituitary gland. As early as 1948, Newton and Newton identified inhibitory effects of lactogenic hormone secretion in response to acute stressful stimuli. Ueda et al. (1994) evaluated implications of acute stress among 22 exclusively breastfeeding women. Participants were assigned to
one of three groups categorized on external stress exposure. Group 1 was the control group in which there was no external stress exposure, group 2 was the mental stress group in which participants were exposed to cognitive stressors of verbal math problem calculations, and group 3 was the noise stress group in which participants were exposed to sounds of building construction at a mean of 70 decibels. Serum oxytocin samples were collected every 2 minutes before and after breastfeeding and during measuring milk transfer pre and post weights. Frequency of pulsatile release of oxytocin was significantly lower in both stress exposed groups. These findings support the hypothesis that stress negatively affects lactogenesis by inhibiting oxytocin release during galactokinesis. Further applications of Ueda et al.’s (1994) findings speculate that suckling induced oxytocin release may be regulated by the central nervous system; indicating that psychological relaxation may be essential for an adequate milk ejection let-down response.

**Mediating Effects of Skin to Skin Contact on Stress Responses**

SSC has been known to promote psychological relaxation by mediating stress and anxiety through attachment and bonding behaviors (Dalbye, Calais, & Berg, 2011; Tessier et al., 1998). In response to stressful stimuli, Heinrichs et al. (2001)
enrolled forty-three lactating women. Prior to stress exposure one group was asked to breastfeed and the other to solely hold their infants for a 15 minute period. Thirty minutes prior the intervention, participants were exposed to a brief psychological stress test measuring anxiety, known as the Trier Social Stress Test. In this procedure, participants were asked to perform or speak in front of a panel and then asked to verbally answer a mathematical question. Salivary or serum cortisol levels were collected pre, post and during the stress test respectively. No differences in baseline cortisol levels were identified between the groups before the stress test. In response to stress exposure, adrenocorticotropic hormone, total plasma cortisol, salivary free cortisol, norepinephrine, and epinephrine were significantly increased in all lactating women \((p < 0.001)\). However, total plasma cortisol and salivary free cortisol responses to stress were less among breastfeeding women \((p = 0.001 \text{ and } p = 0.067)\). Additionally, both breastfeeding and holding interventions led to decreased anxiety \((p < 0.05)\), whereas, stress exposure worsened psychologic symptoms of mood, calmness, and anxiety in total \((p < 0.001)\). Researchers concluded that breastfeeding and tactile stimulation via holding interventions may be further explored as effective mechanisms applicable for reducing psychological stressors.
Among premature infants, SSC mediates neonatal stress responses up to 6 hours post extra-uterine life by stabilizing cardiopulmonary function (Bergman, Linley, & Fawcus, 2004). SSC also mediates breastfeeding outcomes by facilitating priming of the olfactory neuro-pathway (Winberg & Porter, 1998). Like other mammals, newborns exhibit primitive innate reflexes to self-attach to their mother’s nipple. Immediately after birth, newborns take up to 60 minutes to go through nine phases that lead them to self-attach to the breast (Widstrom et al., 2011). Cognitively, preterm infants may not have the capacity to self-attach to the breast but are able to gradually transition to breastfeed with the practice of SSC intervention (Spatz, 2004). SSC position gives neonates the opportunity to smell the scent of their mother’s milk due to the close proximity to the breast. Breast odors from the mother exert a pheromone-like effect where the newborn learns to recognize their own mother's unique odor and locate the mother’s nipple to initiate breastfeeding (Winberg & Porter, 1998). By the time premature infants are ready to be discharged home, premature infants exposed to SSC repeatedly verses infants exposed to conventional incubator care with SSC are more likely to receive exclusive breastmilk feedings (Ghavane et al., 2012). However, due to multiple confounders (neonatal environments, maternal-infant separation, gestation
age, breast pumping frequency, etc) further correlative studies are warranted to fully understand how and why SSC promotes positive breastfeeding outcomes. Further understanding of the relationship of oxytocin secretion and SSC frequency and duration is expected to add to this knowledge.

**Oxytocin and Skin to Skin Contact**

Research synthesized by Kerstin Uvnas-Moberg (1998) suggests that oxytocin plays a role in positive social interaction, stress regulation and neuroendocrine functionality necessary for motherhood and lactation. In this framework positive social interactions between the mother and the newborn are expected to begin immediately after birth where the mother is expected to provide milk, warmth, and protection of her offspring. During this early period of social interaction, oxytocin performs two functions. First it allows for milk to be ejected in response to non-noxious stimuli like infantile suckling. Second it transmits warmth to her young by vasodilating blood vessels in the skin that overlay the mammary gland (Uvnäs-Moberg, 1996). Reciprocal effects of infant social behaviors like breastfeeding and SSC activate maternal touch and smell somatosensory afferents that promote antistress effects that reduce blood pressure (Handlin et al., 2012) and cortisol (Handlin et al., 2009) and promote sedative effects that solicit
a calm mood and less anxiety (Nissen, Gustavsson, Widstrom, & Uvnas-Moberg, 1998; Uvnas-Moberg, 1998b). Physiologically, Uvnas-Moberg supports oxytocin’s antistress and sedative properties by emphasizing that the hormone is produced in the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus. When triggered, oxytocin is released into circulation from PVN neurons found in the pituitary. Beyond the HPA, PVN neurons extend to many other areas like the limbic system, vagal motor and sensory nuclei, spinal cord and preganglionic sympathetic neurons. Receptors that enable oxytocin to act have been found in the uterus with effects that activate the central nervous system (Uvnäs-Moberg, 1996). This allows oxytocin to have multiple functions beyond milk ejection, like stress modulation.

As mentioned previously, antistress effects (Uvnas-Moberg, 1998a) that result in positive mood and decreased anxiety have also been found among mothers with premature infants through tactile stimulation (Ahn, Lee, & Shin, 2010; Holditch-Davis et al., 2014). Additionally, reciprocal effects of maternal-infant behaviors like SSC positively influence human milk volume and lactation (Acuna-Muga et al., 2014; Chatterton et al., 2000; Heidarzadeh, Hosseini, Ershadmanesh, Gholamitabar Tabari, & Khazaee, 2013). To evaluate effects of SSC on human milk volume, Hurst et al. (1997) conducted a retrospective chart review
comparing 24 hour milk volumes of mothers of ventilated low birth weight infants compared to mothers without SSC intervention. Mean 24 hour milk volumes at 2, 3, and 4 weeks after delivery were compared with those of a retrospective control group from the 12 month period immediately preceding the introduction of SSC in the neonatal intensive care unit. Data analysis revealed a strong linear increase in milk volume in the SSC group compared to no change in milk volume in the control group.

These findings speculate that skin to skin contact and oxytocin release are positively correlated. Similarly, oxytocin is thought to be positively correlated with human milk volume because it is essential for the expulsion of human milk from the mammary gland in lactating mothers. While it may be safe to assume that SSC positively correlates with human milk volume, dose responses of human milk volume relative to SSC frequency, duration and oxytocin release have yet to be determined. Restrictions for measuring plasma oxytocin levels require the use of multiple invasive blood draws or an indwelling line due to its pulsatile nature. To circumvent these constraints, the use of salivary oxytocin has recently gained popularity (Horvat-Gordon, Granger, Schwartz, Nelson, & Kivlighan, 2005). Further exploration of basal salivary oxytocin will contribute to the reliability of salivary oxytocin in research and enhance the
knowledge of biobehavioral science associated with oxytocin secretion and SSC.

Summary

Mothers of premature infants experience immediate maternal-infant separation, delayed onset of breastfeeding, poor suckling and biologic effects of maternal stress and anxiety. Compounding effects of these stressors inhibit the secretion of oxytocin and affect the ejection reflex. A poor ejection reflex perpetuates a cycle that negatively affects the amount of human milk expelled from the mammary glands. This in turn leads to altered human milk synthesis and insufficient human milk volume.

Mediating interventions aimed to repair human milk synthesis and galactokinesis like skin to skin contact is suspected to ameliorate maternal psychological stress responses and improve the amount of oxytocin secreted yielding more milk ejected from the mammary gland. However, the true relationship between SSC and basal oxytocin levels have yet to be determined to fully understand the clinical significance of SSC on human milk volume. The proposed logic model (Figure 1) aims to fill this gap by determining how SSC frequency and duration influence basal oxytocin and other co-variants like human milk volume under similar conditions.
References


### Table 1. Physiology of Lactogenesis and Galactokinesis

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Process</th>
<th>Trigger</th>
<th>Physiology</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lactogenesis</strong></td>
<td>Lactogenesis is a two stage process</td>
<td>Infant Suckling</td>
<td>After birth, the placenta is expelled and progesterone levels decline rapidly. This abrupt withdrawal of progesterone removes the inhibitory effect of progesterone and triggers lactogenesis in the presence of high plasma concentrations of prolactin secreted from the anterior pituitary.</td>
</tr>
<tr>
<td></td>
<td>Stage I - The onset of human milk synthesis during the second trimester of pregnancy.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stage II - Within four days postpartum, increasing prolactin levels result in copious milk synthesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Galactokinesis</strong></td>
<td>Galactokinesis is a process responsible for the ejection of human milk from the mammary gland</td>
<td>Infant Suckling, Conditioned Responses like infant crying prior breastfeeding, Breast massage</td>
<td>In response to infant suckling, impulses from sensory stimulation of nerve terminals in the areola travel to the central nervous system triggering a pulsatile release of oxytocin from the posterior pituitary. Oxytocin is then carried through the blood stream to the mammary gland where it interacts with specific receptors on the epithelial cells. Oxytocin binds to receptors located on milk-secreting alveoli in the ducts, initiating contraction of the cells, which resulting in expulsion of milk from the gland.</td>
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</table>
Table 2. Biobehavioral Factors that Influence Human Milk Volume

<table>
<thead>
<tr>
<th>Psychologic</th>
<th>Physiologic</th>
<th>Behavioral</th>
<th>Biologic</th>
<th>Social Determinants</th>
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</thead>
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<td>Grief</td>
<td>Mode of Delivery</td>
<td>Bond/Attachment</td>
<td>Gestational Age</td>
<td>Race</td>
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<tr>
<td>Anxiety</td>
<td>Delivery Trauma</td>
<td>Nipple Stimulation</td>
<td>Maternal Age</td>
<td>Economic Status</td>
</tr>
<tr>
<td>Fatigue</td>
<td>Comorbidities</td>
<td>Feeding Frequency</td>
<td>Parity</td>
<td>WIC* Participant</td>
</tr>
<tr>
<td>Stress</td>
<td>Endocrine Deficiency</td>
<td>Motivation</td>
<td>Body Mass Index</td>
<td>Transportation</td>
</tr>
<tr>
<td>Separation</td>
<td>Medications</td>
<td>Social Support</td>
<td>Breast Anatomy</td>
<td>Education Level</td>
</tr>
<tr>
<td>Pain</td>
<td>Hemodynamic Status</td>
<td>Infant Temperament</td>
<td>Suckle Reflex</td>
<td>Marital Status</td>
</tr>
<tr>
<td></td>
<td>Cardiac Disease</td>
<td>Formula</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Supplementation</td>
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<tr>
<td></td>
<td></td>
<td>Pacifier Usage</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*(WIC) Women Infant and Children Government Nutrition Program
Figure 1. Logic Model: Salivary Oxytocin and Skin to Skin Contact among Lactating Mothers of Premature Infants Model
CHAPTER THREE:

METHODS

Design

This descriptive, repeated measures design explored the relationship between basal salivary oxytocin and SSC among lactating mothers with hospitalized premature infants.

Setting

BayCare Health System is a community based health care system made up of 14 hospitals that serve the Tampa Bay, Saint Petersburg and Clearwater areas. Morton Plant Mease (MPM) Hospital is a 687-bed facility with over 25 specialty services located in Clearwater, Florida. Of these specialty services, obstetric services account for over 3,000 deliveries per year with a 10 bed, semi-private room level II Neonatal Intensive Care Unit (NICU). St. Joseph’s Women’s Hospital (SJWH) is the largest birthing facility within the BayCare network. It is located in Tampa, Florida and accounts for over 6,000 births annually with 49 Level III private room infant beds and 15 Level
II private room infant beds. Both facilities are fully staffed with a neonatologist or nurse practitioners that tend to the NICU 24 hours a day. Mothers with infants admitted to the NICU at both facilities have high breastfeeding initiation rates close to 95%. Sufficient lactation support is available to NICU mothers at SJWH and MPM with daily lactation consultants, adequate hospital grade breast pumps and highly skilled nursing staff trained in lactation support. In addition to lactation support, SSC is routinely practiced within the guidelines outlined by the BayCare SSC evidence based clinical standards and protocols. In addition, MPM and SJWH utilize the BayCare network electronic medical record (EMR) that may be helpful for the research team to verify demographic and outcome measures.

Participants

Twenty lactating mothers and their premature infants born 37 weeks and 0 days – 22 weeks and 0 days of gestational age were enrolled in the study.

Inclusion Criteria

Postpartum lactating females that: 1) are 0 – 7 days postpartum, 2) intend to breastfeed or breast pump, 3) initiate breastmilk expression within 12-24 hours after birth, and 4) have performed SSC with her neonate at least once since delivery
were included in the study. In addition to this inclusion criteria, participants were able to speak and read English or Spanish and have access to a telephone with mode of transportation to and from the hospital until infant discharge. Neonatal infants born 37 weeks and 0 days – 22 weeks and 0 days of gestational age admitted to the NICU were enrolled into the study after parental consent.

Exclusion Criteria

Mothers with maternal diagnoses or behaviors contraindicated in breastfeeding, polydrug use, human immunodeficiency virus (HIV), galactosemia, active tuberculosis (TB), radioactive isotopes, chemotherapy, or active herpes lesions on the breast(2012) or taking medications that may affect salivary oxytocin assay like antidepressants, psychotropic or dopamine agonist/antagonist medications were excluded from the study. Premature infants unable to perform SSC (e.g. umbilical intravenous lines, terminal conditions, surgical emergencies and congenital anomalies) were also excluded from the study.
Measures

Demographic Data and Medical History

Standard socio-economic demographic and medical history data (Appendix A) were collected at baseline in English or Spanish by self-report and verified with the EMR for neonatal and maternal subject information. A chart audit was completed to update demographic or medical history information at all data collection time points (baseline, session 1, and session 2).

Variables

Independent Variable (Skin to Skin Contact)

SSC frequency and duration was documented by each mother in a daily tracking log (Appendix B). Metrics were recorded in minutes per SSC session with start time, stop time, and daily 24 hour total minutes. Additional co-variants, such as infant gestational age, weight, feeding method (bottle, tube feeding, breastfeeding), and nutrition source (breastmilk, formula, donor milk or mixed) and nipple stimulation (pumping or breastfeeding) were also documented in the tracking log by self-report.

Dependent Variable Collection (Salivary Oxytocin)

Salivary samples were collected at 4 time points per participant by the drool method (Appendix C) and handled with the specimen handling procedure in Appendix H. Sample collection
was timed for 5 minutes by participants at each time point and a protease inhibitor (aprotinin) was immediately added prior to centrifugation, aliquotted and frozen in a freezer located in the hospital at -60°C. Frozen samples were batched once a month and then transported to the College Of Nursing Biobehavioral laboratory in a cooler and stored at -80°C. After specimens were thawed and dried down, salivary samples were prepared for assay utilizing the enzyme-linked immunosorbent assay (ELISA) kit manufacture procedure (Enzo Life Sciences Product. Catalog Number ADI-900-153). Each dried sample was reconstituted in assay buffer to concentrate the sample and assayed in duplicate. The assay protocol utilizes a polyclonal antibody to competitively bind oxytocin prior to incubating specimens overnight at 4°C. After incubation, excess reagents were washed and the enzymatic reaction was stopped and read at 405nm immediately. Oxytocin concentration was calculated by immunoassay software utilizing a logistic curve fitting program.

**Procedures**

**Approvals**

Institutional Review Board (IRB) approvals from University of South Florida and BayCare Health System at Morton Plan Mease-Saint Anthony Hospital and St Joseph’s Women’s Hospital was obtained prior to enrolling participants.
Consent, Recruitment and Screening

Twenty-five postpartum lactating mothers were screened for inclusion criteria (Appendix D) by collaborating co-investigators (neonatologist and advanced practice nurses) within 24-48 hours post-delivery and prior to maternal discharge from the hospital. Mothers that met inclusion criteria (N = 20) were approached by co-investigators to evaluate interest in enrollment. Mothers who expressed interested in enrolling in the study were referred to the Principal Investigator (PI) by co-investigators or given a recruitment flyer (Appendix E) to contact the PI directly. The PI provided additional information at the patient bedside prior to obtaining informed consent. Mothers were oriented to the study protocols and consent document by the PI in a quiet and private area using clear and simple language. Adequate time to read the informed consent document was given to allow for all questions to be answered. Comprehension of the research protocol and consent was ensured by querying the individual in regards to salient talking points in the checklist provided in Appendix G. After consent, participants were supplied with data collection materials and assessed for demographic and medical history data. A $20 gift card and honorarium receipt (Appendix I) was offered for participation of the study upon completion to reduce attrition from the study.
Data Collection Procedure

Data collection among participants included the following intervals: baseline, session 1 and session 2. Baseline data collection began on the day of consent where an overview of the benefits of SSC and hospital protocol was provided concurrent with critical study elements defined in the consent form. After obtaining consent, participants were supplied with data collection materials, maternal and infant demographic/medical history questionnaire, instructions on SSC, breastmilk expression and drool method procedures for salivary oxytocin during session 1 and session 2. At the end of session 2 the PI reconciled and compiled the participant’s tracking log data and updated maternal and infant medical history information verbally or via hospital EMR.

Skin to Skin Contact

Mothers were asked to self-report SSC data in their tracking log immediately after consent for up to 7 days depending upon neonatal discharge from the hospital. The PI screened for missing data by verifying entries of SSC frequency/duration and co-variant data after session 1 and session 2 with the mother. Missing data was corrected by the
mother or PI by the end of session 2. A summary of data collection procedures has been provided in Table 3.

Salivary Oxytocin

Samples were collected at 4 time points for all lactating mothers. Participants were asked to arrive at the NICU at 9:00 am each morning of saliva sample collection. Mothers were instructed to eat breakfast from 7:00 – 8:30 am and not to eat, brush, floss or use mouthwash one hour prior to collection. Participants were also notified to avoid caffeine, alcohol, and nicotine smoke and to report any extreme tooth decay or gingivitis. Immediately prior to collecting salivary samples, participants were asked to rinse their mouth with water to remove any food particles. To avoid potential hormone variations relative to diurnal rhythm, samples were collected no earlier than 9:00 am. Salivary oxytocin has been found to be elevated within 30 minutes post breast pumping/breastfeeding or during pumping/breastfeeding (Rosemary et al., 2009). In keeping with these parameters, basal salivary samples were collected prior placing the neonate SSC at least 30 minutes post milk expression or breastfeeding to avoid suckle induced oxytocin release. Samples collected with any nipple stimulation or suckling during SSC was documented on the daily tracking log and considered for
recollection if feasible. Specimens collected under SSC intervention were collected within the last 5-10 minutes of SSC while the infant is still being held by the mother (i.e. 55-60 minute time point of SSC) due to oxytocin’s pulsatile nature and short-half-life.

**Data Analysis**

Power analysis for this exploratory research was not feasible because statistical data on basal oxytocin in comparison to SSC has never been reported. MPM NICU census estimates 20 premature infants were admitted per month with approximately 50% eligible for SSC with a length of stay of seven to fourteen days. Feasibly, these admission rates indicate that 25 mother-infant dyads (5 per month) could be recruited over a six to eight month study period with the anticipation that a minimum of 20 (80 observations = 40 with no SSC and 40 post SSC) would continue through the study for the 7 day time frame.

**Preliminary Analysis**

For testing Specific Aim 1: Explore the relationship between oxytocin and SSC among lactating mothers with hospitalized premature infants, the basal oxytocin measured by saliva sample with and without SSC on each day was computed to
form a new measure. The two differences were used as the repeated measures of the true difference variable. A mixed effects model that fits the true difference variable in the previous step as the dependent variable with other controlled variables including frequency and duration of SSC as the independent variables was determined by the lowest Akaike information criteria (AIC).

Additional Analysis

Further analysis for Specific Aim 1 utilized paired sample t test to compare within-session and within-subject data to determine acute and sustained effects of SSC on basal oxytocin. Acute effects were assessed by determining within-session effects by analyzing the difference between the with and without oxytocin measures per session 1-2. Sustained effects were analyzed by the single measure within-subject data to determine the difference between two time point changes in salivary oxytocin (i.e. session 1 and session 2) over time per subject.

Limitations

Recruitment limitations secondary to the complexity of consenting premature mothers due to environmental and emotional stressors were avoided at time of consent with the inclusion of language and mode of transportation. Additionally, while most
mothers are discharged from the hospital within 2-3 days post-delivery, many return to visit their infants daily to administer hands on care and promote bonding. Offering an honorarium to participants also minimized enrollment limitations by reducing attrition from the study.

Protection of Human Subjects

Human Subjects Involvement, Characteristics and Design

Twenty-five lactating mothers and their premature infant were screened for eligibility for the study. Mothers and infants with maternal diagnoses/behaviors contraindicated in breastfeeding were excluded from the study as well as premature infants unable to perform SSC. Collaborating neonatologists and clinical nurses trained on the study protocol recruited participants prior to meeting the PI. The PI made weekly contact with mothers who consented to monitor compliance of SSC self-report, assure accuracy of salivary sample collection and encouraged continued participation of the study. Confidentiality was maintained by removing identifying information, assigning study identification numbers to all data and securing the code in a locked cabinet. Sources of maternal demographic and medical history data were abstracted from the medical record and documented on case report forms (CRF). Saliva samples and CRFs were only labeled with the subject number and only the PI could
link the study subject with the sample. Data maintained electronically was password protected.

**Potential Risks**

The risks of performing SSC and collecting salivary samples were minimal. Before and after SSC, electrocardiogram and pulse oximeter devices monitored neonatal heart rate, respirations and oxygen saturation. On the occasion that the neonatal vital signs range outside of normal parameters, i.e. bradycardia or oxygen desaturation, will trigger alarms for the attending practitioner or bedside nurse to respond and assess the situation.

**Inclusion of Women and Minorities**

Women were included in the study. Twenty mothers and their premature infant born 37 weeks and 0 days – 22 weeks and 0 days of gestational age were enrolled. Presumably, infants enrolled in the study were of the same ethnicity as their mother. Targeted distribution of subjects included mothers of all ethnicities and infants of both gender types.

**References**

Table 3. Data Collection Procedure Summary

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Session 1</th>
<th>Session 2</th>
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<tr>
<td><strong>Time Frame</strong></td>
<td>0-7 days post</td>
<td>0-3 days post</td>
<td>3 days post</td>
</tr>
<tr>
<td>delivery</td>
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<td>consent</td>
<td>session 1</td>
</tr>
<tr>
<td><strong>Data Collection</strong></td>
<td>Informed</td>
<td>Saliva Sample</td>
<td>Saliva Sample</td>
</tr>
<tr>
<td>consent</td>
<td>SSC tracking</td>
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<tr>
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<td>Medical Hx</td>
<td>Medical Hx</td>
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</table>

# saliva samples 0 2 2
CHAPTER FOUR:

RESULTS

Restatement of the Problem and Study Aim

In the United States, prematurity remains one of the top three leading causes of death annually. Exclusive human milk feedings have been known to reduce infant mortality. Unfortunately, mothers of premature infants are challenged with multiple stressors that affect their ability to provide exclusive human milk feedings to their infants. Compounding effects of these stressors inhibit the secretion of oxytocin hormone and affect the ejection reflex. A poor ejection reflex perpetuates a cycle that negatively affects the amount of human milk expelled from the mammary glands. This in turn leads to altered human milk synthesis and insufficient human milk volume. Mediating effects of skin to skin contact (SSC) between a mother and her neonate is suspected to repair altered lactogenesis and human milk volume but the true relationship between SSC and basal oxytocin levels have yet to be determined.
In this chapter results of the research design discussed in chapter 3 aims to better understand the true relationship between basal salivary oxytocin levels and SSC among lactating mothers with hospitalized premature infants by answering the following research questions:

1a. Does maternal basal salivary oxytocin differ across 7 days in mothers before SSC?
1b. Does SSC frequency affect basal salivary oxytocin levels?
1c. Does SSC duration affect basal salivary oxytocin levels?

**Data Collection Methods**

Variables identified in the logic model (Figure 1, Chapter 2) were measured under similar conditions by limiting inclusion criteria to: 1) postpartum mothers of premature infants born 37 weeks and 0 days – 22 weeks and 0 days of gestational age, 2) mothers that had given birth within 0–7 days, 2) mothers that intended to breastfeed/pump, 3) mothers that initiated breast pumping within 12–24 hours after birth and 4) mothers that implemented SSC with her neonate at least once since giving birth. Those with maternal diagnoses or behaviors contraindicated in breastfeeding, polydrug use, HIV, galactosemia, active TB, radioactive isotopes, chemo therapy, active herpes lesions on the breast or taking medications that
may affect salivary oxytocin assay like antidepressants, psychotropic or dopamine agonist/antagonist medications were excluded from the study.

Sample

Early recruitment at Morton Plant Hospital initially met recruitment goals of 5 per month until mid-recruitment time due to a reduction in NICU census. To increase participant eligibility, recruitment was sought at St. Joseph’s Women’s Hospital after IRB approval. Ongoing recruitment continued for an additional two months at both facilities and ended after nine months of recruitment due to grant funding limitations. A total of twenty-five postpartum lactating mothers were screened by collaborating neonatologists and advanced practice nurses for inclusion criteria at participating neonatal intensive care units (NICU) within the BayCare Health System. Of the twenty-five recruits, one declined to participate because she felt uncomfortable performing SSC with her neonate and four were excluded because of limited transportation, polydrug use or inability to meet inclusion criteria. The remaining twenty that met full inclusion criteria were approached by the PI and consented to participate (N= 20 mother-infant dyads) in the study. Demographic and social determinant information were collected via self-report questionnaire at time of consent and
summarized in Table 4. Additional physiologic and co-variant characteristics discussed in the literature review and outlined in the study logic model are included in Table 5 and Table 6.

Differences among Sample Characteristics

Differences in social determinant and physiologic factors among sample participants were determined via one-way ANOVA using SPSS software. Since maternal age has been known to predict premature birth and early lactation failure (Avery, Duckett, Dodgson, Savik, & Henly, 1998; Beta, Akolekar, Ventura, Syngelaki, & Nicolaides, 2011; Furman, Minich, & Hack, 2002) the sample size was categorized into two groups based on maternal age. Group one (n=10) included post-partum mothers less than 31 years of age and Group two (n=10) included post-partum mothers greater than 31 years of age. Social determinant factors were dichotomized for race (1=white, 0=black), insurance (1=private, 0=Medicaid), marital status (1=single, 0 = married) and WIC eligibility (1= yes, 0=no). There were no outliers, as assessed by boxplot; data were normally distributed for each age group, as assessed by Shapiro-Wilk test \( (p > .05) \); and there was homogeneity of variances, as assessed by Levene's test of homogeneity of variances \( (p = .781 \text{ and } p = .932) \). Differences between age group across marital status \( (F(1,18) = 0.184, p = 0.673) \), race \( (F(1,18) = 0.275, p = 0.660) \), and insurance \( (F(1,18) \)
were not significant. WIC eligibility was the same across both age groups ($p = 1.000$).

Physiologic factor differences (Table 7) were also determined via one-way ANOVA per age group. Mode of delivery was dichotomized ($1 = \text{vaginal delivery}, 0 = \text{cesarean delivery}$), comorbidities were categorized by three levels ($0 = \text{none}, 1 = \text{hypertension/preeclampsia}, 2 = \text{endocrine/thyroid disease}$), first pregnancy status, or primigravida, was dichotomized ($1 = \text{yes and 0 = no}$) and both gestational age (GA) and infant weight were continuous raw score variables. Data based on these parameters were normally distributed among both age groups for infant weight, comorbidities and primigravida by Shapiro-Wilk test ($p > .05$) with no outliers as assessed by boxplots. Mode of delivery did have two extreme outliers identified via box plots for age group one. Errors in data entry were assessed to determine probable cause for outliers with no need for correction.

Homogeneity of variances for infant weight, comorbidities and primigravida were also determined by Levene's test of homogeneity of variances ($p = 0.631, p = 0.192, p = 0.865, \text{and } p = 0.065$). Mode of delivery and GA did not have homogeneous variances ($p = .001$). Continued data analysis with ANOVA testing was not performed with variables that had outliers or non-homogeneous variance (mode of delivery and GA) due to the violation of ANOVA assumptions. For the remaining variables that
met the assumptions of ANOVA, no significant differences were identified between age groups for comorbidities (F(2,17)=0.119, p = 0.888), primigravida (F(1,18)= 0.275, p = 0.660) and infant weight (F(1, 18)= 2.165, p = 0.158.

Dependent Variable Data Collection and Measurement

Maternal saliva samples (n= 76) were collected at 4 time points during two 90-minute sessions at the bedside of the neonate in the NICU. Due to early neonatal discharge for participants 16 and 20 specimen sample sizes differ by two units per session. Salivary samples were collected over 5 minutes by the drool method (Appendix C) in the amount of 3-5 milliliters. To maintain the integrity of the samples repeat freeze and thaw times were avoided with careful specimen handling (Appendix I) as recommended by the Enzo Life Sciences Product enzyme-linked immunosorbent assay (ELISA) oxytocin kit manufacture procedure (Catalog Number ADI-900-153). Immediately after collection ten microliters of Aprotinin was added to each specimen, kept cold on ice and transported to the laboratory for centrifugation X 1500 rpms for 10 minutes at 4 degrees centigrade. Specimens were then aliquoted in 1 ml tubes and promptly frozen at negative 60-70 degrees within one hour of collection. Frozen samples were batched monthly and transported to the College Of Nursing
Biobehavioral laboratory in a cooler with dry ice to be stored at −80°C until ready for assay.

When ready for assay specimens were prepared utilizing the manual’s 14 step ELISA procedure with the exclusion of extraction columns. In exchange for the use of extraction columns, the kit manufacturer advised to follow the methodology utilized by Feldman, Gordon, and Zagoory-Sharon (2010) where thawed specimens were dried down via lyophylizer overnight and reconstituted in enzyme immunoassay (EIA) buffer with a higher concentration. Once reconstituted, specimens were assayed in duplicate with paired standard samples and incubated overnight at 4°C. After incubation all the wells were washed, emptied and prepared for the second one hour incubation period after adding p-nitrophenyl phosphate (pNpp) substrate solution to each well. Post incubation the enzymatic reaction was stopped and the plate was read at 405nm immediately. Oxytocin concentration in picograms per milliliter (pg/ml) was calculated by the immunoassay PRISM software utilizing a 4 parameter logistic curve. Specimens with an intra-assay coefficient of variation (CV) greater than 15 (n=7) were repeated, excluding participant 4 at time point 1 with a CV of 17 percent due to insufficient quantity of specimen available.
Dependent Variable Validity and Reliability

The study’s protocol for specimen handling and ELISA was tested with two control samples within child-bearing age. Control one (C1) was a 24 year old lactating post-partum female and control two was a 36 year old non-lactating female. To account for circadian rhythm and variation associated with day and time both specimens were collected after 9am on the same date and time. Results of both control samples (Table 8) detected normal salivary OT concentration based on previous research and provided reassurance that the study protocol for handling and processing saliva specimens maintain the integrity of the sample.

To determine if the OT ELISA kit was a good measure of OT, assay validity and reliability were tested by the manufacturer’s analysis of sensitivity, precision and cross reactivity. In the product manual the manufacturer references the use of the National Committee for Clinical Laboratory Standards evaluation protocols and guidelines to test for sensitivity by determining the average optical density bound for forty eight wells (M 0.67, SD 0.03) compared to the average optical density for forty-eight wells with standard number 7 (M .61, SD 0.02) with a detection limit determined at 2 SD from the zero along the standard curve that resulted in 15.0 pg/ml (ie.- standard 7 OT concentration).
The manufacturer describes testing for intra-assay precision with 20 replicates of three controls containing OT in a single assay and inter-assay as using controls of varying OT concentrations in 17 assays over several days. Results of the intra-assay test detected OT concentration that ranged 39.9 – 363.7 pg/ml with a CV range of 10-13 percent and the inter-assay tests resulted in OT concentrations that ranged 47-398 pg/ml with a CV range of 11-21 percent.

Cross reactivity test results among 13 related compounds were also included in the kit manual to be sure similar molecular structures like OT did not interfere with accuracy of OT measurement. For this test, the manual describes dissolving each compound in assay buffer first and then serially diluting each compound to concentrations of 10,000 pg/ml to 0.6 pg/ml prior to being measured in the OT assay. Percent cross reactivity for 11 compounds reached less than 0.02% with 2 compounds measuring close to 7%.

**Preliminary and Exploratory Data Analysis**

*Dependent Variable*

Maternal OT salivary samples (n= 76) were collected during two sessions for a total of 4 time points over a 7 day time frame. During session one (n = 20), time point one of salivary OT was collected under basal conditions with no SSC and time...
point two under SSC intervention with a mean of 50 minutes of SSC duration (SD 15.80 minutes). During session two (n=18), time point 3 of salivary OT was again taken under basal conditions with a mean interval of 3 days (SD 1.20) between sessions. The final repeated measure at time point 4 of salivary OT was collected under SSC intervention with a mean of 54.70 minutes of SSC duration (SD 13.20 minutes). Descriptive data analysis performed via SPSS in Table 9 document the differences between OT specimen sample size and OT concentration per time point with further descriptive data for CV and time of collection in Table 10 for the entire sample (n=76).

Other differences among OT concentrations (Table 9) based on conditions at time of collection (neonatal day of life (DOL), time, and interval) are presented in Table 11 and 12. Figures 2 and 3 provide a preview of the relationship OT concentrations (n=76) per time of day with higher values in the afternoon near 1400 military time and a continuous increase in value over time per DOL. To determine the significance of these conditions at time of collection on OT concentration a one-way ANOVA was performed to compare time of collection (1 = AM, 2 = PM), interval between repeated measure category (1 = < 3 days, 2 = 3 days, 3 = > 3 days) and day of life (DOL) at 3 levels (1 = < 5 days, 2 = 5-7 days, 3 = > 7 days). Through boxplots, no
extraneous outliers were detected more than 2 box-lengths from
the edge of the box. Homogeneity of variance was satisfied with
Levene’s test (p = > .05) for all variables. The differences
between OT and DOL (F(2, 73) = 0.3.420, p =0.038) was significant
whereas time of collection(F(1,74) = 2.907, p = 0.092) and
interval between sessions were not. Follow up data analysis via
Post HOC Tukey test among the DOL > 7 days group (M = 310 pg/ml,
SD = 160 pg/ml) compared to the DOL < 5 days group (M = 219
pg/ml, SD = 105 pg/ml), was statistically significant (p = .040)
with a mean increase of 92 pg/ml, 95% CI [-10.2, 162].

To remove pooled sample bias for time and DOL among samples
collected under basal conditions (n=36), the sum of saliva
specimens collected with no SSC (time point 1 and time point 3)
were combined to signify basal OT (M 250 pg/ml, SD 148 pg/ml).
Results of the scatter plots (Figures 4 and 5) replicate peak
centrations of basal OT near 1400 military time and support
an increase in value of OT over time per DOL. Despite these
results, the difference between basal OT and DOL are not
significant (F(2, 36) = 1.860, p = 0.171).

Independent Variable

Daily SSC was collected via self-report diary data over a 7
day time frame. Metrics were recorded in minutes per SSC session
with start time, stop time, and daily 24 hour total minutes.
Missing data was corrected by the mother or verified by electronic medical records by the end of the 7th day study period. Descriptive statistics via SPSS (Table 13) separates SSC frequency by number of SSC sessions (M = 4 sessions, SD = 1 session) measured over the participant’s enrollment period and the average amount of SSC measured in minutes per day (M 68 minutes per day, SD 27 minutes per day); whereas SSC duration (M 291 minutes, SD 165 minutes) accounts for the total amount of SSC measured in minutes over the participant’s enrollment period.

While the study enrollment period was targeted for 7 days, the number of days of diary data reported by participants ranged from 2-6 days and is the reason for inflated SD measurements. To account for this variability, Table 14 shows the adjusted SSC frequency (M 4 sessions, SD 0.1 sessions; M 68 minutes per day, SD 6 minutes per day) and duration (M 291 total minutes, SD 6 total minutes) variables clustered within the number of days of diary data category (1 = less than 5 days, 2 = 5 days and 3 = more than 5 days).

Exposure of the Independent Variable on the Dependent Variable

At time of consent, SSC exposure was controlled by requiring implementation of SSC at least once prior to study enrollment. Post consent, SSC diary data suggest a gradual
increase in SSC exposure over time and time point (Figure 6 and 7). Descriptive statistics in Table 15 display differences among the sample and mean SSC exposure prior to each OT time point collection (M = 141 minutes, SD 81; M 133 minutes, SD 96; M 235, SD 140; M 300, SD 149). Table 16 includes descriptive statistics that compare differences among SSC exposure and OT concentrations per time point. Preliminary data analyses predict how the dependent variable and independent variable are associated via line of fit scatter plot projections in Figures 8-12.

Acute Effects of the Independent Variable

Oxytocin pre and post scores for session 1 (time point 1 vs. time point 2) and session 2 (time point 3 vs. time point 4) were measured per interval of repeated measures via paired t-test to determine acute effects of SSC on OT. Concentrations for both sessions were normally distributed with no outliers. Pre and post paired t test of OT at Session 1 were not significant (Table 17). However, pre and post paired t- test scores for session 2 (n = 10) were significant with a moderate effect size (t(9) = -71.30, p = 0.047, d = 0.7). Session 2 paired means were also correlated with r = 0.935 and p = <0.001 (Table 17 and 18).
**Sustained Effects of the Independent Variable**

Mean scores for time point 1 (n=20) and time point 4 (n=18) were measured to determine if OT had sustained effects of SSC over time. No outliers in the data set were identified as assessed by inspection of a boxplot for values greater than 1.5 box-lengths from the edge of the box per time point. Mean scores for time point 1 (M 236 pg/ml, SD 105 pg/ml) and time point 4 (M 269 pg/ml, SD 170 pg/ml) were correlated (r= 0.483, p = .042) but paired t-test was non-significant (t(17) = -1.708, p = 0.106). Analysis per interval data counter these findings and indicate that paired t-test results for sustained effect time points collected with a 3 day interval are significant with a small effect size (t(9) = 2.425, p = 0.038, d = .15).

**Statistical Analysis for Specific Aim 1**

**Question 1**

To answer question 1 of the specific aim: “Does maternal basal salivary oxytocin differ across 7 days in mothers before SSC?” differences between basal OT concentrations were determined via scatter plots, Pearson correlations and paired-samples t-test to compare the mean difference between paired observations of basal OT at time point 1 and time point 3. Paired OT samples with a CV < 15 were included for analysis
(n=17 pairs). Figure 13 scatter plot suggests that OT concentrations collected under basal conditions are positively associated indicating an increase in means of basal OT from 234 pg/ml (SD 107 pg/ml) at time point 1 to 257 pg/ml (SD 125 pg/ml) at time point 3. Pearson correlations (n=17) confirm that the relationship between both time points are positively associated, but the measures are not correlated (r=.449, p = .071). Paired-samples t-test for basal OT at time point 1 and time point 3 are also non-significant (t(16) = .756, p = .461).

Question 2

To answer question 2 of the specific aim: “Does SSC frequency affect basal salivary oxytocin levels?” Initial data analysis procedures discussed in the original proposal proposed the use of a difference variable (i.e. OT timepoint1 - OT timepoint2) as the dependent variable in a mixed multilevel model analysis, however the use of the difference variable would be an inappropriate measure of SSC frequency or duration on OT because it focuses on the acute affect OT rather than the variance explained of the dependent variable under basal conditions. To remove this bias from the entire OT sample (n=76), the dependent variable was redefined as the group mean of OT at time point 1 and time point 3 (basal OT) to only include OT levels corresponding with no SSC intervention (n=20).
Plot diagrams (Figure 14) describing basal oxytocin and SSC frequency measured in mean minutes per day where categorized as low = < 50 minutes of SSC per day, medium = 50-75 minutes, moderate = 75-85 minutes and high = > 85 minutes of SSC per day. Preliminary analyses with scatter plot interpolation identify peak basal oxytocin under moderate SSC exposure. Elevated mean concentrations of basal OT (Table 18) for moderate measures of SSC frequency per day (M 572.92 pg/ml, SD 451 pg/ml) support this prediction. To determine if these measures of SSC frequency significantly affect basal OT concentrations, a 1 level fixed effect and 4 level random effects multilevel model analysis was structured with an AIC of 226.85. The omnibus test for the model was significant (F(1, 16) = 101, p = <0.001) indicating that average basal OT concentrations of the sample population differ. Fixed effects from the intercept was also significant t(16) = 6.389, p = < 0.001) indicating that the average basal OT concentration was 480 pg/ml with a standard error of 75 pg/ml. While coefficient estimates per each SSC frequency parameter were not significant (p = .814, p = .867, p = .589) coefficient estimates for moderate SSC exposure (92.66 pg/ml, standard error 167 pg/ml) indicate that basal OT concentrations exposed to 75-85 minutes of SSC per day are 92 pg/ml higher than the group mean (480 pg/ml). The variance component explained by the model at level 1 representing SSC frequency within-groups was
significant \((p < 0.005)\). An interclass correlation (ICC) among SSC frequency groups was not calculated because the variance estimate was zero.

To determine if a different measure of SSC frequency better fit the data, SSC frequency per mean number of sessions (Table 14) compared to basal OT concentrations was analyzed in a 1 level fixed effect and 3 level random effects multilevel model analysis with an AIC of 238.45. The omnibus test for the model was significant \((F(1, 17) = 110.99, p < 0.001)\) indicating that average basal OT concentrations of the sample population differ. Fixed effects from the intercept was also significant \(t(17) = 5.955, p < 0.001\) indicating that the average basal OT concentration was 539 pg/ml with a standard error of 90 pg/ml. Coefficient estimates per each SSC frequency parameter were not significant \((p = .313, p = .685)\). The variance component explained by the model at level 1 representing SSC frequency per number of sessions within-groups was significant \((p < 0.004)\). An interclass correlation (ICC) among SSC frequency groups was not calculated because the variance estimate was zero.

While both models resulted in a significant omnibus test indicating mean basal OT differ across all SSC frequency groups, model 1 was the best fitting model that explained the effects and variance within and between SSC frequency groups because it had the lower AIC (Table 20).
Question 3

To answer question 1b of the specific aim: “Does SSC duration affect basal salivary oxytocin levels?”, sustained effects of SSC duration in minutes per enrollment period on OT collected under basal conditions were determined via scatter plots, descriptive data and a mixed effects multilevel model analysis. Preliminary data analysis via scattered plots in Figure 15 and 16 and Table 21 suggest that basal OT levels increase as SSC duration increases over time.

To determine if sustained effects of SSC duration affect basal OT, SSC total duration in minutes clustered within number of days of diary data discussed in Table 14 were structured within a 1 level fixed effect and a 3 level random effects model. The omnibus test for the model was significant \((f(1, 17) = 109.11, p = < 0.001)\) indicating that the average basal OT among the sample population differed. Fixed effects from the intercept was also significant \((t(17) = 6.867, p = < 0.001)\) indicating that the average basal OT was 491.70 pg/ml with a standard error of 71.60 pg/ml. Coefficients of SSC group 1 (M 281 total minutes, SD 80 total minutes) indicate that basal OT concentrations are 48 mg/ml higher than average mean basal OT (491 pg/ml). The variance component at level 1 representing SSC duration between and within-groups was significant \((p = 0.004)\).
An interclass correlation (ICC) among SSC frequency groups was not calculated because the variance estimate was zero.

**Additional Data Analysis**

**Relationship between OT and Covariants**

Preliminary analyses of co-variants were determined via descriptive statistics, scatter plots and Pearson correlations in Tables 22 and 23 and Figures 17, 18 and 19. Results show that basal OT correlates with OT collected under SSC intervention \((r = .575, p = .008)\) indicating that as basal OT increases OT concentrations under SSC intervention continue to increase as well. Similarly, HM volume correlates with SSC frequency and duration \((r = .750, p = < .001; r = .585, p = .007)\), indicating that as SSC increases HM volume continues to increase over time. Beyond this relationship, HM volume does not directly predict or significantly affect OT collected with or without SSC.

**Predictors of Salivary OT under SSC Intervention**

Since inclusion criteria required the implementation of SSC prior OT collection, all basal OT measures were influenced by sustained effects of SSC. The use of regression analysis determined how much variation of OT with SSC intervention can be explained by covariant independent variables in the logic model.
In the first regression analysis, the proportion of variance explained by SSC frequency and duration variables (SSC number of episode per day, SSC mean minutes per day and SSC total duration in minutes) on OT with SSC (time point 2 and time point 4) was 45% with a moderate to large effect size (adjusted $R^2 = .53$). The assumptions of linearity, independence of errors, homoscedasticity, unusual points and normality of residuals were all met. SSC frequency and duration variables were not significant predictors of OT with SSC ($F(1, 3) = 1.353, p = .293$). However, Pearson correlations indicate that OT with SSC and SSC duration have a significant positive correlation ($r = .041, p < .05$). Unstandardized coefficients indicate that for every 1 unit SSC total minutes increases when all other independent variables are held constant, OT with SSC increases 1.225 units. Unstandardized coefficients are in Table 23.

To determine if the influence of OT with SSC would be affected by other covariates identified in the logic model of the study design, a second regression analysis was performed with the inclusion of the following co-variants clustered by number of days of diary data: 1) total human milk volume per milliliters (HM total), 2) nipple stimulation at time of collection, and 3) feeding method (where 1 = nasogastric tube, 2 = oral feeding, 3 = breastfeeding with nasogastric tube, 4 = exclusive breastfeeding), birth weight and gestational age.
Proportion of variance explained by all five variables was 68% with a low effect size (adjusted $R^2 = .271$). The assumptions of linearity, independence of errors, homoscedasticity, unusual points and normality of residuals were met via scattered plots, casewise P-P plot, review of all standardized residual cases less than ±3 SD for outliers and Durbin-Watson statistic of 1.555. Human milk volume, nipple stimulation, method of feeding, birth weight and gestational age were not significant predictors of OT with SSC ($F(1, 5) = 2.409, p = .089$). Pearson correlations indicate that OT with SSC, birth weight, and gestational age have a significant positive correlation ($r = .005, r = .021$). Unstandardized coefficients are in Table 24.

**Summary and Conclusion**

Results of this research were conducted on a homogenous sample with no significant deviations among demographic and biologic factors (race, age, insurance, marital status infant weight, comorbidities and gravidity). Similarly, salivary OT was well controlled for variability associated with time and interval collected with a non-significant $F$ test.

Over time OT was found to increase per day of life ($F(2, 73) = 0.420, p = 0.038$) with a mean increase of 92 pg/ml for the entire sample (n=76). Via scatter plots mean basal OT also increased per DOL and time point (M 234 pg/ml, SD 26 pg/ml time
point 1; M 257 pg/ml, SD 30 pg/ml time point 3) but the relationship between basal OT, DOL and time point was not significant ($F(2, 36) = 1.860, p = 0.171; t(16) = .756, p = .461$). In comparison to SSC exposure, basal OT was found to have a meaningful amount of dependence on SSC frequency ($t(16) = 6.389, p = < 0.001$) and SSC duration ($t(17) = 6.867, p = < 0.001$) with a high level of variance explained by within groups ($p = .005, p = .004$). Coefficient estimates for moderate SSC frequency exposure indicate that basal OT concentrations exposed to 75-85 minutes of SSC per day are 92 pg/ml higher than the group mean of 480 pg/ml. Through regression analysis OT measured with SSC also correlated with SSC duration ($r=.041, p = < .05$). Unstandardized coefficients indicate that for every 1 unit SSC total minutes increases when all other independent variables are held constant, OT with SSC increases 1.225 units. HM volume correlates with SSC frequency and duration ($r = .750, p = < .001; r = .585, p = .007$), indicating that as SSC increases; HM volume continues to increase over time. Beyond this relationship, HM volume does not directly predict or significantly affect OT collected with or without SSC.

Implications of these findings on the true relationship between basal salivary oxytocin levels and SSC among lactating mothers with hospitalized premature infants will be presented in
Chapter 5 including clinical significance and recommendations for further course of study.

References


Table 4. Sample Demographic and Social Determinant Factors

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Note. Social economic status classified via insurance. WIC = Women Infant and Children Government Nutrition Program
## Table 5. Sample Physiologic Factors

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*Note. Gravida = number of pregnancies, Para = number of living children, PNV = prenatal vitamin*
Table 6. Sample Co-Variant Factors

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<td>5</td>
<td>34</td>
<td>4lbs 15</td>
<td>2239</td>
<td>NG</td>
<td>DM/MBM</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>32</td>
<td>4lbs 12oz</td>
<td>2155</td>
<td>Sham</td>
<td>MBM</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>33</td>
<td>3lbs 2oz</td>
<td>1417</td>
<td>NG</td>
<td>MBM</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>33</td>
<td>4lbs 0oz</td>
<td>1814</td>
<td>Mixed</td>
<td>Mixed</td>
<td>Yes</td>
</tr>
<tr>
<td>9</td>
<td>33</td>
<td>3lbs 14oz</td>
<td>1758</td>
<td>Mixed</td>
<td>Mixed</td>
<td>Yes</td>
</tr>
<tr>
<td>10</td>
<td>34</td>
<td>3lbs 6oz</td>
<td>1503</td>
<td>Mixed</td>
<td>Mixed</td>
<td>No</td>
</tr>
<tr>
<td>11</td>
<td>32</td>
<td>5lbs 0oz</td>
<td>2268</td>
<td>Mixed</td>
<td>MBM</td>
<td>No</td>
</tr>
<tr>
<td>12</td>
<td>34</td>
<td>5lbs 7oz</td>
<td>2466</td>
<td>NG</td>
<td>Mixed</td>
<td>No</td>
</tr>
<tr>
<td>13</td>
<td>34</td>
<td>6lbs 7oz</td>
<td>2920</td>
<td>BF</td>
<td>Mixed</td>
<td>Yes</td>
</tr>
<tr>
<td>14</td>
<td>34</td>
<td>4lbs 9oz</td>
<td>2070</td>
<td>Mixed</td>
<td>Mixed</td>
<td>Yes</td>
</tr>
<tr>
<td>15</td>
<td>35</td>
<td>6lbs 3oz</td>
<td>2807</td>
<td>Mixed</td>
<td>MBM</td>
<td>Yes</td>
</tr>
<tr>
<td>16</td>
<td>36</td>
<td>6lbs 10oz</td>
<td>3005</td>
<td>Mixed</td>
<td>Mixed</td>
<td>Yes</td>
</tr>
<tr>
<td>17</td>
<td>34</td>
<td>5lbs 12oz</td>
<td>2608</td>
<td>Mixed</td>
<td>Mixed</td>
<td>Yes</td>
</tr>
<tr>
<td>18</td>
<td>35</td>
<td>4lbs 4oz</td>
<td>1928</td>
<td>Mixed</td>
<td>Mixed</td>
<td>No</td>
</tr>
<tr>
<td>19</td>
<td>31</td>
<td>2lbs 10oz</td>
<td>1190</td>
<td>NG</td>
<td>Mixed</td>
<td>No</td>
</tr>
<tr>
<td>20</td>
<td>37</td>
<td>5lbs 11oz</td>
<td>2580</td>
<td>PO</td>
<td>Mixed</td>
<td>No</td>
</tr>
</tbody>
</table>

Note. GA= Gestational age. Feeding method categories (NG) Nasogastric tube (PO),= Oral or Per os, Sham (breastfeeding and NG),(BF) = Exclusive breastfeeding, (NG/PO/BF)= Mixed. Nutrition source categories MBM = maternal breastmilk, DM = donor milk, mixed = formula with MBM or DM
Table 7. Descriptive Statistics for Normally Distributed Physiologic Factors by Age Group

<table>
<thead>
<tr>
<th>Variable Clustered by Age group</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group &gt; 31 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA (weeks)</td>
<td>10</td>
<td>32</td>
<td>37</td>
<td>34.40</td>
<td>1.430</td>
</tr>
<tr>
<td>Weight (grams)</td>
<td>10</td>
<td>1417</td>
<td>3005</td>
<td>2239</td>
<td>532.64</td>
</tr>
<tr>
<td>Primigravida</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comorbidities</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age group &lt; 31 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA (weeks)</td>
<td>10</td>
<td>29</td>
<td>34</td>
<td>32.80</td>
<td>1.69</td>
</tr>
<tr>
<td>Weight (grams)</td>
<td>10</td>
<td>1190.00</td>
<td>2807.00</td>
<td>1899.40</td>
<td>500.82</td>
</tr>
<tr>
<td>Primigravida</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comorbidities</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. GA = gestational age in weeks, Primagravida = 1 = yes, 0 = no, weight = infant birth weight in grams, comorbidities = 0 = none, 1 = hypertension/preeclampsia, 2 = endocrine/thyroid disease
Table 8. Salivary Oxytocin Controls for Handling and ELISA Procedure

<table>
<thead>
<tr>
<th>ID</th>
<th>Days Post Delivery</th>
<th>Condition</th>
<th>OT (pg/ml)</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>7</td>
<td>Lactating</td>
<td>63.39</td>
<td>3%</td>
</tr>
<tr>
<td>C2</td>
<td>N/A</td>
<td>Non-lactating</td>
<td>168.15</td>
<td>2%</td>
</tr>
</tbody>
</table>

Note. C1 = control one, C2 = control 2
Table 9. Salivary Oxytocin Descriptive Statistics per Time Point

<table>
<thead>
<tr>
<th>Time point</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean (pg/ml)</th>
<th>SD (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OT1</td>
<td>20</td>
<td>113.44</td>
<td>441.77</td>
<td>240.67</td>
<td>96.03</td>
</tr>
<tr>
<td>OT2</td>
<td>20</td>
<td>63.66</td>
<td>471.79</td>
<td>203.70</td>
<td>108.12</td>
</tr>
<tr>
<td>OT3</td>
<td>18</td>
<td>36.03</td>
<td>524.64</td>
<td>262.56</td>
<td>124.21</td>
</tr>
<tr>
<td>OT4</td>
<td>18</td>
<td>65.03</td>
<td>704.02</td>
<td>296.77</td>
<td>170.32</td>
</tr>
</tbody>
</table>

Note. OT1 = basal OT at time point 1 with no SSC, OT2 = OT at time point 2 with SCC, OT3 = basal OT at time point 3 with no SSC, OT4 = OT at time point 4 with SCC
Table 10. Salivary Oxytocin Descriptive Statistics for Coefficient of Variation and Time Collected

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CV</td>
<td>76</td>
<td>0%</td>
<td>17%</td>
<td>5%</td>
<td>4%</td>
</tr>
<tr>
<td>Time</td>
<td>76</td>
<td>910.00</td>
<td>1830.00</td>
<td>1213.61</td>
<td>228 minutes</td>
</tr>
</tbody>
</table>

Note. CV = inter-assay coefficient of variation, Time = military time
Table 11. Salivary Oxytocin Concentration per Interval between Repeated Measures at Time of Collection

<table>
<thead>
<tr>
<th>Interval</th>
<th>Time Point</th>
<th>N</th>
<th>Minimum (pg/ml)</th>
<th>Maximum (pg/ml)</th>
<th>Mean (pg/ml)</th>
<th>SD (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 3days</td>
<td>OT1</td>
<td>5</td>
<td>184.00</td>
<td>429.00</td>
<td>259.40</td>
<td>98.43</td>
</tr>
<tr>
<td></td>
<td>OT2</td>
<td>5</td>
<td>93.00</td>
<td>342.00</td>
<td>173.40</td>
<td>105.79</td>
</tr>
<tr>
<td></td>
<td>OT3</td>
<td>3</td>
<td>207.00</td>
<td>524.00</td>
<td>350.00</td>
<td>160.75</td>
</tr>
<tr>
<td></td>
<td>OT4</td>
<td>3</td>
<td>125.00</td>
<td>380.00</td>
<td>279.66</td>
<td>135.90</td>
</tr>
<tr>
<td>3 days</td>
<td>OT1</td>
<td>10</td>
<td>119.00</td>
<td>441.00</td>
<td>243.10</td>
<td>104.77</td>
</tr>
<tr>
<td></td>
<td>OT2</td>
<td>10</td>
<td>79.00</td>
<td>471.00</td>
<td>226.80</td>
<td>119.73</td>
</tr>
<tr>
<td></td>
<td>OT3</td>
<td>10</td>
<td>130.00</td>
<td>450.00</td>
<td>267.60</td>
<td>110.10</td>
</tr>
<tr>
<td></td>
<td>OT4</td>
<td>10</td>
<td>74.00</td>
<td>704.00</td>
<td>338.90</td>
<td>192.51</td>
</tr>
<tr>
<td>&gt; 3 days</td>
<td>OT1</td>
<td>5</td>
<td>113.00</td>
<td>343.00</td>
<td>200.00</td>
<td>100.14</td>
</tr>
<tr>
<td></td>
<td>OT2</td>
<td>4</td>
<td>64.00</td>
<td>335.00</td>
<td>192.75</td>
<td>110.90</td>
</tr>
<tr>
<td></td>
<td>OT3</td>
<td>4</td>
<td>36.00</td>
<td>239.00</td>
<td>159.75</td>
<td>87.14</td>
</tr>
<tr>
<td></td>
<td>OT4</td>
<td>5</td>
<td>65.00</td>
<td>409.00</td>
<td>222.20</td>
<td>138.40</td>
</tr>
</tbody>
</table>

Note. OT1 = basal OT at time point 1 with no SSC, OT2 = OT at time point 2 with SSC, OT3 = basal OT at time point 3 with no SSC, OT4 = OT at time point 4 with SSC.
Table 12. Salivary Oxytocin Concentration and Time of Collection
Variables per Time Point

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Variable</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>OT1</td>
<td>OT pg/ml</td>
<td>20</td>
<td>113.44</td>
<td>441.77</td>
<td>240.69</td>
<td>96.04</td>
</tr>
<tr>
<td></td>
<td>Time</td>
<td>20</td>
<td>915.00</td>
<td>1800.00</td>
<td>1212.0</td>
<td>237.73</td>
</tr>
<tr>
<td></td>
<td>DOL days</td>
<td>20</td>
<td>2.00</td>
<td>8.00</td>
<td>4.4500</td>
<td>1.57</td>
</tr>
<tr>
<td>OT2</td>
<td>OT pg/ml</td>
<td>20</td>
<td>63.66</td>
<td>471.79</td>
<td>203.76</td>
<td>108.09</td>
</tr>
<tr>
<td></td>
<td>Time</td>
<td>20</td>
<td>1025.00</td>
<td>1830.00</td>
<td>1307.2</td>
<td>223.92</td>
</tr>
<tr>
<td></td>
<td>DOL days</td>
<td>20</td>
<td>2.00</td>
<td>8.00</td>
<td>4.4500</td>
<td>1.57</td>
</tr>
<tr>
<td>OT3</td>
<td>OT pg/ml</td>
<td>18</td>
<td>36.03</td>
<td>524.64</td>
<td>262.68</td>
<td>124.13</td>
</tr>
<tr>
<td></td>
<td>Time</td>
<td>18</td>
<td>910.00</td>
<td>1725.00</td>
<td>1111.6</td>
<td>211.69</td>
</tr>
<tr>
<td></td>
<td>DOL days</td>
<td>18</td>
<td>5.00</td>
<td>11.00</td>
<td>7.4444</td>
<td>1.78</td>
</tr>
<tr>
<td>OT4</td>
<td>OT pg/ml</td>
<td>18</td>
<td>65.03</td>
<td>704.02</td>
<td>296.86</td>
<td>170.28</td>
</tr>
<tr>
<td></td>
<td>Time</td>
<td>18</td>
<td>1010.00</td>
<td>1827.00</td>
<td>1213.2</td>
<td>211.19</td>
</tr>
<tr>
<td></td>
<td>DOL days</td>
<td>18</td>
<td>5.00</td>
<td>11.00</td>
<td>7.4444</td>
<td>1.78</td>
</tr>
</tbody>
</table>

Note. OT1= basal OT at time point 1 with no SSC , OT2= OT at time point 2 with SCC, OT3 = basal OT at time point 3 with no SSC, OT4 = OT at time point 4 with SSC, OT = oxytocin, Time = military time, DOL = days of life.
Figure 2. Trend of Oxytocin Concentration per Time of Day (N=76)
Figure 3. Trend of Oxytocin Concentration per Neonatal Day of Life (N=76)
Figure 4. Trend of Basal Oxytocin Concentration per Time of Day (n=37)
Neonatal Day of Life (DOL) at Time of Maternal Saliva Collection

Figure 5. Trend of Basal Oxytocin Concentration per Neonatal Day of Life (n=37)
Table 13. Skin to Skin Frequency and Duration Descriptive Statistics

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSCTmin</td>
<td>20</td>
<td>30.00</td>
<td>630.00</td>
<td>291.20</td>
<td>163.54</td>
</tr>
<tr>
<td>SSCF</td>
<td>20</td>
<td>1.00</td>
<td>7.00</td>
<td>4.25</td>
<td>1.77</td>
</tr>
<tr>
<td>SSCfmeanperday</td>
<td>20</td>
<td>17.00</td>
<td>117.00</td>
<td>67.90</td>
<td>26.85</td>
</tr>
</tbody>
</table>

Note. SSCTmin = total amount of SSC measured in minutes over the participant’s enrollment period. SSCF = number of SSC sessions measured over the participant’s enrollment period SSCfmeanperday = average amount of SSC measured in minutes per day.
Table 14. Skin to Skin Contact Frequency and Duration
Descriptive Statistics Clustered within Number of Days of Diary Data

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSCTmin</td>
<td>20</td>
<td>281.80</td>
<td>294.50</td>
<td>291.20</td>
<td>5.57</td>
</tr>
<tr>
<td>SSCF</td>
<td>20</td>
<td>4.14</td>
<td>4.40</td>
<td>4.25</td>
<td>.10</td>
</tr>
<tr>
<td>SSCfmeanperday</td>
<td>20</td>
<td>63.75</td>
<td>78.80</td>
<td>67.90</td>
<td>6.48</td>
</tr>
</tbody>
</table>

Note. SSCTmin = total amount of SSC measured in minutes over the participant’s enrollment period. SSCF = number of SSC sessions measured over the participant’s enrollment period. SSCfmeanperday = average amount of SSC measured in minutes per day.
Figure 6. Skin to Skin Exposure by Neonatal Day of Life
Figure 7. Scatter Plot of Skin to Skin Exposure per Time Point
Table 15. Descriptive Statistics for Skin to Skin Exposure prior Oxytocin Time Point Collection

<table>
<thead>
<tr>
<th>Time point</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean (minutes)</th>
<th>SD (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OT1</td>
<td>11</td>
<td>33.00</td>
<td>300.00</td>
<td>141.27</td>
<td>81.23</td>
</tr>
<tr>
<td>OT2</td>
<td>20</td>
<td>30.00</td>
<td>390.00</td>
<td>133.05</td>
<td>95.67</td>
</tr>
<tr>
<td>OT3</td>
<td>18</td>
<td>50.00</td>
<td>510.00</td>
<td>235.11</td>
<td>139.77</td>
</tr>
<tr>
<td>OT4</td>
<td>18</td>
<td>105.00</td>
<td>570.00</td>
<td>300.78</td>
<td>149.23</td>
</tr>
</tbody>
</table>

Note. OT1 = basal OT at time point 1 with no SSC, OT2 = OT at time point 2 with SSC, OT3 = basal OT at time point 3 with no SSC, OT4 = OT at time point 4 with SSC.
Table 16. Descriptive Statistics for Skin to Skin Exposure and Oxytocin Concentration per Time Point Collection

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Variable</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>OT1</td>
<td>SSC minutes</td>
<td>11</td>
<td>33.00</td>
<td>300.00</td>
<td>141.27</td>
<td>81.22</td>
</tr>
<tr>
<td></td>
<td>OT pg/ml</td>
<td>20</td>
<td>113.44</td>
<td>441.77</td>
<td>240.69</td>
<td>96.04</td>
</tr>
<tr>
<td>OT2</td>
<td>SSC minutes</td>
<td>20</td>
<td>30.00</td>
<td>390.00</td>
<td>133.05</td>
<td>95.66</td>
</tr>
<tr>
<td></td>
<td>OT pg/ml</td>
<td>20</td>
<td>63.66</td>
<td>471.79</td>
<td>203.76</td>
<td>108.09</td>
</tr>
<tr>
<td>OT3</td>
<td>SSC minutes</td>
<td>18</td>
<td>50.00</td>
<td>510.00</td>
<td>235.11</td>
<td>139.77</td>
</tr>
<tr>
<td></td>
<td>OT pg/ml</td>
<td>18</td>
<td>36.03</td>
<td>524.64</td>
<td>262.68</td>
<td>124.13</td>
</tr>
<tr>
<td>OT4</td>
<td>SSC minutes</td>
<td>18</td>
<td>60.00</td>
<td>570.00</td>
<td>286.61</td>
<td>159.54</td>
</tr>
<tr>
<td></td>
<td>OT pg/ml</td>
<td>18</td>
<td>65.03</td>
<td>704.02</td>
<td>296.86</td>
<td>170.28</td>
</tr>
</tbody>
</table>

Note. OT1 = basal OT at time point 1 with no SSC, OT2 = OT at time point 2 with SCC, OT3 = basal OT at time point 3 with no SSC, OT4 = OT at time point 4 with SCC.
Figure 8. Relationship between Skin to Skin Exposure and Oxytocin Concentration (n=76)
Figure 9. Basal Oxytocin Concentration and Skin to Skin Exposure at Time Point 1 (n=11)
Figure 10. Skin to Skin Exposure and Oxytocin Concentration at Time Point 2 (n=20)
Figure 11. Basal Oxytocin Concentration and Skin to Skin Exposure at Time Point 3 (n=18)
Figure 12. Skin to Skin Exposure and Oxytocin Concentration at Time Point 4 (n=18)
Figure 13. Relationship between Basal Oxytocin Repeated Measures (n=20)
Table 17. Acute Effects of Skin to Skin on Oxytocin by Interval

<table>
<thead>
<tr>
<th>Interval</th>
<th>Session</th>
<th>N</th>
<th>Correlation</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days between sessions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Session 1</td>
<td>10</td>
<td>.463</td>
<td>.178</td>
</tr>
<tr>
<td></td>
<td>Session 2</td>
<td>10</td>
<td>.935</td>
<td>.00*</td>
</tr>
<tr>
<td>4days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Session 1</td>
<td>4</td>
<td>-.639</td>
<td>.361</td>
</tr>
<tr>
<td></td>
<td>Session 2</td>
<td>4</td>
<td>-.528</td>
<td>.472</td>
</tr>
</tbody>
</table>

Note. * p < .05, session 1 = oxytocin collected at time point 1 vs time point 2, session 2 = oxytocin collected at time point 3 vs time point 4
Table 18. Acute Effects of Skin to Skin on Oxytocin Paired T-Test

<table>
<thead>
<tr>
<th>Interval</th>
<th>Session</th>
<th>Mean (pg/ml)</th>
<th>SD (pg/ml)</th>
<th>T test (+/-)</th>
<th>df</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>1day</td>
<td>Session 1</td>
<td>88.000</td>
<td>103.23</td>
<td>1.205</td>
<td>1</td>
<td>.441</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2days</td>
<td>Session 1</td>
<td>206.00</td>
<td>172.53</td>
<td>1.659</td>
<td>1</td>
<td>.340</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Session 2</td>
<td>10.500</td>
<td>101.12</td>
<td>.147</td>
<td>1</td>
<td>.907</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3days</td>
<td>Session 1</td>
<td>16.300</td>
<td>117.06</td>
<td>.440</td>
<td>9</td>
<td>.670</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Session 2</td>
<td>-71.300</td>
<td>97.75</td>
<td>2.307</td>
<td>9</td>
<td>.047*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4days</td>
<td>Session 1</td>
<td>-8.7500</td>
<td>198.20</td>
<td>.088</td>
<td>3</td>
<td>.935</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Session 2</td>
<td>-75.000</td>
<td>215.58</td>
<td>.696</td>
<td>3</td>
<td>.537</td>
</tr>
</tbody>
</table>

Note. * p < .05, session 1 = oxytocin collected at time point 1 vs time point 2, session 2 = oxytocin collected at time point 3 vs time point 4
Figure 14. Plots Describing Basal Oxytocin and Skin to Skin Contact Frequency
Table 19. Descriptive Statistics for Basal Oxytocin per Skin to Skin Frequency

<table>
<thead>
<tr>
<th>SSC Frequency Category</th>
<th>N</th>
<th>Mean (pg/ml)</th>
<th>SD (pg/ml)</th>
<th>Coefficient of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>low</td>
<td>5</td>
<td>451.26</td>
<td>198.6</td>
<td>44.0%</td>
</tr>
<tr>
<td>medium</td>
<td>5</td>
<td>459.59</td>
<td>209.56</td>
<td>45.6%</td>
</tr>
<tr>
<td>moderate</td>
<td>2</td>
<td>572.92</td>
<td>451.15</td>
<td>78.7%</td>
</tr>
<tr>
<td>high</td>
<td>8</td>
<td>480.26</td>
<td>162.20</td>
<td>33.8%</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>477.11</td>
<td>197.95</td>
<td>41.5%</td>
</tr>
</tbody>
</table>

*Note. low = < 50 minutes of SSC per day, medium = 50-75 minutes, moderate = 75-85 minutes and high = > 85 minutes of SSC per day*
Table 20. Basal Oxytocin and Skin to Skin Contact Frequency
Fixed effects

<table>
<thead>
<tr>
<th>SSC Frequency Parameter</th>
<th>Coefficient (pg/ml)</th>
<th>Std. Error (pg/ml)</th>
<th>df</th>
<th>t</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>480.26</td>
<td>75.06</td>
<td>16</td>
<td>6.398</td>
<td>.000*</td>
<td>321.14 - 639.39</td>
</tr>
<tr>
<td>low</td>
<td>-28.10</td>
<td>121.03</td>
<td>16</td>
<td>-.240</td>
<td>.814</td>
<td>-285.58 - 227.58</td>
</tr>
<tr>
<td>medium</td>
<td>-20.67</td>
<td>121.03</td>
<td>16</td>
<td>-.171</td>
<td>.867</td>
<td>-277.25 - 235.91</td>
</tr>
<tr>
<td>moderate</td>
<td>92.66</td>
<td>167.84</td>
<td>16</td>
<td>.552</td>
<td>.589</td>
<td>-263.15 - 448.48</td>
</tr>
<tr>
<td>high</td>
<td>-</td>
<td>-</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>

Note.* p < .05, low = < 50 minutes of SSC per day, medium = 50-75 minutes, moderate = 75-85 minutes and high = > 85 minutes of SSC per day
Table 21. Basal Oxytocin and Skin to Skin Contact Duration
Descriptive Statistics

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal OT (pg/ml)</td>
<td>20</td>
<td>195.57</td>
<td>891.93</td>
<td>477.11</td>
<td>197.95</td>
</tr>
<tr>
<td>SSC Total Duration in</td>
<td>20</td>
<td>30.00</td>
<td>630.00</td>
<td>291.20</td>
<td>163.53</td>
</tr>
</tbody>
</table>
Figure 15. Skin to Skin Duration per Participant
Figure 16. Relationship between Skin to Skin Duration and Basal Oxytocin
Table 22. Covariant Data Descriptive Statistics

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>OT with SSC (pg/ml)</td>
<td>20</td>
<td>92.54</td>
<td>1001.10</td>
<td>473.166</td>
<td>261.44</td>
</tr>
<tr>
<td>HM total in ml</td>
<td>20</td>
<td>101.00</td>
<td>5978.00</td>
<td>1440.75</td>
<td>1517.93</td>
</tr>
<tr>
<td>Nipple stimulation</td>
<td>20</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Feeding method</td>
<td>20</td>
<td>1</td>
<td>5</td>
<td>3</td>
<td>1.90</td>
</tr>
</tbody>
</table>

Note. OT with SSC = salivary oxytocin collected at time point 2 and 4) HM total = total human milk volume per milliliters (ml), nipple stimulation (1= yes, 0= no at time of collection), feeding method (1= nasogastric tube, 2= oral feeding, 3= breastfeeding with nasogastric tube, 4 = exclusive breastfeeding)
Figure 17. Scatter Plot of Basal OT and Human Milk Volume
Figure 18. Scatter Plot of OT with SSC and Human Milk Volume
Figure 19. Scatter Plot of Basal OT and OT with SSC
Table 23. Covariant Data Correlations

<table>
<thead>
<tr>
<th></th>
<th>OTnoSSC</th>
<th>OTwithSSC</th>
<th>SSCTmin</th>
<th>SSCF</th>
<th>HMtotal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OTnoSSC</strong></td>
<td>Pearson</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation</td>
<td></td>
<td>1</td>
<td>.575**</td>
<td>.091</td>
<td>-.026</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.008</td>
<td>.081</td>
<td>.000</td>
<td>.007</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td><strong>OTwithSSC</strong></td>
<td>Pearson</td>
<td>.575**</td>
<td>1</td>
<td>.399</td>
<td>.228</td>
</tr>
<tr>
<td>Correlation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.008</td>
<td>.081</td>
<td>.000</td>
<td>.007</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td><strong>SSCTmin</strong></td>
<td>Pearson</td>
<td>.091</td>
<td>.399</td>
<td>1</td>
<td>.842**</td>
</tr>
<tr>
<td>Correlation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.702</td>
<td>.081</td>
<td>.000</td>
<td>.007</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td><strong>SSCF</strong></td>
<td>Pearson</td>
<td>-.026</td>
<td>.228</td>
<td>.842**</td>
<td>1</td>
</tr>
<tr>
<td>Correlation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.913</td>
<td>.335</td>
<td>.000</td>
<td>.000</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td><strong>HM total</strong></td>
<td>Pearson</td>
<td>-.223</td>
<td>.141</td>
<td>.585**</td>
<td>.750**</td>
</tr>
<tr>
<td>Correlation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.344</td>
<td>.554</td>
<td>.007</td>
<td>.000</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

Note.* p < .05. OTnoSSC = salivary oxytocin at time point 1 and 3; OTwithSSC = salivary oxytocin collected at time point 2 and 4; SSCTmin = total amount of SSC measured in minutes over the participant’s enrollment period. SSCF = number of SSC sessions measured over the participant’s enrollment period. HM total = total human milk volume per milliliters (ml), nipple stimulation (1 = yes, 0 = no at time of collection), feeding method (1 = nasogastric tube, 2 = oral feeding, 3 = breastfeeding with nasogastric tube, 4 = exclusive breastfeeding)
Table 24. Unstandardized Coefficients for Skin to Skin Frequency and Duration

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unstandardized Coefficients</th>
<th>t</th>
<th>Sig.</th>
<th>Partial Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Constant)</td>
<td>398.298</td>
<td>180.465</td>
<td>2.207</td>
<td>.042</td>
</tr>
<tr>
<td>SSCTmin</td>
<td>1.225</td>
<td>.750</td>
<td>1.633</td>
<td>.122</td>
</tr>
<tr>
<td>SSCF</td>
<td>-52.980</td>
<td>61.307</td>
<td>-.864</td>
<td>.400</td>
</tr>
<tr>
<td>SSCfmeanperday</td>
<td>-.835</td>
<td>3.349</td>
<td>-.249</td>
<td>.806</td>
</tr>
</tbody>
</table>

Note. SSCTmin = total amount of SSC measured in minutes over the participant’s enrollment period. SSCF = number of SSC sessions measured over the participant’s enrollment period
Table 25. Covariant Unstandardized Coefficients on OT with SSC

<table>
<thead>
<tr>
<th>Variables Clustered by diary data groups</th>
<th>Unstandardized Coefficients</th>
<th>t</th>
<th>Sig.</th>
<th>Partial Correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Constant)</td>
<td>-106.19</td>
<td>220.92</td>
<td>-.481</td>
<td>.638</td>
</tr>
<tr>
<td>HM total</td>
<td>-.02</td>
<td>.04</td>
<td>-.600</td>
<td>.558</td>
</tr>
<tr>
<td>Nipple stimulation</td>
<td>69.79</td>
<td>153.04</td>
<td>.456</td>
<td>.655</td>
</tr>
<tr>
<td>Feeding method</td>
<td>35.64</td>
<td>30.95</td>
<td>1.151</td>
<td>.269</td>
</tr>
<tr>
<td>Weight group</td>
<td>325.13</td>
<td>144.97</td>
<td>2.243</td>
<td>.042*</td>
</tr>
<tr>
<td>GA group</td>
<td>121.03</td>
<td>85.05</td>
<td>1.423</td>
<td>.177</td>
</tr>
</tbody>
</table>

*Note.* *p* < .05
CHAPTER 5:
CONCLUSIONS

Methods and Procedures

This one-group, repeated measures design involved daily SSC via self-report diary data and maternal salivary OT with and without SSC at 4 time points over a 7 day time frame.

Major Findings

Mean levels of OT under basal conditions increased over time with a mean concentration of 234 pg/ml (SD 108 pg/ml) at time point 1 and mean concentration of 257 pg/ml (SD 125 pg/ml) at time point 3. Pearson correlations confirmed that the relationship between basal OT time points were positively associated, but the two were not significantly correlated (r=.449, p = .071) and were also non-significant via paired sample t-test (t (16) = .756, p=.461. Through mixed effects multilevel model data analysis, basal OT levels were found to have a meaningful amount of dependence on SSC frequency (t(16) = 6.389, p = < 0.001) and SSC duration (t(17) = 6.867, p = < 0.001) with
coefficient estimates that indicate that basal OT concentrations in women exposed to moderate levels of SSC (75-85 minutes of SSC per day) were 92 pg/ml higher than the group mean of 480 pg/ml. In addition, basal levels of OT were found to correlate with OT collected under SSC intervention (r = .575, p = .008) indicating that as basal OT increases OT under SSC intervention continues to increase as well. Similarly, HM volume correlated with SSC frequency and duration (r = .750, p < .001; r = .585, p = .007), indicating that as SSC increases HM volume continues to increase over time. Beyond this relationship HM volume did not directly predict or significantly affect OT collected with or without SSC.

**Discussion**

Results of this research suggest that lactating mothers with premature infants sustain positive effects of SSC that increase basal OT secretion over time. Prior to this research salivary OT had never been measured during SSC in order to explore the relationship between basal OT and SSC among lactating mothers with hospitalized premature infants. These findings are unique and narrow the gap in the literature by adding a better understanding of the physiology of galactokinesis by satisfying three research questions. In relation to question 1a, maternal basal salivary oxytocin did
differ across 7 days in mothers before SSC but the relationship was non-significant. For questions 1b and 1c, SSC frequency and duration did positively affected basal salivary oxytocin levels over the enrollment period.

Exposure of term infants to SSC intervention for 25 to 120 minutes after birth, with or without early suckling, positively influences mother-infant interaction at 1 year of age compared to standard care that separates mother and infant at birth (Bystrova et al., 2009). For premature mother-infant dyads, separation at birth is common and often triggers episodes of maternal sadness, depression and anxiety (Carvalho, Martinez, & Linhares, 2008). Positive effects of OT secretion elicit maternal “feel good” responses that improve mood, reduce symptoms of stress, depression and anxiety while promoting social attachment (Pratt et al., 2015; Samuel et al., 2015; Tops et al., 2013; Uvnas-Moberg, 1996; Weisman, Zagoory-Sharon, & Feldman, 2013). SSC has also been known to reduce maternal stress and depression in NICU environments (Flacking, Thomson, Ekenberg, Lowegren, & Wallin, 2013; Holditch-Davis et al., 2014). In this research, maternal benefits of OT secretion and SSC intervention are likely to manifest with routine SSC exposure (75–85 minutes per day) when OT levels are markedly higher and encouraged continuously (24 hours/7 days a week) in high tech neonatal environments (Nyqvist et al., 2010).
Findings of this dissertation research also provide new insight on the relationship of SSC on oxytocin and human milk volume. Due to lack of lactogenic hormone measurement in previous research (Hill et al., 1999; Hurst, 1997), gaps in the literature presumed that SSC yielded more HM volume because SSC increased oxytocin secretion thus allowing the expulsion of HM from the mammary gland. However, evidence from this research showed that OT did not correlate with HM volume and suggests that SSC serves as a mediator for HM volume in response to an increase in OT secretion. Recent findings by Acuna-Muga et al. (2014) published that mean human milk volumes were significantly higher during (107.7 mL) and after SSC (117.7 mL) compared to milk volumes obtained at the beside of the incubator (96.9 mL). Ongoing research aims to determine if milk expression conducted in proximity to the infant, particularly prior and during SSC is associated with higher milk volume via secondary data analysis.

Limitations

Measurement Error

Large standard deviations among mean OT concentrations raise areas of concern regarding the accuracy, reliability and stability of OT measurement. McCullough, Churchland, and Mendez (2013) agreed that plasma OT measurement lacked reliability depending upon methodology and technique and suggested that an
accurate, specific, and readily available method for measuring OT be standardized in the field. Likewise, Horvat-Gordon, Granger, Schwartz, Nelson, and Kivlighan (2005) reported that meaningful oxytocin levels may not be detected in saliva assay. However, more recent studies have successfully measured salivary oxytocin with a modified concentration or extraction procedure technique mentioned (Carter et al., 2007; Feldman, Gordon, & Zagoory-Sharon, 2011; Grewen, Davenport, & Light, 2010; White-Traut et al., 2009). Results of this research contribute to salivary OT technique recommended by the manufacturer. While noticeable discrepancies were noted per basal OT time point with a standard deviation of 26 for time point 1 and standard deviation of 30 for time point 3, assay wells that measured standard controls of OT were within normal limits for each EIA kit assayed. Since OT is labile and pulsatile in nature (Ueda, Yokoyama, Irahara, & Aono, 1994) with a 5 minute half-life, such factors may contribute to discrepancies noticed among OT salivary concentration. Although important, these factors are difficult to control and require more rigorous research beyond the scope of this study.
Sample Size

Power analysis to determine sample size was not attainable because statistical data (i.e., sample means, alpha and effect size) on basal oxytocin in comparison to SSC had never been published. Feasible sample size was determined by NICU census data and budget restraints for a total of twenty participants yielding a sum of 76 observations. However, depending upon the test performed for data analysis, smaller sample sizes were required to answer each research question.

For this research data analysis for a sample size of 17, paired sample t-test resulted in a non-significant t test. In hypothesis testing, a non-significant t test would indicate rejecting the null hypothesis where basal OT at time point 1 is not equal to basal OT at time point 3. Rejecting the null would then indicate that the alternative hypothesis would be true indicating that basal OT at time point 1 and time point 3 are the same. To determine the probability of rejecting the null hypothesis in error, both the effect size and power are considered given a certain sample size. Via G*Power 3.1.9.2 (Faul, Erdfelder, Lang, & Buchner, 2007) the paired samples t test where OT at time point 1 mean was 234 pg/ml (SD 108 pg/ml) and OT at time point 3 was 257 pg/ml (SD 125 pg/ml) calculated an effect size of d=0.2 with a power level of 20%. Implications
of a small effect size and low power level suggest that results of the study were under powered for determining differences in basal OT over time and increases the probability of committing a type II (false-negative) error. To reduce the likelihood of type II error, findings of this research should be replicated in future studies with a larger sample size. An increase in sample size reduces the likelihood of results differing from the population, resulting in a significant t-test indicating that basal OT significantly differs over time (Banerjee, Chitnis, Jadhav, Bhawalkar, & Chaudhury, 2009).

Design

Inclusion of a comparison or control group was also not feasible due to budget limitations of this research. Future studies comparing basal OT concentrations among mother-infant dyads that do not perform SSC would offer an unbiased estimate of basal OT concentrations over time. These findings would better explain trends of OT during the day, over time and by neonatal day of life. In addition, the exclusion of maternal stress also limits clear interpretations of SSC on OT and human milk volume. Psychological and physiological measures of stress are expected to decrease over time at 2, 4, and 6 weeks postpartum with no significant differences in cortisol and
oxytocin among mothers with non-nursing infants less than 1500 grams (Chatterton et al., 2000). Measuring acute and sustained stress responses in the NICU will validate the use of SSC as a means to deregulated maternal stress responses to increase OT and possibly HM volume.

**Conclusions**

Preliminary findings of this study have a high level of significance for nursing science because it provides insight on the relationship between SSC and oxytocin hormone. This new understanding adds to the importance of continued SSC intervention to promote sustained effects of SSC on the physiology of galactokinesis and maternal mental health that improve mood and attachment behaviors. Preliminary data analyses also suggest that SSC mediates human milk volume in response to an increase in OT over time. Immunologic benefits of breast milk, consumed by the premature infant via exclusive human milk feedings, offers a significant contribution to neonatal health care outcomes by reducing the incidences of neonatal complications associated with premature delivery, such as sepsis, NEC, and death.
**Recommendations**

Continued research comparing maternal salivary cortisol and OT with and without SSC will determine if SSC physiologically deregulates maternal stressors that influence OT secretion and HM volume. Presumably, OT increases under moderate levels of SSC exposure and SSC exposure positively correlates with human milk volume. Dose responses of SSC frequency, duration relative to human milk volume and oxytocin release are pending on-going data analysis.

Funding for this research was provided by the Southern Nurse’s Research Society Dissertation Grant Award.

**References**


APPENDICES
Appendix A: Demographics and Medical Information Questionnaire

Participant ID: ___________ Date of Birth: ___/___/_____ Age ___

Ethnicity
☐ Hispanic or Latino ☐ Not Hispanic or Latino

Race:
☐ White ☐ Black /African American ☐ American Indian/Alaska Native
☐ Asian ☐ Pacific Islander ☐ Other______________________________

Marital Status: ☐ Single ☐ Married ☐ Divorced ☐ Widowed

Insurance: ☐ Private ☐ Medicaid ☐ Self Pay

Pregnancy and Birth Information:

Date of Delivery: ___/___/_____ Time of Birth: ______

Type of delivery: ☐ Vaginal ☐ Cesarean

Gestational age at delivery: ______ weeks

How many pregnancies have you had? ____ How many living children? ___

Did you take any medications during your pregnancy? Yes ☐ No ☐
If yes please name the medications ________________________________

Are you still taking these medications? Yes ☐ No ☐
If yes please name which medications______________________________
Did you experience any prenatal or delivery complications (for example - high blood pressure, preterm labor, severe illness, etc)? Yes □ No □
If yes please explain ____________________________________________
________________________________________________________________

Do you have any extreme tooth decay or gingivitis? Yes □ No □

**Infant Information:**

Sex of baby □ Male □ Female

Baby’s Birth Weight _______ lbs _______ oz (or) _________ gms

Why is your baby receiving care in the NICU?
________________________________________________________________
________________________________________________________________
________________________________________________________________

Will your baby be receiving benefits form WIC? Yes □ No □

Have you breastfed your baby or pumped your milk since giving birth to your baby? Yes □ No □
If yes what date & time? Date: ___/___/_____ Time: ____:_____ am/pm

Have you initiated skin-to-skin contact or kangaroo care since giving birth to your baby? Yes □ No □
If yes when? Date: ___/___/_____ Time: ____:_____ am/pm

Did you feel comfortable holding your baby skin-to-skin? Yes □ No □
If no, please explain________________________________________________

Please provide any further information you would like to share about your experience.
________________________________________________________________
________________________________________________________________
________________________________________________________________

Thank you for your time in completing this questionnaire.
Appendix B: Tracking Log Instructions

After enrollment into the study please begin to track activities you perform related to skin-to-skin, saliva collection and infant feedings. Below is an example to follow:

Tracking Log Day 1: 2/12/14

<table>
<thead>
<tr>
<th>Activity</th>
<th>Start Time</th>
<th>End Time</th>
<th>Feeding Type</th>
<th>Amount (ml/min)</th>
<th>Minutes of SSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP</td>
<td>6:00 am</td>
<td>6:20 am</td>
<td>PM</td>
<td>10 ml</td>
<td>-</td>
</tr>
<tr>
<td>SSC</td>
<td>8:00 am</td>
<td>9:35 am</td>
<td>-</td>
<td>-</td>
<td>95 minutes</td>
</tr>
<tr>
<td>BF</td>
<td>10:00 am</td>
<td>10:15 am</td>
<td>BF</td>
<td>15 minutes</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>10:30 am</td>
<td>10:45 am</td>
<td>DM</td>
<td>30 ml</td>
<td>-</td>
</tr>
<tr>
<td>S</td>
<td>9:00 am</td>
<td>9:05 am</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

Totals: 95 minutes

Activity Legend:
- SSC – Skin-to-Skin Contact
- BF – Breastfeed
- BP – Breast pump
- S – Saliva Collection
- B – Bottle Feeding
* - nipple stimulation during SSC

Feeding Legend:
- BF - Breastfed
- PM – Pumped milk
- DM – Donor milk
- F – Formula
- TB – Tube feeding
Appendix C: Instructions for Drool Method Saliva Collection

Supplies to be given the day of collection:

2 Collection tubes (2ml Cryovials)
Cup of ice

Collection instructions:

1. On the day of saliva collection arrive at the NICU at 9am.
2. If upon arrival your baby is hungry and needs to breastfeed, nurse your baby or pump. Wait 30 minutes after the feeding prior to saliva collection.
3. When ready for saliva collection, place both cryovials in a cup of ice.
4. Next, rinse your mouth with water and spit it out in a sink or cup for disposal. Mark the time of disposal in your tracking log.
5. Over the next 5 minutes spit out all of the saliva that collects in your mouth into the collection tube without coughing or clearing your throat. Mark the time you finish on your tracking log.
6. Place the cap on the collection tube and place it back in the cup of ice.
7. The PI will immediately label the tube with the collection date and time and transport the specimen to the laboratory for processing.
8. Place your baby skin-to-skin (try to avoid any nipple stimulation, breastfeeding or pumping during skin-to-skin). If nipple stimulation does occur during sample collections merely indicate it on your tracking form with a star. These samples may need to be recollected.
9. Right before removing your baby in skin-to-skin contact (at 55 or 60 minutes of SSC) repeat steps 3-8 for the second saliva sample
Appendix D: Recruitment Screening

Inclusion Criteria Screening: (no to any will be excluded)

1. Mother is 0-7 days Postpartum □ Yes □ No
2. Intends to breastfeed or breast pump □ Yes □ No
3. Initiated breastmilk expression within 12-24 hours after birth □ Yes □ No
4. Performed SSC with her neonate once since delivery □ Yes □ No
5. Speaks and reads English or Spanish □ Yes □ No
6. Access to a telephone □ Yes □ No
7. Has a mode of transportation to and from the hospital □ Yes □ No
8. Neonate born 37 – 22 weeks gestation & admitted to NICU □ Yes □ No

Exclusion Criteria Screening: (yes to any will be excluded)

1. Mother with maternal diagnoses/behaviors contraindicated in breastfeeding (polydrug use, HIV, galactosemia, active TB, radioactive isotopes, chemo therapy, active herpes lesions on the breast) □ Yes □ No
2. Taking medications contraindicated in salivary oxytocin assay (antipsychotic medications) □ Yes □ No
3. Premature infants unable to perform SSC (umbilical lines, terminal conditions, surgical emergencies, congenital anomalies) □ Yes □ No

Eligible for study □ Yes □ No – place in PI box and text 813-469-3129

Consented to participate? □ Yes □ No
Date: ___/____/_____

Participant ID: __________
Appendix E: Recruitment Flyer

Do you plan to breastfeed or hold your preemie skin to skin in the NICU?

If your answer is YES, we are interested in working with you!

Nurse researchers want to better understand how holding your preemie skin to skin affects the release of the breastfeeding hormone oxytocin.

Research participants will be asked to:

- Track daily infant feedings & skin to skin contact for 7 days
- Attend two 90 minute sessions while visiting their preemie in the NICU
- Collect 2 saliva samples each session

All volunteers will receive a $20.00 Baby’s R Us gift card for their participation.

All information is strictly confidential.

For further information please contact:
Jessica Gordon, USF College of Nursing
PhD Student at 813-489-3139
or
Attend an information session held in the NICU consult room.

Date & Time TBA
Appendix G: Check List and Participant Instructions

☐ Meet with the Principal Investigator _____/_____/_____

☐ Complete Consent Form _____/_____/_____

☐ Complete Demographic Questionnaire_____/_____/_____

☐ Receive Participant Instructions & Supplies _____/_____/_____ 

☐ Collect and Store Saliva Samples (within 0-7 days after delivery and 3 days later)

   Day 1_____/_____/_____ ☐ before SSC ☐ after SSC

   Day 2_____/_____/_____ ☐ before SSC ☐ after SSC

☐ Complete Daily Tracking Log

   Day 1 ☐ Day 2 ☐ Day 3 ☐ Day 4 ☐ Day 5 ☐ Day 6 ☐ Day 7 ☐

☐ Submit Tracking Log, Forms and Samples to Principal Investigator by: _____/_____/_____ 

☐ Receive your $20.00 Gift Card to Baby’s R Us_____/_____/_____
Instructions for Mothers Performing Skin-to-Skin Care

Skin-to-skin contact is a safe practice that involves placing your naked baby on your bare chest. Premature babies benefit from skin-to-skin care because it increases their oxygen saturation, calms the baby, and regulates their heart rate, breathing and temperature. The hospital supports skin-to-skin care and routinely promotes when performed within their hospital guidelines.

To perform skin-to-skin:

1. First consult your baby’s nurse to see if it is safe to position your baby skin-to-skin.
2. When safe, prepare for skin-to-skin position by find a chair or recliner with pillows and footstool if available.
3. Wear an open-front shirt or gown to allow direct skin-to-skin contact.
4. Dress your newborn in only a diaper (a hat may be worn as well).
5. Allow the nurse to switch the incubator temperature control from servo-control to air temperature to prevent incubator cooling while newborn is being held.
6. Remove your baby from the crib or isolate and place your baby chest to chest on your bare skin with your baby’s head in chin-tilt position.
7. Hold your baby securely as you recline into a 45 degree angle.
8. Wrap your gown or garment around the newborn and place a cover or blanket over your chest and the newborn.
9. After five minutes if newborn’s feels too warm or temperature is greater than 99.0 degrees F remove the cover blanket.
10. Hold your newborn as long as desired (preferably a minimum of 1 hour) if newborn remains stable.
11. Attempt to perform routine skin-to-skin contact when able.
12. Record each time you perform skin-to-skin with your baby in your tracking log.
13. After skin-to-skin, dress your baby and place them back in his/her crib or isolate.
Instructions for Mothers Pumping in the NICU

A hospital grade breast pump is preferred to help you build a good milk supply for your baby. Single and double pump setups are available. Double-pump setups will allow you to pump both breasts at the same time, but are not required.

After you deliver ask a nurse to provide you with a hospital grade breast pump for your use during your hospital stay if you do not already have one. You should also ask for plastic storage bottles and lids to collect your milk in at each pump session. These storage bottles are for single use only and easily attach to the hospital pumps for your convenience.

Each time you pump keep a daily record of the following information:

- the date of each session,
- start and end time of each pumping session,
- amount of milk pumped each session, preferably in millimeters (ml)
- daily 24 hour total of the amount of milk expressed.

This will help you be more certain that you are producing an adequate milk supply for your baby.

When to pump

It is important for you to begin to pump your breast within four to six hours after delivery, or as soon as you are physically able, especially if you are unable to breastfeed your baby right away. This, along with continued frequent pumping, will help stimulate your breast to produce milk and encourage adequate milk production. The best time to pump is the same time you expect to feed your baby, at least every 2-3 hours. This usually averages out to be 8-10 times a day.

Pumping Instructions

1. Wipe down the pump and wash your hands prior each pumping session.
2. Attach a clean setup with storage bottles to the pump (each bottle should only
collect expressed milk from one single pumping session.)

3. Place yourself in a comfortable position

4. Set the pump on minimum pumping force to begin, adjusting strength as needed.

5. Pump breast for approximately 10-15 minutes. Remember this is just a rule of thumb. Mothers should concentrate more on their own milk flow and are encouraged to pump at least two minutes after your milk flow stops to completely empty the breast.

6. When finished pumping, take the breast pump setup apart and wash all parts in luke-warm soapy water, rinse, air dry and store until next use.

7. Sterilize the pump accessories, excluding the tubing, by boiling them for 15 minutes or putting them through the dishwasher once a day. Microwaveable sterilization bags may also be used as well.

**Labeling Your Milk**

Use a waterproof pen to label each bottle with your baby’s name and the date & time of collection. If the hospital provides preprinted labels please use them but be sure to verify that the name on the label is your baby’s each time you place a label on a bottle you just expressed.

**Storing Your Milk**

Unless there is a medical reason, you can save your breast milk to be used to feed your baby until he/she is ready to eat. During your hospital stay, properly label the provided storage bottles and deliver the breast milk to the NICU immediately after pumping to be placed in the refrigerator or freezer. Hospital freezer-storage containers for long term use are usually available, but may be limited to one container per baby. Any additional milk unable to be stored in the freezer container should be stored at home.
Appendix H: Salivary Oxytocin Assay Specimen Handling Procedure

1. Wear gloves and prepare supplies for collection.
2. Assist patient collect 3-5 ml of saliva via drool method for 5 minutes.
3. Immediately place saliva sample in a cup of ice and add 10 microliters (UL) of Aprotinin (PI) to 3-5 ml of saliva and transport on ice to the laboratory.
4. Spin saliva samples in centrifuge at 4 x 1000 rpms for 10 minutes at 4 degrees C.
5. Label 3 1.5 cryovials (clean tube) according to the labeling guidelines below with permanent marker and fill each vial with 1ml of supernatant.
6. Freeze immediately at -70 degrees C and store in freezer box located in the Microbiology Section of the laboratory within 30-60 minutes of collection.
7. Batch samples once per month and transport to the CON laboratory via dry ice.
8. Store samples in CON freezer at -80 degrees C until ready for Assay.

Specimen labeling guidelines:

001 – Participant ID
XX/XX – Date
0900 – time in military time
#1-4 – time point of collection
   Session 1
    #1 No SSC
    #2 With SSC
   Session 2
    #3 No SSC
    #4 With SSC
Appendix I: Honorarium Receipt

Study: Skin to Skin Contact and Basal Salivary Oxytocin among Lactating Mothers of Premature Infants

PI: Jessica M Gordon, MS, ARNP, CPNP-PC, CLC

Funded By: Southern Nurses’ Research Society Dissertation Award

BayCareIRB # - 2014.001
USF IRB # - 00017904

Participant reimbursement for time:
You will be reimbursed for the time you are providing in order to complete your participation. A one-time receipt of a $20 gift card will be provided.

I, ________________________________________________________________________________________________, (print name)
agree to this reimbursement for participation.

I confirm that I have completed my participation in the study and a $20 gift card payment has been provided to me as reimbursement for my time. I understand that payment will only be received one-time and the amount received will be equal to the amount outlined on this form.

______________________________________________________________________________________________
(print name) (date of birth)

______________________________________________________________________________________________
(signature) (date)

______________________________________________________________________________________________
(street address)

______________________________________________________________________________________________
(city) (state) (zip code)

______________________________________________________________________________________________
(witness signature) (date)
Appendix J: Institutional Review Board Letters

6/24/2014

Jessie Gordon
USF St. Petersburg - College of Arts and Sciences
Family Study Center
140 7th Ave S, Building 1 Suite 100
Saint Petersburg, FL 33701

RE: Expedited Approval for Initial Review
IRB#: Pro00017904
Title: Skin to Skin Contact and Basal Salivary Oxytocin among Lactating Mothers of Premature Infants

Study Approval Period: 6/24/2014 to 6/24/2015

Dear Ms. Gordon:

On 6/24/2014, the Institutional Review Board (IRB) reviewed and APPROVED the above application and all documents outlined below:

Approved Item(s):
Protocol Document(s):
Gordon_SSCandOxytocin Protocol

Consent/Assent Document(s)*:
Gordon_SSCandOxytocin-Version 4_stamped consent.pdf.pdf

*Please use only the official IRB stamped informed consent/assent document(s) found under the "Attachments" tab. Please note, these consent/assent document(s) are only valid during the approval period indicated at the top of the form(s).

(3) Prospective collection of biological specimens for research purposes by noninvasive means.

(5) Research involving materials (data, documents, records, or specimens) that have been collected, or will be collected solely for nonresearch purposes (such as medical treatment or
(7) Research on individual or group characteristics or behavior (including, but not limited to, research on perception, cognition, motivation, identity, language, communication, cultural beliefs or practices, and social behavior) or research employing survey, interview, oral history, focus group, program evaluation, human factors evaluation, or quality assurance methodologies.

Per CFR 45 Part 46, Subpart D, this research involving children was approved under the minimal risk category 45 CFR 46.404. Research not involving greater than minimal risk.

As the principal investigator of this study, it is your responsibility to conduct this study in accordance with IRB policies and procedures and as approved by the IRB. Any changes to the approved research must be submitted to the IRB for review and approval by an amendment.

We appreciate your dedication to the ethical conduct of human subject research at the University of South Florida and your continued commitment to human research protections. If you have any questions regarding this matter, please call 813-974-5638.

Sincerely,

V. Jørgensen MD

E. Verena Jørgensen, M.D., Chairperson
USF Institutional Review Board
IRB Study#: 2014.001

Altln: Jessica Gordon MS, ARNP, CPNP-PC, CLC
10232 Meadow Crossing Dr.
Tampa, FL 33647

Date: 23 April 2014

Submission Type: IRB Progress Report/ Continuing Review Follow Up Resolution

Date on Form: 3/10/2014

Protocol Title: Skin to Skin Contact and Basal Salivary Oxytocin among Lactating Mothers of Premature Infants

PI: Jessica Gordon MS, ARNP, CPNP-PC, CLC

Initial Review Date: 17 April 2014

Review Type: Expedited Review Approval

The submitted documents for the above titled research have been reviewed.

Approval* to commence study has been granted. IRB oversight expires in 12 months.

Previously Approved Document(s): 17 April 2014 Full Board Approval

Research Design Protocol Skin to Skin Contact and Basal Salivary Oxytocin among Lactating Mothers of Premature Infants

Appendix A: Demographics and Medical Information Questionnaire
Appendix B: Tracking Log Instructions
Appendix C: Instructions for Drool Method Saliva Collection
Appendix D: Recruitment Screening
Appendix E: Recruitment Flyer
Appendix G: Check List and Participant Instructions
Instructions for Mothers Performing Skin-to-Skin Care
Instructions for Mothers Pumping in the NICU
Honorarium Receipt

Resolutions Received: Approved 23 April 2014 Expedited Review
Appendix F: Informed Consent Version 4; 9 pgs
The Investigator is responsible for complicity to IRB requirements. Progress reports to maintain continuing IRB review must be approved before oversight expires; an application to renew oversight or close a study should be received 60 to 90 days prior to the end of the review cycle indicated in this letter.

*Implementation date(s) of protocol, informed consent other resources and activities requiring IRB approval cannot be prior to the date indicated in the IRB determination letter.

All requested resolutions must include a letter indicating that the submission is follow up; this will ensure that resolutions are processed appropriately and in a timely manner.

Regards,

IRB Chairperson

Date