

EFFECT OF BABYWEARING ON ACUTE REACTIVITY OF CORTISOL AND
OXYTOCIN IN INFANTS WITH NEONATAL ABSTINENCE SYNDROME: A
FEASIBILITY STUDY

by

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ABSTRACT

Purpose: The purpose of this study was to examine feasibility and test the study protocol for a babywearing intervention aimed at reducing withdrawal symptoms in infants with Neonatal Abstinence Syndrome (NAS) through mechanisms of comfort and connection.

Background: NAS continues to affect thousands of infants every year. Nonpharmacological treatment options are the first line of therapy for NAS, and babywearing is one nonpharmacological intervention that has been shown to decrease heart rate in infant withdrawal.

Methods: A prospective cohort feasibility study design was used to compare three interventions (babywearing, Skin-to-Skin Care or SSC, and walking with a stroller) in two groups (infants with NAS and infants without NAS). A convenience sample of infants was taken from an active parent study examining outcomes in mothers. The infants participated in a 20-minute reactivity lab at baseline (~ 2 weeks of age) and at 3 months to assess the acute reactivity of cortisol and oxytocin in relation to the intervention.

Results: Twenty-five (n=25) infants were enrolled, and eighteen (n=18) completed participation to three months, translating to a 72% retention rate. The groups differed in that the NAS group was more often born by c-section and admitted to the Neonatal Intensive Care Unit (NICU) more often (71% vs. 11%; t-value=2.94, df=14, p = 0.01). Feasibility results included a low intervention compliance (8 – 33%) among both groups and all three interventions. Saliva samples successfully collected with ≥ 1 mL at baseline was 6.8% and at Post-partum month 3 (PPM3) was 51.4%. Exploratory results for cortisol changes from baseline to PPM3 by intervention included a decrease of 0.206 $\mu\text{g/dL}$ (SD 0.28) for babywearing, a decrease of 0.272 $\mu\text{g/dL}$ (SD 0.46) for SSC, and an increase of 0.163 $\mu\text{g/dL}$ (SD 0.38) for the stroller-walking

group. Exploratory results for oxytocin changes from baseline to PPM3 included decreases in all three interventions: babywearing by 20.99 pg/mL (SD 23.26), SSC by 83.79 pg/mL (SD 48.96), and stroller-walking by 237.35 pg/mL (SD 303.62).

Implications to Practice: Babywearing may be effective at decreasing cortisol and minimizing the decrease in oxytocin in infants with NAS, but further research is needed with improvements in intervention compliance and methods for adequate saliva collection.

Abbreviations:

EIA = Enzyme Immunoassay

HPA Axis = Hypothalamic-Pituitary-Adrenal Axis

NAS = Neonatal Abstinence Syndrome

NICU = Neonatal Intensive Care Unit

OUD = Opioid Use Disorder

PPM3 = Post-Partum Month 3 Visit

SSC = Skin-to-Skin Care

CHAPTER I: INTRODUCTION

This dissertation was the culmination of years of work focused on improving the care of neonates as a nurse scientist and advanced practice nurse, pursuing both the Doctor of Philosophy (PhD) and Doctorate in Nursing Practice (DNP) degrees. The research focus was to produce and test a feasibility study examining babywearing as a nonpharmacologic intervention for infants with Neonatal Abstinence Syndrome (NAS). NAS, or infant withdrawal, affects over two thousand infants every month nationally and, despite recent advances in care, is utilizing precious Neonatal Intensive Care Unit (NICU) resources (Leech et al., 2020). Babywearing is an easy-to-use, inexpensive, nonpharmacologic intervention that can be used with almost any infant with NAS by any caregiver, including nurses (Williams, Grisham, et al., 2020). Similar to Skin-to-Skin Care (SSC), babywearing can potentially reduce stress and increase connection, as measured by decreased salivary cortisol and increased salivary oxytocin (Vittner et al., 2019). The lifelong effects of reduced stress and improved interactions for this high-risk population can reduce gaps in health equity by reducing illness and disability from birth (Feldman, 2015). This dissertation is titled “Effect of Babywearing on Acute Reactivity of Cortisol and Oxytocin in Infants with Neonatal Abstinence Syndrome: A Feasibility Study.” The acronym BEAT-NAS is used throughout this dissertation and stands for “Babywearing Exploration of Assays Trial for infants with Neonatal Abstinence Syndrome”. This study is the first step in a program of research to shift paradigms of care for infants with NAS. This first chapter includes the significance and background of the problem, the philosophical underpinnings of this research, the problem statement, purpose, and specific aims of this study designed to address this problem.

Significance

Increasing Incidence of Neonatal Abstinence Syndrome (NAS)

Due to the increasing prevalence of Opioid Use Disorder (OUD), Neonatal Abstinence Syndrome (NAS) has become a national epidemic. The number of infants requiring treatment for NAS has increased from 1.2 in 2002 to 6.8 infants per 1000 live births in 2021, with some states as high as 40.8 per 1,000 live births HCUP (HCUP Fast Stats, 2024) The actual incidence of NAS may be higher than reported in the literature, as not all infants experience moderate to severe withdrawal or receive the diagnosis and medical treatment for their symptoms. It is estimated that every 24 minutes, an infant is born who will experience NAS (Centers for Disease Control and Prevention, n.d.). NAS consists of a spectrum of withdrawal symptoms due to increased adenylyl cyclase activity leading to increases in some neurotransmitters (e.g., norepinephrine, acetylcholine, and corticotropin) and decreases in others (e.g., dopamine and serotonin), resulting in withdrawal symptoms in up to 80% of opioid-exposed newborns (Gomez-Pomar & Finnegan, 2018; Kocherlakota, 2014; Wexelblatt et al., 2018). NAS results in extended Neonatal Intensive Care Unit (NICU) hospitalizations and high costs to the healthcare system as charges can exceed \$90,000 per patient, with Medicaid insuring over 75% of infants with NAS (Patrick et al., 2015). There has been a paradigm shift in the last six years to include parents in the treatment of their infant and optimization of non-pharmacologic care, including the Eat, Sleep, Console approach (Blount et al., 2019; Grisham et al., 2019; Grossman et al., 2017; Wachman et al., 2018). These alterations in practice have demonstrated a decreased need for pharmacologic treatment, reduced length of stay, and increased likelihood of the infant being discharged home with the family (Blount et al., 2019; Grisham et al., 2019; Grossman et al., 2017; Holmes et al., 2016; Miller &

Willier, 2021; Ponder et al., 2021; Wachman et al., 2018). Given this shift in treatment approach, additional non-pharmacologic treatments (such as babywearing) are being sought to aid the infant through their withdrawal symptoms.

Background

Neonatal Abstinence Syndrome (NAS) is the name given to the collection of withdrawal symptoms an infant may experience after birth from in-utero exposure to opioids (Kocherlakota, 2014). The incidence of NAS has continued to rise over the past 20 years, matching the ongoing opioid epidemic (Patrick et al., 2020). Sixty to eighty percent of infants with this exposure will develop moderate to severe symptoms requiring medical treatment. For almost five decades, infants with NAS have been treated with scheduled medication (e.g., morphine every 3 hours) to control the withdrawal symptoms and then weaned slowly as tolerated. This results in large amounts of postnatal opioids being administered to manage the withdrawal symptoms, prolonged hospitalizations for the newborn, and extensive costs to the family and healthcare system (Patrick et al., 2015; Ramphul et al., 2020). Additionally, the long-term neurological effects of extended postnatal opioid treatment for newborns to manage NAS are unknown and could present a risk to normal development.

A new approach to managing infants with NAS began to emerge around 2015. This approach focused on maximizing non-pharmacologic treatment therapies, utilizing the family as part of the treatment, changing the assessment tool used for withdrawal symptoms in the newborn, and changing from scheduled postnatal opioid treatment to as-needed (Grossman et al., 2017). This bundled approach is called Eat, Sleep, Console, or ESC. However, ESC is the simplified assessment of withdrawal by asking if the infant can do three functional tasks. First,

can the infant eat a normal amount for their age? Second, can the infant sleep for at least an hour without their withdrawal symptoms waking them up? And third, can the infant be consoled within 10 minutes? If the answer is yes to any of these three questions, the infant should receive a dose of an opioid to address their withdrawal symptoms that are interfering with normal functional skills. This approach has spread across the country through quality improvement initiatives and, more recently, in a large randomized controlled trial (Blount et al., 2019; Grossman et al., 2018; Young et al., 2023). The results showed a significant reduction in the number of postnatal opioids needed to treat NAS, as well as a decreased length of stay (LOS) and lower healthcare costs (Young et al., 2023).

To maximize non-pharmacologic interventions, clinicians have come up with some new solutions, such as weighted blankets (Summe et al., 2020), smart bassinets that respond to infant crying (Gellasch et al., 2023), and babywearing (Williams, Gebler-Wolfe, et al., 2020). Babywearing is the use of a cloth or infant carrier to secure the infant to the caregiver's body. Babywearing may be preferred to the weighted blankets and smart bassinets, as the infant can remain in close contact with the caregiver, and the caregiver has full use of both hands instead of in-arms carrying (Williams, 2020). These interventions appear to be effective in the clinical setting but lack foundational evidence of the mechanisms involved that aid an infant through their withdrawal symptoms (Williams, Grisham, et al., 2020).

Babywearing as an Intervention for NAS

Babywearing is a low-cost, easy-to-use, nonpharmacologic intervention that facilitates contact and proximity to the mother and has numerous behavioral and biological benefits to the mother and infant (Grisham et al., 2023) Babywearing may also reduce the infant's experience of

stress (as a result of comfort) and may improve neurodevelopmental outcomes in infants with NAS by decreasing the need for additional postnatal opioid treatment. During skin-to-skin care, both cortisol and oxytocin (OT) have successfully been measured in newborns and their parents, demonstrating positive effects of decreased cortisol and increased OT (Cong et al., 2015; Cong et al., 2011; Pados, 2019; Pados & Hess, 2020; Vittner et al., 2019). Babywearing is similar to skin-to-skin care regarding prolonged contact but different in that there are often 1-2 layers of clothing between the mother and infant. There is a paucity of research on the effect of babywearing on cortisol and OT. The BEAT-NAS feasibility study aims to test the integrity of the study protocol and provide preliminary data to lay the groundwork for future research to address this gap in the literature. Both cortisol and OT can be reliably measured through saliva, which is non-invasive and often preferred by parents (Vittner et al., 2019).

Philosophical and Theoretical Underpinnings

A sound theoretical framework underpins scholarly nursing research and provides the connection between practice and science (Hartrick, 2012). The nursing metaparadigm includes the four essential elements of person, health, environment, and nursing practice (Reed, 2021b). This metaparadigm is foundational in developing one's philosophical perspective on the science of nursing (Fawcett, 1984). Many philosophers, including Thomas Kuhn, Lorraine Walker, Yura & Torres, and Jacqueline Fawcett, have informed this disciplinary perspective over the last century (Reed, 2021b). My nursing philosophical view integrates the nursing metaparadigm with my practice experience as a Neonatal Nurse Practitioner (NNP). It reads:

Nursing is a profession that engenders and values knowledge expansion through exploration while incorporating experience, which becomes the link between a person

and their health. Through nursing research and practice, the nurse can create an optimal healing environment and help the patient achieve optimal health by providing evidence-based interventions delivered with comfort, care, and connection.

Epistemology

Epistemology examines *how* knowledge is developed (Chinn & Kramer, 2018). For this work, the three philosophies of science used for the development of scientific knowledge were abstractionism, empiricism, and rationalism (Reed, 2021a). Although developed at different historical times, these systems function best in combination. This research started empirically with the principal investigator's previous experience using babywearing as a tool to comfort an irritable infant with severe gastroesophageal reflux. Second, when working with irritable infants with neonatal abstinence syndrome (NAS), the previous knowledge of babywearing as a tool for fussy infants presented itself as a potential solution through abstractionism. The last step was to rationalize the process by studying the variables' comfort and connectedness to ensure they were logical and reasonable from a theoretical perspective (Reed, 2021a). The epistemological process was crafted through constructionism, where the meanings of comfort and connectedness were constructed about reality (fussy infants) and influenced by beliefs and experiences with babywearing.

Ontology

Environment and Florence Nightingale

One of the first principles to evolve in this philosophical view was the concept that by altering the environment, one can facilitate healing to occur. Florence Nightingale first described this as she identified the critical role that the environment plays in health and wellness (Hegge,

2013). Nightingale could improve soldier health and healing during the Crimean War by ensuring clean water, fresh air, healthy food, sanitary spaces, clean bedding, and decreased noise. Her methods relied heavily on empirical generalizations, which became the basis for a paradigm shift in nursing science in the 1800s (Reed, 2021c). The observations by Nightingale were essential to future work by nurse theorists using abstraction to make the connection between why changes in the environment could improve one's health.

Pepper and the Environment

In the 1900s, several theorists built upon the work of Nightingale, incorporating the environment into their theories and worldviews, all of which contribute to my worldview. Most notable is Pepper's Developmental Mechanistic-Person-Environment Worldview. Pepper theorized that patients react to their environment mechanistically and that the ability to create change lies within the environment, creating a stimulus (Reed, 2021b). For example, the nurse can change the environment with pillows and warm blankets, and with this stimulus, the patient can positively react to the comfort to promote healing.

Comfort and Caring

Two additional theories tied the pieces together in developing this nursing philosophical view. The first was Swanson's Theory of Caring, where the nurse needs to be able to relate to the patient (Swanson, 2010). This occurs through adjustments to the environment and delivery of interventions involving comfort. Kolcaba's Comfort Theory was the second theory, which combines context (physical, social, psychosocial, and environmental) with the senses (ease, relief, and transcendence) to describe different concepts of comfort (Kolcaba, 2003; Kolcaba, 1991, 1994, 1995). Comfort Theory guides the nurse to create or alter the environment to provide

comfort, which promotes healing. Through caring, comfort, and adjustments in the physical and psychosocial environment, the nurse can aid the patient through recovery to health and wellness.

Kolcaba's Comfort Theory

Kolcaba's Comfort Theory is the guiding framework behind the principles defining the effect of babywearing on infants with NAS (Kolcaba & DiMarco, 2005). Comfort Theory emphasizes physical and environmental properties and is characterized as free from pain or anxiety-provoking situations (Kolcaba & DiMarco, 2005; Kolcaba & Kolcaba, 1991). Kolcaba defines comfort as the absence of pain and as something reflective of the person-environment relationship; it can be physical, mental, or both (Kolcaba, 1992). The variables of stress and connection are associated with comfort theory in that increased comfort may facilitate decreased stress and increased connection. Babywearing can increase comfort and be considered a moderator for effects on stress and connection, influencing the strength and direction of these relationships. The primary action of babywearing is altering the environment to create comfort to promote healing and well-being using elements from Kolcaba's comfort theory (Kolcaba, 1994).

Bio-behavioral Synchrony Model

A second and closely aligned theory is the Bio-behavioral Synchrony Model (Feldman, 2012). In this model, the presence of behavioral and biological processes during contact helps to form attachment and bonding. This attachment bond describes the synchrony that occurs between the infant and the parent, developing into a connection that is more significant than relationships with other caregivers. With mother-infant synchrony, there are behaviors (gaze, affect, vocal, and touch) that influence synchrony (Bell et al., 2018). Biological elements such as heart rate, oxytocin, and cortisol responses are mirrored when there is strong synchrony.

Synchrony may be related to connection in that when there is a tangible connection between the infant and the caregiver; there is synchrony.

Calming Cycle Theory

The third theory used to form the basis of this work is the Calming Cycle Theory by Marth Welch (Welch, 2016; Welch & Ludwig, 2017). This theory stems from work done on attachment by John Bowlby and Mary Ainsworth, which built on ideas and concepts from Sigmund Freud (Welch & Ludwig, 2017). Attachment Theory operates based on the central nervous system learning through operant conditioning, and behaviors such as anxious-avoidant and disorganized were the result of deficits in self-regulation and cognitive mechanisms. Calming Cycle Theory hypothesizes that the learning mechanism is through the autonomic nervous system, and Pavlovian conditioning can change an emotionally disconnected behavior to an emotionally connected behavior through interpersonal co-regulation between the mother and the infant. This co-regulation begins in utero and continues long after parturition. Interventions such as babywearing may facilitate this emotional connection, which aids in visceral/autonomic co-regulation, resulting in the resolution of discomfort and distress and producing a subsequent state of calm experienced by the dyad (Welch, 2016).

Statement of the Research Problem

As the incidence of NAS continues to rise, new treatments are being sought to optimize care while minimizing the need for postnatal pharmacologic treatment. Current literature demonstrates babywearing as an effective intervention for reducing symptoms of NAS, but the mechanisms of action behind this intervention are not well studied.

Statement of the Research Purpose

The purpose of this study was to examine feasibility and test the study protocol for a babywearing intervention aimed at reducing withdrawal symptoms in infants with NAS through mechanisms of comfort and connection. Two other interventions, skin-to-skin care (SSC) and walking with a stroller, served as comparison and control groups. The study protocol included a reactivity lab with infants at 2 weeks and 3 months of age. The reactivity lab provided the opportunity to measure cortisol and oxytocin before and after the 20 minutes of activity for the assigned intervention (babywearing, SSC, and walking with a stroller). Cortisol and oxytocin assays from the morning of, the evening after, and during the reactivity lab allowed an opportunity to explore preliminary differences between interventions and groups (infants with NAS and without NAS). Along with the reactivity lab, the Modified Infant Behavior Questionnaire and the Modified Brief Infants Sleep Questionnaire were collected at baseline and three months (Gartstein & Rothbart, 2003; Sadeh, 2004). The feasibility of the collection of these instruments is in this dissertation.

Specific Aims with Research Questions

Aim 1: Feasibility

Examine the feasibility of collecting salivary cortisol and oxytocin in infants with NAS and without NAS.

Research Question #1

How feasible is saliva collection in infants at 2 weeks and 3 months of age?

We will assess intervention compliance (goal $\geq 80\%$), retention rates through 3 months of age (goal $\geq 80\%$), and percentage of acceptable saliva samples obtained (goal $\geq 80\%$ for

collection of 8 samples per participant with $\geq 80\%$ having a quantity of ≥ 1 mL at 2 weeks of age and $\geq 90\%$ having a quantity of ≥ 1 mL at 3 months of age).

H1: Study findings will include $\geq 80\%$ for intervention compliance and retention through 3 months of age.

H2: There will be $\geq 80\%$ of 2-week saliva samples containing ≥ 1 mL of saliva.

H3: There will be $\geq 90\%$ of 3-month saliva samples containing ≥ 1 mL of saliva.

Aim 2: Protocol Acceptability

Test the integrity of the study protocol.

Research Question #2

Will adherence to the study protocol be achieved 80% of the time during study activities?

We will measure the frequency of protocol variations (goal $< 20\%$) and the percentage of participants that reach completion of all study activities (goal $\geq 80\%$) to assess study integrity.

H1: There will be $\geq 80\%$ completion of study activities (IBQ-R, BISQ-R, and reactivity lab) in participants who complete the study.

Aim 3: Exploration of Biological Markers

Explore differences in salivary cortisol and oxytocin in infants in three interventions (stroller, SSC, and babywearing) by study groups (NAS and non-NAS).

Research Question #3

What differences are shown between baseline values of salivary cortisol and oxytocin in infants with NAS compared to baseline values in infants without NAS?

Research Question #4

What differences are seen in salivary cortisol and oxytocin between the stroller (control) group, the babywearing (intervention) group, and the SSC (comparison) group at 2 weeks and 3 months of age?

We will explore cortisol and oxytocin at three time points (8am, 1pm, and 8pm) and compare the means to estimate effect sizes 1) between groups, 2) between interventions, and 3) for changes from baseline to 3 months of age.

H1: There will be a difference in salivary cortisol and oxytocin in infants, 1) between groups, 2) between interventions, and 3) from baseline to 3 months of age.

Summary

This dissertation was conceptualized and designed as a feasibility study to address a challenging neonatal syndrome using evidence, empiric observations, and theoretical underpinnings. NAS has been at epidemic levels for over a decade, and new treatment approaches are needed to aid clinicians in caring for these infants. Babywearing is one proposed intervention for the management of NAS, but concrete evidence on the mechanism(s) behind the effectiveness of babywearing for infants with NAS is lacking. Guidance and countless rounds of feedback from mentors have led to the culmination of this dissertation research, which is the first step in understanding the impacts of babywearing as a tool for improving the care of neonates with NAS.

CHAPTER II: LITERATURE REVIEW

To begin the research process, a thorough literature search was conducted. First, the search included the incidence, pathophysiology, and treatment approaches for NAS. Second, a scoping review on the behavioral and biological effects of babywearing was completed (Grisham et al., 2023). Finally, this review included examining the literature on cortisol and oxytocin, physiological changes in infants, and collection methods for infants.

Search Strategy

Articles on NAS were collected primarily through a search via PubMed. Search terms included Neonatal Abstinence Syndrome, NAS, Neonatal Opioid Withdrawal Syndrome, treatment for NAS, nonpharmacologic treatment of NAS, and ESC, or “Eat, Sleep, Console.” The search method used for the scoping review on the biological and behavioral effects of babywearing followed the systematic process outlined by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) (Grisham et al., 2023; Tricco et al., 2018). This review included a search of seven academic databases (PubMed, CINAHL, Embase, PsycINFO, Sociological Abstracts, SCOPUS, and Google Scholar) using the search terms “babywearing,” “baby wearing,” or “infant carrying.”

Inclusion and Exclusion Criteria

Given the relatively small number of publications on babywearing, no limitations were placed on the dates of publications for the scoping review. Inclusion criteria included peer review, full-text, and availability in the English language. Literature searching for articles on NAS, cortisol, and oxytocin was done manually through PubMed, with this author screening the title and abstracts for relevance to the topic.

Description of the Literature and Research on the Topic

Neonatal Abstinence Syndrome

Neonatal Abstinence Syndrome (NAS) is the diagnosis given to an infant experiencing withdrawal symptoms after birth due to passive in-utero exposure to opioids (Kocherlakota, 2014). The symptoms are categorized as autonomic nervous system, central nervous system, and gastrointestinal symptoms. The physiology behind these symptoms is complex- complicated by the immature neurologic processes in neonates and the pharmacokinetics between the placenta and fetus. Additionally, opiates readily cross the placenta due to their water-solubility properties and low molecular weight. This transfer across the placenta increases with gestational age due to increased maternal blood volume and flow, decreased thickness, and increased surface area of the placenta (Nanovskaya et al., 2008; Sachdeva et al., 2009). One factor that can affect the transfer of opioids across the placenta is when drugs are used in combination (e.g., methadone and heroin), which increases permeability, resulting in greater opioid exposure for the fetus (Kocherlakota, 2014). In addition to the combination of drugs, individual drugs such as fentanyl, are now contributing to more severe and prolonged symptoms of withdrawal in neonates (Rana et al., 2024). Other elements that can affect the degree of withdrawal are genetic and epigenetic factors including variations seen in the mu opioid receptor (OPRM1) gene (Wachman & Farrer, 2019). Due to these factors, the degree of NAS and infant experiences can be widely variable and unpredictable.

The physiology of NAS occurs primarily in the brain but extends into the peripheral nervous system (Pergolizzi et al., 2020). The primary opioid receptor in neonates is the Mu or μ -receptor (Kocherlakota, 2014). Neonatal μ -receptors are concentrated throughout the brain and

extend to parts of the body, including the peripheral nervous system and gastrointestinal tract (Pergolizzi et al., 2020). Repeated exposures to opioids in utero result in well-saturated μ -receptors, and the fetus develops a tolerance to the opioid. When the baby is born, and there is no longer access to these opioids, the μ -receptors in the brain and body become hyperactivated, causing G proteins to release adenylyl cyclase from cell membranes (Kocherlakota, 2014). The release of adenylyl cyclase starts a chain reaction, converting adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP). Increased cAMP results in increased protein kinase, which increases transcription factors that affect the release of several neurotransmitters from within the brain (Raffaelli et al., 2017).

Neurotransmitters

The primary neurologic response to withdrawal comes from the brainstem, specifically the locus coeruleus (LC), located in the pons within the brainstem (Kocherlakota, 2014; Pergolizzi et al., 2020). The LC is also known as the noradrenergic nucleus of the brain, acting as the control center for many of the associated chemical changes that occur during withdrawal. This includes the release of norepinephrine. Norepinephrine is responsible for the fight or flight response and functions to increase heart rate and blood pressure in physical or psychosocial high-stress situations (Bari et al., 2020). In NAS, increases in noradrenaline are responsible for symptoms of tachycardia, hypertension, hyperthermia, and tremors (Kocherlakota, 2014).

A second part of the LC is the ascending pathway, which includes the ventral tegmental area (VTA), hippocampus, hypothalamus, thalamus, and prefrontal cortex (Cai & Tong, 2022; Pergolizzi et al., 2020). This VTA is located in the midbrain and provides storage for dopamine, a hormone that regulates mood. During withdrawal, the LC inhibits dopamine release, causing

symptoms of hyperirritability and anxiety. The ascending pathway of the LC also controls the hypothalamic-pituitary-adrenocortical axis (HPA axis), which regulates the stress response (Herman et al., 2016; Miller et al., 2022). The HPA axis initiates the release of neurotransmitters, which gives a person the energy to handle stressful situations. The hypothalamus releases corticotrophin-releasing hormone (CRH). CRH signals the anterior pituitary to release adrenocorticotrophic stimulation hormone (ACTH). The ACTH travels to the adrenal glands and binds to the zona fasciculata (the second layer of the adrenal gland), which is responsible for cortisol production. Cortisol can give a person a large amount of energy to respond to the acute stressor, but there are also adverse effects of increased cortisol, especially when it is persistent. When stress persists, cortisol continues to be produced until the stress is reduced, removed, or the HPA axis becomes desensitized to the stimulation. Thus, the response is muted through the feedback loop (Herman et al., 2016). In response to stress and stimuli, such as the shortage of opioids in chronically stimulated μ -receptors, this increased cortisol and stress leads to hyperphagia, where the infant eats significantly more than they would if they did not have NAS (Kocherlakota, 2014).

Serotonin (5-HT) is the fourth neurotransmitter regulated within the brainstem. Serotonin is produced by hydroxylation of L-tryptophan with the enzyme tryptophan hydroxylase and is primarily stored within the gastrointestinal tract in the enterochromaffin cells (Berger et al., 2009). Serotonergic neurons control the regulation of serotonin in the raphe nuclei, located within the brainstem, but they extend throughout the central and peripheral nervous systems. When an infant experiences withdrawal, there is modulation (a decrease) in circulating serotonin, and the resulting symptoms include difficulties with sleep (Kocherlakota, 2014). In adults,

mental health problems such as depression have been reported with decreased levels of circulating serotonin (Berger et al., 2009).

Acetylcholine is the last major neurotransmitter that contributes to symptoms of NAS. Acetylcholine is released by neurons in the basal forebrain and mesopontine tegmentum located within the brainstem (Bari et al., 2020). Acetylcholine primarily affects the autonomic nervous system within the brain and other body organs. When this neurotransmitter is increased, as in the case of NAS, neonatal symptoms include diarrhea, frequent emesis, sweating, yawning, and sneezing (Kocherlakota, 2014).

Treatment of NAS

The American Academy of Pediatrics (AAP) has recommended non-pharmacologic interventions as the primary treatment for infants with NAS (Kocherlakota, 2014; Patrick et al., 2020). Holding, swaddling, dimly lit rooms, and decreasing noise and stimulation are some of the non-pharmacologic techniques used with infants experiencing withdrawal. Recent research has demonstrated rooming in, parental presence, and breastfeeding as additional interventions that have significantly reduced the need for medication and decreased length of stay for infants with NAS (Crook & Brandon, 2017; Holmes et al., 2016; Howard et al., 2017). Despite maximizing non-pharmacologic interventions, some infant's withdrawal symptoms remain severe and require pharmacologic treatment (Patrick et al., 2020). Several validated tools are available to assess the degree of withdrawal, and when scores exceed predefined thresholds, medication can be administered (D'Apollito & Finnegan, 2010). Scheduled opioids such as morphine or methadone are commonly used for severe NAS, as well as adjunct medications, including clonidine or phenobarbital. More recently, treatment modalities have trialed the use of opiate

therapy on an as-needed basis, and results demonstrated great success in the reduction of postnatal opioids needed to treat NAS as well as a significant decrease in LOS (Grossman et al., 2017; Young et al., 2023).

Babywearing

A comprehensive and systematic scoping review on the biological and behavioral effects of babywearing was conducted by a research team led by this author as part of the literature review (Grisham et al., 2023). Seven scholarly databases were searched, which yielded over 200 articles that resulted in 29 full-text peer-reviewed articles in English that met inclusion criteria. Findings from the scoping review were categorized into eight themes: (1) increased contact, responsiveness, and secure attachment; (2) physiologic effects; (3) biomechanics and positioning; (4) facilitating and empowering; (5) comfort; (6) maternal benefits; (7) speech, vocalizations, and tempo; and (8) beliefs and perceptions about babywearing. Effects were generally positive (i.e., secure attachment, decreased heart rate), although some were noted to be negative (i.e., discomfort after prolonged babywearing).

Increased contact, responsiveness, and secure attachments were noted in several studies (Anisfeld et al., 1990; Little et al., 2018; Williams, 2020). One of the physiologic effects of babywearing in infants with NAS included decreased heart rates for both the infant and the caregiver (Williams, Gebler-Wolfe, et al., 2020). Babywearing was found to improve posture and positioning as compared to carrying and infant in-arms, but prolonged babywearing was associated with discomfort for the caregiver (Williams et al., 2019). Comfort and decreased crying were noted in several studies, including a landmark study done by Hunziker and Barr

(1986), revealing a significant reduction in the amount of crying in the evening hours for infants less than 3 months of age (Lela Rankin Williams & Patricia R. Turner, 2020).

Babywearing is a feasible nonpharmacologic intervention that facilitates contact and proximity to the mother to support mother-infant interaction and comfort (Reynolds Miller et al., 2020), decreases negative repetitive maternal thinking (Schoppmann et al., 2021), results in better positioning for developmental dysplasia of the hip (Sidharthan et al., 2020; Vaidya et al., 2021), and is empowering for new mothers (Grisham et al., 2023; Moran, 2017; L. R. Williams & P. R. Turner, 2020). Babywearing may also reduce the infant's experience of stress (a result of comfort) and may improve neurodevelopmental outcomes in infants with NAS by decreasing the need for additional opioid treatment postnatally. However, this is currently a gap in the literature and warrants further research in this area.

Cortisol in Neonates

Despite the recent influx of literature on NAS, there remains limited literature on infants' physiologic stress response during withdrawal. Cortisol is a common biological marker that can be measured in saliva to quantify physiologic stress (Rodriguez et al., 2020). Cortisol is released by the hypothalamic-pituitary-adrenal (HPA) axis, which aids humans in responding to physical or psychosocial stressors (Dismukes et al., 2018). The diurnal cortisol rhythm (DCR) or diurnal cortisol slope is the natural daytime pattern of cortisol spiking approximately 30 minutes after waking, followed by a downward trend throughout the day to when cortisol reaches a low point in the evening around bedtime (Adam et al., 2017). The DCR may be altered in those with stress (both acute and chronic). Although infants do not have an established diurnal cortisol rhythm (DCR), when researchers have used lab challenges to evoke stress responses (e.g., "reactivity")

lab), infants are able to mount a cortisol response to stress that can be detected in saliva samples (Adam et al., 2017; Bajgarova & Bajgar, 2020). Given this knowledge, this feasibility study aimed to explore differences in saliva concentrations of cortisol between three interventions (babywearing, SSC and stroller) utilizing a reactivity lab on two occasions. Flattened diurnal cortisol slopes and responses to stress have been associated with mental and physical health conditions in adults (Adam et al., 2017). Saliva in infants with and without in-utero opioid exposure and NAS was collected to measure cortisol to inform clinicians on HPA axis activity in infants with exposure and ultimately direct clinical interventions to prevent long-term mental health conditions (Dismukes et al., 2018).

Oxytocin in Neonates

Oxytocin (OT) is a neuropeptide produced in the hypothalamus that exerts action throughout the brain and body in numerous ways, including acting as a buffer of stress and a facilitator of social connection (Feldman et al., 2014; Norholt, 2021). As mentioned above, the infant responds to stress through the release of glucocorticoids (cortisol) from the HPA axis (Herman et al., 2016). Immediate effects of cortisol are necessary to manage acute stress, but prolonged levels of elevated cortisol can have negative effects on metabolism, memory, immune function, and the cardiovascular system (Sheng et al., 2020). The HPA axis is also responsible for regulating the release of oxytocin (by the paraventricular nucleus) as part of the response to stress (Smith & Wang, 2014). OT has a modulating effect on the cascade of neurotransmitters responsible for producing glucocorticoids, which then yields a decreased sympathetic response (decreased heart rate and blood pressure) and increased parasympathetic response (rest and digest) (Gamer & Büchel, 2012). This “tend-and-befriend” theory on survival suggests that women

respond to stress by tending to their young and integrating socially (befriending) within a group as a protective mechanism in which the collaboration provides safety, ensuring the survival of the species (Taylor et al., 2000). OT responses in infants are not well studied; thus, it is not known whether infants have this protective mechanism as part of the HPA axis response to stress.

Challenges in Saliva Collection and Assay Analysis

Although saliva collection in infants is feasible, several challenges should be considered. The primary challenge with infants is the lack of a regular schedule. For example, many infants do not eat on a schedule and may need to eat frequently (i.e., sometimes less than one hour between feedings). In a research design using a reactivity lab, it can be challenging to collect multiple samples if the infant feeds frequently. Residual milk left in the infant's mouth can affect assay results by cross-reacting with the antibodies (Salimetrics, n.d.-a). Residual milk in the mouth can also cause a dilutional effect, resulting in a falsely low cortisol result. Therefore, it is necessary to wait 30-60 minutes before collecting saliva after a feeding, which will reduce the chance of these errors but does not eliminate them entirely.

A second challenge related to the lack of a schedule is infant sleep. Infants sleep for most of the day, especially in the first few months of life. The ability to collect saliva in some states of alertness (asleep, crying) is nearly impossible or requires an extended dwell time of the swab in the infant's mouth (Granger et al., 2007; Rodriguez et al., 2020). Aside from the inability to collect adequate samples during sleep and crying states, there are inherent interruptions that can disrupt the reactivity timeline. One example is the infant having a soiled diaper. The infant may become upset with the soiled diaper, requiring a diaper change to get the infant back to a calm, quiet state where saliva samples can be collected.

If an adequate saliva sample is obtained, cortisol can be quantified accurately using an enzyme-linked immunosorbent assay (ELISA). However, oxytocin is a complex biological molecule that makes accurate measurement of the endogenous form challenging (MacLean et al., 2019; Tabak et al., 2023). Two reasons why the measurement of oxytocin is challenging include the short half-life of 1-5 minutes in plasma and oxytocin's affinity to bind to other molecules, especially proteins, making it invisible or discarded during standard assay procedures (MacLean et al., 2019). Although the half-life of oxytocin in saliva is unknown, it is thought to be longer than the half-life of oxytocin in plasma, given elevated results hours after administration (Martins et al., 2020). However, researchers recognize challenges in oxytocin assay analysis and MacLean et al. (2019) state the predicament the most eloquently with the analogy of the blind men and the elephant. The moral of the story is that researchers may be looking at oxytocin from different perspectives, and comparisons and methodologies need further research before a best method can be picked.

Evidence Synthesis

NAS is a complex disorder that alters normal levels of neurotransmitters and hormones, causing uncomfortable and even painful withdrawal symptoms, and it continues to rise in incidence and severity due to the continued opioid epidemic. Known factors, including exposure to fentanyl and polysubstance use by the mother, are associated with more severe withdrawal symptoms, and the literature supports that genetic factors may also play a role in the degree to which an infant may experience withdrawal symptoms. Feasible and effective interventions are needed to treat this neonatal epidemic, and babywearing may be one such intervention. The biological effects of babywearing demonstrate a decreased heart rate in infants with NAS

(Grisham et al., 2023; Williams, Gebler-Wolfe, et al., 2020). Salivary cortisol and oxytocin have been studied in neonates in relation to SSC in terms of reduced stress and increased connection (Cong et al., 2011; Vittner et al., 2018). However, the collection of infant saliva for cortisol and oxytocin remains challenging. Even with sufficient saliva collection, several factors may affect assay results, presenting challenges in producing reliable results.

Summary

The literature available on NAS is abundant and points toward the need for new treatment approaches for this long-standing neonatal ailment. There is evolving evidence on babywearing as a potential intervention that could be effective in mitigating the withdrawal symptoms an infant with NAS experiences. Understanding the mechanisms behind this effect may be explained through a better understanding of the autonomic system and HPA axis. For this reason, cortisol and oxytocin, as measures of stress and connection, emerge as the starting point for this research.

CHAPTER III: METHODS

The research design, sample, setting, procedures, and other methods for this dissertation are described in this chapter. The BEAT-NAS was conducted under the scope of the “Role of Our hormones in Bonding with Infants and New Moms (ROBIN)” project, led by principal investigator Alicia Allen, PhD, MPH. Details on the parent study, recruitment, and identification of the target population for this present study are included in this chapter.

Review of Purpose

ROBIN Project – Parent Study

The ROBIN Project is the parent research study funded by NIH/NICHD (DP2-HD105541; PI: Allen), with the overarching aim to understand the hormonal response to infant caregiving and to utilize infant caregiving activities to prevent opioid relapse during the postpartum period. The ROBIN Project is one sub-study as part of the DP2 funding, with the aim of assessing the feasibility of a study directed at understanding hormonal patterns in mothers in relation to three infant caregiving activities: babywearing, skin-to-skin care (SSC), and walking with a stroller. The ROBIN Project only studied maternal outcomes, whereas the BEAT-NAS study measured infant outcomes.

In the ROBIN Project, data were collected during daily and weekly surveys, as well as five in-person visits. The in-person visits included enrollment (32-40 weeks gestation), baseline (<4 weeks postpartum), Postpartum Month 1 (PPM1), Postpartum Month 2 (PPM2), and Postpartum Month 3 (PPM3). As mentioned previously, in August 2024, a modification was made to the study protocol. For the ROBIN Project, the project procedures were amended to decrease from five in-person visits to four total visits (enrollment, Baseline, PPM1, and PPM3),

with the enrollment and PPM1 visit preferentially conducted virtually. This was done to decrease participant burden and increase recruitment and retention. For BEAT-NAS, the modification added the SSC intervention to allow for increased opportunity to assess the feasibility of saliva collection. Reactivity labs were incorporated into the baseline, PPM2, and PPM3 visits before the modification and during the baseline and PPM3 visits after the modification.

Participants in the ROBIN Project were compensated for their participation at each in-person visit if 80% of surveys were completed, and the visits had increased compensation to incentivize completion for full study participation. It was estimated that the ROBIN Project would collect over 9,000 data points to discover underlying trends in hormones, caregiving activities, and relapses. The design of the ROBIN Project provided the ideal opportunity for the BEAT-NAS study to collect data on newborns in two groups (with and without NAS) and in three interventions (babywearing, SSC, and stroller) to assess for differences in the effect of babywearing.

BEAT-NAS – Current Study

The purpose of this study was to examine the feasibility and test the study protocol for a babywearing intervention aimed at reducing withdrawal symptoms in infants with NAS through mechanisms of comfort and connection. The *central hypothesis* is that babywearing as an intervention would increase comfort for infants with NAS, similar to SSC mechanisms of reducing stress and increasing connection, which could lead to a decreased need for postnatal pharmacologic treatment of NAS symptoms. To test this hypothesis, babywearing was identified as the experimental intervention, walking with a stroller was used as a control intervention (given the little to no physical contact during the activity), and SSC was added as a third

intervention for comparison. The addition of the SSC intervention occurred in August 2024 to increase participant numbers, which would increase the ability to evaluate the feasibility of saliva collection, cortisol and oxytocin results, and protocol adherence. SSC is also similar to babywearing in prolonged contact, but different in that there is a clothing layer present between the caregiver and the baby with babywearing, which is not present with SSC. The ability to compare these groups in a full-scale study to assess whether babywearing is at least as effective as SSC in comforting infants provides valuable information to address this gap in the literature. To accomplish the purpose of the study and test the central hypothesis, three specific aims were created.

Specific Aims with Research Questions

Aim 1: Feasibility

Examine the feasibility of collecting salivary cortisol and oxytocin in infants with NAS and without NAS.

Research Question #1

How feasible is saliva collection in infants at 2 weeks and 3 months of age?

We will assess intervention compliance (goal $\geq 80\%$), retention rates through 3 months of age (goal $\geq 80\%$), and percentage of acceptable saliva samples obtained (goal $\geq 80\%$ for collection of 8 samples per participant with $\geq 80\%$ having a quantity of ≥ 1 mL at 2 weeks of age and $\geq 90\%$ having a quantity of ≥ 1 mL at 3 months of age).

H1: Study findings will include $\geq 80\%$ for intervention compliance and retention through 3 months of age.

H2: There will be $\geq 80\%$ of 2-week saliva samples containing ≥ 1 mL of saliva.

H3: There will be $\geq 90\%$ of 3-month saliva samples containing $\geq 1\text{mL}$ of saliva.

Aim 2: Protocol Acceptability

Test the integrity of the study protocol.

Research Question #2

Will adherence to the study protocol be achieved 80% of the time during study activities?

We will measure the frequency of protocol variations (goal $< 20\%$) and the percentage of participants that reach completion of all study activities (goal $\geq 80\%$) to assess study integrity.

H1: There will be $\geq 80\%$ completion of study activities (IBQ-R, BISQ-R, and reactivity lab) in participants who complete the study.

Aim 3: Exploration of Biological Markers

Explore differences in salivary cortisol and oxytocin in infants in three interventions (stroller, SSC, and babywearing) by study groups (NAS and non-NAS).

Research Question #3

What differences are shown between baseline values of salivary cortisol and oxytocin in infants with NAS compared to baseline values in infants without NAS?

Research Question #4

What differences are seen in salivary cortisol and oxytocin between the stroller (control) group, the babywearing (intervention) group, and the SSC (comparison) group at 2 weeks and 3 months of age?

We will explore cortisol and oxytocin at three time points (8am, 1pm, and 8pm) and compare the means to estimate effect sizes 1) between groups, 2) between interventions, and 3) for changes from baseline to 3 months of age.

H1: There will be a difference in salivary cortisol and oxytocin in infants, 1) between groups, 2) between interventions, and 3) from baseline to 3 months of age.

Research Design

The BEAT-NAS study was a prospective cohort quasi-experimental feasibility study designed to compare three interventions (babywearing, SSC, and walking with a stroller) in two groups (infants with NAS and infants without NAS). Mothers of infants in the babywearing and SSC interventions were instructed to perform their assigned activity for a total of one hour a day. Mothers of infants in the stroller walking group were instructed to walk with the infant in the stroller five times a week for 30 minutes each time after being cleared for exercise by their maternity provider. Parents could use the stroller provided by the research study or a different stroller they preferred. Reactivity labs (described below) were conducted at baseline (around 2 weeks) and at 3 months of age to assess feasibility and test the study design.

The BEAT-NAS study aligns with the ROBIN Project in that the purpose of the BEAT-NAS was to examine feasibility and test the study protocol for a babywearing intervention aimed at reducing withdrawal symptoms in infants with NAS through mechanisms of comfort and connection. Babywearing was one of the included interventions, and the infants born to mothers with OUD were all diagnosed with NAS. The stroller group was utilized as the control group because there was little to no physical contact during the stroller-walking intervention. The SSC group was added during a modification in August 2024 to help establish the feasibility of saliva collection in infants and protocol adherence by the mothers and infants. The ‘study within a study’ was ideal, given that the ROBIN Project was collecting data on the mothers, and this BEAT-NAS study was collecting data on the infants.

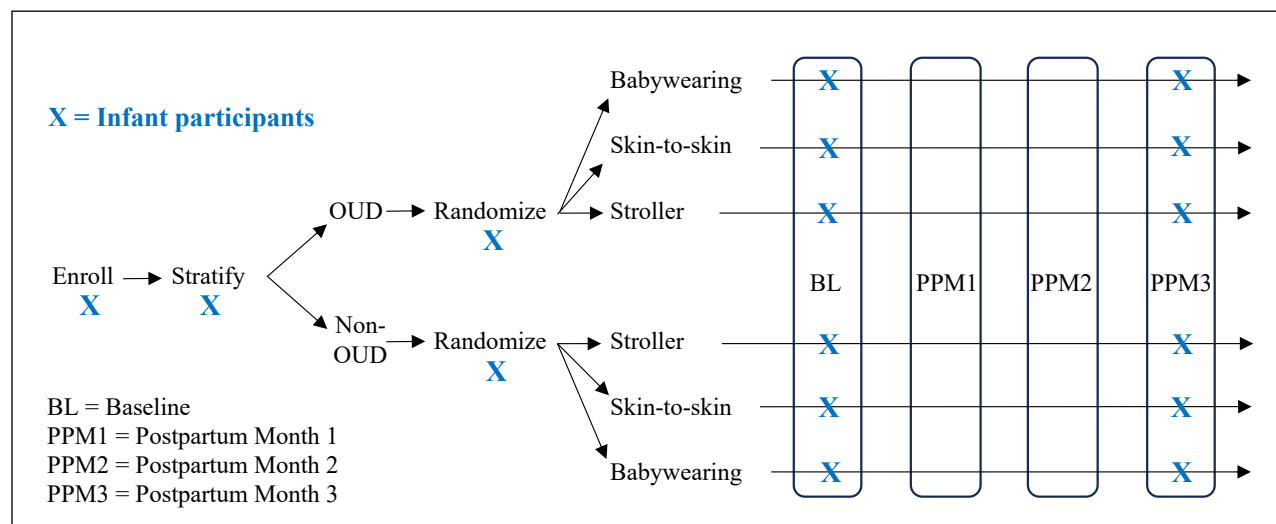
Sample

The target population for the BEAT-NAS study was infants with Neonatal Abstinence Syndrome. Infants without NAS were also recruited to serve as a control population. A convenience sample of infants was recruited through the parent study. Recruitment for the parent ROBIN Project started in the Summer of 2023. Previous successful recruitment strategies from the DP2 precursor study were used (Allen et al., 2024). In addition, information on the ROBIN Project was given to potential participants at Banner University Medical Center Tucson as identified by this PI and research team members. The intended ratio of infants with NAS to those without was to mimic the parent study at a rate of 2:1. Due to low enrollment, a modification was made to the ROBIN Project in August 2024 to change enrollment to a 1:1 ratio of mothers with OUD to mothers without OUD (control). This resulted in a new goal of a 1:1 ratio for this BEAT-NAS study.

The final sample consisted of 25 infants. As part of the parent study, infants born to mothers with Opioid Use Disorder (OUD) were stratified into one group, and those infants were assessed for NAS. All infants born to mothers with OUD were either diagnosed with NAS in the hospital or met the criteria as defined by Jilani et al. (2022). Infants born to mothers in the non-OUD group were also assessed for NAS using the same criteria. No infants in this group met the criteria for NAS. Thus, there were no exclusions. Figure 1 illustrates the convenience sample of infant participants recruited from the parent study.

Figure 1

Randomization and Data Collection for Infants at the Baseline and PPM3 Visits



Inclusion Criteria

1. Infant is < 4 weeks old
2. Infant has either:
 - a. Diagnosis of NAS and/or exposure to opioids in utero with two or more symptoms of NAS (excessive crying, fragmented sleep, tremors, increased muscle tone, and gastrointestinal dysfunction) as defined by Jilani et al. (2022)
 - b. Does not have exposure to opioids in utero and does not display symptoms of NAS
3. Is currently living with the mother
4. Mother is fluent in English

Exclusion Criteria

1. Infants with significant congenital birth conditions (e.g., hydrocephalus, congenital heart disease)

2. Mother not enrolled in the DP2 ROBIN parent study

Sample Size Calculation

This convenience sample consisted of infants ($n=25$) enrolled in the ongoing parent study in an urban medium-sized city in the Southwest United States. Given the paucity of literature on the biological effects of babywearing, effect sizes were difficult to estimate. Given this study's feasibility and exploratory nature, a small sample was used to address the aims. If we had hypothesized a medium effect size between groups, a sample size of 210 participants would have been necessary (105 in each group) to have 0.95 power to detect a medium effect ($d = 0.5$) with a two-tail t-test at $\alpha=0.05$. For a small effect size ($d = 0.3$), we would have needed 290 participants per group (two-tail t-test, at $\alpha=0.05$). However, because this was a feasibility study and the outcomes of interest were focused on feasibility, a sample size of 25 was scientifically justifiable (Julious, 2005; Lancaster et al., 2004; Sim & Lewis, 2012).

Setting

Most of this research occurred at the Collaboratory, a 20,000-square-foot space on the 3rd floor of the Abrams Public Health Building, which is leased for UA research. In this space, there was a dedicated interview room that was furnished by the parent study with a rocking glider chair, a playpen with a changing table, an infant rocking chair, and a playmat to increase parent and infant comfort during the visits. Enrollment and baseline visits could also occur at Banner University Medical Center – Tucson (BUMC-T) if the infant was still a patient within the first several weeks of life. Only two enrollment visits were conducted at BUMC-T; one was held in a private “lullaby” room, and the second was in a private patient room. In both situations, the

parents had access to comfortable chairs and care items for the infant. After the August 2024 modification, the enrollment and month 1 visit for the parent study could be conducted virtually.

Recruitment Approach

Maternal and infant participants were recruited through the ROBIN Project and included flyers, social media, and community outreach (e.g., tabling events), mimicked after the ORCHID study. Recruitment and enrollment occurred in two steps. First, participants who expressed interest or were amenable to receiving more information about the study were directed to complete an online REDCap® interest form (<https://redcap.link/robin>). On the interest form, potential participants could choose to see more information online or have a research assistant call and provide study information over the phone. In this initial online interest survey, there was a check box for the participant to consent to answering the initial screening questions (e.g., age, pregnancy status, gestation of pregnancy, language spoken, substance use, etc.). There was also information regarding the details of the ROBIN Project, compensation, confidentiality, and inclusion of the infant in the study if the infant met the criteria. If the participant met eligibility criteria based on the given information, then additional boxes populated on the online form to allow the participant to provide contact information (e.g., first name, phone number, email). At that point, a research assistant was notified by email and contacted the participant to do a phone screening to provide additional information and confirm eligibility. If the participant met the inclusion criteria, they were emailed a copy of the consent form, and the enrollment visit was scheduled.

Procedures

Enrollment

At the enrollment visit, all eligibility criteria were reviewed for accuracy. The study was described in detail, and the informed consent form (ICF) was provided to the participants to read through. An electronic version of the ICF was available on the iPad, as well as a paper copy, whichever the participants preferred. After the ICF was reviewed and all questions were answered, consenting research staff asked five questions to ascertain whether the participants fully understood their participation in the study. The questions asked about the purpose of the study, who was funding the study, what would be asked of the participants during the study, risks of participation, and who to contact if they had questions. After these questions were answered, the ICF was completed by collecting an electronic signature from the participant and the research staff, who completed the consenting process.

At the enrollment visit, maternal participants in the ROBIN Project were stratified into OUD or non-OUD groups. Enrollment usually occurred before the birth of the baby, so assessment for the presence of NAS was not completed at the enrollment visit. Data collected at this visit was primarily for the ROBIN Project and included surveys on anxiety, stress, and depression and information on demographics (race, ethnicity, insurance, marital status, etc.), pregnancy, and substance use. A stress, trauma, and resiliency interview was also completed and recorded at this visit.

Baseline Visit

At the baseline visit, the maternal participants were randomized to one of the three caregiving activities (Figure 2) by a computer-generated randomization process embedded within

REDCap®. Mothers were educated on their assigned activity and instructed on the frequency and duration of the activity, which were similar among the three interventions. During this visit, the reactivity lab was completed, along with two validated measures. The first was the Infant Behavior Questionnaire – Revised (IBQ-R), which measures infant temperament, and the second was the Modified Brief Infant Sleep Questionnaire – Revised (BISQ-R), which assesses sleep quality as perceived by the caregiver.

Infant Behavior Questionnaire – Revised (IBQ-R)

Two additional measures were collected to supplement the biological assay data collected from the infants. The first was the IBQ-R, a 14-scale instrument that measures infant temperament (Gartstein & Rothbart, 2003). The IBQ-R was initially validated for infants between 3 and 12 months of age (Gartstein & Rothbart, 2003; Putnam et al., 2014), but has more recently been validated in infants as young as two weeks old (Dias et al., 2021), and in racially diverse populations as well as low-income households (Van Schagen Johnson et al., 2016). Items on the measure include approach, vocal reactivity, high-intensity pleasure, low-intensity pleasure, smiling and laughter, activity level, perceptual sensitivity, sadness, distress to limitations, fear, falling reactivity/rate of recovery, cuddliness, duration of orienting, and soothability. A higher score in these areas indicates a greater tendency to demonstrate that behavior. The inter-reliability of the IBQ-R for infant reactivity was .73 and the kappa for discrete behaviors was between .72 and .93 (Gartstein & Rothbart, 2003; Parade & Leerkes, 2008). The IBQ-R was collected at the baseline and month three visit before the reactivity lab. In instances where the mother did not finish the IBQ-R before it was time to start the reactivity lab,

the survey was paused and resumed after the activity. Data on the feasibility of completion of the IBQ-R at the reactivity labs was collected and is reported in the results.

Brief Infant Sleep Questionnaire – Revised (BISQ-R)

The second validated instrument collected at the baseline and month three visit was the BISQ-R. The BISQ-R is a 33-question instrument that measures infant sleep quantity and quality, napping, nighttime sleep habits, the environment (place and people), and parents' perception of infant sleep (Mindell et al., 2019; Sadeh, 2004). The higher the score, the better the sleep quality. The BISQ-R demonstrated good test-retest reliability ($r .82-.95$) and a significant correlation between survey data and actigraph measures of sleep ($p < .0001$) (Sadeh, 2004). The underlying biological mechanism for using infant sleep as a measure is that babywearing may regulate dopamine and serotonin to promote regular sleep-wake cycles (Monti & Jantos, 2008). Dopamine and serotonin have both been recognized as disrupted when an infant experiences NAS, and thus, sleep may be an additional measure of withdrawal and infant comfort (Kocherlakota, 2014). Data on the feasibility of completion of the BISQ-R at the reactivity labs was collected and is reported in the results.

Saliva Collection

Infant saliva samples were collected at five time points (AM, pre-activity, post-activity, post-activity 30 min, and PM) on two different occasions (baseline and PPM3) to determine concentrations of cortisol and oxytocin. See Reactivity Lab in the next section for a complete description of the activity. The AM, pre-activity, and PM samples were collected to establish whether diurnal rhythms/patterns existed, given that newborns often do not have regular DCR. These samples were also collected to evaluate differences between groups for cortisol and

oxytocin. The post-activity and post-activity 30 min samples were collected following the reactivity lab to assess the short-term effects of the intervention. Given that this is a feasibility study, we recognized that statistically significant findings were unlikely. However, testing the design was essential in developing a full-scale study to be conducted later.

Mothers were given verbal and written instructions on collecting and storing infant saliva samples at home, which included weighing the sample to aim for a goal of 1 gram, and immediate storage in their home freezer until research staff came to pick up the sample. Additionally, research staff demonstrated infant sample collection at the baseline visit. Automated text reminders were sent from REDCap® to the participants' mothers in the morning (time determined by the participant) and at 8pm, reminding participants to collect the infant's saliva closest to that time but at least 30 minutes after a feeding. This survey contained questions on activities in the last hour, the ability to complete the saliva collection, challenges with the collection, the time of collection, and the time the sample was placed in the freezer. See Table 1 for collection time points for cortisol and oxytocin.

Table 1

Cortisol and Oxytocin Time Points for Collection

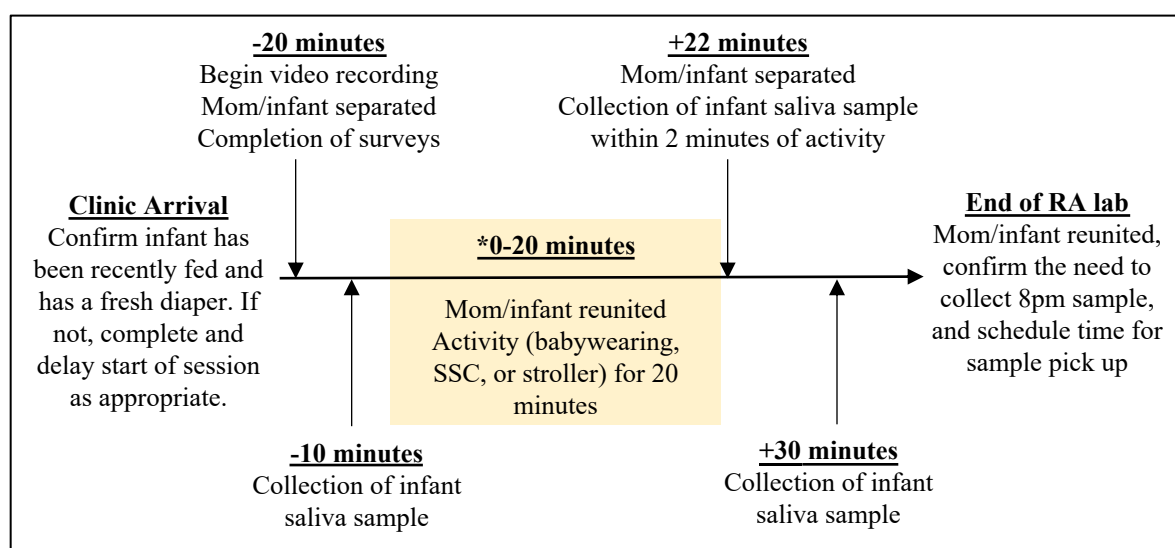
| Time Point | Baseline | PPM3 |
|----------------------|-----------------|-------------|
| AM | F + OT | F + OT |
| Pre-activity | F + OT | F + OT |
| Activity for 20 min | ----- | ----- |
| Post-activity | F + OT | F + OT |
| Post-activity 30 min | F + OT | F + OT |
| PM | F + OT | F + OT |

Reactivity Lab

The purpose of the reactivity lab was to assess for an acute change to stress and connection as measured by cortisol and oxytocin. During the reactivity lab, the mother and infant were first separated, so no contact was present for 20 minutes. The infant did stay within visual contact with the mother during this time. Just before the activity, saliva was collected from the mother and infant. The assigned intervention (aka activity) was performed for 20 minutes, and repeat samples were collected immediately after the activity and again 30 minutes later (Figure 2).

Figure 2

Timeline of Reactivity Lab



We anticipated and appreciated the challenges in adhering to this timeline as infants often have needs that come up randomly (e.g., hunger, dirty diaper, sleepy). We checked to see if the infant had been fed recently and had a clean diaper before starting the reactivity lab. If the timeline changed due to the infant's needs, we prioritized the care of the infant over study activities. In these

situations, we proceeded as able, documenting infant activities and the times samples were collected. Sometimes, samples were not collected per the mother's request (e.g., the infant sleeping after being fussy).

After the reactivity lab, IBQ-R, and BISQ-R were completed, the mother and infant were assessed for well-being, and the mother was provided water and snacks. The mother was reminded to collect the evening saliva sample from the infant around 8pm and at least 30 minutes after a feeding, and a time was arranged for the research staff to pick up the sample the next day. Payment was provided as part of the ROBIN Project, and the participant was escorted to the lobby of the Abrams building.

Post-Partum Month 3 Visit (PPM3)

The PPM3 visit was similar to the Baseline visit, with morning and evening saliva samples, IBQ-R and BISQ-R questionnaires, and another reactivity lab to assess the feasibility of completing the reactivity lab, which could potentially measure the effects of the intervention compared with the control and comparison interventions. The PPM3 visit also included a ten-question study satisfaction survey to gather data on participants' perspectives of the feasibility of the ROBIN Project.

Human Subjects Protection

The BEAT-NAS Feasibility study abided by the rules and regulations of the University of Arizona Institutional Review Board (IRB) and was conducted between May 2023 and May 2025. The ROBIN Project was IRB-approved (STUDY00002022) by the University of Arizona (Appendix D) and included this dissertation work within the application.

Infant data was collected and transmitted only in de-identified form. Our approach was to store the coded list within REDCap®, which also maintained the security of the survey responses. Any data removed from REDCap® was stored on password-protected servers, reducing the risk of disclosure of confidential information. Researchers did not attempt to identify any subject.

A potential benefit of participating in the BEAT-NAS study was the knowledge that one was contributing to science and aiding in the production of knowledge on the mechanisms underlying the comforting effect of babywearing. There were minimal risks to infants participating in the BEAT-NAS study. Risks included discomfort, waking a sleeping infant, and potentially a fall. The risk of fall was no greater than that of any individual using a commercially available stroller or infant carrier; nonetheless, the research team took extra precautions to avoid infant falls. There were no falls during to this study, and the only adverse events that were reported to the IRB included illnesses that occurred outside of the study (e.g., infant diagnosed with influenza at 1 month of age).

Analysis

The BEAT-NAS feasibility study primarily used descriptive statistics to describe the groups and address Aims 1 and 2. An independent sample t-test was performed to assess for differences in the NAS and Non-NAS groups. For Aim 3, the Kruskal-Wallis, a non-parametric one-way ANOVA, was used to assess intervention compliance. The Mann-Whitney U, non-parametric independent samples t-test was used to assess time spent in other activities, and the paired samples Wilcoxon Signed Ranks Test was used to analyze the percentage of saliva collected. The latest version of SPSS (30.) was used for all statistical analysis.

Aim 1: Feasibility

Examine the feasibility of collecting salivary cortisol and oxytocin in infants with NAS and without NAS.

The feasibility analysis was performed with descriptive statistics for intervention compliance (percentage; compliance divided by total enrolled), retention rates (percentage; complete through three months divided by total), and acceptable saliva samples (percentage; number of samples with ≥ 1 mL at 2 weeks and 3 months of age divided by the total number of samples provided at each respective time point).

Aim 2: Protocol Acceptability

Test the integrity of the study protocol and provide preliminary data.

The number of study activities and protocol variations (e.g., baby required adjustments like feeding or diaper change) was quantified for each participant, and protocol acceptability was calculated by looking at overall compliance with study activities. Participant study activities included the IBQ-R and BISQ-R surveys, daily intervention activity for babywearing and SSC, and stroller walking five times a week, and saliva collection at five time points (am, pre, post, 30 min, and pm) on two occasions (baseline and PPM3). Activity completion was monitored through REDCap®. Protocol variations were assessed for frequency and primary and secondary causes for the variation.

Aim 3: Exploration of Biological Markers

The differences in salivary cortisol and oxytocin were explored in infants in two study groups (NAS and non-NAS), with three interventions (babywearing, SSC, and stroller).

The Kruskal-Wallis, a non-parametric one-way ANOVA, was used to assess intervention compliance. The Mann-Whitney U, non-parametric independent samples t-test was used to assess time spent in other activities, and the paired samples Wilcoxon Signed Ranks Test was used to analyze the percentage of saliva collected.

Cortisol

Cortisol concentrations at Baseline and PPM3 were averaged separately by group and intervention at each time point (8am, 1pm, 8pm) for analysis according to the time point (baseline and PPM3) to calculate a within-group average and were analyzed using the Wilcoxon Rank Sum test. Cortisol values were assessed for the presence of a diurnal cortisol rhythm (DCR) slope from morning to evening at baseline and PPM3. Concentrations (mean \pm SD) were evaluated for normality and found to be nonparametric. Differences in cortisol concentrations were compared using the Mann-Whitney U - non-parametric independent samples t-test. The last analysis was the Kruskal-Wallis, non-parametric one-way ANOVA to assess for differences in cortisol from baseline to PPM3 by intervention.

Oxytocin

Oxytocin analysis was similar to the cortisol in that oxytocin concentrations were averaged separately by group and intervention at each time point (8am, 1pm, 8pm) for analysis according to the time point (baseline and PPM3) to calculate a within-group average and were analyzed using the Wilcoxon Rank Sum test. Differences in oxytocin concentrations were compared using the Mann-Whitney U - non-parametric independent samples t-test and the Kruskal-Wallis, non-parametric one-way ANOVA was used to assess for differences from baseline to PPM3 by

intervention. Oxytocin values were also assessed for any patterns from morning to evening at baseline and PPM3.

Assay Analysis

Cortisol

To analyze salivary cortisol levels, an Enzyme Immunoassay (EIA) test from Salimetrics® was used, and samples were run in the Biological Core Laboratory at the College of Nursing, University of Arizona (Salimetrics, n.d.-b). The EIA followed a multi-step process using a microtiter plate covered with antibodies, as outlined by Salimetrics (n.d.-b). Before running the assays, we determined plate layouts to ensure all samples from one participant were run on the same plate to avoid variability from minor differences in antibody plates. Once the plate layout was determined, 25 µL was placed into each well (standards, controls, and saliva samples). The third step was to dilute the enzyme conjugate with 24mL of assay dilutant and add 200 µL to each well. Fourth, we mixed the plate on the plate rotator for 5 minutes at 500 rpm. Fifth, we washed the plate 4 times with 1X buffer on the plate washer. Sixth, we added 200 µL of TMB Substrate Solution to each well with a multichannel pipette. Seventh, we mixed the plate on a plate rotator for 5 minutes at 500 rpm and incubated it in the dark for 25 minutes. Eighth, we added 50 µL of stop solution with the multichannel pipette. Ninth, we mixed the plate on the plate rotator for 5 minutes at 500 rpm until the green color turned yellow. Finally, we read the plate in the plate reader at 450 nm within 10 minutes of adding the stop solution.

Oxytocin

To analyze salivary oxytocin levels, we used the enzyme immunoassay (EIA) kit from Arbor Assays®. The EIA uses an antibody to bind to the oxytocin molecule for measurement.

The participant's sample (saliva) is added to each well in a plate coated with oxytocin antibodies. For each participant, this can be done twice (in duplicate) or three times (triplicate) to compare results and increase accuracy. Theoretically, the participant's oxytocin binds to the antibody on the plate until there is no more free-floating oxytocin. Then, a specific solution with a known amount of enzyme-labeled oxytocin is added to the well, which binds to the remaining antibody sites. The plate is washed to remove the remaining unbound material, and a transparent substrate is added to the well. This reacts with the enzyme-labeled oxytocin and produces a color indicative of how much enzyme-labeled oxytocin is in the well. The lighter color indicates less enzyme-labeled oxytocin and more participant oxytocin. The darker the color, the less participant oxytocin. The color must be read by a calibrated microplate reader or spectrophotometer (Gnanadesikan et al., 2021). A BioTek Synergy LX plate reader was used for this study.

The strengths of plasma ELISA assay are that immunoassays can measure oxytocin in multiple forms (including when it is degraded) and when it is bound to other components (MacLean et al., 2019). This allows the inclusion of oxytocin molecules that would otherwise be discarded in the washing process or missed during the detection phase. The limitation of an ELISA is that we cannot be sure it measures just the oxytocin molecule. This is due to the wide range of interference, cross-reactivity, and heterophilic interference, which generally result in a false positive or reading higher than the actual amount of oxytocin in the sample.

Summary

The BEAT-NAS prospective quasi-experimental feasibility study, conducted under the ROBIN Project, provided a prime opportunity to collect infant data on the effect of babywearing on the acute reactivity of cortisol and oxytocin in infants with NAS. The objectives of feasibility,

protocol acceptability, and exploration of biological markers were designed to assess the capability to conduct this study with two groups and three interventions before progressing to a full-scale study. Additional components tested in the study design included saliva collection at home by parents, the capacity to complete a 20-minute reactivity lab, saliva collection at three time points during the reactivity lab, completion of two validated measures, and participation in the study for three months. Refer to chapter four for the results of the BEAT-NAS study.

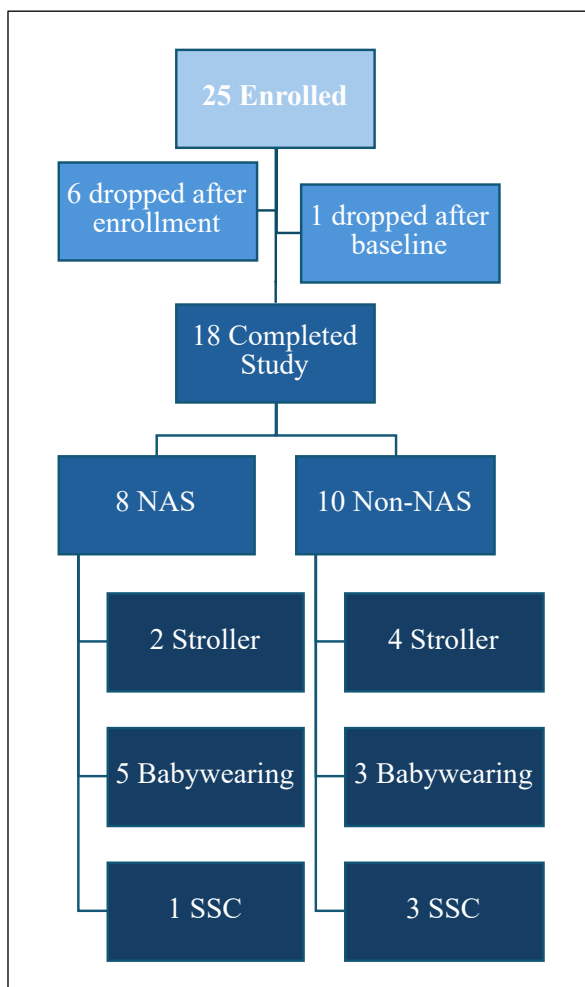
CHAPTER IV: RESULTS

This chapter contains results to address Aims 1-3 of the BEAT-NAS feasibility study. Demographics and sample characteristics are described in detail, followed by the results associated with each specific aim, which addresses the overarching purpose of testing the feasibility and acceptability of the study design.

Description of Sample

Mothers

Twenty-five mother-infant dyads were enrolled in the parent study, from which the BEAT-NAS convenience sample was taken. Of the 25, six dyads dropped after the enrollment visit and one after the baseline visit. Eighteen infants completed the study with participation through the PPM3 visit. There was no selection bias evident as the participants were evenly divided between the NAS and Non-NAS groups. See Figure 3 for the enrollment and randomization allocations.

Figure 3*Enrollment and Randomization Flowchart*

Demographics from the mothers in the parent study were used to describe similarities and differences in the infant NAS and Non-NAS groups. The groups were similar in age, gravida, para, race, ethnicity, education level, marital status, and housing (Table 2). The mean maternal age in the OUD group was 31.33 years (SD 5.08), while the mean age in the Non-OUD group was 30.92 years (SD 3.65). A chi-square test revealed no statistical significance between groups, $\chi^2(1, N = 25) = 13.11, p = 0.44$. In the OUD group, 10 participants (83%) identified as White,

compared to 11 participants (85%) in the Non-OU D group, $\chi^2(1, N = 25) = 2.19, p = 0.54$.

Additionally, 9 participants (75%) identified as Hispanic in the OU D group as compared with 7 (54%) participants in the Non-OU D group, $\chi^2(1, N = 25) = 2.16, p = 0.14$.

No significant differences were observed in the number of pregnancies (gravida), births (para), and housing in the two groups. For housing, it was noted that the OU D group had 4 participants who either lived in transitional housing or reported being at risk of losing their housing, while none were in transitional housing or at risk for losing housing in the non-OU D group.

Although not statistically significant, the OU D group was more likely to have public insurance (n=11, 92%) compared to the Non-OU D group (n=9, 69%, $\chi^2(1, N = 25) = 3.11, p = 0.08$). The start of prenatal care and education levels were similar in the two groups. Prenatal care in the OU D group started at an average of 11.42 weeks (SD 5.13) compared to 7.31 weeks (SD 3.47) for the Non-OU D group, $\chi^2(1) = 10.31, p = 0.59$. Education level for the OU D group ranged from some high school to some college (e.g., 2-year degree), compared to the Non-OU D group ranging from completing high school to having a graduate degree, $\chi^2(1) = 7.205, p = 0.13$.

Table 2*Maternal Demographics (n=25)*

| Mother | OUD N=12 (48%) | Non-OUD N=13 (52%) | χ^2 | <i>p</i> |
|-------------------------------------|---------------------------|-------------------------------|----------|----------|
| Age (years) | | | | |
| Mean (SD) | 31.33 (5.08) | 30.92 (3.64) | 13.114 | 0.439 |
| Range | 21-40 | 23-36 | | |
| Gravida | | | | |
| Mean (SD) | 3.33 (2.27) | 2.62 (1.50) | 10.119 | 0.120 |
| Range | 1-7 | 1-6 | | |
| Para | | | | |
| Mean (SD) | 1.83 (0.39) | 1.69 (0.48) | 0.680 | 0.409 |
| Range | 1-7 | 1-2 | | |
| Start of prenatal care (weeks) | | | | |
| Mean (SD) | 11.42 (5.13) | 7.31 (3.47) | 10.310 | 0.589 |
| Range | 5-22 | 2-12 | | |
| Race | | | | |
| Native American or Alaskan Native | 1 (8%) | 1 (8%) | | |
| Asian | 0 | 0 | | |
| Native Hawaiian or Pacific Islander | 0 | 0 | 2.186 | 0.535 |
| Black or African American | 0 | 1 (8%) | | |
| White | 10 (83%) | 11 (85%) | | |
| More than one race | 3 (25%) | 1 (8%) | | |
| Prefer not to answer | 0 | 1 (8%) | | |
| Ethnicity | | | | |
| Hispanic | 9 (75%) | 7 (54%) | 2.163 | 0.141 |
| Non-Hispanic | 3 (25%) | 6 (46%) | | |
| Insurance | | | | |
| Private | 1 (8%) | 5 (38%) | 3.105 | 0.078 |
| Public | 11 (92%) | 8 (62%) | | |
| Education | | | | |
| 8 th grade or less | 0 | 0 | | |
| Some high school | 3 (25%) | 0 | | |
| High school degree or equivalent | 4 (33%) | 3 (23%) | 7.205 | 0.125 |
| Some college/2-year degree | 5 (42%) | 6 (46%) | | |
| College graduate/4-year degree | 0 | 3 (23%) | | |
| Graduate/professional degree | 0 | 1 (8%) | | |
| Marital Status | | | | |
| Never married | 4 (25%) | 3 (23%) | | |
| Partnered but not married | 6 (50%) | 5 (38%) | | |
| Married for the first time | 1 (8%) | 3 (23%) | 2.197 | 0.700 |
| Separated | 0 | 0 | | |
| Divorced | 1 (8%) | 1 (8%) | | |
| Remarried | 0 | 1 (8%) | | |
| Widowed | 0 | 0 | | |

Table 2 – Continued

| Mother | OUD N=12 (48%) | Non-OUD N=13 (52%) | χ^2 | <i>p</i> |
|--------------------------------|---------------------------|-------------------------------|----------|----------|
| Housing | | | | |
| Rent/Own | 6 (50%) | 9 (69%) | | |
| Living with husband or partner | 7 (58%) | 7 (54%) | 5.796 | 0.123 |
| Living with a family member | 1 (8%) | 2 (15%) | | |
| At risk for losing housing | 1 (8%) | 0 | | |
| Living in transitional housing | 3 (25%) | 0 | | |

Infants

Eighteen infants completed the study through three months of age. Two maternal participants missed the birth interview, and thus, some data on birth weight, NICU admission, and delivery mode is missing. The NAS and Non-NAS groups were similar in gestational age at birth, with the NAS group average birth at 39 5/7 weeks (SD 8 days) and Non-NAS at 38 6/7 weeks (SD 7.36 days), about a week earlier, $\chi^2(1) = 11.25, p = 0.51$. The NAS group had more males (n=6, 75%), and the Non-NAS group had more females, but this did not reach statistical significance (n=6, 60%), $\chi^2(1) = 2.21, p = 0.12$. Birth weight was also comparable between groups, with the NAS group having an average birth weight of 2905 grams, 12.3% less than the Non-NAS group, $\chi^2(1) = 10.83, p = 0.46$.

The groups differed in NICU admission and delivery mode. The NAS group had a higher rate of admission to the NICU at 71% (n=5) as compared to 11% (n=1), $\chi^2(1) = 6.11, p = 0.013$, and were more often born by c-section (71% vs 11%), $\chi^2(1) = 6.11, p = 0.013$. See Table 3 for the infant demographics.

Table 3*Infant Demographics (n=18)*

| Infant | NAS N=8 (44%) | Non-NAS N=10 (56%) | χ^2 | <i>p</i> |
|-------------------------|--------------------------|-------------------------------|----------|----------|
| Gender | | | | |
| Female | 2 (25%) | 6 (60%) | 2.205 | 0.138 |
| Male | 6 (75%) | 4 (40%) | | |
| Gestation | | | | |
| Mean (SD) | 39 5/7 (7.36) | 38 6/7 (8.00) | 11.250 | 0.508 |
| Birth Weight (g) | N=6 | N=9 | | |
| Mean (SD) | 2905 (370.4) | 3313 (385.7) | 10.833 | 0.457 |
| Range | 2381-3487 | 2863-4111 | | |
| NICU Admission | N=7 | N=9 | | |
| | 5 (71%) | 1 (11%) | 6.112 | 0.013 |
| Delivery Mode | N=7 | N=9 | | |
| Vaginal | 2 (29%) | 10 (89%) | 6.112 | 0.013 |
| C-section | 5 (71%) | 1 (11%) | | |

Results by Aim

Aim 1: Feasibility

To examine the feasibility of the study intervention and activities, we measured intervention compliance, retention, and the number of acceptable saliva samples collected at baseline and PPM3.

Intervention Compliance

To measure intervention compliance, maternal participants received daily surveys in the evening around 8pm asking how much time they spent that day doing each activity. Compliance was calculated by completing their assigned daily activity (e.g., one hour a day of babywearing and SSC, and walking with a stroller five times a week for 30 minutes each time), divided by the total number of daily opportunities to complete their activity. These results were analyzed using the Kruskal-Wallis, a non-parametric test. Overall, intervention compliance was low (from 8 – 33%) among both groups and all three interventions (Table 4).

Table 4*Intervention Compliance Scores*

| Intervention | n | M (SD) | H (df) | p |
|--------------------------|----------|---------------|---------------|----------|
| Babywearing Non-NAS | 3 | 0.30 (.24) | 4.28 (2) | 0.51 |
| Babywearing NAS | 5 | 0.33 (0.27) | | |
| Skin-to-Skin Non-NAS | 3 | 0.14 (0.04) | | |
| Skin-to-Skin NAS | 1 | 0.11 (n/a) | | |
| Stroller Walking Non-NAS | 3 | 0.08 (0.03) | | |
| Stroller Walking NAS | 2 | 0.21 (0.30) | | |

The percentage of activity completion is calculated as mean and SD. Babywearing and SSC interventions were to complete the activity daily, and the stroller intervention was five times a week. Kruskal-Wallis (Non-Parametric One-Way ANOVA) Analysis

It was also noted that participants in assigned groups often engaged in the other activities for similar durations (e.g., those assigned to babywearing regularly participated in SSC and stroller walking). See Table 5 for the percentage of time spent in all three activities by participants assigned to the babywearing intervention.

Table 5*Time Spent on Activities for Babywearing Intervention (n=17)*

| Intervention | NAS (n=8) | Non-NAS (n=9) | U | Z | p |
|-----------------------------|------------------|----------------------|----------|----------|----------|
| | M (SD) | M (SD) | | | |
| Babywearing Compliance | 0.28 (.24) | 0.17 (.15) | 34.00 | -.19 | 0.85 |
| Skin-to-Skin Compliance | 0.38 (.14) | 0.52 (.22) | 23.00 | -1.25 | 0.21 |
| Stroller Walking Compliance | 0.38 (.33) | 0.56 (.33) | 27.00 | -.87 | 0.39 |

For participants in the babywearing intervention, similar amounts of time were spent performing SSC and stroller-walking activities.

Mann-Whitney U – Non-Parametric Independent Samples T-Test

Retention

To measure participant retention through PPM3, the total number of participants who completed the study (18) was divided by the total number of participants (25), yielding a retention rate of 72%.

Acceptable Saliva Samples

To calculate the total number of acceptable saliva samples, we calculated the number of samples with ≥ 1 mL at baseline and PPM3. We divided this number of samples by the total number of samples collected at each respective time point. For baseline, 4 out of 59 (6.8%) samples were successfully collected with ≥ 1 mL, and at PPM3, 36 out of 70 (51.4%) samples contained ≥ 1 mL of saliva.

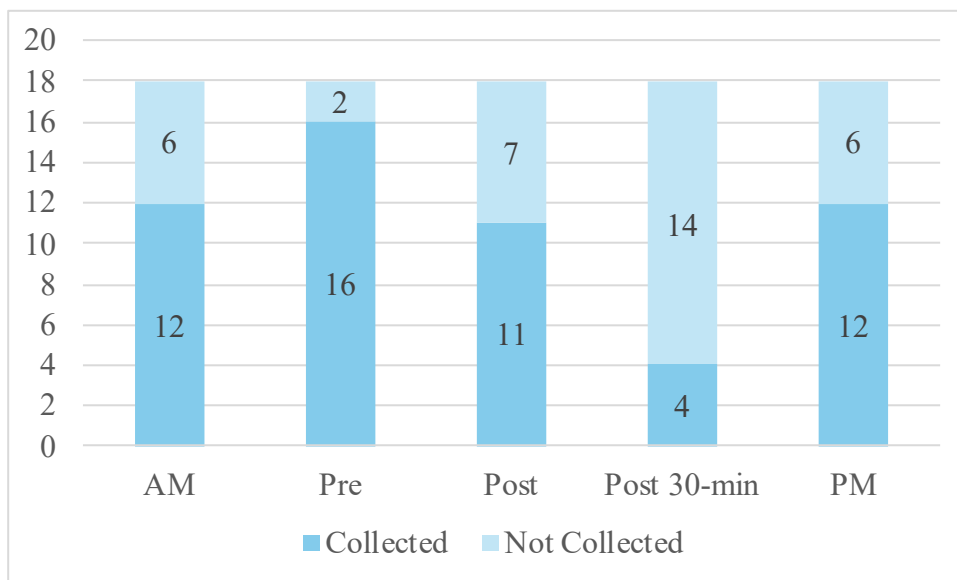
Aim 2: Protocol Acceptability

To test the integrity of the study protocol, research activities and the number of protocol variations were quantified. Participant study activities included the IBQ-R, BISQ-R, completion of reactivity lab, and saliva collection at five time points (AM, Pre, Post, Post 30min, and PM) on two occasions (baseline and PPM3). Activity completion was monitored through REDCap®.

Baseline

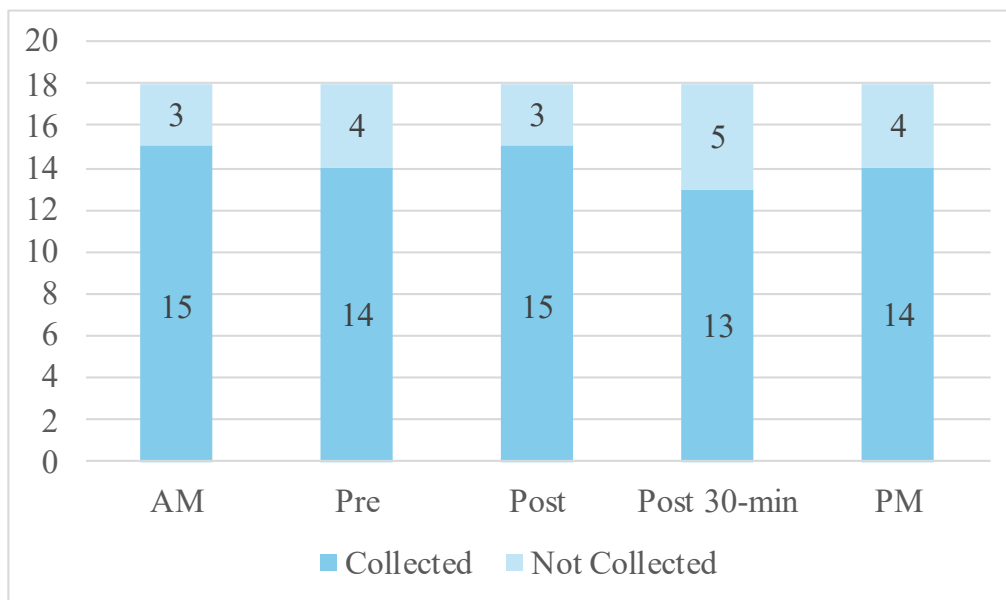
IBQ-R, BISQ-R, and Reactivity Lab Completion. The completion rate of the IBQ-R and BISQ-R surveys was high. At baseline, 17 of the 18 participants completed both surveys. All 18 participants completed the reactivity lab at the baseline visit. All 18 participants also had at least one protocol variation, which was usually documented as the inability to collect saliva at one or more points. The most variations any one participant had was three. The second most common variation was a break in activity to either feed, console, or change the infant's diaper.

Saliva Collection. Fifty-five out of ninety possible saliva samples were collected from infants at the five different time points during the baseline visit. This percentage of saliva samples collected at baseline (61%) was less than the goal of 80%. See Figure 4 for a graph of collected samples.

Figure 4*Number of Saliva Samples Collected at Baseline****Post-Partum Month 3 Visit (PPM3)***

IBQ-R, BISQ-R, and Reactivity Lab Completion. The completion rate of the IBQ-R and BISQ-R surveys continued to be high at the PPM3 visit. Sixteen of the eighteen participants completed the IBQ-R. For the BISQ-R, all 18 participants completed the survey. All participants completed the reactivity lab and again had at least one protocol variation (but no more than two per participant). The inability to collect saliva at one or more points was the primary reason for a variation, and the secondary reason was a break in activity.

Saliva Collection. Seventy-one out of ninety possible saliva samples were collected from infants at the five different time points during the PPM3 visit. This percentage of saliva samples collected (79%) falls below the goal of 90%. See Figure 5 for a graph of collected samples.

Figure 5*Number of Saliva Samples Collected at PPM3***Aim 3: Exploration of Biological Markers*****Cortisol***

Comparison of Groups at Baseline. To compare NAS with Non-NAS groups, infants at baseline with an AM, pre, and PM sample were compared. The average of all three time points for all infants in the group was calculated and analyzed for comparison. The mean cortisol for infants in the NAS group was 0.542 (SD 0.500) compared to the mean value for the Non-NAS group of 0.695 (SD 1.135), which was not statistically different ($p = 0.655$). Salivary cortisol was processed in the Biological Core Laboratory at the College of Nursing, University of Arizona; the intra-assay CV was 7.66%, and the inter-assay CV was 2.3%.

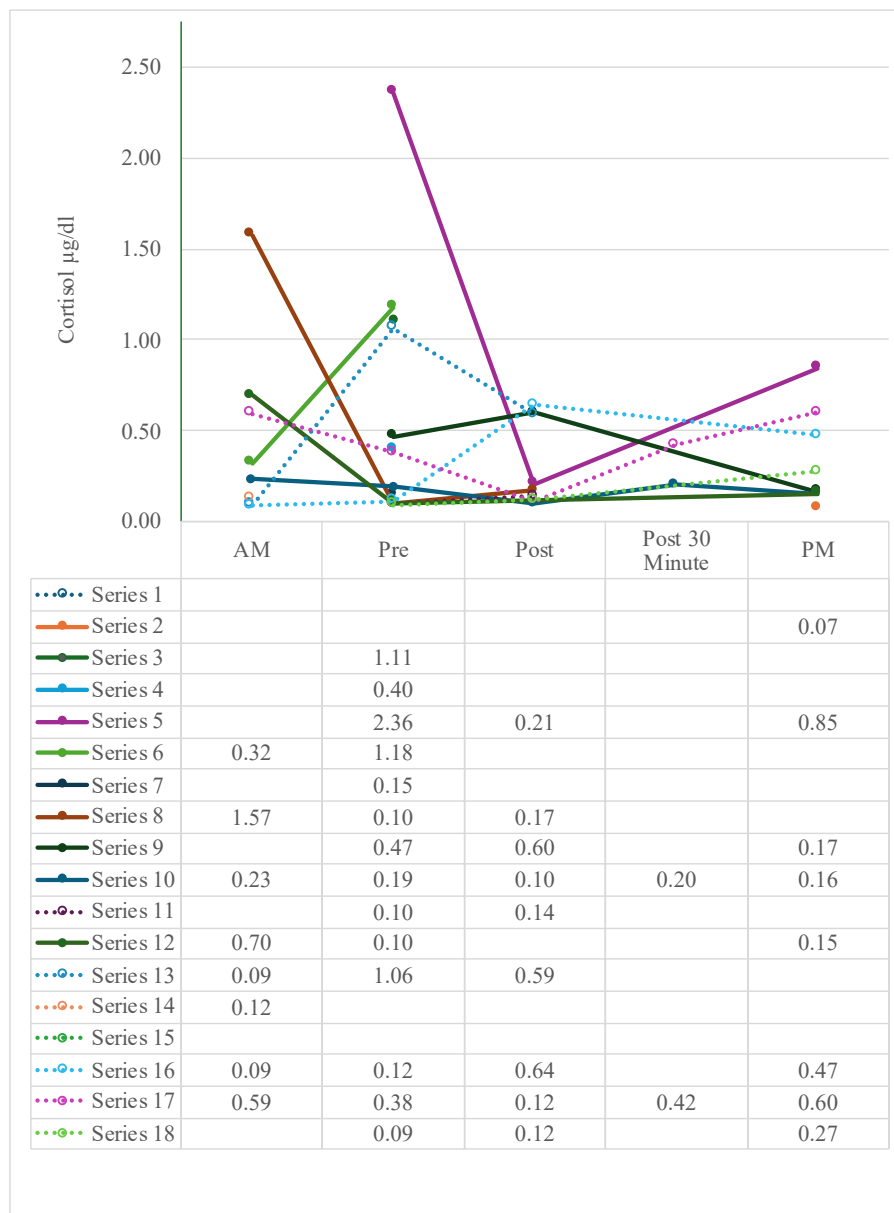
See Table 6 for a comparison of the groups at baseline.

Table 6*Comparison of Cortisol in Groups at Baseline (N=8)*

| Group | N | M | SD | SE | <i>p</i> |
|--------------|----------|----------|-----------|-----------|-----------------|
| NAS | 3 | 0.542 | 0.5 | 0.289 | 0.655 |
| Non-NAS | 5 | 0.695 | 1.13 | 0.507 | |

Analyzed by Wilcoxon Rank Sum Test

Cortisol Patterns at Baseline and PPM3. Given the low likelihood of a diurnal cortisol rhythm in newborns and statistically significant findings due to the small sample size, graphs of available cortisol results were plotted at baseline and PPM3 to evaluate for patterns from morning to evening, including the reactivity lab timed at 1 p.m. Baseline results were available for around half of the possible samples and the results lacked a consistent pattern (Figure 6).

Figure 6*Baseline Cortisol Results*

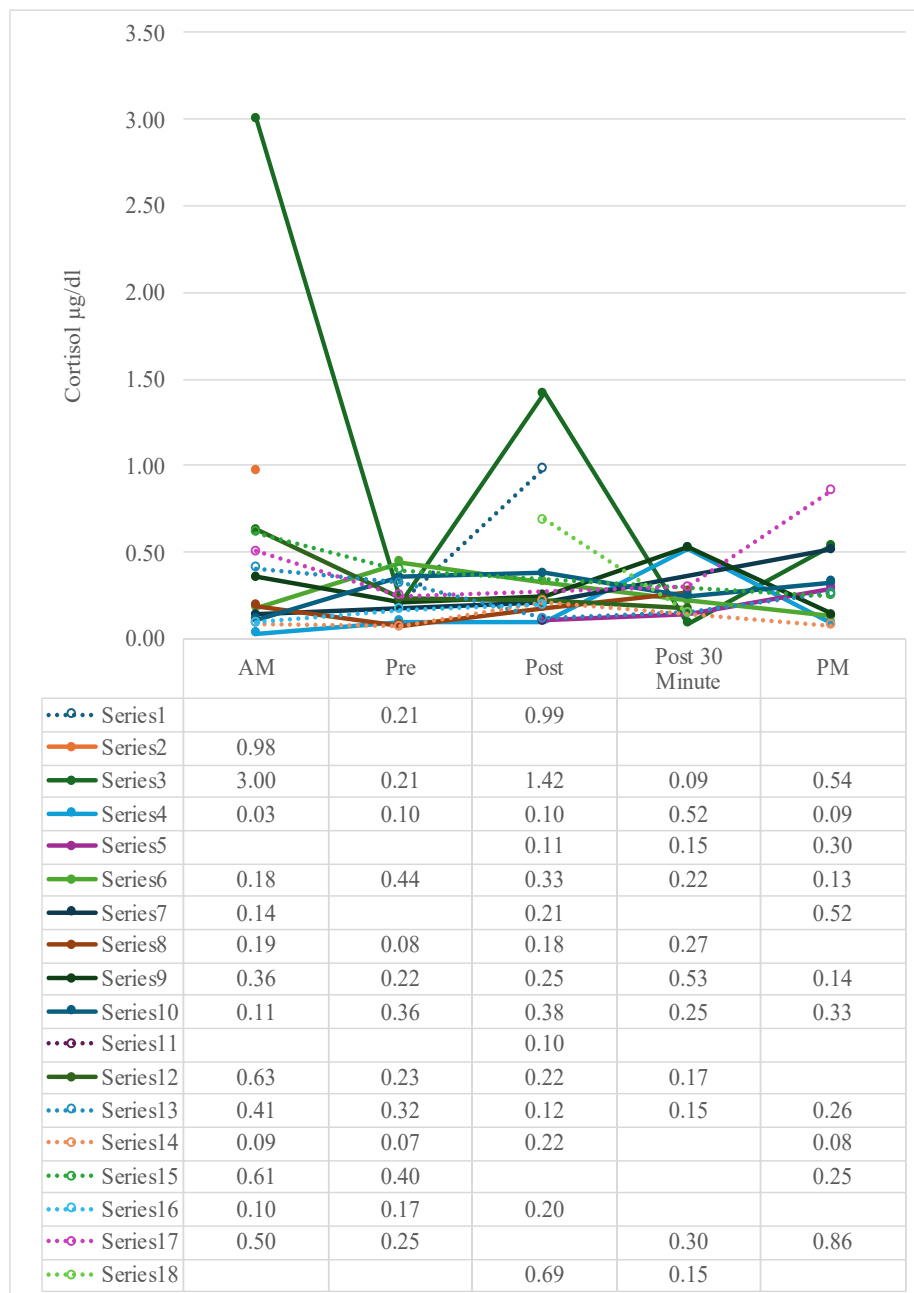
Solid circles and lines are infants without NAS, and open circles with dotted lines are infants with NAS.

Patterns in cortisol at the PPM3 visit were more consistently low from morning to evening. There was a pattern with several participants where the cortisol would drop from the

AM to Pre sample and then increase from the post to PM sample. There was also an outlier result for one participant with a high variability. Since the significance of this finding is unknown, this result was included and reported below. The cortisol started at $3\mu\text{g/dL}$ in the morning, dropped to $0.21\mu\text{g/dL}$ at Pre, increased to $1.42\mu\text{g/dL}$ at Post, dropped again at the 30-minute post-reading to $0.09\mu\text{g/dL}$, and increased to $0.54\mu\text{g/dL}$ by the evening sample. Notes from the PPM3 AM and PM sample were not collected, but for the pre, post, and 30-minute reading the infant was noted to be calm pre, calm post, and sleeping before the 30-minute sample. See Figure 7 for PPM3 Cortisol results on the day of the reactivity lab.

Figure 7

PPM3 Cortisol Results



Solid circles and lines are infants without NAS, and open circles with dotted lines are infants with NAS.

Comparison of Interventions. There were no significant differences in interventions when looking at the change in cortisol levels from morning to evening at the baseline visit. The babywearing intervention decreased by 0.2057 $\mu\text{g/dL}$ (SD 0.284), similar to the SSC intervention decrease of 0.2716 $\mu\text{g/dL}$ (SD 0.457). In contrast, the stroller intervention increased by 0.1628 $\mu\text{g/dL}$ (SD 0.381). See Table 7 for changes in cortisol from morning to evening at the baseline visit as compared by intervention.

Table 7

Change in Cortisol from AM to PM at Baseline Visit by Intervention (N=16)

| Intervention | N | M | SD | SE | 95% Confidence Interval for Mean | | Min | Max | p |
|--------------|---|-------|-------|-------|-------------------------------------|-------------|-------|------|-------|
| | | | | | Lower Bound | Upper Bound | | | |
| Babywearing | 6 | -.206 | 0.284 | 0.116 | -.503 | .092 | -0.49 | 0.26 | |
| Skin-to-Skin | 4 | -.272 | 0.457 | 0.228 | -.999 | .456 | -0.95 | 0.00 | 0.147 |
| Stroller | 6 | .163 | 0.381 | 0.156 | -.237 | 0.563 | -0.18 | 0.90 | |

Oxytocin

Comparison of Groups at Baseline. To compare oxytocin levels between the NAS and Non-NAS groups, we analyzed infants at baseline with AM, pre, and PM samples. The average of all three time points for each infant in the group was calculated for comparison and then the average of the infants in each group were calculated. The mean oxytocin level for infants in the NAS group was 51.937 (SD 90.55), while the mean for the Non-NAS group was 214.722 (SD 263.08), showing no statistically significant difference ($p = 0.149$). The intra-assay CV for oxytocin was 36.9%, and the inter-assay CV was 4.9%; there were challenges with oxytocin results as not all samples outside of an acceptable range were able to be repeated, and this is

discussed further in Chapter 5. Refer to Table 8 for a comparison of oxytocin between the groups at baseline.

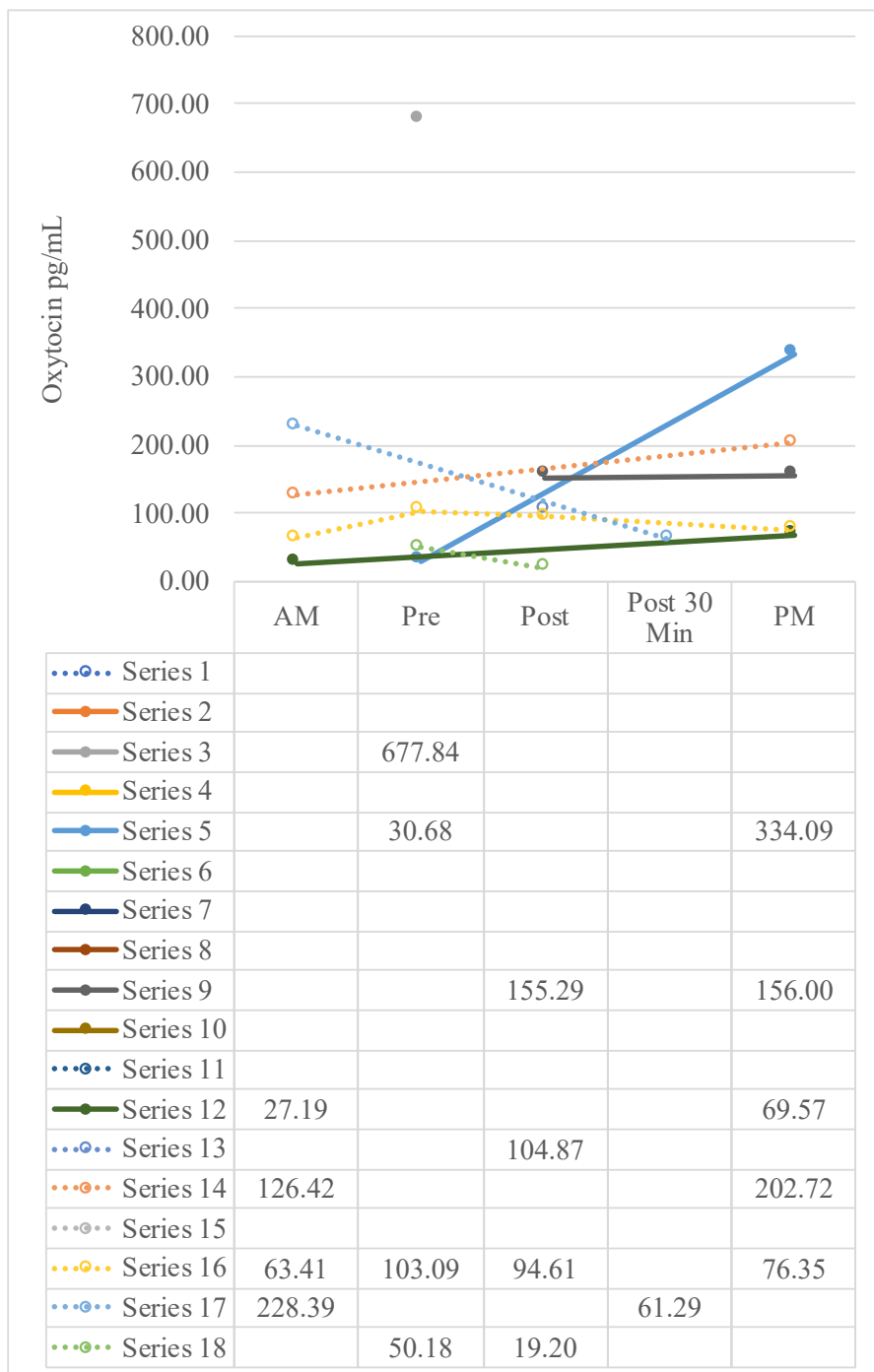
Table 8

Comparison of Oxytocin in Groups at Baseline (N=8)

| Group | N | M | SD | SE | <i>p</i> |
|--------------|----------|----------|-----------|-----------|-----------------|
| NAS | 4 | 51.937 | 90.55 | 45.28 | 0.149 |
| Non-NAS | 4 | 214.722 | 263.08 | 131.54 | |

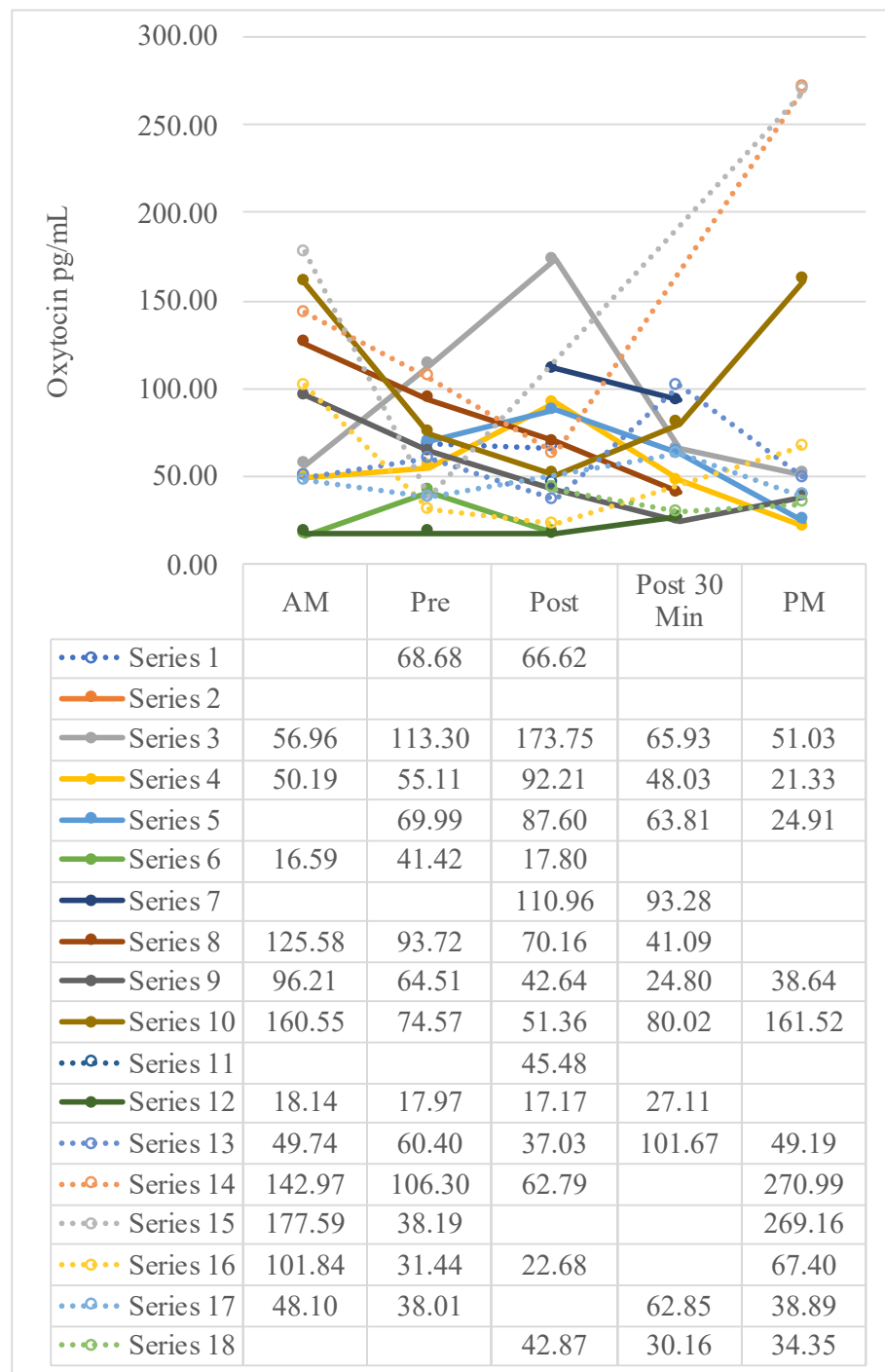
Analyzed by Wilcoxon Rank Sum Test

Oxytocin Patterns at Baseline and PPM3. Patterns of oxytocin from morning to evening are not well established in infants. To address Aim 3 and explore oxytocin results in infants, graphs of available oxytocin results were plotted at baseline and PPM3 to evaluate for patterns from morning to evening, including the reactivity lab timed at 1 p.m. Baseline results were sparse and thus we were not able to evaluate for patterns from morning to evening (Figure 8).

Figure 8*Baseline Oxytocin Results*

Solid circles and lines are infants without NAS, and open circles with dotted lines are infants with NAS.

Oxytocin results from the PPM3 visit were distributed in a heterogeneous pattern and varied widely across all samples (Figure 9). One participant (series 10 – Non-NAS) had an inverse bell curve with higher oxytocin levels in the morning and evening and lower levels in the middle of the day. The opposite was observed in three participants (series 3, 4, 5 – all Non-NAS). However, the curve was not as dramatic in these participants. Two participants had decreasing trends from morning to afternoon and evening (series 8 and 9 – both Non-NAS). Infants in the NAS group had more variation and lack of appreciable trends from morning to evening. Similar to the cortisol, the significance of these findings is unknown, so all results are included and reported below.

Figure 9*PPM3 Oxytocin Results*

Solid circles and lines are infants without NAS, and open circles with dotted lines are infants with NAS.

Comparison of Interventions. When examining the change in oxytocin levels from morning to evening at the baseline visit, there were no significant differences between interventions. The babywearing intervention decreased by 20.99 pg/mL (SD 23.26), the SSC intervention decreased by 83.79 pg/mL (SD 48.96), and the stroller intervention decreased almost three times that of the SSC intervention (237.35 pg/mL, SD 303.62). See Table 9 for changes in oxytocin from morning to evening at the baseline visit as compared by intervention.

Table 9

Change in Oxytocin from AM to PM at Baseline Visit by Intervention (N=9)

| Intervention | N | M | SD | SE | 95% Confidence Interval for Mean | | Min | Max | p |
|--------------|---|---------|--------|-------|----------------------------------|-------------|--------|-------|-------|
| | | | | | Lower Bound | Upper Bound | | | |
| Babywearing | 3 | -20.99 | 23.26 | 13.43 | -78.78 | 36.81 | -45.3 | 1.11 | 0.193 |
| Skin-to-Skin | 3 | -83.79 | 48.96 | 28.27 | -205.4 | 37.82 | -120.8 | -28.3 | |
| Stroller | 3 | -237.35 | 303.62 | 175.3 | -991.6 | 516.9 | -585.7 | -28.5 | |

Summary of Results

Results on the feasibility of this study protocol indicate that modifications are necessary to achieve adequate compliance to study activities and the ability to collect sufficient amounts of saliva. Intervention compliance was low (8-33%), and results are confounded by the amount of time participants spent in the other research activities that they were not assigned to. Although below the goal of 75%, retention of 72% of participants may be reasonable for a high-risk study population of infants with NAS. Adequate saliva collection was not achieved at baseline for most participants (95%) and was significantly more challenging at baseline compared to PPM3 (45% were successfully collected). Revisions to the design are necessary to be able to run duplicate assays on both cortisol and oxytocin and have extra saliva for repeats when necessary. No

significant changes were appreciated in cortisol or oxytocin between groups, interventions, or from baseline to PPM3, as was expected given the feasibility design of this study. The design with the reactivity lab and collection of the IBQ-R and BISQ-R were largely successful and could be carried forward in a future full-scale study.

CHAPTER V: DISCUSSION

The BEAT-NAS study provided valuable information on the feasibility, protocol acceptability, and preliminary findings of biological markers in the acute reactivity to babywearing in infants with NAS. The main findings, study strengths, limitations, implications for practice, and implications for research are discussed in this chapter. The connection to the DNP Essentials will also be expanded in this chapter, given my program of study with the dual PhD/DNP degree.

Main Findings

Although this was a feasibility study with a small sample size, meaningful information was gleaned from the results. The two groups were comparable with non-significant differences observed in maternal age, race, ethnicity, insurance, housing, education level, and start of prenatal care. The OUD group did have an education level that was not as high as the Non-OUD group (college graduates and graduate degrees), which is consistent with the literature. There are barriers and social determinants of health affecting pregnant women with OUD, including financial challenges and lack of a consistent income, that can prevent them from achieving higher education levels (Lee et al., 2019). Barriers also exist that contribute to preventing pregnant women from starting early prenatal care. Some barriers include having access to prenatal care as well as transportation. Additionally, many women with OUD face the fear of repercussions for having OUD, including being reported to child welfare and losing custody of their child (ACOG, 2017). Also not statistically significant, mothers in the OUD group had a higher rate of unstable housing, which has also been reported previously between 2 and 30% of unhoused pregnant people (Admon et al., 2021).

Differences observed between the infants with NAS and those without included delivery mode and NICU admission. Infants in the NAS group were born by c-section more often than infants in the Non-NAS group. This is also consistent with the literature in that women with OUD sometimes have challenges in pain control during labor and complications related to OUD that lead to the need for a c-section (Tobon et al., 2019). Infants with NAS are also more likely to require admission to the NICU for treatment of withdrawal symptoms or birth-related complications, which was consistent with the results from this study (Patrick et al., 2020).

Feasibility

Consistent with its purpose, a feasibility study aims to test a research protocol, acceptability, appropriateness of the population, methods, instruments, or some other aspect of a study before time and resources are used for a full-scale study (McGrath, 2013). The primary aim of this study was to test the feasibility of a protocol aimed at testing a babywearing intervention in two groups (NAS & Non-NAS) with three interventions (babywearing, SSC, and stroller). Overall, the design resulted in a 72% retention rate, and all participants who completed the study, completed the two reactivity labs at baseline and PPM3. The goal was $\geq 80\%$ retention, and thus this feasibility study fell short of the goal. One potential way to increase retention is to decrease the burden on the participants. Given that the BEAT-NAS study fell under the parent ROBIN Project, there was a considerable number of surveys (19) and data (daily surveys, saliva collection at five time points for mother and infant, and dried blood spots collected several times on mother) that were collected as part of the ROBIN Project. It may be that the newborn period is just too challenging for everyone to participate at the level requested by the parent study, but decreasing the burden may help. It also may be reasonable to expect a

30% dropout rate for a high-risk population of mothers with OUD and infants with NAS, but then researchers need to assess for similar attrition between groups to ensure internal validity (Overgaard et al., 2024). For this study, of the seven that stopped participation, three were Non-OUD and four were in the OUD group, indicating that the reason for dropping out is not likely associated to the challenges of having OUD, but rather the challenges of having a newborn.

Some elements of feasibility were found to be less than adequate and would need revisions before running a full randomized controlled trial (RCT). The first of these revisions would be for intervention compliance. Compliance with the assigned activity was less than 35% for all three interventions in both groups. All participants also spent a comparable amount of time on the non-assigned activity. Participants were instructed to focus on completing their assigned activity daily (or five times a week for stroller walking). They were not asked to avoid the other two activities but to ensure the assigned activity was completed. Data on the amount of time spent in each activity was collected from the daily surveys of the parent study, and it was revealed that participants completed similar amounts of time in each activity throughout the study. This hinders the ability to assess the effects of the intervention on cortisol and oxytocin over time (from baseline to PPM3), given the lack of emphasis on one specific activity. A future design could include abstaining from the non-assigned activities. However, that may not be reasonable given that someone assigned to walking with a stroller may want to participate in SSC, and it would be unethical to prevent them from doing SSC given the large amount of data supporting benefits to both mom and baby from SSC. An alternative is a crossover design where participants can participate in each activity for a predetermined time during the early weeks of

life. This may capture the short-term effects of the activities but would not capture the long-term effects of one specific activity.

A second element of feasibility that would need revisions is the collection of saliva. Collecting at least one milliliter of saliva at each time point (AM, pre, post, 30-minute, & PM) was the goal for both baseline and PPM3 visits. The successful collection of this volume was the minimum needed to allow the enzyme immunoassay (EIA) testing of both cortisol and oxytocin hormones to provide information on stress and HPA axis reactivity. The percentage of saliva samples that yielded 1 mL of usable saliva (after centrifuging & removing the supernatant) was far less than the goal of 80% at baseline and 90% at PPM3. Passive drool is the ideal collection method for saliva, but this can be challenging in infants. For this reason, we used the infant swabs from Salimetrics, which absorb the saliva while being held by the mother or research assistant. Despite other studies reporting adequate saliva collection in newborns, we found challenges with infants either sleeping or being awake and crying, both of which were impossible for saliva collection. In two of the other studies, radioimmunoassay was used, and the volume of saliva needed was 70 μ L compared to the 500 μ L that was needed for our assay processing (Vittner et al., 2023; Vittner et al., 2018). The additional two studies did not specify how much saliva was collected, just that it was a sufficient amount (Cong et al., 2011; Congdon et al., 2020). An alternative saliva collection device called the Muddler has been created and tested in adults and infants, which may prove advantageous for saliva collection in newborns (Takagi et al., 2013). The Muddler device is a flat plastic plate covered with gauze. The device is small enough to fit in the infant's mouth, and has holes in the middle of the plate, allowing saliva to pool in this area until removed through centrifuging. A second device, the pediatric

sialometer, works to collect saliva by using low levels of suction (Costa et al., 2020). This could be another alternative approach to saliva collection, especially in infants at two weeks of age, as these infants are often just returning to birth weight and are dry regarding hydration status (Paul et al., 2016).

Protocol Acceptability

Acceptability of the study protocol was shown by high completion rates on the IBQ-R and BISQ-R surveys, and all participants completed the reactivity labs at baseline and three months. Concerning protocol adherence during the reactivity lab, every participant had at least one variation. The variation was most commonly reported as the inability to collect the infant saliva sample. However, saliva collection was noted to have been collected for more than 60% of the samples at the baseline visit and 79% of samples at the PPM3 visit. Although there is room to improve these numbers, which were below targets of 80% and 90%, most infants tolerated the attempt at saliva collection. This indicates that the more significant challenge is in the saliva collection technique, which could be addressed with solutions like the Muddler or pediatric sialometer as mentioned above. Saliva collection was easier in infants at three months, likely due to their increased development in oral muscles and saliva production (Tryphonopoulos et al., 2014)

The second most common reason for a variation was a break in activity. Conducting a reactivity lab that lasted over two hours presented challenges with keeping the infant happy while avoiding feeding for saliva collection purposes. The breaks in activity were almost always related to the infant needing to be fed or have a diaper change. Changes to the reactivity timeline, which include some flexibility in the times samples were collected, could increase the percentage

of samples collected. However, variability in the timing of sample collection could confound the results and decrease the reliability of the saliva results.

Exploration of Biological Markers

All saliva samples collected at home were kept in the freezer in the home until picked up by research staff (usually within 1-3 days of collection). Saliva samples collected during the reactivity lab were placed on ice, then moved to the lab freezer within approximately 30 minutes of collection. All samples were stored in a -80°C freezer until they were moved for processing.

Salivary cortisol was processed in the Biological Core Laboratory at the College of Nursing, University of Arizona. Except two samples having a high coefficient of variation (CV), the results all fell within the curve of the respective plates and can be considered reliable. The intra-assay CV was 7.66%, and the inter-assay CV was 2.3%.

The oxytocin samples were processed at the Laboratory of Evolutionary Endocrinology of Primates at the University of Arizona. Of the four plates with initial oxytocin results, one (plate 2) had high variability in the percent binding, and one (plate 3) had high and low pool results far above the expected ranges. About half of the samples from these two plates were available for re-run, and those results were used in the final analysis. For the samples that were not re-run due to inadequate amounts of saliva, we used the original values for the academic purpose of being able to run statistical analysis as part of this dissertation learning experience. The oxytocin results need to be re-run before definitive conclusions could be drawn regarding outcomes. The intra-assay CV for oxytocin was 36.9%, and the inter-assay CV was 4.9%.

Cortisol Results

Comparison of Groups at Baseline. Cortisol values at baseline were compared between groups and found that the mean cortisol in infants in the NAS group was lower than those in the Non-NAS group. This non-significant difference is likely due to the small sample and needs to be validated with a larger sample size. However, exposure to opioids has been shown in adults to contribute to a blunted cortisol response due to opioid induced adrenal insufficiency or OIAI (Rice & Yoshida, 2024). OIAI has not been studied in neonates, but similar physiology with the HPA Axis and cortisol levels in infants with NAS has been reported and this may indicate a blunted cortisol response (Rodriguez et al., 2020).

Cortisol Patterns at Baseline and PPM3. The results of this study did not consistently show any discernable cortisol patterns from morning to evening or DCRs. Previous research demonstrates that newborns do not have a well-established DCR, given their sporadic wake-sleep cycles. The DCR begins to emerge with regular wake-sleep cycles during the first year of life (de Weerth et al., 2003). There was also no recognizable cortisol response to the reactivity lab, which could indicate that the activities had no effect on the HPA axis or that the activity was not long enough to produce a discernable effect.

Comparison of Interventions. The babywearing and SSC interventions had a decrease in mean cortisol levels when averaged as a group at the baseline visit. The implication(s) of these decreasing values are unclear, as “normal” cortisol levels in newborns vary widely and depend on factors like the time of day, stress, and gestational age. One article gives normal serum ranges of 1.7-14 µg/dL on day of life three, 2-11 µg/dL on day seven, and 2.8-23 µg/dL for infants between 1 and 11 months, indicating that cortisol levels have a growing range over the first year

of life. Given this information, relatively low values may be considered normal. Serum samples contain higher cortisol concentrations as they measure bound and unbound forms, whereas salivary cortisol only measures unbound forms. The decreases observed in this study were small and may be within normal physiologic parameters. However, if these small decreases have clinical significance, the decrease for both babywearing and SSC groups was similar, indicating that babywearing may have similar effectiveness in calming and comforting the infant. Stroller walking had a slight increase in cortisol from morning to evening. This could be in the normal range of expected variation for an infant around two weeks of age, or it could indicate that being walked in a stroller is less comforting to the infant than babywearing and SSC. Further research is needed to determine which is the more likely explanation.

Oxytocin Results

Comparison of Groups at Baseline. Oxytocin mean levels across collection timepoints at baseline in infants with NAS were one-fourth of the levels of infants without NAS. The challenge is that only four infants in each group had a morning, pre, and evening oxytocin result at both baseline and PPM3 to draw these comparisons, which may be limiting the reliability of the results. Although this was not statistically significant, the potential for clinical significance could point toward a blunted oxytocin response in infants with NAS, given that adults with OUD have lower levels of serum oxytocin compared to those without OUD (Mellentin et al., 2023). This information could guide clinicians to seek directed interventions to increase connection and oxytocin levels, such as affective touch (Schneider et al., 2023). This result should be interpreted with caution, given the possibility of a type 1 error exists, and these results are due to the lack of an adequate sample size.

Oxytocin Patterns at Baseline and PPM3. Data at baseline was insufficient to see patterns present from morning to evening. Data from the PPM3 visit was more available but had wide variability in the patterns observed. Literature on oxytocin in newborns indicates higher levels in infants born by vaginal delivery and those infants who are breastfed (Uvnäs Moberg & Prime, 2013). The mechanism for increased oxytocin during breastfeeding was previously thought to be a transfer of oxytocin through the breast milk to the infant. However, this recent research has pointed towards the action of sucking by the infant as triggering oxytocin production and release in the infant. Oxytocin patterns in adults can be observed and influenced by stress, social interactions, touch, and daily rhythms. It has been well documented that oxytocin increases in both the mother and infant during SSC interactions and results from contact and stimulation of sensory nerves in the skin (Bigelow & Power, 2020). As mentioned above, there were no discernible patterns among the groups of infants in relation to the reactivity lab. This is likely due to the small sample size and possibly the short duration of the activity.

Comparison of Interventions. All three interventions had a decrease in mean cortisol levels when averaged as a group at the baseline visit. The implication(s) of these decreasing values are unclear and may be within normal physiologic parameters. However, the SSC decrease was almost four times that of the babywearing group, and the stroller walking group was ten times that of the babywearing group. If these decreases have clinical significance, the smaller decrease in oxytocin from morning to evening for babywearing could indicate that babywearing may have similar or better effectiveness in calming and comforting the infant than SSC and walking with a stroller. Further research is needed to validate these results.

Interpretation of Results

The interpretation of this study's feasibility results provides essential information on the need for revisions to the study protocol to improve intervention compliance. Along with increased intervention compliance, the protocol needs to address the amount of time participants spend in non-assigned activities, as it confounds the findings when similar amounts of time are spent in all three activities (or interventions). Results on recruitment and retention also indicate that enrolling and retaining participants for this population is challenging and changes in design to increase both would prove valuable in a full-scale study.

Although the cortisol results are accurate based on standards, controls, and high and low pools, the interpretation of the findings between groups, interventions, and time points should be made with caution. Statistical significance was low, indicating no observable differences between groups, interventions, and time points, likely due to the small sample size and missing saliva samples/results. However, if the trend seen with cortisol within interventions bore out, then it could be said that babywearing and SSC effectively decrease salivary cortisol from morning to evening, in contrast to walking with a stroller, which increased infant cortisol levels. This could affect the ability to see discernable differences between groups, interventions, and time points, resulting in a type II error.

Salivary oxytocin results had similar challenges concerning low statistical significance and the inability to collect all saliva samples, making it hard to tell if there were differences present but just not detected due to these challenges. Additionally, the oxytocin results on two of the four plates could not be trusted, given the high values on the percent binding on one plate (plate 2) and higher than expected values for the high and low pools on another plate (plate 3).

There was saliva available to repeat the assays for about half of the samples of each plate. The results of the repeat analysis did show a marked difference compared to the original results. If this study were to be published, all results from plates 2 and 3 that were not repeated would have to be discarded. Given the purpose of this dissertation, these results were included so that analyses could be run with as many results as possible. Therefore, the oxytocin results are not valid, nor should they be used to draw conclusions on the results of the study or with differences between groups, interventions, and timepoints.

Study Limitations

As mentioned above, this study had limitations with the ability to draw conclusions from the cortisol and oxytocin results, given the small number of samples and the challenges with the oxytocin assays on plates 2 and 3. Also mentioned previously was the inability to collect enough saliva volume and all samples at each time point and guarantee intervention compliance without participants spending similar amounts of time in the non-assigned research activities. While we recognize that having the BEAT-NAS study housed within the parent ROBIN Project was a strength in terms of recruitment and enrollment, it was also a limitation in that the participant burden was considerable given the daily surveys, daily activities, reactivity labs on three occasions with five in-person visits, 18 surveys at each visit, and collection of saliva and dried blood spots multiple times on visit days. Maternal participants were compensated through the ROBIN Project, which was a clear motivator for some participants. However, one participant stated that the surveys were too long, and it was really hard to break up the different amounts of time spent with the baby (referring to the last question on the IBQ-R) as she felt like she was holding the baby all the time. Other reasons given from participants who did not complete the

study included that they couldn't because the baby was still in the hospital (2), it wasn't a good fit (1), or no response (4).

Study Strengths

There are several strengths of the BEAT-NAS study design. The first is that it is coupled with a parent studying maternal outcomes, and thus, the convenience sample of infants was relatively easy to recruit compared to recruiting infants where mothers did not have a vested interest in the research. The second strength of this study was the design, which compares two groups and three interventions. In a full-scale study, with intervention compliance and saliva collection methods worked out, there is the ability to look at differences between groups (NAS and Non-NAS), interventions (babywearing, SSC, & stroller walking), combined group and intervention (e.g., babywearing with NAS & babywearing without NAS), and between time points (baseline & PPM3). The third strength is that two validated questionnaires were collected by almost all of the participants, and the information collected from the IBQ-R and BISQ-R surveys may also provide valuable information on infant behaviors in relation to the cortisol and oxytocin hormones.

DNP Essentials

In the most recent version of "The Essentials: Core Competencies for Professional Nursing Education," ten domains are identified to guide entry-level and advanced practice nursing education (American Association of Colleges of Nursing [AACN], 2021). The domains most relevant to this work as a dual degree student include scholarship for the nursing discipline, population health, knowledge for nursing practice, and quality and safety. All domains include competencies and sub-competencies, which apply to patients of any age from diverse

backgrounds and can be applied in the delivery of care through prevention, promotion, acute care, chronic care, and palliative or hospice-type settings. This section will discuss the four domains most relevant to this work.

The third domain is *Nursing Scholarship*, which can be defined as any activity that advances the science, knowledge, and practice of the nursing profession (Smith, 2012). This dissertation focused on designing a research study that would provide information to address a gap in knowledge on the mechanism(s) behind babywearing and its empirical effectiveness at comforting infants with NAS. By designing a study, testing the protocol, and working to revise areas of weakness, the first steps to generating new knowledge are complete. The next step is to test revisions and, if successful, perform a full-scale study and disseminate the findings. By sharing the results, clinicians have access to the knowledge and can implement the interventions in their practice to advance care provided for this population of infants with NAS.

The second domain relevant to this research is *Population Health*. Population health is care delivery that focuses on improving outcomes through prevention or disease management of specific populations. It is generally accomplished by collaborating with community partners to develop a well-informed and all-encompassing effort through the work of many. The work in this dissertation is aimed at a specific population (infants) with a specific disease (NAS). This study provides preliminary information on the feasibility of running a full-scale study that will provide results telling whether babywearing is an effective intervention for reducing symptoms of NAS through comfort. If outcomes are positive, this intervention is low-cost, easy to implement, and could be an additional tool for clinicians to use for infants with NAS.

The third domain, *Knowledge for Nursing Practice*, involves obtaining and integrating knowledge as a guide for one's practice. As discussed in Chapter I, epistemology is the 'how' of knowing, and ontology is the 'what' of knowing. The generation of knowledge for this work came through empiricism, abstraction, and constructionism processes. There was an experience where babywearing worked to soothe a fussy infant, and this idea was abstracted to use in infants with NAS who are also very fussy. The concepts of comfort and connectedness were constructed through the ontological information available from nursing work and the environment by Florence Nightingale, Pepper's Developmental Mechanistic-Person-Environment worldview, Swanson's Theory of Caring, Kolcaba's Comfort Theory, and Feldman's Bio-Behavioral Synchrony Model. In this work, we are using knowledge to create a feasibility study to test a protocol that will produce new knowledge and aid clinicians in caring for infants with NAS.

The last domain with significant relevance to this work is *Quality and Safety*. In this domain, the Advanced Practice Registered Nurse (APRN) is well poised to be a leader in quality improvement (QI) projects. The aim of QI work is to improve care and decrease risks for patients and healthcare workers through individual and system approaches (American Association of Colleges of Nursing [AACN], 2021). Currently, nurses and families provide most of the care to infants with NAS. Often, the nurses have to hold the infant while charting, which means the infant is balanced on the nurse's chest or lap so the nurse can have their hands available to chart. This presents the risk of an infant fall, which would be devastating for everyone involved. This feasibility study has the potential to lead to a full-scale study with positive outcomes. Once that happens, babywearing could be implemented in NICUs and other hospital settings caring for

infants with NAS to provide a safe and effective way to keep infants comfortable and decrease the chance of an infant fall while being able to complete non-patient-related tasks (i.e., charting).

The Doctor in Nursing Practice (DNP) utilizes the ten domains in “The Essentials: Core Competencies for Professional Nursing Education” to provide the foundation for a comprehensive educational program to position the Advanced Practice Registered Nurse (APRN) as a leader in practice and quality improvement. The Doctor of Philosophy (PhD) emphasizes research and the generation of new knowledge in a field of study. For the PhD in Nursing, this new knowledge can be taken by the DNP and implemented in the practice setting. A person holding both degrees can generate and implement new knowledge in the clinical setting and is well-poised to bring research questions based on clinical experiences, creating a cycle of research, implementation, research, etc.

Implications for Practice

Current evidence for babywearing as an intervention for infants with NAS supports this practice in NICUs (Williams, Gebler-Wolfe, et al., 2020; Williams, Grisham, et al., 2020). This research aimed to develop a feasibility study to test a protocol that, if run as a full-scale study, would provide answers on the mechanisms and size of the effect from babywearing. Once this information is known, guidelines could be developed to optimize the amount of time babywearing is used. For now, further research is needed before concrete implications for practice can be made.

Implications for Research

This study laid the foundational framework to test babywearing as an intervention for infants with NAS. It includes a control group of infants without NAS, a control intervention of

infants being walked in a stroller, and a comparison intervention of infants participating in SSC with their mothers. Given that there are positive effects from babywearing on postpartum depression, future research combining the dyad could be beneficial for confirming these findings and assessing for additional benefits to the dyad (Grisham et al., 2023). Although not statistically significant, the clinical implications of decreased cortisol and oxytocin in infants at baseline between the groups (NAS & Non-NAS) may be meaningful in that infants with NAS may have a decreased capacity to respond to the stress of withdrawal because of suppressed activity from the HPA axis and oxytocinergic systems (Grahm et al., 2021; Karin et al., 2020). Interventions directed at decreasing stress, either by external actions (e.g., holding, swaddling, providing a dark, quiet environment) or internal actions (administration of postnatal opioids), need further research to facilitate better understanding of the mechanisms of action. Additionally, administration of intranasal oxytocin as an intervention in adults with OUD has demonstrated favorable results (Gully et al., 2025). Investigation of this intervention in infants with NAS, with the aim of replenishing this neurotransmitter and hormone is another area of potential research.

With some modifications to the protocol to improve recruitment and retention, intervention compliance, and saliva collection methods, a full-scale study can provide valuable information to address gaps in knowledge on the mechanisms behind babywearing and the effects on stress and connection hormones, cortisol and oxytocin. Current literature suggests detrimental neurodevelopmental effects from in-utero opioid exposure, including changes to systems related to oxytocin (Conradt et al., 2019). Further testing on intervention compliance, identifying ways to measure the effects of each intervention, and saliva collection methods should be conducted before initiating a full-scale study to address these gaps in the literature.

Ideally, a full-scale study would include infants from all racial groups and include several locations throughout the United States (US), to increase generalizability of the findings.

Conclusion

The purpose of this study was to examine feasibility and test the study protocol for a babywearing intervention aimed at reducing withdrawal symptoms in infants with Neonatal Abstinence Syndrome (NAS) through mechanisms of comfort and connection. A quasi-experimental feasibility design was created and tested with 25 infants. The results included positive numbers on participant completion of two validated study questionnaires and two reactivity labs. Recruitment, retention, and methods for saliva collection need to be optimized prior to running a full-scale study. Preliminary cortisol and oxytocin results were explored. However, the results were not enough to provide definitive data on the effect size of babywearing. Preliminary cortisol results point towards similar effectiveness as SSC, but further research is needed to validate these results.

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