Co-bedding as a comfort measure for twins undergoing painful procedures

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Abstract

Background. Maternal skin contact during a tissue breaking procedure lowers pain reactivity and enhances physiological recovery. It is uncertain if this comfort is derived solely from maternal presence or from stabilization of regulatory processes from direct skin contact. No studies have examined whether the contact or presence of a twin would have a similar comforting effect.

Purpose. To compare the comfort effect of co-bedding by contrasting preterm twins who are co-bedding and those who are not on pain response during a tissue breaking procedure (heel lance).

Methods. Following consent, 67 eligible twin sets, admitted to the Neonatal Intensive Care Unit (NICU) were stratified in pairs by gestational age (≤ 31 6/7 weeks or ≥ 32 weeks) and site and then randomly assigned to a co-bedding group, n=36, (cared for in the same incubator or crib) or a standard care group, n=31, (cared for in a separate incubator or crib). Pain response was determined by physiologic and videotaped facial reaction in accordance with the Premature Infant Pain Profile (PIPP). Additional outcomes included physiologic time to recovery, alterations in salivary cortisol, heart rate variability, frequency of additional 24% sucrose doses required, and response of the co-twin. Sample size was calculated using a 2-sided alpha error of 0.05 and a power of 80 percent. Sixty four sets of twins or a total of 128 infants were needed to detect a difference of 1 point or greater change (SD 2.0) in the PIPP scores if such a difference is in fact caused by co-bedding. Analysis was based on the intention-to-treat principle and compared the means in the two groups before and after treatment and contrasted the mean difference between groups using 95 percent confidence intervals and a 2 sided P value of 0.05.

Results. Mean PIPP scores were highest at 30 seconds post lance and not significantly different between the groups, 7.1 (SD 2.8) in the co-bedding group and 7.2 (SD 3.4) in the standard care group, P=0.91. Nor were they significantly different at 60 or 120 seconds. At 90 seconds, mean scores were higher in the co-bedding group, 6.0 (SD 3.0) vs. 5.0 (SD 1.8), P=0.04, [95% CI ¯1.99 to ¯0.02] in the standard care group. Recovery time post lance was over a minute shorter, M=75.6 seconds (SD 70.0), in the co-bedding condition compared to standard care, M=142.1 seconds (SD 138.1), *P*=0.001, mean difference of 64.5 seconds (95% CI. 25.6-103.3). No group differences were noted in baseline cortisol levels (0.36 ug/dl if assigned to receive co-bedding and 0.43 ug/dl in the standard group) while cortisol levels 20 minutes post lance were significantly lower in the co-bedding group, 0.28 ug/dl (SD 0.25) versus 0.50 ug/dl (SD 0.73). Similarly, mean change in cortisol from baseline was lower in the co-bedding group, -0.06 ug/dl compared to the standard care group, 0.14 ug/dl, P=0.05. Co-bedding infants were significantly less likely to receive any form of additional non-pharmacologic strategy (non-nutritive sucking, swaddling or facilitated tucking), 58.2% versus 95%, P<0.001. Heart variability, frequency of additional sucrose dosages, co-twin response and incidence of adverse events were not significantly different between the groups.

Conclusions. The results of this randomized controlled trial provide evidence that co-bedding enhances physiologic recovery and diminishes the stress response of preterm twins undergoing heel lance in the NICU but did not lead to lower pain scores. Co-bedding did not decrease the frequency of additional 24% sucrose doses. Nor did co-bedding contribute to higher adverse effects for the twin undergoing heel lance or his/her co-twin.

Clinical Trial Registry - NCT00917631

Abrégé

Introduction. Durant une procédure causant un dommage tissulaire, le contact de peau entre un bébé prématuré et sa mère diminue les réactions de douleur de celui-ci et l'aide à récupérer plus rapidement au niveau physiologique. Il est incertain si ce réconfort est dû seulement à la présence maternelle ou à une stabilisation des processus régulateurs provoquée par le contact directe de la peau. Aucune étude n'a examiné si le contact ou la présence d'un jumeau prématuré pourrait avoir un effet réconfortant sur son jumeau.

Objectif. Lors d'une procédure causant un dommage tissulaire (ponction au talon), comparer l'effet réconfortant du partage de lit entre jumeaux, le *co-bedding*, sur la réponse à la douleur en contrastant les bébés qui sont en *co-bedding* avec ceux qui ne le sont pas.

Méthodes. Après avoir obtenu le consentement, 67 ensembles de jumeaux éligibles admis à l'unité des soins intensifs de néonatalogie furent stratifiés en pairs par âge gestationnel (≤ 31 6/7 semaines ou ≥ 32 semaines) et le site. Ceux-ci furent randomisés au groupe de *co-bedding*, n=36 (même incubateur ou petit lit) ou au groupe de soins standards, n=31, (incubateur ou petit lit séparés). La réponse douloureuse fut déterminée par les réactions physiologiques et faciales (captées par vidéo) en lien avec l'échelle de douleur *Premature Infant Pain Profile* (PIPP). Des résultats additionnels furent collectés tels que le temps de récupération physiologique, altérations du niveau de cortisol salivaire, variabilité du rythme cardiaque, nombre de doses de sucrose 24% administrées, ainsi que la réponse de l'autre jumeau. La taille de l'échantillon fut calculée en utilisant une marge d'erreur à 5% et une puissance de 80%. Soixante-quatre ensembles de jumeaux ou un total de 128 nourrissons étaient nécessaires pour détecter une différence de 1 point ou plus (écart type (ÉT) 2.0) du score PIPP afin de déterminer si ce changement est bel et bien dû au *co-bedding*. Les

analyses furent basées sur le principe de l'intention-à-traiter et ont comparé les moyennes des deux groupes avant et après le traitement, ainsi que les différences moyennes en utilisant un niveau de confiance de 95% et une valeur bilatérale de P à 0.05.

Résultats. Les moyennes des scores PIPP furent à leur plus haut 30 secondes suivant la ponction au talon et ne furent pas significativement différentes entre les deux groups, 7.1 (ÉT 2.8) pour le groupe *co-bedding* et 7.2 (ÉT 3.4) pour le groupe de soins standards, P=0.91. Ces scores ne furent pas significativement différents à 60 ou 120 secondes. À 90 secondes, les scores moyens furent plus élevés dans le groupe de *co-bedding*, 6.0 (ÉT 3.0) vs. 5.0 (ÉT 1.8), P=0.04, [CI ¯1.99 à ¯0.02] pour le groupe de soins standards. Le temps de récupération après la ponction au talon fut de plus de 1 minute, M=75.6 secondes (ÉT 70.0), pour le groupe en *co-bedding* comparativement au groupe en soins standards, M=142.1 secondes (ÉT 138.1), *P*=0.001, différence moyenne de 64.5 secondes (CI. 25.6-103.3). Aucune différence entre les groupes fut notée entre les niveaux de bases de cortisol (0.36 ug/dl si assigné au groupe de *co-bedding* et 0.43 ug/dl pour le groupe de soins standards). Les niveaux de cortisol 20 minutes après la ponction au talon furent significativement plus bas dans le groupe du *co-bedding*, 0.28 ug/dl (ÉT 0.25) versus 0.50 ug/dl (ÉT 0.73). Similairement, la moyenne du changement du niveau de base du cortisol était plus basse dans le groupe du *co-bedding*, -0.06 ug/dl et 0.14 ug/dl, pour le groupe de soins standards, P=0.05. Les bébés en *co-bedding* ont eu moins tendance à recevoir des traitements non-pharmacologiques additionnels (succion avec tétine, enveloppement toucher), soit 58.2% versus 95%, P<0.001. La variabilité du rythme cardiaque, la fréquence de doses additionnelles de sucrose, la réponse de l'autre jumeau, ainsi que l'incidence d'événements néfastes ne furent pas significativement différentes entre les deux groupes.

Conclusions. Les résultats de cette étude contrôlée randomisée démontrent que le *co-bedding* entre jumeaux prématurés accroît leur récupération physiologique et diminue leur réponse de stress lors d'une ponction au talon mais sans mener à une réduction de leurs scores de douleur. Le *co-bedding* n'a pas réduit le nombre de doses de sucrose additionnelles ou engendré plus d'événements nocifs chez le jumeau subissant la ponction au talon ou son jumeau.

Registre de l'étude clinique [NCT00917631]

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Dedication

In loving memory of my father Walter

(1923-2006)

Preface

Thesis format

According to the Faculty of Graduate and Postdoctoral Studies guidelines, with the approval of her committee, the candidate chose to submit the thesis in the traditional style. The first chapter of the thesis provides an introduction and background to the research problem leading to the main objective statement. The second chapter presents a comprehensive review of the relevant literature. This chapter closes with the presentation of the study's research objectives, questions and hypotheses. Chapter three describes the research method utilized to meet the study objectives. The results of the primary and secondary research questions are presented in chapter four and chapter five consists of a discussion of the findings of this dissertation study. The thesis concludes with a description of the study's strengths and limitations, contributions, and implications for practice, theoretical considerations and future research directions.

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Chapter 1: Problem Statement

Recent increase in twin births and preterm births among twins

During the past two decades, significant advances in medical technology have contributed to the increased survival of critically ill, preterm, and very low birth weight infants (Stoelhorst et al., 2005). As mortality rates have declined, the focus has shifted to decreasing morbidity and adverse neurodevelopmental outcomes for these high-risk infants (Luke & Brown, 2007). An additional change in the surviving population within neonatal intensive care units (NICU's) is that the numbers of twins admitted has escalated as the occurrence of multiple births is continuing to rise in North America (Millar, Wadhera, & Nimrod, 1992). A Health Canada report indicated that multiple births increased from 2.1 per 100 total births in 1991 to 2.7 per 100 total births in 2000, an increase of 29% (Minister of Public Works and Government Services Canada, 2003) and a continued rise to 3.0 per 100 total births in 2004 (Minister of Public Works and Government Services Canada, 2008). Similarly, the rate of twin admissions to Canadian neonatal units rose by 7% between 2003 and 2008 (Bassil, Shah, Barrington, Harrison, da Silva, & Lee, 2011). Delayed childbearing, advanced maternal age, increased fertility treatments and interventions augmenting conception have been reported as the main reasons for this increase (Blondel & Kaminski, 2002; Millar et al., 1992; Schieve et al., 2002; Wilcox, Kiely, Melvin, & Martin, 1996). These factors have been primarily associated with a rise in dizygotic twins. Although race has some effect on the incidence of dizygotic twinning (10-40 per 1000 live births among those of African descent compared to 7-10 per 1000 among Caucasians), higher maternal age and assisted reproductive technologies are strongly associated with multiple gestations (Goldenberg, Culhane, Iams, & Romero, 2008). Occurrence of monozygotic twinning has remained stable at 4 per 1000 total births worldwide.

Preterm birth is the leading cause for hospitalization during the neonatal period and is responsible for more than 75% of all cases of perinatal morbidity and mortality (Ladden, 1990). In 2004, 57.0% of twins and 96.1% of higher order multiple births were delivered preterm (Minister of Public Works and Government Services Canada, 2008). The incidence of preterm birth among multiples has risen substantially over the past several decades in North America. In the United States, preterm birth rates increased by 14.1%, from 40.9% in 1981 to 55% in 1997 (Kogan et al., 2000). In Canada, the rate of preterm birth among twin live births increased by 14.5% , from 42.5% between 1985 and 1987 to 49.6% between 1994 and 1996 (Joseph et al., 2001) to 53.0% in 2000 (Minister of Public Works and Government Services Canada, 2003) and 57% in 2004 (Minister of Public Works and Government Services Canada, 2008). Furthermore, assisted twin pregnancies were more likely to result in lower gestational age (33.1 versus 34.2 weeks) and birth weight (2,029 versus 2,177g for the first twin and 1,897 versus 2,136g for the second twin) than those occurring spontaneously, with longer associated NICU stays (Kanat-Pektas, Kunt, Gungor, & Mollamahmutoglu, 2008). In a similar study in 2002, examining the effect of multiple births on perinatal indicators over two decades in Canada, the British Isles, France and the Unites States, twins contributed a disproportionate share of preterm deliveries and low birth weight newborns (Blondel et al., 2002). Maternal and neonatal complications associated with twin pregnancies have also contributed to the increased likelihood of NICU admission and prolonged hospitalization. Maternal risks include gestational hypertension, placental abruption, and placenta previa, all which are positively correlated with adverse neonatal sequellae (Allen, Wilson, Cheung, 2006). In a 2005 Canadian review of 3,242 infants born at or before 32 weeks of gestational age and admitted to 24 Canadian NICUs, twins had approximately similar mortality when gestational age and severity of illness were accounted for (adjusted

odds ratio 1.3, 95% confidence interval 1.0-1.6) (Qiu et al., 2008). Weight discordance, chorionicity, and gestational age at birth were more closely associated with adverse outcomes than plurality of pregnancy (Wen et al., 2005). However, the higher likelihood of these factors in twin versus singleton birth created the additional risk associated with multiple births (Ballabh et al., 2003). Twin infants born at 30 to 32 weeks have higher severity of respiratory distress syndrome and those delivered from 27 to 29 weeks were more likely to have at least one of the following complications: patent ductus arteriosus, intraventricular hemorrhage, necrotizing enterocolitis, or retinopathy of prematurity, when compared to matched singletons (Nielsen, Harvey-Wilkes, MacKinnon, & Hung, 1997).

Ubiquitous pain exposure in the neonatal intensive care unit

Given the higher likelihood of preterm birth and associated morbidity leading to the need for NICU admission, twin infants often face increased medical challenges and can be neurodevelopmentally less prepared to cope with multiple stimuli after birth when compared to healthy full-term infants (Als, 1986). Data from several countries have consistently shown that neonates undergo multiple painful and stressful procedures during hospitalization in the NICU. Canadian (Johnston, Collinge, Henderson, & Anand, 1997), European (Cignacco et al., 2007), Scandinavian (Simons et al., 2003) and British (Barker & Rutter, 1995) studies report an average of 10-15 procedures daily with extremely preterm neonates (< 28 weeks) undergoing as many 700 painful procedures during their hospital stay (Porter, Grunau, & Anand, 1999). Recently, Carbajal (2008) conducted a 2 week prospective chart review of 431 infants in several NICUs in the Paris region and showed that these infants endured 30,174 painful procedures and a mean of 26 exposures (range 0 to 62) of painful or stressful procedures per day. Pain management for infants undergoing procedural pain associated with most frequent procedures such as tracheal suctioning, heel lance, tape removal,

venipuncture and intravenous line insertions although improved was suboptimal. Two thirds of infants undergoing heel lance for blood collections, the most commonly performed tissue breaking procedure in the NICU setting, did not receive any form of non-pharmacologic or pharmacologic intervention and 46% of infants underwent tracheal intubation without the benefit of any pain relieving strategies (Carbajal et al., 2008). A similar one week survey of 14 NICU's across Canada, reported a total of 3508 tissue damaging and 14,085 non-tissue damaging procedures, with average of 5.8 (SD 12) and 25.6 (SD 15) respectively (Johnston, Barrington, Taddio & Filion, 2011). Half of the procedures (46% tissue damaging, 57% non-tissue damaging) had no analgesic interventions. Opiates were used for 14.5 % of tissue-damaging procedures and sweet taste was used for 14.3% of the tissue-damaging procedures. Parental presence predicted use of sweet taste or non-pharmacological analgesia for tissue-damaging procedures but practices varied widely among the sites.

Animal studies have linked pain to adverse developmental changes in the brain (Anand, 2000; Porter et al., 1999) and in the spinal dorsal horn (Grunau, Holsti, & Haley, 2005; Ruda, Ling, Hohmann, Peng &Tachibana, 2000). In human infants, immediate responses to untreated pain such as physiological elevations in heart rate, blood pressure, and oxygen requirements, can lead to fluctuations in intracranial pressure, possibly leading to intraventricular hemorrhage (IVH) and periventricular leukomalacia (Scanlon, 1991; Stevens, Johnston, & Horton, 1993). Increased stress hormone release triggered by pain impedes normal regulation of growth and tissue repair (Morelius, Theodoursson, & Nelson, 2005) and has adverse effects on cognition, memory, and behaviour systems (Holsti, Grunau, Whifield, Oberlander, & Lindh, 2006). Stress associated with pain can lead to prolonged structural and functional alteration in pain pathways that lasts into adult life, permanently altering normal or common responses to pain (Fitzgerald & Beggs, 2001; Hermann, Hohmeister, Demirakca,

Zohsel, & Flor, 2006). Given the extreme plasticity of the preterm brain and immature regulatory processes, it is not surprising that exposure to repeated skin breaking procedural pain may disrupt the normal development of physiological, hormonal, behavioural and hypothalamic-pituitary-adrenal (HPA) axis that may contribute to long term effects. Recently, Grunau and colleagues reported a blunting of hypothalamic-pituitary-adrenal (HPA) axis response in infants who had undergone numerous painful procedures in the NICU (Grunau et al., 2005; Grunau, Oberlander, Whitfield, Fitzgerald, & Lee, 2001). Preterm infants in contrast to infants born at term (Taddio, Katz, Ilersich, & Koren, 1997; Taddio, Shah, Gilbert-MacLeod, & Katz, 2002) appear to experience a down-regulation of behavioural responses and a decrease in sympathetic recovery contributing to higher physiological instability.

Despite a surge in the literature illustrating various methods to accurately assess and manage pain and the provision of consensus practice guidelines (American Academy of Pediatrics Committee on Fetus and Newborn et al., 2006), minimal improvement in the treatment of pain associated with routine NICU procedures has ensued. The reasons for this lack of practice change are unclear. Issues related to research utilization and lack of agreement regarding optimal pain management strategies for routine procedural pain is the most likely cause. Evidence that morphine (a commonly used neonatal analgesic) is known to attenuate postoperative and severe pain, is less effective for pain associated with mechanical ventilation and heel lance (Carbajal et al., 2005) and given possible adverse outcomes associated with its prolonged use (Anand et al., 2004) has led to further inquiry regarding the role of non-pharmacologic measures and environmental context in the minimization of acute pain response and long-term impact on immature regulatory pathways of at risk infants.

Environmental context and comfort for alleviation of pain

Infants have been shown to have cortical perception (Bartocci, Bergqvist, Lagercrantz, & Anand, 2006; Slater, Boyd, Meek, & Fitzgerald, 2006) and memory of pain, both exhibited by peripheral hypersensitization (Taddio et al., 2002) and behavioural response (Grunau et al., 2005; Johnston, Stevens, Yang, & Horton, 1995; Taddio & Katz, 2005). Recently, two studies using near infrared spectroscopy (NIRS) to measure pain experience in preterm infants, revealed that infants as young as 28 weeks of gestation exhibit cortical response during heel lance (Bartocci et al., 2006; Slater et al., 2006). Functional MRI imaging of adults have demonstrated that pain perception and inhibitory mediation appears to involve multiple areas of the brain, referred to as the "pain matrix" (Rainville, 2002), and that perception and response can be mediated by visual cues and relational factors (Jackson, Rainville, & Decety, 2006). Although not yet proven with neonatal neuroimaging, the assumption that neonates may also perceive and respond to pain and distress in a similar interlinked manner is highly plausible. It is known that pain in newborns can be soothed with alterations in environmental context and provision of non-pharmacologic strategies involving orogustatory, vestibulokinesthetic, and/or olfactory and tactile systems (Johnston, Fernandes & Campbell-Yeo, 2010). Sweet tasting solutions, breastfeeding and nonnutritive sucking thought to be regulated through endogenous opiate and serotonin systems have been shown to diminish pain response associated with procedural pain (Cignacco et al., 2007; Shah, Aliwalas, & Shah, 2006; Shah, Aliwalas, & Shah, 2007; Stevens, Yamada, & Ohlsson, 2010). Containment, felt to enhance regulation of infant state via swaddling and facilitated tucking have also been shown to be beneficial (Cignacco et al., 2007). Although the benefits of music and vestibular action may be less promising in isolation (i.e., without the mother), these results have helped us better understand the importance of maternal presence and

relationship with respect to pain response (Johnston et al., 2008; Johnston, Filion, & Nuyt, 2007).

Both term and preterm infants have olfactory memory. They not only show preference for their own mother's amniotic fluid and breastmilk, but this recognition has been shown to diminish crying during maternal separation and pain response during heel lance (Marlier, Schaal, & Soussignan, 1998; Rattaz, Goubet, & Bullinger, 2005; Schaal, Marlier, & Soussignan, 1998; Varendi, Christensson, Porter, & Winberg, 1998). Interestingly, olfactory recognition of a familiarized smell can elicit a similar comforting response (Goubet, Rattaz, Pierrat, & Bulinger, 2003; Goubet, Strasbaugh, & Chesney, 2007) indicating both memory and ability to learn, remember and have emotional connections even in young, very preterm infants. Kangaroo mother care (KMC) or skin-to-skin contact care (SSC) provides a multisensorial context encompassing tactile, olfactory, and relational systems (Campbell-Yeo, Fernandes, & Johnston et al, 2011). It has been shown to diminish pain response and improve physiological stability in both term and preterm infants (Johnston, Campbell-Yeo, Filion, 2011). Whether the mother is an essential aspect of this comfort during skin-to-skin contact has yet to be proven.

The discovery and practice of new and innovative approaches to minimize the effects of infant pain is a primary nursing focus (Halimaa, 2003). Numerous avenues of possible non-pharmacologic measures or alternative environmental contexts within the NICU have yet to be fully explored as primary or adjuvant methods to relieve pain and diminish potential long lasting effects of pain on the development of regulatory pathways. The increased incidence of multiple gestation births and admission of these fragile babies to neonatal units also raises questions regarding the differences in care of twins and higher order multiples

versus singletons. Despite the ever increasing numbers of at-risk twin infants, specific interventions targeted this population have not been studied.

Co-bedding as a potential comfort measure

At birth, preterm twins are typically separated as individual health needs are met. They then experience the often painful environment of the NICU alone, without their twin with whom they have spent the last several months feeling and hearing the familiar sound of their twin's heartbeat or the memory of previous touch and synchronous movements. Co-bedding of twins is an example of a developmental care initiative. Its purpose is to minimize neurodevelopmental sequellae associated with admission to a NICU (Als,1986; Hayward, 2003; Nyqvist, 2002). The practice of co-bedding is based on the premise that extrauterine adaptation of twin neonates is enhanced by continued physical contact with the other twin rather than sudden deprivation of such stimuli (Byers, Yovaish, Lowman, & Francis, 2003; Hayward, 2003). Maintaining this presence may assist twins to cope with pain associated with routine procedural pain by stabilizing self regulatory pathways.

Twins, the majority of whom are born preterm, are exposed to painful procedures as part of their essential medical care. The adverse effects are both immediate and potentially long-term, affecting future sensation and behaviour (Anand, 2000; Fitzgerald & Beggs, 2001; Grunau, Holsti, & Peters, 2006). Given that the practice of co-bedding simulates numerous aspects of environmental context – proximity, tactile, olfactory, auditory, memory and relationship – that have been shown to provide comfort to newborns, it is reasonable to propose that the contact or presence of a sibling who has shared the same uterine space since conception would have a similar comforting effect.

Purpose

The purpose of this study was to determine the efficacy of twin comfort during a tissue breaking procedure (heel lance) in the neonatal intensive care unit. The primary outcome was pain response determined by physiological and facial reactions to a painful event.

Chapter 2: Literature Review

This literature review on the comfort of co-bedding is divided into four main sections. The first section describes the empirical evidence known for co-bedding twins. The second section presents the theoretical rationale and conceptual framework for the study. The third section describes the context of the co-bedding environment and its relationship to comfort using supporting literature regarding multisensorial stimuli. The last section provides the objectives and hypotheses of the study.

Developmental Care

Preterm birth and subsequent admission into a NICU leads to the sudden deprivation of the infants intrauterine world and disrupts the normal environment in which the preterm infant should mature and develop. The extreme contrast between the womb and the neonatal intensive care context create numerous challenges to these at-risk infants. Developmental care is a concept that encompasses a family centered nursing care philosophy and multiple strategies designed to minimize the stress of the NICU environment for both the infant and his/her family (Aita & Snider, 2003). The interventions provided may include elements such as control of external stimuli (vestibular, auditory, visual, tactile), clustering of nursery care activities, and positioning or swaddling of the preterm infant (Symington & Pinelli, 2006). Developmental care initiatives have been advocated in neonatal intensive care units (NICU) in an attempt to minimize the risk for poor neurodevelopmental outcomes for preterm infants (Als, 1986). Although initially based solely on a theoretical justification, empirical studies with positive outcomes following implementation of developmental care practices have increasingly been reported (Westrup, 2007). The newborn individualized care and assessment program (NIDCAP), the global developmental care program most studied, when provided to preterm infants has been associated with: improved respiratory outcomes; less mechanical

ventilation (mean difference -27.7 days, 95% CI - 43.9 to -7.5 days) and reduced oxygen requirements (mean difference -41.1 days, 95% CI -65.3 to -16.8) (Jacobs, Sokol, & Ohlsson, 2002); a potentially lowered incidence of Grade III or IV intraventricular hemorrhage (relative risk (RR) 0.51, 95% CI 0.23 to 1.10) (Symington & Pinelli, 2006); higher mean mental developmental index (MDI) scores at 9-12 months (Jacobs et al., 2002); and, improved survival without severe disability (odds ratio (OR) for surviving: without abnormal behaviour was 19.9 ; without mental retardation was 3.5; and, without overall disability was 14.7) (Westrup, Bohm, Lagercrantz, & Stjernqvist, 2004). Consistent findings were also reported in a recent Canadian study examining the effect of developmental care practices for very low birth weight infants (Tyebkhan, Peters, Cote, McPherson, & Hendson, 2004). With respect to comfort, NIDCAP was associated with reduced stress and pain behaviours (Sizun, Ansquer, Browne, Tordjman, & Morin, 2002), fewer episodes of cardio respiratory instability and hypoxia during routine nursing care (Catelin, Tordjman, Morin, Oger, & Sizun, 2005), improved sleep patterns (Bertelle, Mabin, Adrien, & Sizun, 2005) and less usage of sedatives and opioids (Heller, Constantinou, VandenBerg, Benitz, & Fleisher, 1997; Tyebkhan et al., 2004).

Co-bedding

Co-bedding, a developmental care practice described within the NIDCAP context, is the practice of caring for twins and higher order multiples in one incubator versus separating and caring for each infant in a separate incubator (Als et al., 1994; Nyqvist & Lutes, 1998). Twins are generally diaper clad and swaddled together within a similar boundary, thus creating the opportunity for enhanced co-regulation via skin-to-skin contact, touch, and auditory and olfactory recognition (Lutes & Altimier, 2001). The practice of co-bedding originated outside of North America (Lutes, 1996) due in part to the higher incidence of bed

sharing and co-sleeping in European countries. Anecdotal reports and emerging empirical evidence contributed to an increased awareness and uptake of this practice in US and Canadian NICUs in the late 1990's (DellaPorta, Aforismo, & Butler-O'Hara, 1998; Gannon, 1999). Studies examining the psychological and social effects of co-sleeping reported positive outcomes described as improved infant and maternal sleep, more robust infant arousal patterns and an increased incidence of breastfeeding (McKenna et al., 1994; McKenna & McDade, 2005). Bed-sharing between mother and infant has also been positively associated with a longer duration of breastfeeding (Horsley et al., 2007). The earliest anecdotal report occurred in 1995 when the Worcester Telegram & Gazette published a story about preterm twins comforting one another when being cared for together in an NICU (Sheehan, 1995). Currently, there is a paucity of empirical research to either support or refute the benefits of co-bedding. As a result, the National Association of Neonatal Nurses and the American Academy of Pediatrics have stated that "co-bedding cannot be fully endorsed until further research is available" (NANN Position Statement, 2009) and " that it may be prudent to not co-bed twins (Tomashek, 2007).

While co-bedding is theorized to enhance twin co-regulation, improve respiratory status, decrease oxygen requirements, increase weight gain, facilitate mutuality in circadian rhythms and sleep/awake patterns (Byers et al., 2003; Hayward, 2003; Lutes & Altimier, 2001; Nyqvist & Lutes, 1998), the current depth of empirical evidence has been limited to eight studies (three randomized controlled trials and five cohort studies - three prospective, two retrospective) in which co-bedding twins or multiples being cared for in NICU settings were compared with non co-bedding infants (Byers et al., 2003; Chin, Hope, & Christos, 2006; Longobucco, Bernstein, & Rossi, 2000; Lutes & Altimier, 2000; Lutes & Altimier, 2001; Polizzi, Byers, & Kiehl, 2003; Stainton, Jozsa, & Fethney, 2005; Touch, Epstein, Pohl,

& Greenspan, 2002). Outcomes measured included physical growth, physiological response, infection rates, sleep patterns, stress reactivity and self regulation. Stress response was measured using physiological parameters (baseline and high activity heart rate, respiratory rate, oxygen saturation) or behavioural stress cues outlined in the NIDCAP model. Self-regulation was evaluated based on variations in heart rate and temperature. Table 1 contains an overview of each the studies. Details are provided in the subsequent section.

Table 1: *Review of Co-Bedding studies in the Hospital Setting*

Adapted from Tomashek, 2007

AUTHOR	STUDY DESIGN	POPULATION	OUTCOMES	RESULTS STANDARD CARE	RESULTS CO-BEDDING	ANALYSIS AND FINDINGS
Chin et al (2006)	Randomized study. Comparison of differences in growth and physiological regulation in co-bedding preterm twins vs. non co-bedding preterm twins in an NICU	Twins 28–34 wk gestation. Stable (off ventilator; O2 if required administered by nasal cannula only; no infection; parental consent) 40 non co-bedding and 42 co-bedding multiples (twins, triplets)	(1) Adjusted mean weight ±SE At baseline, g Week 1, g Week 2, g Week 3, g (2) Median No. of combined apnea, bradycardia, and desaturation events per group per week	1435.8±333 1572±149 1692.8±16.3 1795.8±22.4	1550.5±283 1643±14.5 1754±19.8 1804.5±24.7	No significant difference at baseline Significantly higher weight gain in week 1 (P=0.001) and week 2 (P=0.02) during co-bedding No significant differences adverse events.
LaMar and Dowling (2006)	Retrospective cohort study. Compared incidence of infection (7 d after delivery to discharge for co-bedding and non co-bedding preterm twins	Twins 23–35 week gestation in stable condition 94 co-bedding twin infants delivered between 1997-2001- no hypotension requiring medication; no umbilical lines or chest tubes; no phototherapy; no ventilator support or CPAP (nasal continuous positive airway pressure) 112 separately bedded twin infants born between 1992–1996	1) No. (%) of positive blood cultures	4.5%	3.2 %	Lower incidence of infection in co-bedding P=0.04 Limitation was that incidence of infection from day 7 to discharge but many infants didn't co-bed until near term- not a true reflection of co-bedding influence
Stainton et al (2005)	Cohort study of self-regulatory and stressful responses among twins Comparison of observations- Group A following co-bedding for	Twins preterm 27-36 week gestation Medically stable; (non-ventilated; no infection) all from same-level nursery 10 co-bedding	Mean self-regulatory and stressful responses			Trend towards higher self regulatory behaviours, lower mean infant stress, when co-bedding – did not reach significance.

	≥24 h and Group B no prior co-bedding	9 non co-bedding twins				
Byers et al (2003)	Randomized, repeated-measures. Compared feeding physiological stability and behavioural effects of co-bedding vs. non-co-bedding preterm multiples in incubators	Multiples of< 37 wk gestation; (mean gestation 33 weeks in co-bedding; 31 gestation standard care) Considered medically stable (normal vital signs for 24 h; normal last white cell count; no known infection; non ventilated) 16 co-bedding 21 non co-bedding	5-d average daily weight g ±SD, 5-d average breast milk/ formula ingestion±SD, mL 5-d average highest activity heart rate ±SD, beats per min 5-d average baseline and activity heart and respiratory rates and oxygen saturation, stress cues, and highest-activity respiratory rate	1348±145 195.6±217 182.6±8.1	1439±134 200.6±22.4 180.2±11.3	5 day average using RM-ANOVA; Significant main effects: P<0.001 P<0.001 P<0.001 High-activity heart rate higher on days 1 and 2 for co-bedding infants and lower on days 3–5 No significant differences
Polizzi et al (2003)	Retrospective cohort study of infant and maternal outcomes among co-bedding and non co-bedding multiples	Preterm multiples Mean gestation 33 weeks 39 co-bedding, 116 non co-bedding (71 twin pairs, 3 sets of triplets)	Clinical outcomes Parental satisfaction Co-bedding after discharge to home Compliance with AAP "back to sleep" guidelines	4.4% 83% 38.1%	4.7% 100% 25.5%	No significant differences clinical outcomes Co-bedding group reported: Higher parental perception for support for bonding P=0.05 Higher co-bedding after discharge P=0.04 No significant difference compliance with AAP guidelines at discharge.

Touch et al (2002	Blinded study of cardio respiratory event recordings of twins 12 h before co-bedding and for first 12 h during co-bedding	Twins< 37 wk gestation. Considered stable (no arterial lines; no ventilator support including nasal continuous positive airway pressure; free from known infection) 22 co-bedding twins 22 non co-bedding	Apnea or a pause in respiration of >10 s, No. of episodes Apnea (A) of 15–20 Or >20s; Bradycardia (B) <80 beats per min, A/B, periodic breathing, mean heart rate, respiratory rate, or arterial blood pressure	52	18	Lower incidence of respiratory pause during co-bedding P< 0.05 No significant differences in other outcomes
Lutes and Altimier (2001)	Prospective randomized study. Comparison of weight, head circumference, and length between co-bedding vs. non co-bedding preterm multiples	Multiples <37 wk gestation; birth weight<1500 g; patient in NICU. considered stable (non ventilated; no birth defects; no severe neurosensory defects; no infection). Co-bedding minimum of 2 weeks 59 non co-bedding (30 twins, 21 triplets and 8 quadruplets) 62 co-bedding (46 twins, 12 triplets, 4 quads)	Average weekly: weight gain, kcal/kg, change in Head circumference, and growth in length; No. of medication errors, nosocomial infections, sepsis workups initiated, and thermal insults			No significant difference
Longobucco et al (2000)	Prospective cohort comparison of growth and physiological parameters between co-bedding infants and non co-bedding historical controls matched for gestational age and size at birth	Multiples<37 wk gestation 31 co-bedding 31 non co-bedding multiple-gestation infants	No. of body temperature decreases Daily average weight gain, heart rate, respiratory rate, body temperature, and apnea episodes; incidence of periodic breathing and length of stay; No. of heart rate elevations, respiratory rate elevations			Higher in co-bedding (P<0.02); No significant differences

Benefits of Co-bedding.

The reported benefits of co-bedding include short-term but no long-term improvements in growth (Byers et al., 2003); more stable respiratory patterns (less pauses in breathing lasting > 10 seconds) which contributed to regulated sleep patterns in the co-bedding group (Touch, Epstein, Pohl, & Greenspan, 2002); and reduction in the number of positive blood cultures (LaMar & Dowling, 2006).

Growth.

Of the five studies to compare growth between co-bedding and non co-bedding groups the results of two randomized controlled trials reported a faster although not sustained rate of growth when compared to non co-bedding infants (Byers et al., 2003; Chin et al., 2006; Longobucco et al., 2000; Lutes & Altimier, 2000; Lutes & Altimier, 2001; Polizzi et al., 2003). Sixteen twin infants (average gestational age of 29 weeks and birth weight of 1,150 g), randomly assigned to co-bed had a significantly higher statistical (although not considered clinically important) 5-day average daily weight and breast milk or formula intake when compared to 21 separately bedded twins and triplets (Byers et al., 2003). In a similar population using the same design, which compared 42 co-bedding and 40 non co-bedding twin infants, mean daily weights in the first two weeks but not the third week of the study were significantly higher in the co-bedding group in week 1 and in week 2 (Chin et al., 2006). These findings were not consistent with the results of a larger randomized study that failed to find any significant differences in mean weight (grams), head circumference or length between co-bedding (n=56) and non-co-bedding twins (n=60) (Lutes & Altimier, 2001).

Physiological Stability.

Physiological parameters (baseline or activity heart rate, respiratory rate, oxygen saturation or behavioural indicators of stress) were examined in four of the studies (Byers et al., 2003; Lutes & Altimier, 2000; Stainton et al., 2005; Touch, Epstein, Pohl, & Greenspan, 2002). No significant differences between groups were reported except in a small randomized study comparing 16 co-bedding twins with 21 non co-bedding twins and triplets. Byers et al (2003) reported significantly lower activity heart rate levels in co-bedding infants after co-bedding for 48 hours, which she contributed to a decrease in stress levels positively associated with increasing exposure to co-bedding. In a very small cohort study examining the responses of twins to intermittent periods of co-bedding, twin infants with prior co-bedding experience of no less than 24 hours were compared to infants that had never been co-bedded. Infants' responsive behaviours were then observed and recorded in an infant observation guide, and videotaped in three bedding arrangements on five occasions for duration of 30 minutes in each bedding condition (2 during co-bedding, 2 when separated and one with infant individually wrapped in a warm blanket). Although not statistically significant, Stainton and colleagues (2005) reported a trend toward lowered stress levels and higher self regulatory behaviour in co-bedding twins and a larger effect with increasing duration of co-bedding.

Apnea and bradycardia.

The incidence of apnea and bradycardia were compared between 22 preterm infants (average gestational age of 32 weeks at birth and 33.5 weeks at enrolment). Since co-bedding was considered a routine practice in this setting, infants were monitored for the 12 hours prior to initiation of co-bedding and the first 12 hours after being placed together to co-bed. During the co-bedding period, infants experienced significantly less episodes of central apnea

(defined as a pause in respiration > 10 seconds) (57 vs. 18, $p<0.05$). No differences were reported in longer pauses in respiration > 15 seconds or in other physiologic measures such as heart rate, respiratory rate or blood pressure. The authors concluded that twin contact contributed to improved regulation of sleep and respiratory patterns leading to a reduction in central apnea (Touch, Epstein, Pohl, & Greenspan, 2002).

Parental perception and attitudes towards co-bedding.

Although anecdotal and case reports (DellaPorta et al., 1998; Gannon, 1999; Lutes & Altimier, 2001; Swinth, Nelson, Hadeed, & Anderson, 2000) indicate that parents of co-bedding twins have a higher perceived sense of control in their parental role (DellaPorta et al., 1998), stronger parent-infant bonding and improved nurse-parent communication (Gannon, 1999), consistent findings related to parental benefits were not reported in empirical studies (Byers et al., 2003; Lutes & Altimier, 2001; Polizzi et al., 2003; Stainton et al., 2005). In a small randomized study in which parents were asked to complete instruments measuring maternal satisfaction, parental anxiety or maternal attachment, no significant differences between groups were reported. In contrast, when parental interviews were conducted, three studies found that parents approved of the practice of co-bedding and felt that it enhanced communication with nursing staff and consistency in care (Lutes & Altimier, 2001), that parental satisfaction with co-bedding increased over time (Stainton et al., 2005) and was more likely in older less ethnically diverse mothers (Polizzi et al., 2003)

Risks of Co-bedding.

The theorized risks associated with co-bedding twins have been raised primarily within two distinct groups 1) infants who are co-bedding during hospitalization in the NICU, and 2) infants who are co-bedding at home following discharge. Although this study pertains

only to the first group, a brief discussion of the concerns related to both groups is provided below.

Co-bedding in the NICU.

Theoretical risks associated with in hospital co-bedding include misidentification, cross contamination resulting in higher infection, temperature instability especially in the smaller twin, sleep disruption and accidental dislodgement of medical equipment (DellaPorta et al., 1998; Gannon, 1999). To date, no increased risk for adverse outcome has been reported. Incidence of suspected sepsis, nosocomial infections, pneumonia and necrotizing enterocolitis were not increased during co-bedding (LaMar & Dowling, 2006; Lutes & Altimier, 2001). Only one medication error (missed medication dose) has been reported in an infant assigned to a co-bedding group (LaMar & Dowling, 2006). This occurrence of misidentification was lower than the average reported incidence in the NICU setting and was not noted in any other study. More frequent temperature decreases were noted in 31 co-bedding compared to 31 individually bedded multiple birth infants ($p<0.02$). The authors concluded that this finding was related more to unfamiliarity with co-bedding among nursing staff and differences in nursing practice rather than the condition of co-bedding itself. Specifically, nursing staff were not consistent when placing servo probes on the twin infants while co-bedding in an incubator. Servo probes were alternately placed on the larger infant in some cases and in other twin pairs on the smaller infant. At other times, the probes may have been changed from one twin to another. Although, no other study reported temperature instability as a concern, additional study using clear clinical protocols is warranted.

Co-bedding at home.

Co-sleeping or bed sharing with an adult or older sibling, and prematurity have been reported as potential contributors to an increased incidence of sudden infant death syndrome

(SIDS) (Baddock, Galland, Bolton, Williams, & Taylor, 2006; Lahr, Rosenberg, & Lapidus, 2005; Lahr, Rosenberg, & Lapidus, 2007). Recently the primary risks for SIDS have been attributed to maternal smoking, a lowered socio economic status, parental alcohol use and prone sleep position (Lahr et al., 2005). The risk associated with co-sleeping with a non-smoking, breastfeeding mother who adheres to the "back to sleep" policy is now under debate (Horsley et al., 2007). Co-sleeping with an older sibling has been shown to have a fourfold increase in the incidence of SIDS however; the home environment consisted of concomitant risk factors (Hauck et al., 2003). In a small recent study, the videotape analysis of sleeping term twins less than 3 months old who were co-bedding in a home (n=10) and in a sleep lab environment (n=14) provided preliminary data for co-bedding twins of equal size. No negative effects were associated with co-bedding. Co-bedding twins did not wake more frequently, have higher recorded temperature or have any evidence of airway compression. They did experience greater sleep synchrony when co-bedding compared to sleeping separately (Ball, 2007). To date, although research has not demonstrated or refuted the benefits of co-bedding nor the possible association between the practice of co-bedding twins and SIDS, given the paucity of evidence related to benefits, current practice guidelines (Tomashek el al., 2007) do not recommend co-bedding of twins following discharge.

Summary of Co-bedding

Despite the recent interest in co-bedding studies, empirical information is still lacking regarding the short and long-term effects of co-bedding twins. Limitations in study design, the unblinded nature of the intervention, and small sample size have contributed to the lack of solid evidence related to this topic. Currently, no study has specifically examined the effect of co-bedding on pain response. However, anecdotal evidence and case reports have described several comforting aspects of co-bedding. When placed together, twins appeared to

touch and soothe one another (DellaPorta et al., 1998; Gannon, 1999; Lutes, 1996) and mothers of twins being co-bedded when interviewed reported that their infants appeared to be more restless and irritable when separated and "complained " more when nurses took blood samples and performed other medical procedures (Nyqvist & Lutes, 1998). Hayward (2003) describes co-bedding as a natural extension of the socialization process that allows twins to adjust to the extrauterine environment by co-regulating their body.

Theoretical underpinnings and conceptual framework

Synactive theory and twins.

Als (1986) hypothesized that infants actively communicate how they perceive and cope with their environment. She referred to this communication as the *Synactive Theory of Development* and provided an explanatory model for understanding how co-bedding may assist preterm twins in coping with their extrauterine environment by continued physical contact with the other twin, rather than the abrupt withdrawal of this presence (Als, 1986; Byers et al., 2003; Lutes & Altimier, 2001; Nyqvist & Lutes, 1998).

Extensive research on infant cues and state modulation provides evidence of the newborn infant's ability to respond to his/her external situation and initiate communication through the use of cues (Klaus & Klaus, 1998; Sumner & Spietz, 1996). Behavioural cues encompass specific movements, facial expressions and alterations in state in a way that predicts how infants perceive and cope with their surroundings. Infants interact with their environment in five major systems; autonomic (pattern of respiration, colour changes, visceral signs, and involuntary movements), motor (muscle tone, movements), behavioural state (ability to transition between states), attention/interaction (focus on stimuli such as faces and sounds), and ultimately self–regulation (ability to balance all other systems and transition smoothly between states). These systems are interdependent. Interruption or assault on one

sub-system may lead to disruption in another that may lead to abnormal behavioural and pain responses, alteration in the release of neurochemical mediators or "messengers ", and impairment of inhibitory processes.

Regulation.

Twins can support each other through contact and close proximity. Swaddling multiples in the same blanket and boundary provides them with the opportunity to co-regulate. Physical regulation involves the regulation of state (i.e., levels of arousal) and in the healthy functioning and maintenance of all body systems including physiological stability (temperature, blood pressure, respirations), hunger and stress reactivity. These regulating systems are governed by the mechanism of homeostasis. Derived from Greek words *homeo* = similar and *stasis* = standing, homeostasis is described as regulated mechanisms implicit within a harmonious state that resists change (Hardy, 1983). Regulation has three distinct aspects: (1) the ability of individuals to respond to changes in their environment *(responsiveness),* (2) the communication between and mutual adjustment among different cells, organs, systems, and domains of function *(internal regulation),* and (3) the maintenance of internal state within a given range, *(homeostasis)* ultimately to prevent overload. The combination of these three aspects creates a measurable concept called "regulatory *strength"* (Kahn & Westerhoff, 1993).

All neonates early in gestation have the ability for regulatory functioning. Through experiences, the nature and extent of this neurobiological programming is determined. Optimal operation of functioning regulatory processes or *newborn maturity* have been referred to as a *neurodevelopmental construct* that describes organized and interlinked systems that balance neurophysiologic and behavioural responses to stimuli (Feldman & Eidelman, 2003). Infants with well-developed internal mechanisms have the capacity to

respond to or regulate the effects of these encounters (Brazelton, 1990), which are socially interactive, but when less well developed, the interactions may be stressful. Regulation, essential for human adaptation, is the capacity and means to perceive, react, and recover from changing situations, and environmental inputs that in turn may lead to a reshaping of brain architecture (*From neurons to neighbourhoods: The science of early childhood development,* 2000). This possibility has led to increasing interest in underlying neural mechanisms and their association with socially directed motivational, social attachment and self regulation systems within the brain; and, the role of environmental influences. There is an emerging belief that these neural systems emerge in infancy and continue to modulate affiliative behaviours throughout the life-span (Nelson & Panksepp, 1998).

Following birth, newborns must quickly develop regulatory processes to safely transition into the extrauterine world and elicit protection from others. In most cases this protection is derived from maternal presence. However, during maternal separation such as in the case of hospitalization in an NICU, supporters of co-bedding and synactive theory believe that the continued presence of a twin, may provide a stabilizing presence especially in the case of preterm birth. Being close together, twins may provide a continued link with the familiar intrauterine world in which these infants should still be developing and maturing. The familiar sound of a twin's heartbeat, the memory of previous touch and synchronous movements may create an environment that could modulate the many adverse conditions that these vulnerable infants face during hospitalization. Although the synactive theory provides a theoretical basis for the self-regulatory benefits of co-bedding, this theory provides less guidance to explain the possible mechanisms that may lead to reduction in pain reactivity experienced by for twins undergoing a routine tissue breaking procedure.

Neuromatrix theory of pain.

Conceptually, the neuromatrix theory of pain provides an underlying framework to better understand the efficacy of non-pharmacologic methods of pain relief (Trout, 2004). The concept of a "body-self neuromatrix" suggests that the perception of pain is simultaneously modulated by multiple influences; and, that immediate pain perception and response to sensory stimuli can be altered with differing environmental and contextual factors. Additionally, context may also mediate the longer lasting effects of pain that contribute to abnormal maturation and disruption in intricate neural pathways that control physiological, behavioural, hormonal and sensory pain response.

***Body-self neuromatrix*.**

The n*euromatrix* theory, developed by Melzack, (1999) is an extension to his original "gate theory" of pain developed in 1965 (Melzack & Wall, 1965) and has been most studied as a model to describe the occurrence of phantom limb (Melzack, 1999), chronic pain (Melzack, 2001; Moseley, 2003; Moseley, 2005) and more recently non-pharmacologic interventions (Trout, 2004). The model outlines the importance of both ascending and descending inputs to the conscious experience of pain and includes additional inputs such as the important contributions of memory and context. This theory thus transcends the concept that pain is solely an unpleasant sensory and emotional experience. Melzack (2001) theorized that pain was regulated via an intricate circuit of regulatory networks (neuromatrix) rather than a single centre in the brain. This speculation was supported recently from an accumulation of neuroscience evidence using functional magnetic resonance imaging (fMRI) techniques revealing that a single pain center does not exist. Alternatively, multiple areas of the brain including the anterior cingulate cortex, insular cortex, thalamus, and the sensorimotor cortex, are involved with pain sensory and affective perception and response

(Derbyshire, 2000; Melzack, 2001). The neuromatrix theory is composed of four primary components: 1) The neuromatrix theory proposes that pain is multidimensional and produced by the synthesis of the incoming stimulus via an interconnected neural system entitled – *the body-self neuromatrix* in the brain which is essentially a linked network of interconnected loops between the cortex and thalamus and the limbic systems (Melzack, 1999); 2) Continuous cyclic nerve impulses within the feedback loops creating recognizable patterns or flow referred to as the *neurosignature;* 3) Simultaneous transmission of the neurosignature output is relayed to specific areas in the brain, the *sentient neural hub*, and creates ongoing awareness of pain; and, 4) Concurrently, the patterns of the neuromatrix activate the action centers of the matrix which lead to pain responses (Melzack, 1999). Thus, pain is a result of an accumulation of inputs processed via a widely distributed neural network *in the brain* rather than directly by sensory-evoked injury, inflammation, or other tissue damage. Specific areas of the brain involved in pain perception and response are complex and encompass the limbic somatosensory, visual, vestibular and cognitive systems.

Modulation of pain response

All viable infants have the structural and innate biological capacity to perceive pain, experience stress and respond to painful stimuli. However, those born preterm have a limited ability to self modulate pain experiences. The body-self neuromatrix, one of many regulatory human systems present at birth, although genetically determined can be shaped by sensory, environmental conditions and learning that balance excitatory and inhibitory processes. Mediating factors, such as context, relationships, competing multisensorial inputs and meaning have been shown to modulate the experience of pain in adults (Moseley, 2005). Providing multi sensorial stimuli during co-bedding which are congruent with the twins' past experiences and thus body-self neuromatrix, may modulate the pain stimulus associated with

heel lance and in turn may alter biological regulation leading to a reduction in pain responses and improvement in physiological stability. Evidence supporting this premise is reviewed in the subsequent section.

Effects on maturation of regulatory systems.

Inability to effectively modulate incoming stimuli or mount adequate inhibitory and recovery reactions may also lead to disturbances in the optimal development, functioning capacity, and possible long lasting alterations of regulatory systems. Importantly, the neuromatrix theory proposes that the sensation of pain is not absolute; its effects can lead to disruption of the brain's homeostatic systems and produce a stress response that in turn activates neural, hormonal, and behavioural regulating systems in an attempt to reinstate homeostasis. Thus, the central nervous system, a dynamic system, sorts, selects and mediates incoming stimulus through inhibition, excitation, and modulation (Melzack, 2005). The continued presence of a co-twin during co-bedding, especially for the most at risk preterm infant, may enhance this process.

Stress systems.

The strong correlation between stress and pain has been linked with chronic pain syndromes in adults and has been described by Melzack (2005) as homeostasis regulation patterns that have failed or have become disrupted within a complex, delicately balanced stress regulation system. In infants born preterm, exposure to repeated pain and stress in the neonatal period has been associated with 'system overload' leading to a down regulation of normal hypothalamic pituitary adrenal (HPA) axis functioning, and behavioural reactivity in human infants (Grunau et al., 2005). The HPA axis, an essential regulatory mechanism, helps to coordinate a person's ability to cope with changes in their environment. It appears that this "resetting" of the HPA axis may alter the basal arousal systems in preterm infants causing a

disorganized stress response to occur (Grunau et al., 2006). In addition to the stress associated with repeated painful experiences, animal models have shown that the stress associated with maternal separation and environmental manipulations can permanently alter the development of both behavioural and physiological responsiveness to subsequent stressors (Ladd et al., 2000; Pryce, Bettschen, Nanz-Bahr, & Feldon, 2003). Similarly, in human infants, the hypothalamic-pituitary-adrenal (HPA) axis, has been shown to be adversely effected by maternal separation (Meaney, 2001).

Stress response measured by cortisol levels is not consistent across gestational age. Preterm infants appear to be less able to cope with ongoing stress when compared to more mature newborns. In full term infants, cortisol levels are highest following birth or stressful events and lower over time (Gunnar, Fisch, & Malone, 1984) while in preterm infants consensus on cortisol patterns has not been achieved. Earlier reports indicated that severity of illness and younger gestational age (24-27 weeks) predicted higher cortisol levels (Economou, Andronikou, Challa, Cholevas, & Lapatsanis, 1993). Subsequently, higher severity of illness was correlated with lowered cortisol response (Scott & Watterberg, 1995). This discrepancy in findings may have been associated with cortisol collection techniques or differences in neonatal care practices including the use of postnatal corticosteroids and duration of mechanical ventilation, factors which have been associated with alteration in hormonal stress reactivity (Grunau et al., 2001). Cortisol response is also generally highest following painful procedures and less with handling and routine care. This response is dampened in preterm infants, and in infants born $\leq$ 28 weeks gestational age, repeated pain exposure predicted a similar lowering of cortisol response to routine clustered nursing care such as diapering and turning. Such changes are reflective of system overload and disruption of normal regulatory systems even with minimal sources of stress (Grunau et al., 2005).

The long lasting effects of the reprogramming of regulatory pathways has been further substantiated with longitudinal studies in which infants born at less than 28 weeks continued to have alteration in their HPA axis following discharge from the NICU (Grunau et al., 2006). Despite lowered cortisol levels following birth, these infants had significantly higher cortisol levels at eight (Grunau, Weinberg, & Whitfield, 2004) and eighteen months (Grunau et al., 2007) corrected gestational age (CGA) when compared with full term infants. Comparison between preterm infants in basal cortisol levels during introduction of visual novelty indicate that differences found after controlling for severity of illness, duration of respiratory support and morphine exposure were attributable to the number of prior tissue breaking procedures (Grunau et al., 2004; Grunau et al., 2007).

Neural reprogramming.

From this evidence we know that preterm infants are also highly susceptible to altered stress reactivity and subsequent 'reprogramming' of regulating networks (Matthews, Owen, Banjanin, & Andrews, 2002; Meaney, 2001). Given the degree of neural plasticity, immaturity of brain development, and chronic early exposure to pain and other stressors in these infants, this potential for disruption is easily understood. Neonates as young as 23 and 24 weeks of gestational age are viable. The underlying structure of their brain architecture is present yet virtually all functioning systems remain immature and not yet fully established. Using quantitative 3D-volumetric MRI to determine normal brain development in living preterm infants, Huppi and associates determined that cortical grey matter volume increased four to five fold between 28-40 weeks and an abrupt five-fold increase in myelinated white matter volume occurred between 34 and 41 weeks postconceptional age (Huppi et al., 1998; Huppi, Warfield et al., 1998).

In the case of preterm delivery neural maturation must continue outside the protective environment of the womb, and more specifically for a twin outside an environment in which previous development has occurred in proximity to its twin. Preterm birth adversely effects this development and has been shown to delay the expected acceleration in growth in the cerebellum region in the third trimester (Limperopoulos et al., 2005). Furthermore, in keeping with the work of Grunau and colleagues (Grunau et al., 2005), it appears that stresses associated with birth early in or prior to the third trimester of gestation are likely to disrupt specific areas of brain maturation. When eight-year old children who had been delivered at term were compared with a similar group of children delivered preterm, those children delivered early had reduced volumes of numerous brain regions including the hippocampus which is involved in central regulation of the hypothalamic-pituitary-adrenal (HPA) axis (Peterson et al., 2000). Alterations in early maturation of regulatory systems may also impact the developmental course of later self-regulation. In two studies by Grunau and colleagues, extremely low birth weight (ELBW) neonates (< 1000g) were more likely to complain of physical pain of unknown medical cause at age four; and, at age 8-10 years rated pictures of painful recreational situations significantly higher ($p<.05$) for pain content and perceived these pictures as more emotionally charged, when compared to normal full term counterparts. This preterm group also rated pictures of medically related situations significantly higher ($p<.001$) for pain intensity than depictions of psychosocial situations. Whereas, the children who had been born at term did not rate these situations differently in relation to the degree of perceived pain intensity (Grunau, Whitfield, & Petrie, 1998; Grunau, Whitfield, Petrie, & Fryer, 1994).

Heart Rate Variability

Maturation of biological regulatory systems is thought to also lay the foundation for developing emotional regulation. Heart rate variability is one of the key predictors of optimal development of the prefrontal cortex and limbic structures that are the essential components of the frontolimbic system of the parasympathetic nervous system. Greater parasympathetic inhibition on heart rate is associated with better regulated heart rate variability and greater vagus response, commonly referred to as vagal tone. Under normal conditions, higher baseline vagal tone is an indicator of a healthier, more physically self regulated infant (Porges, 1992). The vagus, the main nerve of the parasympathetic nervous system originates in the brain stem and communicates with different organs in the body. An excellent example of a regulatory feedback loop, the vagus serves as a messenger between brain centers and organs to maintain homeostasis (Porges, 1991). During periods of stress, healthy newborns withdraw parasympathetic control causing heart rate variability to drop. The lowering of heart rate variability is considered to be adaptive and allows for a robust sympathetic response (Porges, 2003). Higher baseline vagal tone has been linked with greater reactivity in infancy (Huffman et al., 1998); improved cognitive and emotional well-being (Feldman & Eidelman, 2003); and higher intensity of pain response (Porter, Porges, & Marshall, 1988). During a painful stimulus such as heel lance, the release of central and peripheral catecholamines, including norepinephrine (NE) and epinephrine (E), in response to pain directly control autonomic activation and lead to rapid changes in heart rate and heart rate variability. Both full term and preterm infants have been shown to have a decrease in heart rate variability and increased sympathetic nervous system activation during heel lance, with the most significant changes seen during the lance and with squeezing (Lindh, Wiklund, Sandman, & Hakansson, 1997; Lindh, Wiklund, & Hakansson, 1999). However, numerous

exposures to untreated painful experiences when a preterm infant has immature and developing regulatory processes may lead to an alteration in the normal maturation process of vagal tone. Extremely low birth weight infants undergoing heel lance had a less intense parasympathetic withdrawal in the lance period and a more sustained sympathetic response during recovery when compared with infants born full term (Oberlander & Saul, 2002). Vagal tone has been shown to be positively impacted by environmental context. Preterm infants between 32 and 37 weeks' gestational age provided with opportunity for skin-to-skin contact with their mothers exhibited higher vagal tone indicating improved maturation when compared to similar aged controls (Feldman & Eidelman, 2003). Use of a topical anaesthetic and sweet tasting solutions appeared to diminish the stress associated with venipuncture in 3 day old full term infants (Lindh, Wiklund, & Hakansson, 2000) and during immunization in three month old infants (Lindh, Wiklund, Blomquist, & Hakansson, 2003),

Summary.

The neuromatrix theory of pain provides a conceptual model to investigative pain management and comfort. During co-bedding, a twin's proximity and skin contact with his/her co-twin provides multiple sensory inputs (closeness, tactile, auditory, olfactory, and relational). Multisensorial stimulation rather than a single sensory stimulus has been shown to provide significantly better pain treatment in infants (Bellieni et al., 2007). When the infant undergoes a heel lance, the multisensory inputs derived from the practice of co-bedding may act on the pain matrix system to modulate and inhibit pain perception and diminish stress response which in turn alters the neurosignature in such a way that pain responses are reduced. Therefore the combination of familiarity and closeness leading to tactile, auditory and olfactory stimulation present when twins are co-bedding together provides excellent promise as a pain relieving and comforting strategy.

Modulation Effects Of Co-Bedding

Within the context of co-bedding twin infants, three primary conditions are met which together create an optimal environment to modulate and buffer the short and long-term effects of repeated pain and stress endured during their NICU stay. The concept of proximity in the practice of co-bedding encompasses three factors. By being placed together, twin *closeness* may reduce the normal stress associated with maternal separation. Opportunities while co-bedding for *tactile stimulation* and *skin contact* is also enhanced. Second, fetal and newborn memory and learning facilitates the recognition and soothing effects of *auditory and olfactory stimuli*. Lastly, the bond between twins creates a relationship that may contribute to the process of comforting through preprogrammed affiliative behaviours. The extent of conscious awareness within this twin relationship is yet to be determined.

Proximity.

Closeness.

Maternal separation in both animal and human newborns induces stress. Rat pups separated from their mothers show diminished growth, an acceleration in neuronal apoptosis, heightened stress reactivity, delayed prefrontal brain growth, and disturbed orientation (Anand, 2000). Maternal proximity and care-giving in the form of grooming and handling appears to modulate HPA-axis responses (Caldji, Diorio, & Meaney, 2000; Francis, Diorio, Plotsky, & Meaney, 2002; Liu, Rovnaghi, Garg, & Anand, 2004; Meaney, 2001; Plotsky, Thrivikraman, & Meaney, 1993), is neuroprotective (Kuhn & Schanberg, 1998; Rojas et al., 2003; Schanberg, Evoniuk, & Kuhn, 1984), promotes optimal learning, memory and regulatory systems (Hofer, 1994; Meaney, 2001), and acts as a buffer on the cumulative effect of pain on stress responsiveness (Walker, Kudreikis, Sherrard, & Johnston, 2003). Interestingly, despite the devastating consequences to rat pups exposed to maternal separation, unfavorable outcomes are lessened by the continued presence of familiar

littermates. Maintenance of proximity with a littermate diminished the immediate effects of protest and despair and adverse effects on learning seen in rat pups separated from their mothers. Pups left in contact with their littermates had less agitation vocalization, searching, inactivity and less ultrasonic vocalization (Hofer, 1994a; Hofer, 1994b) than those in isolation. The proximity of littermates during maternal absence has also been shown to be comforting. Blass, Shide, Zaw-Mon and Sorrentino (1995) examined rat pups responses to different levels of pain stimulus in various contexts including the effect of littermates on pain response in contact or non-nutritive suckling test conditions. Pain response was highest in those pups separated from both mother and littermates. Although pain response was lowest with maternal presence, pups in close proximity to their littermates had significantly higher pain thresholds than those in isolation, in moderate but not high pain conditions in which the mother's presence was more analgesic. These findings support our premise that allowing twins to remain together could potentially modulate response elicited from moderate procedural pain and stress associated with hospitalization.

Touch and skin contact.

From a very early gestation, infants have inborn mechanisms for soothing and comfort (Mooncey, Giannakoulopoulos, Glover, & Acolet, 1997) and all neonates are capable of perceiving, and mounting biological and sensory responses to touch (Feldman & Eidelman, 2003). One of the first senses to develop, tactile awareness occurs in the fetus at 7-8 weeks gestational age (Liaw, 2000) and psycho-neuro-endocrine development occurs in mid-gestation at approximately 20 weeks (Anand, 2007; Walker & Plotsky, 2002). Gentle touch or stroking and massage have been shown to have positive effects on newborns. Infants have been shown to exhibit decreased levels of active sleep, motor activity, and behavioural distress following gentle human touch (Harrison, Olivet, Cunningham, Bodin, & Hicks,

1996; Harrison, Leeper, & Yoon, 1990) and massage (Field, 2002; Field & Diego, 2008). Alteration in vagal tone, a marker for regulatory maturation, secondary to tactile stimulation has been associated with diminished infant stress and improved regulation (Dieter & Emory, 1997; Porges, 1992) and the sensation of touch leads to a release in endorphins, oxytocin and serotonin, which have been associated with modulating pain response (Carden & Hofer, 1990; Nelson & Panksepp, 1998; J. Panksepp, Nelson, & Siviy, 1994; J. Panksepp, Nelson, & Bekkedal, 1997).

Full skin contact and maternal presence have been shown to be beneficial for both term and preterm infants (Conde-Agudelo, Diaz-Rossello, & Belizan, 2003; Moore, Anderson, & Bergman, 2007). Skin-to-skin contact or Kangaroo Care (KC) involves the upright holding of a diaper clad infant at a sixty-degree angle between the breasts of a woman or on the bare chest of a man with an overcover. Advantages for the infant are numerous; stable heart and respiratory rates, balanced thermoregulation, decreased apnea and periodic breathing, improved weight gain, accelerated maturation of the autonomic and circadian systems and analgesia to painful therapeutic procedures (Conde-Agudelo et al., 2003; Engler et al., 2002; Feldman & Eidelman, 2003; Johnston et al., 2003; Moore et al., 2007). A recent systematic review conducted by Johnston and colleagues (Johnston et al., 2011) has reported the positive impact of maternal comfort through holding and skin-to-skin contact during painful procedures. When used with healthy full terms, skin-to-skin contact was reported as a powerful way to decrease crying, grimacing and heart rate during heel lance (Gray et al., 2000). It appears that holding with skin-to-skin contact provides more comfort than holding with clothed body-to-skin contact (Arditi, Feldman, & Eidelman, 2006). The difference in skin-to-skin contact comfort may be related to inborn tactile receptor response and regulation of opiates, oxytocin, beta endorphins, and vagal tone (Michelsson,

Christensson, Rothganger, & Winberg, 1996; Mooncey et al., 1997). The findings of three randomized trials examining late preterm and very preterm neonates have consistently demonstrated the comforting effect of maternal skin-to-skin care in relation to pain reduction and physiological recovery during heel lance. Johnston (Johnston et al., 2003) compared stable 32-36 week gestation infants using a crossover design. Each infant underwent a heel lance in both a maternal skin-to-skin condition (20 minutes) and in an incubator condition. A minimum of 24 hours was required between heel lance to prevent carry over effects and the order of conditions was varied and the preterm infants were randomly assigned. Premature Infant Pain Profile scores across the first 90 seconds from the heel lancing procedure were significantly ($0.002<P<.04$) lower by 2 points in the skin-to-skin contact condition. When this study was replicated with infants 28-32 weeks gestational age, pain response scores and time of physiologic recovery were also significantly lower in groups receiving 15 minutes of skin-to-skin contact (Johnston et al., 2008). In another study examining a similar population receiving longer intervals of 3 hours of skin-to-skin contact (Ludington-Hoe, Hosseini, & Torowicz, 2005) pain response in the skin-to-skin group was significantly lower during heel lance when compared to standard care in incubator group. The mechanism of comfort during skin-to-skin contact appears to be based on a blunting of sympathetic response and an up-regulation of parasympathetic action through biological and hormonal neural regulators (Hofer, 1994a).

By remaining close together during co-bedding twins can freely touch one another and have full body contact similar to that which occurs during maternal skin-to-skin contact. Touch may stimulate the activation of C tactile afferent nerve endings and produce a pleasant sensation. Recent studies using functional magnetic resonance imaging (fMRI) analysis reported that C fibre stimulation led to changes in the insular but not the somatosensory

region of the brain. (Olausson et al., 2001; Olausson et al., 2002). Given the correlation between C tactile fibres, touch and the limbic system, these mechanism may create emotional, hormonal and affiliative responses to both intermittent touch and full body skin contact between twins. The practice of co-bedding creates an opportunity for twins to have ongoing contact, memory recognition and a continued relationship that was initiated in utero.

Memory.

Auditory stimulation.

The human fetus is thought to be capable of auditory perception by 29 weeks gestational age (Hepper & Shahidullah, 1994), and have the ability to learn and remember auditory stimuli from their intrauterine environment. This early experience may have lasting effects on the developing brain and later self-regulation (Fifer & Moon, 1994). When exposed to voices, near term fetuses' had an increase heart rate (Kisilevsky et al., 2003) and more robust vagal tone (Smith, Dmochowski, Muir, & Kisilevsky, 2007) in response to the mother's voice and a decrease in response to a stranger's voice. Infants as young as three days recognize their mothers' voice and heart beat (DeCasper & Fifer, 1980; DeCasper & Prescott, 1984) and this memory has been shown to affect physiological and behavioural responses and have soothing effects (Kurihara et al., 1996).

Numerous studies have determined that maternal heart beat and recorded voice or lullaby can be soothing to both fullterm and preterm newborns. Following birth, infants exhibited heart rate decelerations, increased non-nutritive sucking, more relaxed facial expressions, diminished crying and less body movements when hearing syllables that are paired with the maternal voice than when syllables are paired with another woman's voice or silence (Fifer & Moon, 1994; Nakajima, 1994). Exposure to familiar sounds has been positively associated with improved physiological stability (decreased heart and respiratory

rate and an increase in oxygen saturation) (Caine, 1991; Collins & Kuck, 1991; Zimmer et al., 1993); less agitation (Standley & Moore, 1995) and more time in stable sleep or quiet alert state (Collins & Kuck, 1991). Maternal heart beat has also been shown to blunt the effects of pain associated with a tissue breaking procedure in a study in which 131 full term infants underwent a heel lance while being exposed to either maternal heart rate, a Japanese drum with identical rhythm or no sound. Infants exposed to maternal heart beat had reduced facial response and crying and lower levels of cortisol and dehydroepiandrosterone (DHEA) following heel lance when compared to the other two groups (Kurihara et al., 1996). Similar findings were not observed in a recent study examining the soothing effect of maternal voice in infants between 32 and 36 weeks gestational age where no differences were seen between those infants exposed to a recorded and filtered maternal 'singsong' voice versus no voice during a heel lance procedure (Johnston et al., 2007). These results may have been affected by the high volume of the recorded sound (70 db) or may indicate that familiar sound alone in the absence of additional environmental context such as olfactory stimulus or proximity may not be sufficient to ameliorate the effects of a tissue breaking procedure in younger more immature infants.

Olfactory recognition.

There is now compelling evidence that newborns remember, recognize and prefer smell that is associated with their intrauterine environment and their mothers, and that olfactory stimuli can provide infants with comfort and modulate pain response (Goubet et al., 2003; Goubet et al., 2007; Kawakami et al., 1997; Sullivan & Toubas, 1998; Varendi et al., 1998). Infants less than 4 days of age regardless of being formula fed (Marlier et al., 1998) or breastfed (Schaal et al., 1998) showed preference by head-turning towards familiar amniotic fluid smell versus formula or an unfamiliar amniotic fluid smell, and had decreased

crying and increased sucking bursts when presented with maternal odour versus no odour (Sullivan & Toubas, 1998). Exposure to amniotic fluid smell also diminished stress and crying associated with maternal separation. Babies exposed to maternal amniotic fluid smell cried significantly less (median 29 seconds) than babies in the two other groups (breast odour-301 seconds, no odour -135 seconds) (Varendi et al., 1998).

Newborns also appear to have early learning and memory of olfactory stimuli and this memory can affect both behavioural and hormonal response to a tissue breaking procedure (Goubet et al., 2003; Goubet et al., 2007; Rattaz et al., 2005). To determine the effect of familiar, unfamiliar or no odour on infant pain response during heel lance, 44 breast-fed newborns were randomly assigned to one of four groups: Group 1 was naturally familiarized with their mother's milk odour, Group 2 was familiarized with a vanilla smell, and Groups 3 and 4 did not receive any familiarization. During and after the heel lance, Group 1 was presented with their familiar mother's milk odour, Group 2 was presented with the familiar vanilla, Group 3 was presented with an unfamiliar odour, and Group 4 was a control group. Results revealed that infants who smelled a familiar odour (their mother's milk or vanilla) cried and grimaced significantly less during the recovery phase compared with those infants exposed to a non familiar or no odour condition. Infants exposed to their mother's milk also exhibited significantly less motor agitation during and after the heel lance (Rattaz et al., 2005). These findings were also seen in additional studies in which full term (Goubet et al., 2007) and preterm (average 32.3 weeks gestational age) (Goubet et al., 2003) infants exposed to a familiar vanilla smell during heel lance had significantly less crying and grimacing compared to infants exposed to an unfamiliar odour. In the only study to examine the effect of familiar odour during heel lance on hormonal stress response, Kawakami reported a much higher ($p < 0.05$) cortisol response in 85 five-day old infants undergoing heel lance not

exposed to odour versus exposure to a familiarized lavender smell or mother's milk, demonstrating that olfaction plays a role in the neuroendocrine feedback system (Kawakami et al., 1997).

The mechanism underlying the comforting effects of intrauterine, maternal and familiarized smell remain unknown although it has been postulated that it is an opioid mediated system. The rationale behind this premise is twofold and is derived from animal and human studies. Animal models have demonstrated that the opioid system modulates olfactory learning, odour preference and nociceptive responses in rats (Jahangeer, Mellier, & Caston, 1997; Roth & Sullivan, 2005; Shide & Blass, 1991) and in humans gustatory systems encompassing the beneficial effects of sweet tasting solutions are known to be opioid mediated and are strongly linked with the olfactory system (Stevens et al., 2004).
These studies provide compelling evidence that neonates, even those born preterm, have some ability for auditory and olfactory processing of familiar sound and smell, not just perception. This memory recognition is associated with diminished pain response and may have the potential when combined with other familiar context to help the infant modulate pain experiences. In the case of twins who have shared the same intrauterine space it is highly plausible that the recognition of their twins heart beat and shared familiar scent while being co-bedded could provide a similar soothing effect.

Relationship.

Intrauterine environment of twins.

Twins spend their entire lives before birth in close quarters, growing and developing in the presence of another fetus. Twins have been observed on ultrasound, as early as 14 weeks, sucking on their twin's face and fingers and appearing to be touching and exploring their twin's face (Klaus & Klaus, 1998). These activities may be interpreted as preparation

for self-soothing given the comforting benefits derived from non-nutritive sucking and touch following birth (Cignacco et al., 2007). Mothers of twins have reported that their babies appeared to have similar periods of activity and sleep while in utero (Gallagher, Costigan, & Johnson, 1992). Studies have consistently demonstrated that twins, when monitored by non stress tests, exhibit a remarkably high incidence (58%) of coincident fetal heart rate acceleration. These accelerations appeared to be independent of gestational age, growth patterns, or placental type. Synchronous fetal heart accelerations remained consistent with increasing gestational age (27- 42 weeks) (Devoe & Azor, 1981; Sherer, Nawrocki, Peco, Metlay, & Woods,). In a later study examining fetal heart accelerations, fetal movements and behaviour patterns, twins were found to have synchronous behaviour (sleep or awake patterns) 94.7% of the time (Gallagher et al., 1992). Synchronous behaviour between twins have been related to a form of 'interfetal communication' and may provide a basis for twin comforting and improved physiologic stability (recovery) following painful procedures if twins are placed in contact with each other (Arabin, Gembruch, & von Eyck, 1993). Gottfried, Seay and Leake (1994) examined the attachment relationship of twins (aged 18-34 months) and found that when separated from their mothers, the twins showed minimal distress if their twin remained present. In contrast, separation from both their twin and mother created a high level of distress. Furthermore, when reunited with their mothers, twins that had not been separated from each other were able to quickly restore normal social behaviours. Among twins separated from their mother and their twin, twins remained distressed during reunion and both solicited physical contact and comfort from their mother. This ability for twins to comfort each other may begin in utero and be linked with conscious memory and affiliative behaviour.

Summary of body-self neuromatrix

Preterm infants have complex neural networks (body-self neuromatrix) present at birth to perceive and respond to incoming painful stimuli and to be responsive to environmental conditions to modulate stressful experiences. The body-self neuromatrix, although genetically predisposed, is further shaped and developed by prior experiences, memory, learning and sensory inputs. This maturation establishes networks that allow the infant to perceive and regulate incoming stimuli. A twin pregnancy creates a unique intrauterine environment as the development of a twins' body-self neuromatrix occurs in close proximity of its sibling. Therefore, it is possible that a twins' ability to adapt and respond to stress may be enhanced by the continued presence of a twin sibling and lack of disruption in their body-self neuromatrix systems following preterm birth. Additionally, co-bedding may not only minimize immediate distress but may also protect vulnerable immature pathways from developing abnormally (Figure 1).

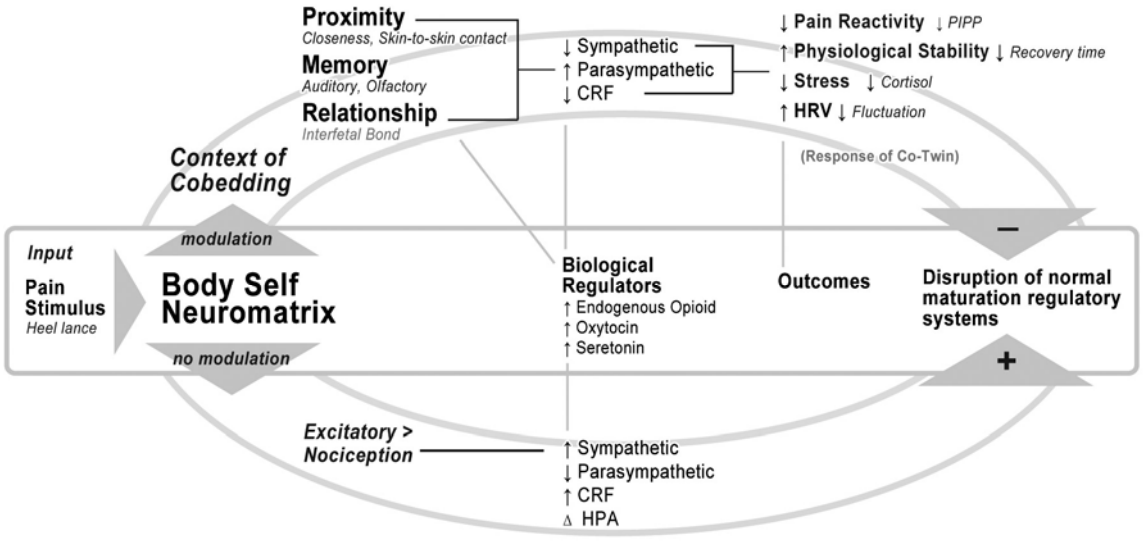


Figure 1: Body-self neuromatrix and hypothesized modulation of pain response in the context of co-bedding

A heel lance procedure leads to an excitatory nociceptive pain stimulus that triggers autonomic, hormonal and biobehavioural responses. Co-bedding via the continued proximity of the twins, stimulation of auditory and olfactory memory and continued twin relationship is thought to contribute to a greater release of biological regulators (endogenous opioids, serotonin, oxytocin) and subsequent modulation of the response to the heel lance. This modulation is linked with a diminished sympathetic and more robust parasympathetic response and lower stress, which is associated with a lower pain response, faster physiologic recovery, less salivary cortisol release, fewer fluctuations in heart rate variability, reduced 24% sucrose exposure and ultimately less disruption in normal maturation of regulatory systems.

CRF = corticotrophin releasing factor; PIPP = premature infant pain profile; # sucrose = number of 24% sucrose doses administered during heel lance procedure; HR = heart rate; RR= respiratory rate; SaO2- oxygen saturation; HRV= heart rate variability; ΔHPA= alteration in hypothalamic pituitary adrenal axis.

A priori consideration of possible confounding variables

In light of the literature review, zygosity, gestational age, postnatal age, gender, duration of co-bedding exposure, and previous experiences of pain and inflammation could potentially affect pain response. However, the evidence is relatively sparse, or the conclusions that can be drawn are limited due to theoretical and methodological problems. These a priori factors are reviewed to identify which should be studied or controlled for with respect to this study.

Zygosity and gender.

Twin pregnancy may be dizygotic (fraternal) when 2 sperm fertilize 2 ova, generally leading to separate (or di) amnions, chorions, and placentas. Monozygotic (identical) twins occur following the splitting of a single fertilized ovum within the first 2 weeks after conception. In identical twins, the timing of the separation of the ova following fertilization is associated with placentation and related adverse outcomes. Division between days 3-8 is most commonly seen and occurs in 70% of monozygotic twins resulting in monochorionic/diamniotic placentation. Earlier division prior to day 2 is seen in almost a third of cases resulting in completely separate or dichorionic/diamniotic placentas. The rarest condition, monochorionic/monoamniotic, occurring in less than 1 % of identical twin pregnancies, results from delayed splitting between days 9-12. These twins share a common placenta and sac and are at the highest risk for death, twin-to-twin transfusion syndrome (TTTS), prematurity, necrotizing enterocolitis, vascular accidents and anomalies (Hack et al., 2008).

The initiation of ‘interfetal’communication via tactile stimulation in monochorionic-monoamniotic twins when compared to dichorionic-diamniotic twins has been reported to occur 3 weeks earlier in gestation at approximately 9-12 weeks in monochorionic-monoamniotic twins and at approximately 12-15 weeks in dichorionic-diamniotic twins (Arabin et al., 1993). This is most likely due to the lack of an inter-twin membrane between the monochorionic-monoamniotic

groups. However at 25 weeks no distinct differences have been reported (Piontelli, Bocconi, Boschetto, Kustermann, & Nicolini, 1999). The speed at which initiation and reaction to contact (such as touching a leg, arm or head) occurs between twin pairs appears to be higher in male pairs when compared to females, while the degree of contact (full body or embrace or mouth contact) appear to be highest in female pairs (Arabin et al., 1993). Twins have a higher incidence of in utero tactile contact compared to their singleton counterparts. It is uncertain if this contact accelerates the maturation of specific regulatory mechanisms in twins or if tactile stimulation is more important in the maintenance and continued development of these systems following birth. Similarly in the postnatal period, stimulation from one twin does not always initiate a reaction from the other twin, and sleep or a resting state decreases the response of the co-twin.

In summary, although twin interfetal response appears to equalize near viability, the possible effect of zygosity should be accounted for in studies involving twins.

Gestational age, postnatal age, gender and co-bedding exposure.

The relationship between specific subject characteristics and pain response may potentially confound study findings. Preterm neonates show lower tactile thresholds to painful stimuli (Fitzgerald & Beggs, 2001), increased sensitivity to non-noxious stimuli (Grunau et al., 2001; Johnston et al., 1995); and higher baseline heart rate and lower heart rate variability (Sahni et al., 2000) when compared to more mature newborns. Gender influences are less well understood and only one study has demonstrated that postnatally female infants regardless of gestational age show higher facial response to pain stimuli than male infants (Guinsburg et al.,). Additionally, duration of co-bedding exposure is felt to be positively correlated with maturation of regulatory pathways and lower levels of stress (Byers et al., 2003; Stainton et al., 2005). Therefore, every effort should be made to ensure that the amount of time infants spend together in the co-bedding condition is recorded.

Previous experiences of pain and inflammation.

Early and repeated pain exposure especially in preterm infants can result in altered behaviour and autonomic reactivity (Als et al., 1994; Gibbins et al., 2008; Grunau et al., 2006; Johnston et al., 1995; Johnston & Stevens, 1996; Morison et al., 2003; Taddio et al., 2002) and structural and functional changes to pain pathways (Torsney & Fitzgerald, 2003). This potential influence on subsequent pain responses and regulation should be accounted for in any study examining these outcomes in this population.

Conclusion

It is clear that an increasing number of multiple pregnancies and increasing obstetric intervention at preterm gestation has led to a rising number of preterm twin infants being admitted into NICUs. Infants in an NICU undergo repeated and often untreated procedural pain that can contribute to immediate stress and may have a long term impact on the normal maturation of regulatory systems. Pain and stress are perceived via a complex interaction of multiple neural networks and feedback loops that can be modulated by environmental contexts that have been shown to blunt pain response and facilitate regulatory stability through modulation of biological and hormonal regulatory systems. The practice of co-bedding twins simulates various aspects of the intrauterine environment. Co-bedding allows twins to remain in close proximity and have contact with each other, thus creating opportunity for familiar recognition of auditory and olfactory stimuli and for a continuation of twin relationship that has begun in utero. Given the potential benefits of co-bedding, theoretical and conceptual underpinnings, and compelling evidence related to the effects of environmental context, it is important to examine the possibility that twins allowed to remain together could provide comfort and protection against the numerous stressful procedural assaults experienced during hospitalization.

Study objectives and hypotheses

The intent of this study was to compare the comfort effect of co-bedding between twin infants who are co-bedding versus those who are not on pain response during a tissue breaking procedure (heel lance). Pain response was measured in all infants. Reactivity was determined by validated pain scores (Premature Infant Pain Profile, PIPP scores). Findings were compared between groups. Secondary outcomes compared included: recovery from the procedure, heart rate variability; hormonal stress response (cortisol); frequency of additional dosages of 24% sucrose given during painful procedures; and, the response of the twin not receiving the tissue breaking procedure.

Primary Hypothesis - A twin undergoing a painful procedure in a co-bedding environment when compared with a twin undergoing a painful procedure cared for in a standard NICU environment (in a separate incubator/crib) will have:

a. less pain reactivity exhibited by a decrease in a validated pain score

Secondary Hypothesis - A twin undergoing a painful procedure in a co-bedding environment when compared with a twin undergoing a painful procedure cared for in a standard NICU environment (in a separate incubator/crib) will have:

a. faster physiologic recovery exhibited by a shorter duration of time required to return to baseline parameters.

b. lower hormonal stress response (salivary cortisol)

c. less fluctuation in heart rate variability

d. a lower frequency of 24% sucrose administration

Exploratory examination of Co-twin response

Although it is accepted that animals and infants are capable of prosocial behaviour and emotional contagion (Preston & de Waal, 2002) which are precursors to empathy, the possibility that animals or the very young could have true self-awareness and thus be empathetic is not

currently supported in the literature (Kunyk & Olson, 2001). Recent evidence supports the possibility that some element of empathetic response may exist in infants and animals. In a recent experiment examining the social modulation of pain in mice, Lanford (2006) reported higher pain sensitivity in mice who could visually observe their cagemates versus those who watched strangers undergoing painful situation. These findings which were not altered even when basic identifying stimuli such as olfactory and auditory cues were blocked. The authors speculated that this response was linked with innate survival tendencies and social affiliations which may be precursors to empathetic response.

Although speculative, the possibility that a form of basic empathetic response may occur between co-bedding twins when one twin sees another twin undergo a painful procedure is plausible. Data encompassing behavioural, physiological and hormonal responses of a twin not receiving a painful stimulus but exposed to his/her twin undergoing a heel lance during co-bedding will provide preliminary data regarding the unique relationship between twins.

Exploratory research question: What was the response of the twin not receiving the painful procedure when his/her co-twin underwent a tissue breaking procedure when cared for in a co-bedding environment compared to a standard NICU environment (in a separate incubator/crib)?

Chapter 3: Methods

Design

A randomized controlled trial design was used to examine the effect of co-bedding on infant pain response following a heel lance. Secondary outcomes included time to recovery, cortisol, heart rate variability, and frequency of 24% sucrose dosages during painful procedures, and the twin response to his/her twin sibling experiencing a painful procedure. Twin pairs were stratified by gestational age (≤31 6/7 weeks or ≥ 32 weeks) and study site and randomly assigned to a co-bedding group (cared for in the same incubator or crib) (Appendix A) or a standard care group (cared for in a separate incubator or crib) (Appendix B). Twins who were randomized to standard care were cared for in incubators located beside each other in a nearby intensive care site. Differences in nursing care practices were controlled by ensuring that regardless of group assignment, each set of twins were cared for by the same nurse each shift. Twins were randomized as a pair but each twin was considered a study participant. Each infant underwent a medically indicated heel lance done in a separate isolate/crib (standard care) or in the same isolate/crib as the other twin (co-bedding). The heel lance procedure was completed in either condition following no less than 24 hours and no greater than 10 days of that condition (ie. Co-bedding or Standard care).

Study setting and population

A detailed description of the study design has been published previously (Campbell-Yeo, Johnston, Joseph, Feeley, Chambers, Barrington, 2009). The study was conducted in three tertiary level university affiliated NICU's in Eastern Canada. The reason for admission could have been medical or surgical in nature and consultative paediatric services were available. The practice of co-bedding twins or multiples was not considered a standard of care in any of the NICU's.

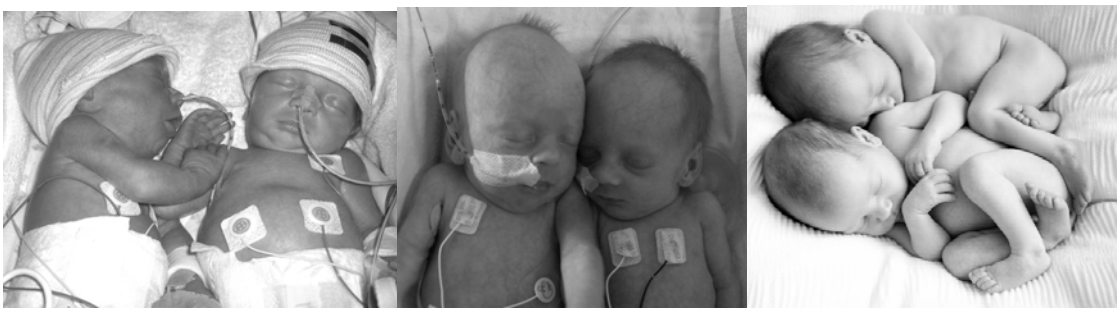
The study was approved by the Ethics Review Board of each of the three hospitals and McGill University, Montreal, Canada. Written informed consent was obtained from the parent(s) of all twin participants before study enrolment. Parents were approached if they understood verbal and written English or French. Between November 2008 and March 2011, we enrolled 67 sets of twins, a total of 134 infants that were considered medically stable and would require at least one medically indicated heel lance for blood procurement. Medically stable was defined as being (1) free from infection and (2) breathing room air or receiving oxygen via nasal prongs. Twins receiving feeds via gavage tubes, IV therapy via peripheral or central line, or experiencing occasional periods of apnea were included. Twins were considered ineligible if they (1) weighed less than 1000 grams, (2) were receiving mechanical ventilator support, (3) had a chest tube or umbilical catheter in situ, (4) had a major congenital anomaly or chromosomal aberration or (5) only one of the twin pair required treatment with overhead phototherapy.

Intervention

For this study, a description of the intervention of co-bedding and of standard care was clearly articulated. Once enrolled, specific nursing care measures for the condition assigned were posted at the twins' bedside after randomization to improve the likelihood of adherence to the protocol (Appendix A and B).

Co-bedding care.

Following randomization to the co-bedding group, twins were placed together in a Giraffe Incubator or crib lying side-by-side. Twins were diaper clad and nested together in boundaries consistent with neonatal care practices. Larger twins were partially clothed if in an open crib but still able to freely touch each other and remain nested together. Twins were positioned close to each other (lying face-to-face, side-by-side, or in a spooning position), permitting them to touch each other.



All twins had cardio-respiratory monitoring while co-bedding. One side of the incubator/crib was labeled for twin ‘a’ and the other side for twin ‘b’ and the twins and their equipment were colour coded. Incubator temperatures were maintained based on either anticipated neutral thermal environmental needs from pre-calculated charts available in the NICU or individual incubator settings prior to initiation of co-bedding. If servo temperature regulation was required, in the case of younger twin pairs or significant discordance in infant weights, the servo probe was placed on the larger infant. Infant temperatures were closely monitored and recorded throughout the co-bedding condition to maintain axilla readings between 36.8 and 37.2 degrees Centigrade.

All twins were co-bedding for no less than 24 hours prior to heel lance to allow for stabilization following transfer. The heel lance being studied occurred no greater than 10 days following initiation of co-bedding. Duration of co-bedding was recorded. Limiting the length of co-bedding duration decreased the degree of variance associated with duration of co-bedding yet still allowed adequate time for a heel lance to be ordered as part of routine care.

Monitoring (using the Somté and Massimo oxygen saturation systems) and video-tape recording took approximately 15-24 minutes - a baseline period (1-2 minutes), warming (3-5 minutes), heel lance (2-6 minutes), and recovery phase (up to 11 minutes) and were carried out by a research nurse or principal investigator.

The heel lance was collected by one of six experienced nurses who have performed heel lance procedures in previous studies in the NICU in a standardized manner according to the

institutional and NICU policies. The nurse assigned to care for the twins assisted with the heel lance procedure. Their role was to provide non-pharmacologic measures as per the NICU pain guidelines as they would do normally. All non-pharmacologic strategies including the number of 24 % sucrose doses given were recorded and confirmed with video data.

All data were collected following randomization and data collection continued until completion of the heel lance. For example, prior painful procedures included all procedures from birth until the time that the twin underwent the heel lance procedure. The one exception was the surveillance of infection following co-bedding. Based on parent's wishes, infants assigned to the co-bedding group could continue to co-bed until 48 hours prior to discharge at which time monitors were discontinued and the twins were separated. Therefore infection was recorded until co-bedding was discontinued.

All staff and parents were informed that the purpose of our research was to examine the effects of co-bedding while in an NICU setting only. We did not intend for this research to indicate support of co-bedding after discharge. All parents were provided information regarding the "Back-to-Sleep Program" recommendations and were counselled to refrain from smoking (American Academy of Pediatrics Task Force on Sudden Infant Death Syndrome, 2005). In addition, all mothers were encouraged to breastfeed or provide breast milk to their twins.

Standard Care.

For the twins randomized to receive standard care, each twin remained in a separate incubator as per current NICU policy. Twins were nested in boundaries consistent with neonatal care practices. The heel lance occurred at any time following randomization (within 10 days) to maintain consistency between the groups. Twins underwent a medically indicated heel lance in the incubator or crib in an identical fashion as the co-bedding twins. A research nurse or principle investigator monitored (using the Somté and Massimo oxygen saturation systems) and

video-taped the twins for a baseline period (1-2 minutes prior to heel lance), warming (3-5 minutes) heel lance (2-6 minutes) and recovery phase (up to 11 minutes).

NICU nursing clinical leaders were made aware of the study and agreed when possible to assign the same nurse to care for a set of twins regardless of which group they had been assigned. Adherence to this aspect of the study protocol was recorded daily by the research team until the study heel lance has been completed using an intervention fidelity checklist.

Sample Size

Previous studies examining the effect of maternal contact or the effect of sucrose on pain response during heel lance have revealed a greater than 2 point difference in PIPP scores (Johnston et al., 2003; Stevens et al., 2004). In those studies the intervention of maternal contact or sucrose administration were compared to no intervention. With respect to this proposed study, each of the NICU's, in accordance with recommended practice guidelines changes, had instituted standing orders that all infants undergoing painful tissue breaking procedures receive oral 24% sucrose. Because it is known that sucrose has a moderate effect on pain response, the intervention of co-bedding was considered an additional comfort measure. A one-point additional decease in PIPP scores was therefore considered clinically significant over and above the decrease in pain score expected with the administration of 24% sucrose.

We based our PIPP score assumptions on previous studies which reported PIPP scores of 10.7 (2.3) vs. 12.9 (2.5) from a study on kangaroo skin-to-skin contact in an older age group of preterm infants (32-36 weeks) (Johnston et al., 2003) and PIPP scores 8.87 (95% *CI* 7.85-9.89) versus Incubator 10.68 (95% *CI* 9.56-11.79) in a younger group of infants (28-32 weeks). Based on these reported 0.5 and 0.6 standard deviation pain scores (Johnston et al., 2008) and the reported values in the above studies, we used a conservative standard deviation estimate of 2.0 as our proposed study population encompassed both groups of infants. Sample size was calculated

using a 2-sided alpha error of 0.05 and a power of 80 percent. We designed the study to detect a difference of 1 point or greater change (SD 2.0) in the PIPP scores. Sixty four sets of twins were required to identify this variation in the PIPP scores if such a difference was in fact caused by co-bedding. (http://stat.ubc.ca/~rollin/stats/ssize/n2.html). With this sample size, we also had over 95% power to detect a > 15 second difference in physiological recovery (heart rate and oxygen saturation) between groups. In a recent study of skin-to-skin contact (Johnston et al., 2008), the time to return to baseline heart rate following the application of the band-aid signifying the end of the procedure was significantly different, 123 seconds (95% *CI* 103-142) for the Kangaroo Mother Care condition and 193 seconds for Incubator condition (95% *CI* 158-227, $p < .001$). Since all infants received 24% sucrose, we did not expect that the differences seen in time to recovery would be this large. Therefore, by using the larger sample, and conservatively accounting for the use of regression techniques, we planned to recruit 64 sets of twins, 32 assigned to the co-bedding group and 32 to the standard group for a total of 64 infant participants in each group.

Procedure

Parents of infants who met the inclusion criteria were approached to participate in this study by the research nurse or principal investigator. The study was explained and informed consent and authorization was obtained.

Randomization

Eligible infants whose parent(s) provided consent were randomized by a computerized off-site website accessed by the research nurse or principal investigator. Allocation was made from randomly permuted blocks of two, four or six to receive either co-bedding or standard care. Infants' ≤31 6/7 weeks were randomized separately from those twins greater than or equal to 32 weeks. Each site was also randomized separately to ensure identical proportions within the co-

bedding and standard care groups (i.e. randomization were stratified by gestational age and site at study entry).

Successful randomization gives each participant a known (usually equal) chance of being assigned to any of the groups. In smaller studies, simple randomization may not ensure a balanced distribution of participants in the study groups. A random number sequence is used to choose a particular block, which sets the allocation order for the first two, four or six subjects. Treatment group is allocated to the next two, four or six patients in the order specified by the next randomly selected block. Block randomization ensures treatment group numbers are evenly balanced at the end of each block. Alternating both sequence (permuted) and number of participants per block diminishes the risk of unblinding and allocation bias that can occur in trials where the intervention cannot be blinded to participants (Schulz & Grimes, 2002). Use of stratification can also improve the credibility of a trial as it ensures a balanced distribution of known risk factors which could confound the results of the study if they were unequally distributed between groups (Campbell-Yeo, Ranger, Johnston, Fergusson, 2009).

Outcomes

i. Primary outcomes– pain response measured using PIPP score.

ii. Secondary outcomes- time to recover (duration of time in seconds for heart rate and oxygen saturation to return to average baseline values for no less than 5-7 beats); hormonal stress response (cortisol); heart rate variability (low, high and low/high frequency ratio); frequency of additional doses of 24% sucrose administration; response of the co-twin not receiving the painful procedure when his/her twin undergoes a tissue breaking procedure.

iii. Other outcomes- clinical stability; infection rates; caregiver error

Measures

The measurement of the four main outcomes of the study relied on four strategies: video recording of facial actions, monitoring of cardio-respiratory and oxygen saturation, collection of salivary cortisol and chart review (Table 2). This section presents details regarding each measure.

Table 2: *Key variables, measures proposed, data sources, and time of administration*

Variables	Measures	Method / Sources	Time of administration
Pain response	PIPP (Stevens, 1996)	Videotape facial Monitoring	Baseline, heel lance and recovery
Physiologic Recovery	Monitors	Monitoring	Baseline, heel lance and recovery
Heart rate variability	Somte	Compumedics/Somte software	Baseline, heel lance
Hormonal stress response	Salivary Cortisol	Sorbette oral swab	Prior to heel lance (basal) and 20 minutes after the heel lance (stress)
Frequency of 24% sucrose Administration	Count	Chart medication record and video	Following intervention
Co-twin Response	Same as twin undergoing heel lance except sucrose	All above measures except sucrose	Baseline, heel lance and recovery
Safety surveillance	Caregiver error Infection rate	Institutional reports	Quarterly
Clinical stability	Supplemental oxygen Incidence of apnea/ bradycardia	Chart review	Baseline, heel lance and recovery

Pain Response.

The heel lance procedure was selected as the pain stimulus for four reasons: 1) it is the most common tissue-damaging procedure that preterm infants undergo, with reports of frequency ranging from several times per week to three times per day (Anand, Selankio, & NOPAIN Study Group, 1996; Barker & Rutter, 1995; Johnston et al., 1997) ; 2) the procedure can be relatively standardized across time and staff; 3) it is used in other studies of preterm infant pain, making some cautious comparisons across studies possible, and, 4) it is an event that occurs as part of routine care of preterm infants and is not an artificial stimulus.

When one of the twins underwent a routine heel lance, data were collected simultaneously on the other twin. Using two cameras, each twin was videotaped, during a baseline period, heel warming, lance, blood collection (squeeze) and recovery period, to record facial pain responses. The facial actions of the PIPP (Premature Infant Pain Profile, PIPP Score)(Stevens, Johnston, Petryshen, & Taddio, 1996) were scored by independent (blinded) reviewers (Appendix C) according to the Neonatal Facial Coding System (NFCS) (Grunau & Craig, 1987) (Appendix D) and heart rate and oxygen saturation measured using the Somte' system (Compumedics, Inc.) and a pulse oximeter (Masimo) placed on the hand or unaffected foot of the neonate (Appendix E).

The primary outcome was pain response measured by the Premature Infant Pain Profile (PIPP), a reliable and well validated composite measure developed specifically for procedural pain in preterm neonates (Anand et al., 1999; Ballantyne, Stevens, McAllister, Dionne, & Jack, 1999; Schiller, Stevens, Sidani, Ballantyne, & McNair, 1999; Stevens et al., 1996) (Appendix F). In a recent review (Stevens, 2010), it was reported that the PIPP has been used in 59 studies, with 14 evaluating its psychometric properties. The indicators (gestational age, infant state, heart rate, oxygen saturation, brow bulge, eye squeeze, and nasolabial furrow) are physiological,

behavioural, and contextual in nature. Contextual factors known to influence pain response in newborns, namely gestational age and sleep state are used to weight scores. Each indicator is evaluated on a 4-point scale with possible scores of 0, 1, 2, and 3. A total score of 6 or less generally indicates minimal or no pain, a score of 6-12 indicates mild to moderate pain and scores of greater than 12 indicate moderate to severe pain (Schiller et al., 1999). Given that co-bedding may improve infant regulation and enhance the likelihood of quiet sleep which may inadvertently increase PIPP score, data were analyzed using total PIPP scores and the individual components.

Heart rate was collected using four ECG leads connected to a data acquisition system (Compumedics E-series) with a sampling rate of 100 Hz averaged on a beat-to-beat basis. Transcutaneous oxygen saturation was collected via infrared oximeter (Massimo Radical) placed on a hand or the unaffected foot of the infant and connected to the data acquisition system. The physiological data were analyzed using the software in the system (Compumedics E-series Profusion PSG II) that allowed minimum, maximum, mean, and standard deviation to be calculated. Artifacts were removed according to the standard protocol in our laboratory, which deleted sections in which HR was below range for 4 or more consecutive beats before analyzing. The *three facial actions* (brow bulge, eye squeeze and naso-labial furrow) of the PIPP were continuously recorded by a digital video camera (Panasonic KS162) and physiologic parameters were input into a time synchronous data acquisition system. The investigator or site research nurse flashed colour coded cards in front of the camera to mark visual phases of the heel lance procedure and simultaneous times of phases were recorded. The selected facial actions were scored on a second-to-second basis according to the Neonatal Facial Coding System (Grunau, Oberlander, Holsti, & Whitfield, 1998; Grunau, Johnston, & Craig, 1990). The video-recordings

were viewed in real time on Windows Media Player which allows viewing of the Panasonic AG-1970 default screen with a clock to the 4th decimal place. Three recorded sessions were completed, once for each of the facial actions. A laptop computer using BASIC software to record the scores was used to organize scores. A final score based on percentage of time the facial action was present was calculated for each 30 second time block throughout each phase of the procedure, specifically baseline, warm, and every 30 seconds following the lance until the application of the band-aid signifying the end of the lance and beginning of recovery which lasted until the twin's physiologic parameters returned to baseline signifying the end of the procedure. At baseline, the neurobehavioural state indicator was determined according to Prechtl's categories (Prechtl, 1974; Prechtl & Beintema, 1977). The lowest score of zero was assigned to an infant in an active/awake state with scores increasing by one for quiet/awake and then active /sleep to a high score of three for a quiet/sleep state at baseline. Gestational age was abstracted from the chart, based on menstrual dates and early ultrasound (< 20 weeks) dating.

Recovery Time.

Time to recovery was considered to be the amount of time in seconds that elapsed until the twins' heart rate and oxygen saturation returned to baseline average values for no less than 5-7 beats. The point at which the baseline heart rate and oxygen saturation were reached after the heel lance indicated recovery. Time for recovery was recorded following the heel lance and verified using physiologic data recordings.

Hormonal Stress Response.

Cortisol is considered a primary marker indicating stress in both adults and newborns (Davis & Emory, 1995; Kurihara et al., 1996). Although adult levels have been shown to reflect consistent diurnal rhythm of secretion, with high levels found in the morning upon awakening and lower levels in the evening close to bed time, this has not been the pattern observed in the ill

preterm neonate (Kidd, Midgley, Nicol, Smith, & McIntosh, 2005). Additionally, although cortisol response to pain in very young and ill neonates is clearly linked with gestational age, severity of illness and repeated exposure to pain, the exact short and long term predictive correlation is still under investigation (Grunau et al., 2006).

Salivary collections which are non-invasive and less likely to initiate a non specific stress reaction have been found to accurately reflect the unbound, biologically active cortisol, in the general circulation (Riad-Fahmy, Read, Walker, Walker, & Griffiths, 1987; Vining, McGinley, & Symons, 1983). Cortisol enters saliva via diffusion through the acinar cells into the saliva at a rate high enough to maintain a concentration of equilibrium between unbound plasma and saliva, independent of saliva flow rate (Vining et al., 1983). The swab (Sorbettes, Salimetrics, Inc) was placed into the buccal area of the oral cavity of each twin, briefly held in place while the cheek was gently massaged to allow the swab to absorb the saliva (Appendix H). Saliva was collected prior to (basal) and 20 minutes after the heel lance (stress) from both infants. Every effort was made to ensure that morning samples were collected. Since non urgent blood collection blood work occurred between 0700 and 1000 am, this was generally not an issue. Once a specimen had been collected, the swab was spun and the saliva collected in an Eppendorff tube. Samples were frozen and kept at -20°C prior to being sent to a McGill University laboratory for analysis of salivary cortisol concentrations by specific radioimmunoassay. Salivary cortisol concentrations were measured using a sensitive enzyme immunoassay kit (Salimetrics, State College, PA, USA) as specified in the kit instructions. Briefly, 25 µl of standard or saliva was incubated with assay buffer and conjugate in the antibody-coated well for 1hour at room temperature. All assays were done in duplicates. After several washes, assay plates were incubated with the color developing reagent for 30 minutes at room temperature (protected from light). Three minutes after stopping

the reaction, plates were shaken and the optical density of each well was read on a spectrophotometer set at 450nm and 492nm. The difference in optical density between the two wavelengths was used to calculate salivary cortisol concentration using the Assayzap software program (Biosoft Inc.). The limit of detection of this assay was 0.012 ug/dl for a range of 0.012 to 3 µg/dl. The intra- and inter-assay coefficient of variation was 2.14% and 2.47%, respectively.

Heart Rate Variability.

Heart rate variability is considered a measure of the maturation of the autonomic system. Two measures of a heart rate variability (HRV) period were derived. The first during baseline and second during the heel lance until recovery was reached (return to average baseline heart rate and oxygen saturation). HRV was calculated using the Somte' system (Compumedics, Inc.) cardiac software through the ECG tracing feeding directly into a computer.

Heart rate variability, a predictor of stress following pain response, is primarily an indicator of the balance of sympathetic and parasympathetic control on heart rate (Chatow, Davidson, Reichman, & Akselrod, 1995; Lindh et al., 1999; Lindh et al., 2000). The heart rate, oxygen saturation and ECG tracing were taken from a Somte physiologic data acquisition system and infrared oximeter (Massimo Radical) probe which was placed on a hand or the unaffected foot of the infant. A minimum of 3 leads were applied to capture these measures. These extra leads were made especially for preterm infants and did not cause any additional stress or pain. However, to ensure that removal of extra leads did not affect the findings, removal was delayed until after collection of final cortisol specimens. Facial videotaping was done during the heel lance procedure only. Phases of the heel lancing procedure were recorded by the research nurse. The physiological data were analyzed using the software in the system (Compumedics E-series Profusion PSG II) that allows minimum, maximum, mean, and standard deviation to be calculated. ECG artefacts, generally related to infant movement, were removed before analyzing.

HRV measured from the cyclic changes or fluctuations in the R-to-R interval that occur with respiration (Cowan, 1995), when analyzed provide a specific yet non-invasive measure of autonomic input to the sino-atrial node of the heart. Parasympathetic and sympathetic components of autonomic control are best analyzed with frequency domain analysis using spectral power (Cowan, Pike, Burr, Cain, & Narayanan, 1993). The spectral power can range from a high-frequency (HF) band (.15 -1.0 Hz) to a low-frequency (LF) band (.04 - .10 to .15 Hz). The HF band is representative of parasympathetic activity related to respiratory sinus arrhythmia. The LF band is a reflection of primarily sympathetic activity with some parasympathetic input (Cowan, 1995). The low to high frequency ratio (LF/HF) indicates the balance between sympathetic and parasympathetic activity and has been referred to as an index of *sympathovagal balance* (Oberlander & Saul, 2002). An increased ratio generally suggests an elevated sympathetic cardiac response and decreased parasympathetic action. An overview of the empirical research regarding infant pain response and HRV can be found in Appendix G.

Frequency of additional 24% sucrose administration.

Sweet tasting solutions have been shown to be effective in diminishing newborn pain response related to tissue breaking procedures such as heel lance (Stevens et al., 1996). This evidence has led to the creation of standing medical orders in the NICU's in which 24 % sucrose is given to infants in specific doses based on weight (Appendix I). The peak effect of sucrose occurs 2 minutes after administration and this effect can be very brief in duration (Blass & Shah, 1995). Additional doses of 24% sucrose could be administered every 2 minutes up to a total of 3 doses as required during the procedure. All infants received no less than one dose of 24% sucrose 2 minutes prior to the heel lance. The number of any additional doses given as clinically indicated and based on infant pain response and PIPP scores calculated by neonatal nurses caring for infants as per standard guidelines in the NICU setting were monitored. Total number of doses

given were recorded and confirmed during review of the video tapes. Mean scores were compared between groups. Sucrose administration was viewed as a co-intervention in this randomized trial; this is an important issue given the lack of blinding regarding the intervention.

Stability /Prior pain experience/maternal and infant characteristics

Incidence of apnea or bradycardia, and need for supplemental oxygen prior, during and following heel lance (recovery period)(Appendix J), the number of painful procedures experienced by the neonate prior to the heel lance procedure (Appendix K), and maternal and infant characteristics were collected by chart review (Appendix L). Mean scores were calculated and compared between groups.

Safety surveillance.

Safety surveillance throughout the study included monitoring incidence of infection (determined using three measures: a. incidence of septic work-up; b. treatment with antibiotics; and c. confirmed incidence of sepsis as defined by a positive blood culture or X-ray findings; recording episodes of apnea or bradycardia and, caregiver error that were collected from readily available quarterly institutional reports.

Data Collection

Once the research nurse or Principal Investigator approached the parents and obtained informed consent and authorization from the mother or father, the twins were randomized to a co-bedding or standard care group, stratified by site and gestational age ($\leq$31 6/7 weeks and $\geq$32 weeks). Study data were collected during the next medically indicated heel lance procedure. Blood collections were performed by one of six experienced neonatal nurses who have worked with the Principal Investigator on previous studies to control for variation in practice. The twin's nurse was present during the heel lance. Their role was to provide usual non-pharmacologic pain strategies as per standard NICU practice including the administration of sucrose as they deemed

necessary. Co-bedding or standard care conditions were standardized across groups. Videotaping of both twins during heel lance conditions, via a Panasonic digital camera (model KS162), focused solely on the neonate's face without audio to ensure coders remained blinded to the condition. Two coders trained on previous data sets coded the videotaped facial responses. One coder scored only infants in the co-bedding condition and one coder scored only infants in the standard care condition to ensure diminished risk of unblinding. Following study completion, previously assigned group coders were asked to code a random selection of tapes (n=6) from the alternate group to ascertain any subtle differences between coders that may have accounted for group differences. Inter-rater scores were correlated. Heart rate, oxygen saturation and ECG tracing of the infant were transferred directly from the Somté system into a Pentium computer with Compumedics software.

Reducing bias

The multifaceted nature of most nursing interventions makes nursing intervention research particularly vulnerable to bias. Due to the unblinded nature of the co-bedding intervention, it was important to strictly adhere to rigorous methods to eliminate potential bias. For example, intervention or exposure biases can occur when there are differences in how the treatment or intervention is carried out, or how subjects are exposed to the factor of interest. To minimize the impact on validity of outcomes, it was essential to specify the features of the intervention being tested. In our study, the intervention was clearly described in the protocol and details were made readily available to the neonatal staff (Appendix A and B). Inadequate intervention definition has been reported as the most common problem associated with intervention bias in a review of 47 randomized control trials of nursing interventions (Lindsay, 2004). Description of interventions was found to be suboptimal following the recent review of 141 research articles published in 27 journals. Intervention descriptions averaged 7.3% of total

article space compared to 20.7% of space allocated to non-intervention methodological descriptions. Additionally, only 38 (27.0%) articles reported enough detail to potentially replicate the study or translate the intervention into practice (Conn, Cooper, Ruppar, & Russell, 2008).

Precisely describing the intervention allows the study to be easily replicated, improves generalizability and facilities clinical utility of the findings (Campbell-Yeo, et al., 2009). In addition, adherence to the protocol was monitored and recorded daily on an intervention fidelity checklist.

The use of an off site computerized website for group allocation decreased the risk of allocation bias. In an attempt to blind the data collection procedure, individuals coding facial reponse and PIPP scores remained blinded. The Prinicpal Investigator or research nurse approached the parents, explained the study and obtained consent prior to enrolment. The video camera set to focus only on the infants face was set up and controlled by the investigator or research nurse. Coding of facial responses and PIPP scores were carried out at McGill University at Dr. Johnston's lab and at the IWK Health Centre. Specific coders (Group A & B) coded data on infants who were co-bedding (A) or coded data on infants who were receiving standard care (B). Coders did not enter the unit or compare data sets (i.e. PIPP and individual facial scores were estimated without knowledge of group assignment), thus minimizing observer bias. Research coders A and B were trained by the Principal Investigator or a member of Dr. Johnston's research lab team. Training was standarized and coding performances assessed so that a minimum interclass correlation coeffecient (ICC) of 0.85 was reached between coders and standarized scores for state and each of the individual facial behaviours. Following the initial training, coders were retested quarterly using standarized videotapes to ensure inter-rater

reliability. Re-training and re-coding would have been undertaken if the ICC fell below 0.75. However, this was not the case. ICC inter-rater scores remained > 0.85. This reliability check conducted with standarized tools minimized the likelihood of measurement bias. Coders A & B were asked to re-code four randomly selected videotapes from the first weeks of coding to ensure intra-rater reliability. ICC's remained > 0.85, with ICC's of 0.75 as the pre-determined cutoff point of acceptability. Following study completion, previously assigned group coders were asked to code a random selection of approximately 20% (n=6) of the tapes from the alternate group. Inter-rater scores were correlated to ensure that observed differences were related to the intervention of co-bedding and not to systematic error between the two coders scoring techniques. ICC comparison between Group A and Group B were 0.88.

Data analysis

Data were analyzed using descriptive statistics and parametric, non-parametric tests. This section provides information on how hypotheses were tested. Information on data coding and data editing strategies are also discussed.

Hypothesis testing

Analysis and inference were based on the intention-to-treat principle. Efforts were made to ensure that follow-up was complete for all subjects and that there were no missing values for any of the subjects for any variable. Blinding of independent coders was retained until after the completion of the analysis. Baseline characteristics of study subjects were contrasted to ascertain that randomization had in fact produced comparable groups with respect to all variables that effect pain response and physiologic stability. Data were compared using chi-square for nominal data and independent group t tests for continuous variables. For the differences noted in baseline characteristics, additional inferences were made based on observed and (linear regression) adjusted differences between groups.

Primary Hypothesis -A twin undergoing a painful procedure in a co-bedding environment when compared with a twin undergoing a painful procedure cared for in a standard NICU environment (in a separate incubator/crib) would have a decrease in pain response. The primary outcome of interest was the pain response of the infant experiencing a tissue breaking procedure while co-bedding with his/her twin when compared to a twin experiencing a tissue breaking procedure receiving standard care (alone in incubator or crib). This analysis compared the mean PIPP scores calculated in 30 second blocks throughout the procedure (beginning at baseline, during warming of the heel, heel lance, heel squeeze and until recovery determined by return to baseline heart rate and oxygen saturation). The mean difference in PIPP scores at each time point was contrasted between groups using 95 percent confidence intervals and a P value using a student t test. Facial actions were also analyzed separately from PIPP scores. A facial action score (based on percentage of time the facial action was present) was calculated at baseline and for each 30 second time block following the heel lance procedure. Mean scores were compared between groups using 95 percent confidence intervals and a P value in a similar fashion as above.

The stratified nature of the randomization was accounted for in the analysis. Also, since twin pairs were randomized together (i.e. to co-bedding or standard care), the analysis was corrected for potential non-independence of outcomes between twin pairs. This involved appropriate variance adjustment which was carried out using Generalized Estimating Equations (GEE) procedures using SAS software (Proc Genmod, SAS 9.1, SAS Institute Inc. Cary, NC (Zeger & Liang, 1986). Twin studies provide a particular challenge in that the circumstance of being a twin creates a situation that threatens the integrity of the assumption of independence required for many statistical models (Ananth, Platt, & Savitz, 2005). Independence implies that every observation is statistically independent of all other observations. Twins who have shared

the same intrauterine environment and related genetic makeup have similarities that fail to meet the criteria for independence. And thus alternative models must be used to compensate for this *intra cluster* or *within cluster correlation* (Ananth et al., 2005). Reponses from twins are correlated and need to be considered "clustered" within a pregnancy. Failure to account for this potential lack of independence may result in inaccurate estimates of variance and incorrect inferences made. Introduced by Zeger and Liang (Zeger & Liang, 1986), Generalized Estimating Equations (GEE) is a method of estimation of regression model parameters that can effectively deal with correlated data. A particular strength of this methodology is that GEE does not require a constant number of repeated measures or an equal time interval, and can be used with either discreet or continuous outcome data (Twisk, 2003). Additionally, because the method of GEE assumes a 'working' correlation between dependent data, specific distribution of response are not required. Estimation is completed on a method of quasi-likelihood (Zeger & Liang, 1986).

Secondary Hypotheses - A twin infant undergoing a painful procedure in a co-bedding environment when compared with a twin infant undergoing a painful procedure cared for in a standard NICU environment (in a separate incubator/crib) would have: a quicker physiologic recovery; a decrease in hormonal stress response; lower parasympathetic withdrawal and more balanced heart rate variability; and, a lower frequency of 24% sucrose administration. The secondary outcomes of interest were time to recovery, cortisol levels, heart rate variability response and number of 24% sucrose doses of a twin infant experiencing a tissue breaking procedure while co-bedding with his/her twin when compared to a twin infant experiencing a tissue breaking procedure receiving standard care (alone in incubator or crib). These analyses include:

a. recovery time, defined as the period of time measured in seconds, elapsed following the application of the adhesive bandage signifying the end of blood sampling until the heart rate and oxygen saturation return to an average baseline condition for a minimum of 5-7 beats mean. Mean time to recovery was compared and contrasted between groups using 95 percent confidence intervals and a P value;

b. basal (collected prior to procedure) and stress (20 minutes following procedure) salivary cortisol levels. Mean salivary cortisol levels at time basal and time stress were compared and contrasted between groups using 95 percent confidence intervals and a P value. Differences in basal and stress levels were calculated within groups and comparison made between groups of the mean change using independent t tests. Mean difference from baseline to post lance was calculated and compared between groups;

c. mean HRV indices (low frequency (LF), high frequency (HF) and low to high frequency ratio LF/HF, was calculated for the duration of baseline and for heel lance (commencing at the time of the lance until application of the bandaid). HRV was compared and contrasted between groups using 95 percent confidence intervals and a P value (independent t test). Change from baseline HRV was calculated and compared between groups to determine differences over time for the repeated values for LF, HF and LF/HF throughout the procedure;

d. mean number of sucrose doses given throughout procedure. The number of sucrose doses was recorded by the nurse and verified using video tapes. The mean number of dosages administered in each group was compared and contrasted between groups using 95 percent confidence intervals and a P value. All secondary outcomes were further adjusted for the potential non-independence of the twins and differences at baseline in a similar fashion to the primary outcome using GEE analysis.

***Exploratory research question*:** What is the response of the infant not receiving the painful procedure when his/her co-twin undergoes a tissue breaking procedure when twins are cared for in a co-bedding environment compared to twins cared for in a standard NICU environment (in a separate incubator/crib)? In both groups, video recording of facial reactions, heart rate, and oxygen saturation levels were collected from the twin not receiving the painful stimulus considered to be the observer twin. Salivary cortisol at time basal and time stress were collected and heart rate variability was calculated in the same manner as described above. Each variable result was compared within twin pairs and in addition mean scores of each variable were compared between those twin infants undergoing the heel lance and the twin that was in proximity to his/her twin undergoing a heel lance using 95 percent confidence intervals and a P value (independent t test).

Data editing

Data editing included inspection of means and frequency distributions to identify any outliers and implausible values. The detection of an outlier defined as "scores more than three times the standard deviation beyond the mean" (Kline, 2005) were manually reviewed to ensure that no error had been made during data entry. Data were corrected accordingly if the information was available.

Ethical considerations

The study protocol was submitted to the Ethics Review Board of the IWK Health Centre, Ste. Justine Hospital, Jewish General Hospital and McGill University Institutional Research Ethics Board for ethics approval prior to study initiation. Heel lance procedures are an aspect of routine care for infants in the NICU and were not conducted solely for the purpose of this study. Co-bedding twins is not considered to be a standard of care in any of the NICU's. Authorization and informed consent was obtained from a parent(s) of eligible twins prior to study entry. The

Principal Investigator or site research nurse explained the research study to the parents if twins met the study criteria. At that time parents were asked to read the Consent/Authorization Form (Appendix M) and any questions they had concerning the research study was answered. The Consent/Authorization form contained information on potential risks and benefits to the participants, research rights of the participant, and information on how to contact the invetigator or study nurse. Participation in the study was voluntary. Parents were made aware of their right to withdraw their children at any point in the course of the study. Consent forms were provided in English or French and a copy was provided to participants once signed. Contact information for the local research department was provided for information concerning the rights of study participants. This study provided no direct benefit for the parents or infant's enrolled. Compensation was not offered.

All twins had continuous cardio-respiratory monitoring and ongoing surveillance for any adverse effects. If a co-bedding infant showed clinical signs of infection, twins were separated until the infecton was ruled out or treated, following which they were returned to the co-bedding condition and the study resumed. It was predetermined that if the incidence of co-infection among co-bedding twins increased significantly above the unit norm, the trial would be discontinued but this was not the case. Routine strategies for pain relief including sucrose administration and non-pharmacologic measures were provided as per standard IWK Health Centre NICU care. Study enrolment did not interfere with routine care practices. Confidentiality of all data collected were maintained. All information gathered was coded before analysis and data were stored in a secure, locked location accessible only to the Principal Investigator and research nurse. The list of code numbers and names was stored separately from the coded data. When the study results are published or presented at a scientific Meeting or health care

conference, the information shared will not contain any personal identifiers. The salivary cortisol samples, coded with a number were kept frozen in a locked freezer located at each of the hospitals. They were couriered in batches for analysis to the McGill University laboratory. Cortisol samples would not be used for any other purpose and any remaining samples will be destroyed following dissemination of the study findings. All videotapes were encrypted. Master copies of research data will be kept secure in a locked location until twenty-five years past the age of majority of the infants. At the conclusion of the study, group results will be shared with the parents of the participants involved and the institution; these results may be useful for teaching and improvement in care initiatives both at the unit and hospital level.

Chapter 4: Results

This chapter will begin with a flow diagram showing recruited participants (in accordance with CONSORT guidelines (http://www.consort-statement.org), baseline characteristics of each group at randomization and potential confounder that could bias the study result. The results for the primary outcome of pain response will follow. A comparison was made between the Premature Infant Pain Profile (PIPP) scores over the duration of the heel lance between groups. Unadjusted and adjusted findings are presented. In addition, the components of the PIPP were analyzed separately, specifically neurobehavioural state, gestational age at procedure, HR (average and maximum), SpO2 (average and minimum), and individual facial scores (eye squeeze, brow bulge and nasolabial furrow). Lastly, in a similar fashion, comparisons are presented for each of the secondary outcomes: time to recovery, salivary cortisol response, heart rate variability, frequency of 24% sucrose dosing, adverse events and response of the co-twin.

Participant enrolment

Participants were recruited in three tertiary level neonatal intensive care units in Eastern Canada. Of the 178 sets of twins that were screened during the study period (November 2008 through March 2011), 91 sets of twins were eligible for the study and 67 sets had parental consent and were recruited (Figure 2). Of these, 36 sets (72 infants) were randomly assigned to receive co-bedding and 31 sets (62 infants) to receive standard care. The majority of the twins were enrolled at one site (58 sets). Only 1 set was recruited from one site just prior to an unanticipated change in referral patterns that led to the majority of twins being transferred prior to eligibility and subsequent discontinuation of the study at this site. A major reason for exclusion of potential subjects was transfer to a different unit prior to eligibility (44%).

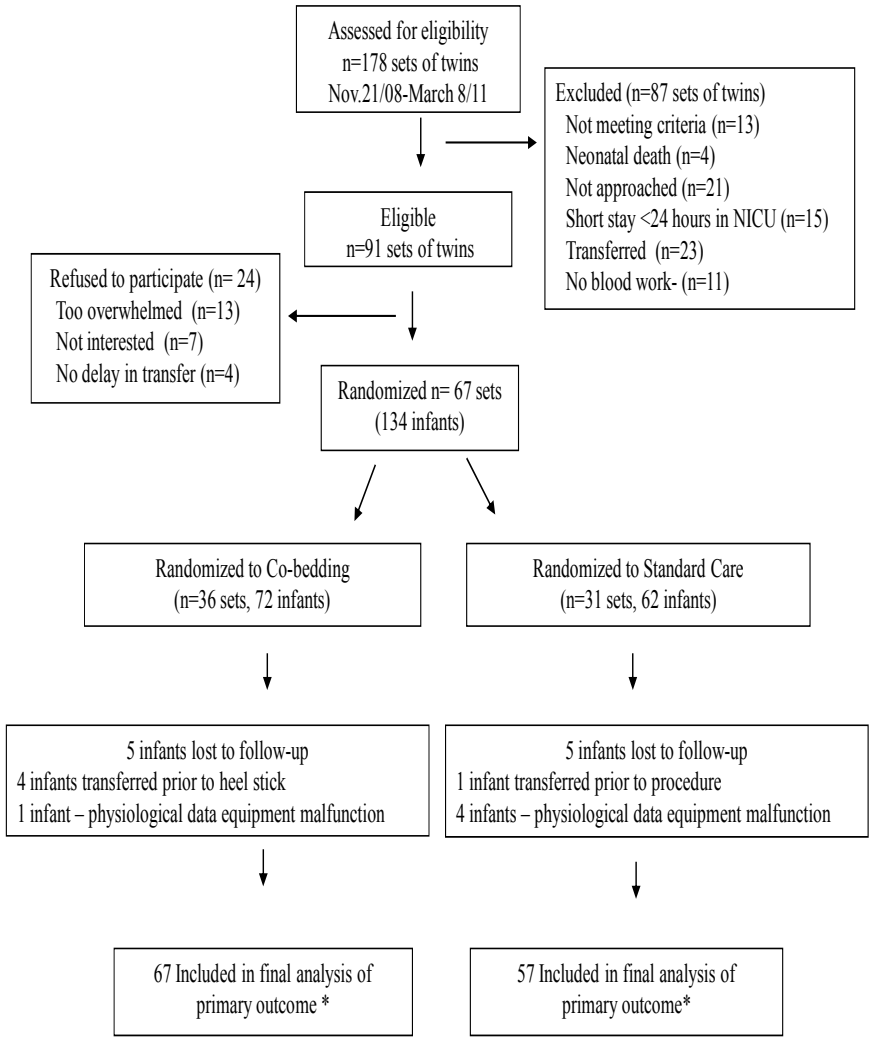
Additional reasons were that parents were not approached (24%), that inclusion criteria was not met or an infant died (19%) or a blood test was not ordered (13%).

The refusal rate was 26%. Primary reasons for those who refused were that the mothers felt too overwhelmed (54%) or that the parents were not interested in participating in any research (29%). Another reason was that parents did not want the study to delay potential transfer (17%). On three occasions, only one infant of the twin set required a blood collection (two in the co-bedding group and one in the standard care group). There was physiological equipment failure in 1 infant in the co-bedding and 4 in the standard care that made it impossible to calculate PIPP scores in these twins. Therefore, the final sample included in the primary analysis of PIPP score was based on 67 infants in the co-bedding group and 57 in the standard care group. There were some instances where one or more physiological data points were lost from the overall heel lance procedure due to infant movement or other artefact, which occurred in 10 infants in the co-bedding group and 8 infants in the standard care group.

Most maternal and infant characteristics were not significantly different between the co-bedding and standard care groups at randomization (Table 3). Maternal age averaged 30.1 years for the co-bedding twins and 29.6 years for standard care (range 16-38) and about half were first time mothers (52.8% and 48.8%, respectively). About one third of the twins (36.1% co-bedding, 25.8% standard care) were identical. Approximately two thirds of the co-bedding group was delivered by caesarean section (63.9%) and almost one half (41.9%) of the standard care group. All of the co-bedding twins and 87.1% of the standard care twins were Caucasian and the majority of their parents in both groups had attended a university or college (77.8% and 80.0%, respectively). All of the twin parents were in a 2-parent family relationship and few mothers smoked (none of the co-bedding twins and only 3 mothers (9.7%) in the standard care group).

Overall, the infant's in both groups had an average birth weight of 1751 (range 640-2675) grams and they were delivered between 31 and 32 weeks of gestational age (range 24.3-36.3), approximately equal numbers delivered at less than 32 weeks (50% co-bedding, 41.9% standard). Just over half were male (58.3% co-bedding, 54.8% standard care) and overall the infants would be considered quite stable. Mean 5 minute Apgar was 7.8, mean days receiving mechanical ventilation were 4.3, mean number of confirmed episodes of sepsis was 0.32 and only 2 twins had been diagnosed with a grade 3 or 4 intraventricular haemorrhage in the co-bedding group compared to 8.2, 3.6, 0.92 and 1 in the standard care group. On average, all twins had undergone 98 prior painful procedures (128.5 co-bedding, 91.3 standard care), such as heel lance, venipuncture, intramuscular injection, insertion of intravenous catheter, oral/tracheal suctioning (Appendix L), before the study heel lance, although there was a wide range from as few as 4 to as high as 1152 prior exposures.

Despite the group's relative homogeneity, there were some significant differences and the major outcome was adjusted for these in the analysis (see below). The number of infants less than 32 weeks corrected gestational age at the time of the heel lance was greater in the standard care group ($p=0.04$), 8 versus 2, $p=0.04$; however the overall corrected gestational age at heel lance was only marginally different between the groups, mean age of 34 2/7 weeks in the co-bedding group and 33 6/7 weeks in the standard group, $p=0.06$. Postnatal age at the time of the heel lance and 5 minute Apgar < 7 were also marginally different ($P = 0.06$ and 0.05, respectively). Infants in the co-bedding group were on average 6 days older (18.7 versus 12.6) than the standard care group and 20.8% versus 8.1% of the infants assigned to co-bedding had a 5 minute Apgar less than 7.



* 8 infants in the standard group and 10 infants in the co-bedding group had at least one missing physiological data point over the duration of the heel lance procedure due to movement artefact.

Figure 2: Flow diagram of subject enrolment, treatment allocation, follow-up and inclusion in final analysis

Table 3: - *Comparison of Maternal Characteristics between Co-bedding and Standard Care Groups at Randomization*

Maternal Characteristics	Co-bedding (n=36)	Standard (n=31)	P value
Mean age in years (SD)	31.1 (5.5)	29.6 (6.2)	0.28
Primiparous (%)	19 (52.8)	15 (48.4)	1.00
Monochorionic (%)	13 (36.1)	8 (25.8)	0.60
Caesarean delivery (%)	23 (63.9)	13 (41.9)	0.09
Antenatal steroids (%)	28 (77.8)	21 (67.7)	0.42
Caucasian (%)	36 (100.0)	27(87.1)	0.18
Family arrangement, 2 parent (%)	36 (100.0)	31(100.0)	1.00
Education (some college or university, %)	28 (77.8)	24 (80.0)	1.00
Smoking (%)	0 (0.0)	3 (9.7)	0.09
Infant Characteristics	**Co-bedding (n=72)**	**Standard (n=62)**	**P value**
Mean gestational age in weeks (SD)*	31.6 (2.5)	32.1 (2.7)	0.48
Gestational age < 32 weeks (%)	36 (50.0)	26 (41.9)	0.39
Mean birth weight g (SD)	1719.2(477.9)	1787.9 (508.8)	0.42
Gender, male (%)	42 (58.3)	34 (54.8)	0.73
Mean Apgar at 5 minutes (SD)	7.8 (1.7)	8.2 (1.3)	0.11
Apgar at 5 minutes <7 (%)	15 (20.8)	5 (8.1)	0.05*
Postnatal Age at heel lance in days (SD)	18.7 (20.6)	12.6 (16.1)	0.06
Corrected gestational age at heel lance	34.2 (1.6)	33.6 (2.1)	0.19
Corrected gestational age <32 weeks at heel lance	2 (2.8)	8 (12.9)	0.05*
Mean total painful procedures (SD)	128.5 (226.0)	91.3 (209.4)	0.34
Mean days mechanical ventilation (SD)	4.3(13.6)	3.6(12.0)	0.73
Mean no. confirmed sepsis (SD)	0.32(0.92)	0.24 (0.92)	0.63
Grade 3 or 4 Intraventricular haemorrhage (%)	2(2.7)	1(1.3)	1.0

Denotes *SD-standard deviation

Potential confounders - infant characteristics and factors associated with heel lance

Volume of blood, need for additional Lance and lance order.

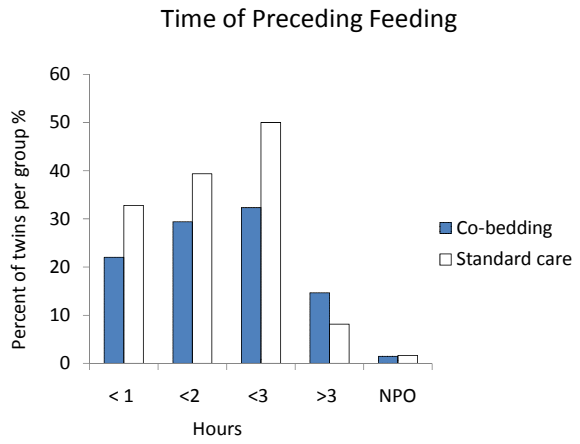
Since the blood collection via heel lance was a medically indicated procedure that was related to individual patient clinical status, amount of blood volume obtained could not be dictated by the study protocol. Therefore, amount of blood volume collected was recorded and a comparison was made between the groups. The average blood collected was not significantly different between the co-bedding or standard care group, 0.63 ml and 0.56 ml respectively, p=0.88.

Five of the infants in the co-bedding required the heel lance to be repeated (7.5%) while none of the twins in the standard care group required the lance to be repeated, which was nominally significant $\chi^2(1)=4.7$, P=0.06.

Order of heel lance was primarily based on clinical implications and data were collected on each infant as blood work was requested. However, if both infants in the twin required blood work then random selection using coin toss was used to decide order. Order in which twins underwent the heel lance (A-B or B-A) was not significantly different. Order A-B occurred in 46.8% of the standard care group and 52.2% in the co-bedding group.

Timing and method of feeding prior to heel lance.

Time of last feeding prior to heel lance was similar between the groups, $\chi^2(4)=2.69$, p=0.61. However, the method of feeding was significantly different, $\chi^2(3)=8.6$, p=. 03. Three-quarters of the co-bedding infants (75%) compared to half (53.5%) of the standard care group received their preceding feeding prior to heel lance via gavage tube. More infants in the standard care group, 17.2% versus 8.6%, nipple-fed their preceding feed and the number of infants breast-feeding was < 5% in both groups (Figure 3).



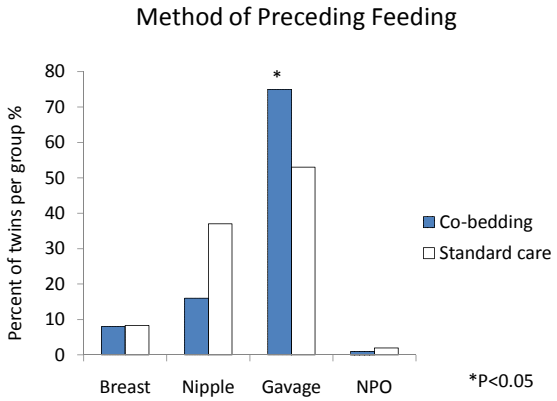


Figure 3: Timing and method of preceding feeding

Timing and type of last painful procedure prior to heel lance and exposure to sucrose within preceding 24 hours.

In addition to cumulative pain exposure, the most recent painful procedure that each infant underwent prior to the heel lance procedure was recorded. Procedures were coded as tissue breaking (e.g. venipuncture, heel lance, intravenous insertion) or non tissue breaking (e.g. NG insertion, tape or dressing removal). Whether the infant received 24% sucrose within 24 hours of the heel lance was also recorded. Although there were no significant differences between the groups regarding overall timing of last painful procedure, $\chi^2(5) = 7.76$, P=0.17, 51% of infants in the standard care group and 30.8 % of the co-bedding infants had undergone a prior painful procedure within 24 hours of the heel lance being studied (Figure 4). The infants in the standard care group were significantly more likely to have had a tissue breaking rather than a non-tissue breaking procedure, 78.7% versus 60 %, $\chi^2(1) = 5.09$, P=0.04 (Figure 5); and to have received at least one dose of sucrose (34.4% versus 12.1%) compared to the co-bedding infants in the preceding 24 hours prior to heel lance $\chi^2(1) = 5.95$, P=0.003 (Figure 6).

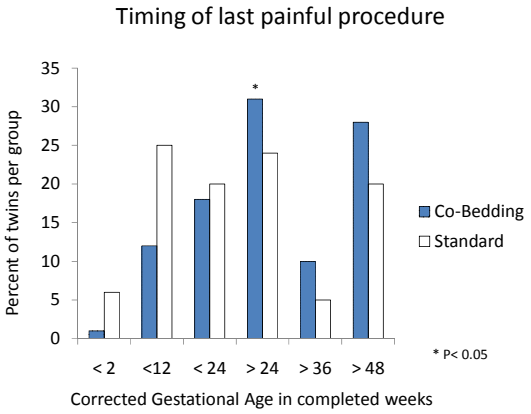


Figure 4: Timing of preceding painful procedure

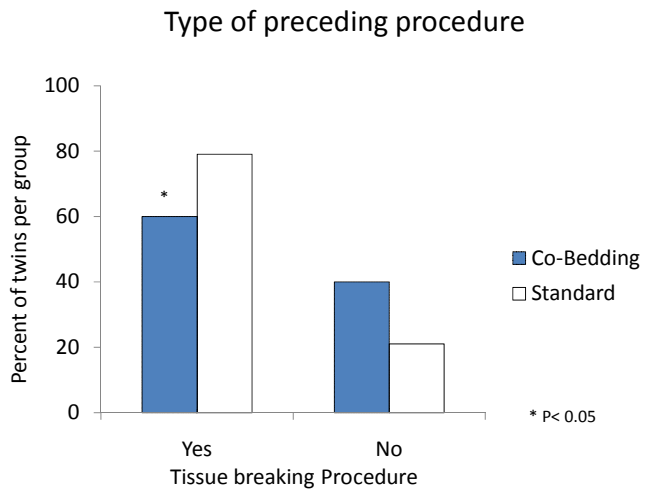


Figure 5: Type of preceding procedure

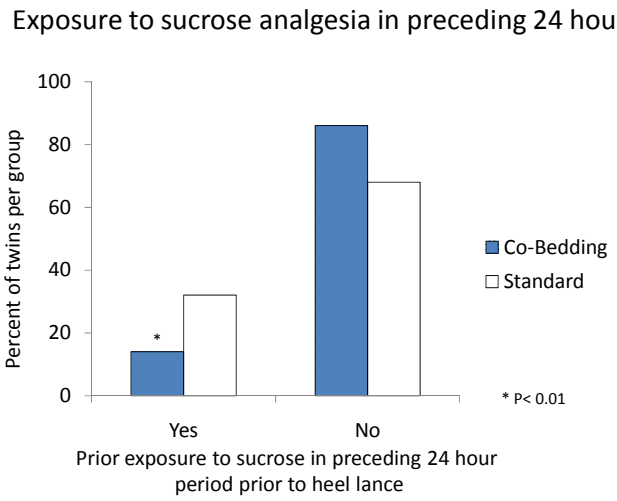


Figure 6: Comparison of prior exposure to 24% sucrose in preceding 24 hours

Additional non-pharmacologic strategies

Additional non-pharmacologic strategies, specifically non nutritive sucking, swaddling and facilitated tucking were not prohibited during the heel lance procedure and could be provided by the staff caring for the twins proactively in preparation of the heel lance and/or reactively based on infant response and their clinical judgment. Each strategy was recorded during the procedure and verified by video recordings. Non nutritive sucking (pacifier) was offered to all infants following sucrose administration as part of the sucrose intervention, but recorded as a strategy used only if it initiated a sucking response by the twin. Swaddling was considered any form of bundling or wrapping with a blanket while facilitated tucking was any form of containment that was provided by a care provider or parent by using their hands. Comparisons were made regarding any form of additional strategy and for each specific strategy using the Pearson Chi-Square test (or Fisher Exact test for small counts of less than 5 per cells when necessary) and significant group differences were noted. Mean differences were compared using independent t tests, a P value of <0.05 and 95% confidence intervals. Ninety-five percent of the infants in the standard care group received at least one additional non- pharmacologic strategy, and just over half (58.2%) of the co-bedding group received such a treatment, χ^2 (1) =23.65, P<.001. Similar findings were reported for the co-twin observing the heel lance, 86.5% of the infants in the standard condition received at least one additional non-pharmacologic strategy compared to only 27.7% of the co-bedding observer infants, χ^2 (1) =44.8, P<0.001. The mean total of additional non-pharmacologic strategies for twins undergoing heel lance was 1.88 in the standard care group and 0.74 in the co-bedding group, P<0.001 and for the observer twins the same means were 1.38 compared to 0.33, respectively (Table 4).

Table 4: *Comparison of total additional non-pharmacologic strategies between groups*

Factor	Group assignment	N	Mean	Std. Deviation	P value	Mean Difference	95% CI Lower	Upper
Total NonPhar Heel Lance Twin	standard care	61	1.88	.73	<0.001			
	co-bedding	68	0.74	.66	<0.001			
						1.15	.907	1.39
Total Non Phar Observer twin	standard care	61	1.38	.78	<0.001			
	co-bedding	66	0.33	.48	<0.001			
						1.04	.819	1.27

Infants in the standard care group were significantly more likely to initiate non nutritive sucking at baseline and during the heel lance, 86.9% versus 46.3% compared to the co-bedding infants, χ^2 (1) =44.8, P<0.001. This was also the case for swaddling, 65.6% versus 16.4%, χ^2 (1) =32.2, P<.001; and facilitated tucking 34.4% versus 4.5%, χ^2 (1) =18.8, P<0.001(Figure 7).

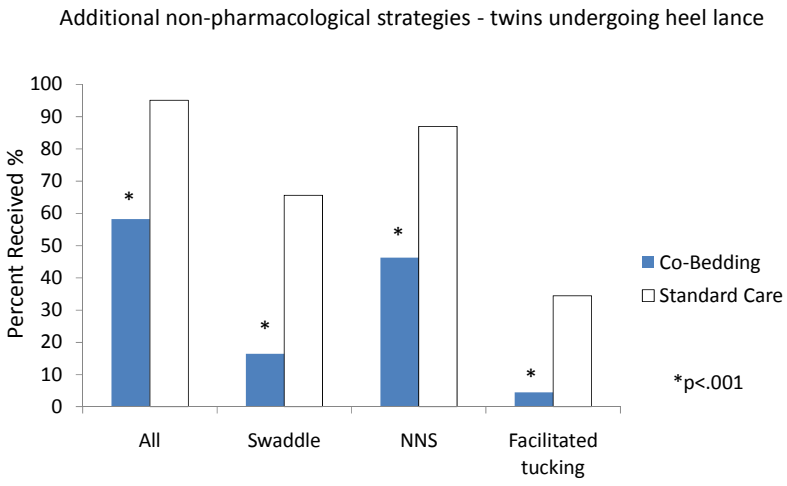


Figure 7: Additional Non-pharmacologic Strategies -Twin undergoing heel lance

Co-twin observers in standard care had a higher incidence of non nutritive sucking compared to co-bedding observers (80.3% vs.19.7%, χ^2 (1) = 48.2, P<0.001.); swaddling (41.0% vs. 7.7%, χ^2 (1) =19.2, P<0.001.); and facilitated tucking 19.7 vs.3.1%, χ^2 (1) =8.8, P<0.004) (Figure 8).

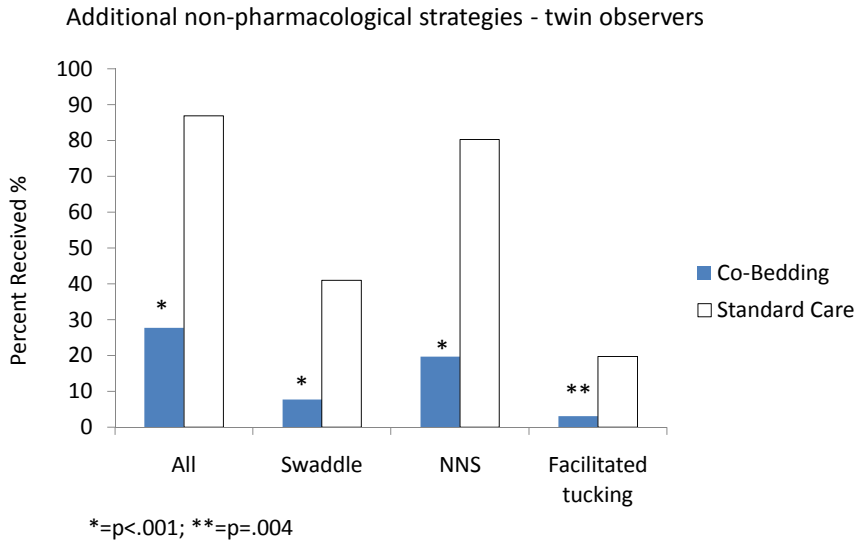


Figure 8: Additional Non-pharmacologic Strategies – Twin Observer

Summary

Co-bedding infants were more likely to have received their prior feed by gavage instead of nipple feeding. It was less likely that the co-bedding twin's prior painful procedure was tissue breaking and they were less likely to have received a dose of sucrose in the preceding 24 hours before heel lance and marginally more likely to need a repeated lance during procedure. Additionally, infants in the co-bedding condition were significantly less likely to receive additional non-pharmacologic strategies during the heel lance procedure.

Adjustment for group differences

Before conducting the hypothesis testing, group differences in baseline characteristics and potential confounders associated with the heel lance were examined in relationship to hypothesized outcomes (PIPP, time to recovery, salivary cortisol, HRV) using either bivariate correlations for continuous variables (postnatal days since birth at heel lance) or one-way analysis of variance (ANOVA) for dichotomous (yes/no) variables: 5 minute Apgar < 7; corrected gestational age <32 weeks at heel lance; gavage method as last feeding prior to heel lance; sucrose analgesic dose in preceding 24 hours; most recent painful procedure preceding lance was tissue breaking; need for additional heel lance or use of non-pharmacologic strategies.

Although co-bedding twins were almost 3 times less likely to have received 24% sucrose in the 24 hours prior to the heel lance, neither prior sucrose or prior type of procedure were associated with any of the outcomes. To preserve parsimony, these variables were excluded from further analyses and only variables significantly associated with each outcome were included (see below).

Corrected gestational age less than 32 weeks was associated with all outcomes and was adjusted for in all analyses. Postnatal age in days was linked with PIPP scores and the individual components of the PIPP and heart rate variability. Apgar less than 7 at 5 minutes was related to

pre and post cortisol and heart rate variability and were included in the analysis of these variables. Method of preceding feeding was associated only with heart rate (average, maximum). Need for additional heel lance was linked with PIPP score at 30 seconds and use of additional non-pharmacologic strategies was associated with PIPP score. Since it is difficult to discern if need for additional heel lance and use of additional non-pharmacologic strategies were in fact related to the intervention rather than baseline group differences, these variable were not included in adjusted values. They were, however, added later into the model to understand their association with all outcomes.

To control for the potential non-independence between twins and to account for significantly associated group differences, regression analysis using generalized estimating equation (GEE) was conducted for all major outcomes.

Primary hypothesis

Premature infant pain profile (PIPP) scores.

The primary outcome of pain response following heel lance was compared using Premature infant pain profile (PIPP) scores. The overall PIPP scores were obtained by adding the scores of its seven components (described previously). It was hypothesized that the PIPP scores would be lower across the heel lance procedure in the co-bedding group. Data were collected on all infants up to and including 240 seconds following the initiation of the heel lance; however, for the majority of the infants, the procedure lasted 120 seconds or less. Thus, mean PIPP scores were compared and reported across the procedure in 30 second epochs for the first 2 minutes of the procedure contrasting the mean difference between groups using the t test, 95 percent confidence intervals and a P value. In both the groups, peak mean scores were as expected, highest in the first minute following the heel stick and decreased over time. The mean PIPP

scores were similar at 30 seconds, 7.1 (SD 2.8) in the co-bedding group and 7.2 (SD 3.4) in the standard care group, [95% CI ¯1.13 to 1.26], P=0.91. Nor were they significantly different one minute following the heel lance, 6.1 (SD 3.0) versus 5.2 (SD 2.0) in the co-bedding compared to the standard care group, [95% CI ¯1.86 to 0.04], P= 0.06. There was however, a significant one point higher mean score in the co-bedding group, 6.0 (SD 3.0) compared to the standard care group, 5.0 (SD 1.8), [95% CI ¯1.99 to ¯0.02], P=0.04, at 90 seconds post lance (Figure 9). The remaining PIPP scores were not significantly different.

Generalized estimating equation modeling adjustment for the potential non-independence of the twins, corrected gestational age <32 weeks and postnatal age at heel lance, did not alter our findings and resulted in a P value of 0.03 for mean PIPP score at 90 seconds post heel lance. Being <32 weeks corrected gestation at the time of the heel lance was associated with higher PIPP score at 30 seconds (Mean difference 1.97, P=0.02). Further analysis including need for repeated lance during procedure and use of additional non-pharmacologic strategies did not change our findings related to group differences at 90 seconds, P=0.02. Need for additional lance was associated with lower PIPP score at 30 seconds (Mean difference -1.98, P<0.001), 60 seconds (Mean difference -1.79, P=0. 01), 90 seconds (Mean difference -2.94, P<0.001) and 120 seconds (Mean difference -1.17, P<0.001). Use of additional pain relieving strategies were associated with higher PIPP score at 30 seconds (Mean difference 1.94, p<0.001), 60 seconds (mean difference 1.70, p=0.01) post lance but not after one minute (Figure 9).

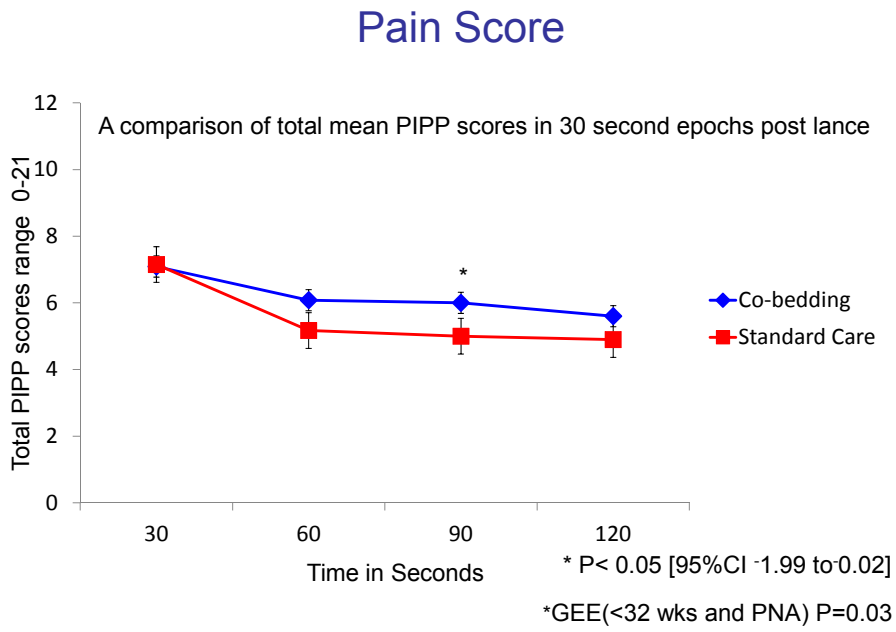


Figure 9: Comparison of mean PIPP scores at 30, 60, 90, and 120 seconds post lance between groups.

Individual components of the PIPP.

In this section, the individual components of the PIPP score (neurobehavioural state, gestational age, HR, O2 saturation and facial action) were contrasted between groups at baseline and where applicable mean change from baseline to each of the phases throughout the heel lance were compared using independent t test, 95% confidence intervals and P value of 0.05.

Neurobehavioural state.

Neurobehavioural state was recorded at baseline prior to undergoing the heel lance for all infants and a comparison was made between the groups. Four neurobehavioural states are considered in the PIPP scoring tool: active/awake; quiet/awake; active/asleep; and quiet/asleep. No differences were seen between the groups, Pearson Chi-square P=0.84. Since differences in either sleep or wake state were relevant to the findings, the categories were dichotomized to either a sleep or an awake condition at baseline prior to the heel lance. The groups did not differ

significantly, with the majority of the infants in a sleep condition, 83.8% of the co-bedding and 82% of the standard care group, Fisher Exact test P =0.82.

Gestational age at heel lance.

Corrected gestational age of the twin was recorded prior to undergoing the heel lance and a comparison was made between the groups. Mean gestational age as reported earlier using the t test was 34.2 (SD 1.6) weeks in the co-bedding group and 33.6 (SD 2.1) in the standard care group, F (127) 1.92, P=0.06. Four categories of age range are considered in the PIPP scoring tool from a 0 score to 3 with decreasing gestational age: $\geq$ 36 weeks; 32-35 6/7 weeks; 28-32 weeks and < 28 weeks. Categorical data were analyzed using Pearson Chi-square. Consistent with the above findings, marginally significant differences were found between the groups, χ^2 (2) =0.84, P=0.06. The majority of the infants were in the 32-35 6/7 weeks age range (PIPP score of 1), 83.8% and 73.8% in the co-bedding versus standard care group. The primary variation occurred in category 3 where more infants (12.9% vs. 2.8%) in the standard care group were less than 32 weeks (Figure 10). No twins were less than 28 weeks corrected gestational age at the time of heel lance.

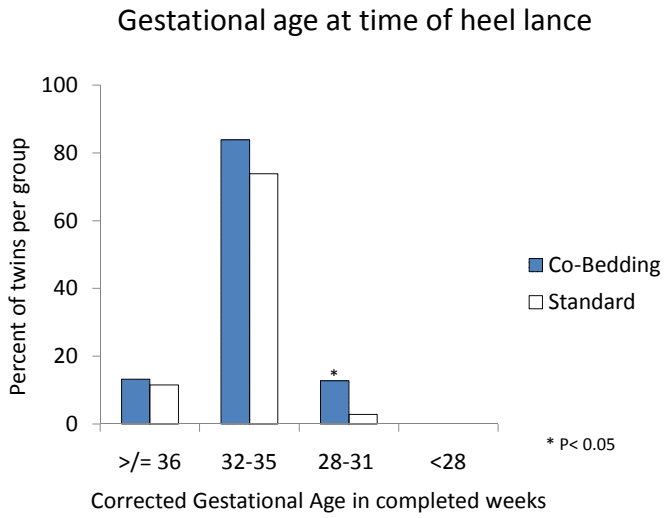


Figure 10: Corrected gestational age at time of heel lance

Physiologic parameters.

Heart rate and Oxygen Saturation at baseline.

Maximum and average heart rate and minimum and average SpO2 values prior to heel lance were compared between groups using t tests, 95% confidence intervals and a P value. Significant differences were found for the 30 second baseline epoch immediately prior to the initiation of the heel lance procedure for both the maximum and average heart rate, with higher values found in co-bedding group compared to the standard care group, 172 (SD 16.2) versus 164 (SD16.1) beats per minute (bpm), mean difference -8.75 [95% CI ¯14.1 to ¯3.4], P<0.001; and, 160 versus 151bpm, mean difference -8.31 [95% CI-13.9 to 2.7], P=0.005,respectively (Table 5).

Table 5: *Comparison of Maximum and Average Heart Rate at Baseline*

		N	Mean	Std. Dev.	P value	Mean Diff	Std. Error Diff	95% Confidence Interval Lower	Upper
Maximum	Standard	59	163.8	16.2					
	Co-bedding	67	172.6	14.0					
					0.001	-8.6	2.7	-14.1	-3.4
Average	Standard	59	151.5	18.1					
	Co-bedding	67	159.8	13.6					
					0.005	-8.3	2.9	-14.0	-2.6

There were no significant differences with respect to oxygen saturation at baseline, mean minimum of 91.6 versus 92.6, mean difference 0.98 [95% CI ¯1.0 to 3.0), P=0.34 and mean average of 94.1 versus 94.9, mean difference 0.76, [95% CI ¯0.8 to 2.3], P=0.328 in the co-bedding compared to standard care group (Table 6).

Table 6: *Comparison of Minimum and Average Oxygen Saturation at Baseline*

		N	Mean	Std. Dev.	P value	Mean Diff	95% Confidence Interval Lower	Upper
Minimum	Standard	58	92.6	6.4				
	Co-bedding	68	91.6	5.1				
					0.34	.98	-1.02	3.00
Average	Standard	58	94.9	4.6				
	Co-bedding	66	94.1	3.9				
					0.33	.76	-.77	2.28

Average and Maximum Heart Rate across phases of the heel lance procedure.

To test if there was an effect of the intervention on heart rate and oxygen saturation, mean differences in heart rate and oxygen saturation levels from baseline were calculated and compared between groups using a t test, 95% confidence intervals and a P value for each phase of the heel lance procedure, specifically from heel warming and every 30 seconds thereafter until 120 seconds post lance. Both average and maximum heart rate and average and minimum oxygen saturation were analyzed in this fashion.

The rise in average heart rate from baseline was lower in the co-bedding group compared to the standard care group at all phases of the heel lance (Figure 11). However, this rise in average heart rate was statistically significant between the groups during the warm phase only, mean increase of 3.54 bpm (8.9) vs. 8.35 bpm (SD 13.4), P=0.02 [95% CI 0.73-8.89] respectively (Table 7).

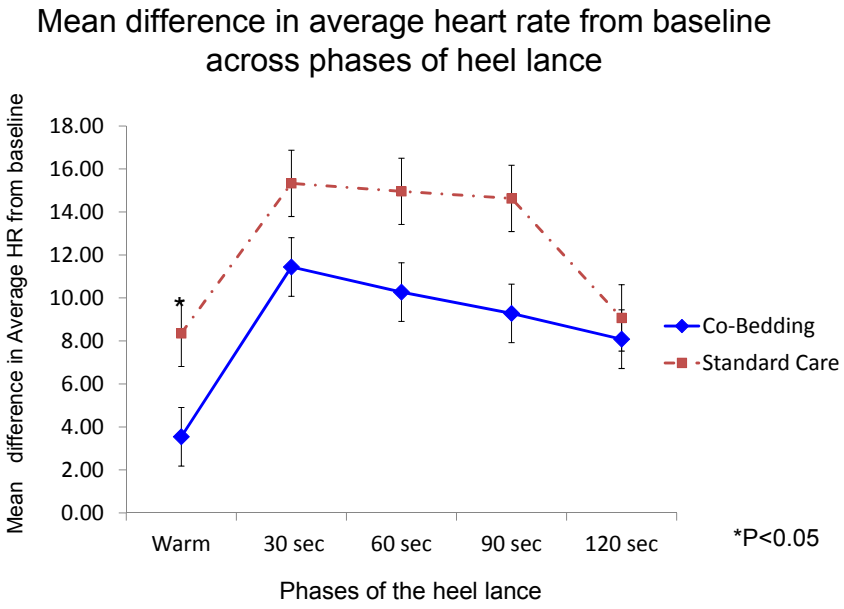


Figure 11: Mean difference in average heart rate from baseline across phases of heel lance

Table 7: *Comparison of mean difference in average heart rate from baseline*

Mean difference from baseline		N	Mean	Std. Dev.	P value	Mean Diff	95% Confidence Interval of the Difference	
Warm	Standard	59	8.4	13.4				
	Co-bedding	66	3.5	8.9			Lower	Upper
					0.02	4.8	0.7	8.9
30 Seconds	Standard	59	15.3	15.1				
	Co-bedding	67	11.4	12.9				
					0.12	3.9	-1.1	8.8
60 Seconds	Standard	59	14.1	15.0				
	Co-bedding	66	10.3	13.8				
					0.14	3.8	-1.3	8.9
90 Seconds	Standard	58	11.1	14.6				
	Co-bedding	65	9.3	14.3				
					0.49	1.8	-3.4	7.0
120 Seconds	Standard	52	9.1	14.0				
	Co-bedding	58	8.1	14.4				
					0.72	1.0	-4.0	6.4

Similarly, although not statistically significant, the changes in maximum heart rate from baseline for all phases of the heel lance were lower in the co-bedding twins. The largest magnitude of

change in maximum heart rate occurred during the heel warming for both groups, mean increase of 10.57 (SD 16.57) during co-bedding and 12.03 (SD 16.75) in standard care (Figure 12).

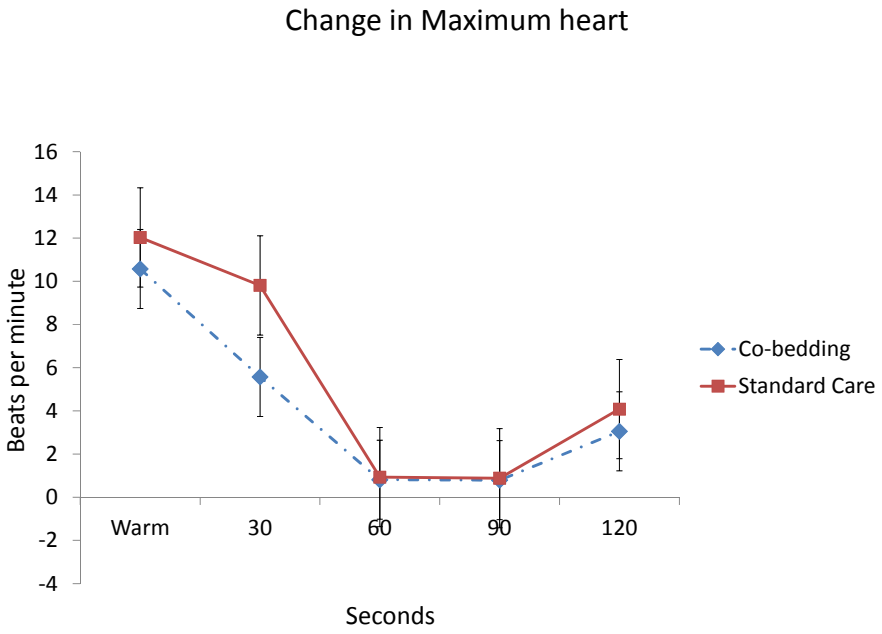


Figure 12: Mean difference in maximum heart rate from baseline

Average and Minimum Oxygen Saturation levels across phases of the heel lance procedure.

There were no significant differences noted between groups for the change from baseline to any of the phases of the heel lance procedure for average or minimum oxygen saturation. Mean average oxygen saturation was stable, with a range from 92-95% with little deviation among groups during heel lance (Figure 13).

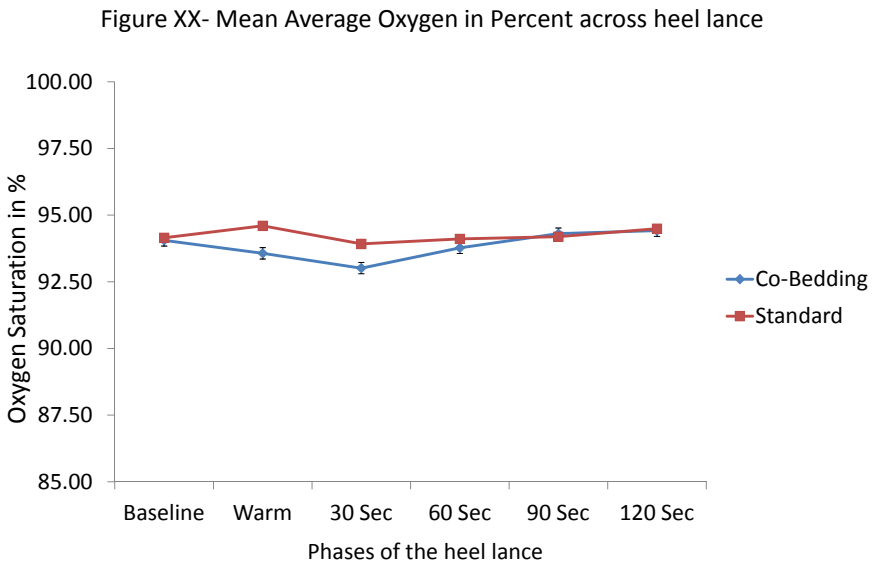


Figure 13: Mean Average Oxygen in Percent across heel lance

Facial Actions.

In this section, individual facial actions specifically brow bulge, eye squeeze or nasolabial furrow, all highly correlated, (Pearson correlation coefficient > 0.94), are presented as a combined score and compared between the groups at baseline and as percent change from baseline across the phases of the heel lance procedure. Individual facial actions are expressed as the mean percentage of time (0-100%) that the infants' displayed the specific facial action coded every second and averaged over 30 second epochs (Individual facial scores can be seen in (Appendix M). Hence, the combined additive scores of the three facial actions are a value ranging from 0-300%. Independent student t test using a P value cut off of 0.05 and 95% confidence intervals were used to assess group differences.

Baseline Total Facial Actions.

Although scores were slightly higher in the co-bedding group, no significant differences were noted at baseline for the combined three facial actions (possible display of 0-300%) between groups for twins undergoing heel lance. Mean percent time displaying facial response was low for groups, 22.33% in the co-bedding and 11.24% in standard care (Table 8). Following the heel lance, Tte highest magnitude of the total display of the three facial actions (averaged over 30 second epochs) was similar between groups and occurred at 30 seconds post lance, 45.8 (SD 79.0) in co-bedding and 43.9 (SD 84.5) in the standard care condition, P=0.90. At 60, 90 and 120 seconds post lance, although co-bedding twins showed greater change in facial display higher although no statistical significance was reached (Figure 14).

Table 8: *Comparison of combined facial action at baseline between groups*

		N	Mean	Std. Dev.	P value	Mean Diff	95% Confidence Interval of the Difference	
Total Facial Action	Standard	60	11.2	31.1				
	Co-bedding	68	22.3	43.5			Lower	Upper
					0.104	-11.1	-24.5	2.3

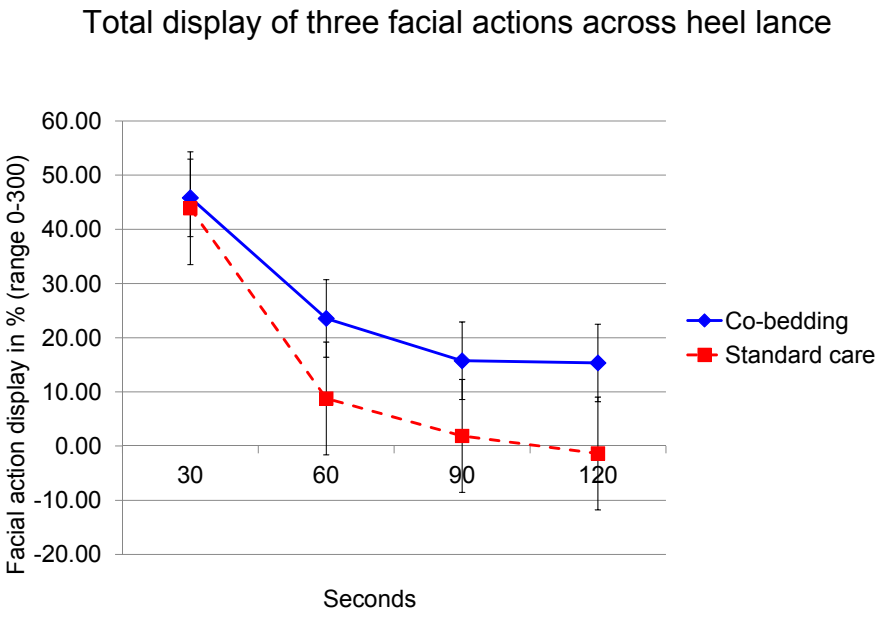


Figure 14: Total display of combined three facial actions across heel lance

Secondary Hypotheses

Time to recovery.

Recovery time, defined as the period of time measured in seconds, elapsed following the application of the adhesive bandage signifying the end of blood sampling until the heart rate and oxygen saturation return to an average baseline condition for a minimum of 5-7 beats, was over a minute shorter, M=75.6 seconds (SD, 70.0), in the co-bedding condition compared to standard care M=142.08 seconds, (SD 138.1), t(86.0)=3.30, $P = 0.001$, mean difference of 64.5 [95% CI. 25.6-103.3] (Table 9).

Table 9: *Comparison of mean time to recovery between groups*

	N	Mean	Std. Dev	P value	Mean Diff	95% Confidence Interval of the Difference	
Standard care	61	142.1	138.1				
Co-bedding	68	75.6	68.8			Lower	Upper
				.001	64.5	25.6	103.4

Adjustment using GEE for potential non-independence among twins and corrected gestational age less than 32 weeks at heel lance did not change these findings and resulted in a P value of 0.005 indicating faster recovery following heel lance in the co-bedding group compared with the standard care group. Further adjustment for use of additional non-pharmacologic strategies yielded a P value of 0.01 for the same difference.

Hormonal response – salivary cortisol

Baseline (pre) salivary cortisol levels obtained just prior to the heel lance procedure were not significantly different between the 2 groups, 0.36 ug/dl, if assigned to receive co-bedding, and 0.43 ug/dl, if assigned to standard care (Table 10). However, cortisol levels obtained 20 minutes post heel lance were significantly lower in the co-bedding group compared to the standard care group, mean levels 0.28 ug/dl (SD 0.25) versus 0.50 ug/dl (SD 0.73) (Table 11).

Table 10: *Comparison of Pre Salivary Cortisol levels between the groups*

	N	Mean	Std. Deviation	P value	Mean Diff	95% Confidence Interval of the Difference	
Standard care	40	0.43	0.50				
Co-bedding	49	0.36	0.25			Lower	Upper
				0.39	.07	-0.09	0.23

Table 11: *Comparison of Post Salivary Cortisol levels between the groups*

	N	Mean	Std. Deviation	P value	Mean Diff	95% Confidence Interval of the Difference	
Standard care	55	0.50	0.73				
Co-bedding	58	0.28	0.25			Lower	Upper
				0.04	0.22	0.01	0.43

Generalized estimating equation adjustment for potential non-independence between twins, corrected gestational age <32 weeks at heel lance, and 5 minute Apgar <7 did not change this finding and resulted in a P value of 0.03 for the difference in post-cortisol 20 minutes following heel lance for twins who were co-bedding compared to twins receiving standard care. Further adjustment for use of additional non-pharmacologic strategies yielded a P value of 0.10 for the same difference.

Change in salivary cortisol from baseline pre to post levels were also calculated and compared between the groups. Mean difference was less in the co-bedding group, -0.06 compared to the standard care group, 0.14, P=0.05 (Table 12).

Table 12: *Comparison of the change in salivary cortisol 20 minutes post heel lance from baseline between groups*

	N	Mean	Std. Deviation	P value	Mean Diff	95% Confidence Interval of the Difference	
Standard care	37	0.14	0.57				
Co-bedding	45	-0.06	0.23			Lower	Upper
				0.05	0.20	0.01	0. 40

Adjustment using Generalized Estimating Equation (GEE) for corrected gestational less than 32 weeks at heel lance and 5 minute Apgar less than 7, indicated that the change in post cortsiol from baseline remained significantly different between the groups, P=0.03. Further adjustment of use of additional non-pharmacologic strategies resulted in a marginally significant P value of 0.05 for the same change.

Heart rate variability.

Initial analysis comparing heart rate variability consisting of mean low frequency (LF), high frequency (HF) and low to high frequency ratio (LF/HF) at baseline and throughout the heel lance procedure using independent t tests and 95% confidence intervals did not show any significant differences based on group assignment (Table 13).

Table 13: *Comparison of mean low frequency (LF), high frequency (HF) and low to high frequency ratio (LF/HF) at baseline and throughout heel lance between groups*

Measured in milliseconds squared (ms^2)	Group	N	Mean	Std. Dev.	P value	Mean Diff	95% C I of the Difference Lower	Upper
Base LowF	standard	56	183.6	198.8				
	co-bedding	62	186.6	277.3	0.95	-3.0	-91.8	85.9
Base HighF	standard	56	44.7	50.2				
	co-bedding	62	41.5	79.4	0.80	3.2	-21.3	27.7
Base Ratio	standard	56	5.4	3.3				
	co-bedding	62	6.7	5.0	0.11	-1.3	-2.8	0.3
Lance LowF	standard	59	132.8	223.0				
	co-bedding	64	124.2	226.0	0.83	8.7	-21.3	27.7
Lance HighF	standard	57	26.2	48.8				
	co-bedding	61	23.8	34.9	0.75	2.4	-13.0	17.8
Lance Ratio	standard	59	8.3	7.3				
	co-bedding	64	9.8	13.1	0.46	-1.4	-5.2	2.3

The change in HRV indices (LF, HF, LF/F) from baseline to heel lance were calculated and group differences were analyzed by comparing mean differences using t test, 95% confidence interval and P value of 0.05 and adjustment for possible non-independence of the twins, baseline group differences and confounders were completed using GEE.

Low frequency.

Low frequency (LF) heart rate variability was lower during heel lance when compared to baseline in both groups with no significant differences reported, mean decrease of 65.8 ms^2 during co-bedding and 55.3 ms^2 for twins receiving standard care, P=0.86 (Table 14). GEE adjustment for potential non-independence between twins, postnatal age in days and corrected

gestational age <32 weeks at heel lance, preceding gavage feeding, and 5 minute Apgar <7 did not change this finding and resulted in a P value of 0.68 for the change in LF at heel lance from baseline for twins who were co-bedding compared with twins receiving standard care. Further adjustment for the use of additional use of non-pharmacologic strategies (yes/no) in the model did not change the results, P=1.00. Older postnatal age in days was marginally associated with a larger mean decrease in LF, P=0.06.

High Frequency.

High Frequency (HF) variability also decreased during the heel lance from baseline and this change was similar in both the co-bedding and standard care group, mean decrease of 19.8 ms^2 (SD 82.43) and 23.58 ms^2 (SD 52.41) respectively, P=0.78. Adjusting for potential non-independence between twins, postnatal age in days and corrected gestational age <32 weeks at heel lance, preceding gavage feeding, and 5 minute Apgar <7 using GEE analysis did not change this finding and resulted in a P value of 0.07 for the change in HF at heel lance from baseline for twins who were co-bedding compared with twins receiving standard care. Several of the covariates in the GEE model were significantly associated with the change in HF. Twins who were nipple fed rather than gavage fed prior to the heel lance (P=0.03); twins who at the time of the heel lance were less than 32 weeks corrected gestational age (P=0.01); and, twins who had a higher postnatal age in days since birth (P=0.03) were more likely to have a greater decrease in HF at heel lance. Inclusion of the additional use of non-pharmacologic strategies in the model did not alter our non-significant findings between groups for change in LF at heel lance from baseline, P=0.10.

LF/HF Ratio.

The change in the LF/HF ratio from baseline at heel lance was not different between the 2 groups (mean 3.2,SD 7.9) versus 3.1, SD 13.4). GEE adjustment for potential non-independence

between twins, postnatal age in days and corrected gestational age <32 weeks at heel lance, preceding gavage feeding, and 5 minute Apgar <7 did not alter this result and yielded a P value of 0.92 for the change in LF/HF Ratio at heel lance from baseline for twins who were co-bedding compared with twins receiving standard care. The use of additional non-pharmacologic strategies when added to the model did not change our results, P=0.81.

Table 14: *Comparison of the mean difference in the change in low frequency (LF), high frequency (HF) and low to high frequency ratio (LF/HF) from baseline to heel lance*

Change from Baseline (ms^2)	Group	N	Mean	Std. Dev.	P value	Mean Diff	95% C I of the Difference Lower	Upper
Lance LowF	standard	55	-55.3	278.8				
	co-bedding	61	-65.7	340.1	0.86	10.5	-104.6	125.7
Lance HighF	Standard	53	-23.6	52.4				
	co-bedding	58	-19.8	82.4	0.77	-3.8	-29.6	22.0
Lance Ratio	standard	55	3.2	7.9				
	co-bedding	61	3.1	13.4	0.98	0.06	-4.0	4.2

Frequency of sucrose doses.

There were no significant differences between the need for additional doses of 24% sucrose between the groups. The majority of twins received only the initial dose of 24% sucrose two minutes prior to the heel lance, 58 (85.3%) infants in the co-bedding group and 51 (83.6%) of the standard care infants (Figure 15). Ten infants in each group, 14.7% of the co-bedding group and 16.4% of the standard care group received up to three additional sucrose doses. Five of the 10 twins received additional sucrose preemptively because they required a second heel lance to complete the blood collection.

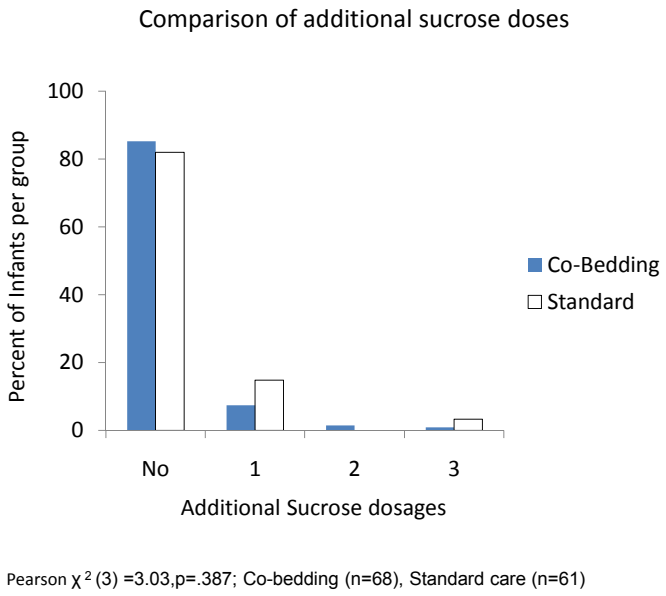


Figure 15: Comparison of additional sucrose doses

Adverse events.

Despite a lack of empirical evidence, theoretical risks associated with co-bedding during hospitalization include temperature instability especially in the smaller twin, physiological instability and sleep disruption leading to respiratory compromise and higher likelihood of apnea and bradycardia, cross contamination resulting in higher infection, and misidentification (DellaPorta et al., 1998; Gannon, 1999). No significant concerns arose in our study in either condition regarding temperature instability. Infants were observed during the heel lance for the incidence of apnea, bradycardia, or an increased need for supplemental oxygen. A total of six infants, three from each group experienced an episode of bradycardia. All events occurred following administration of oral sucrose. The infants recovered quickly without any long lasting effects. Five of the twins in the standard care group (8%) and 10 (13.9%) of the twins in the co-

bedding group had an episode of confirmed sepsis prior to participation in the study. Following randomization, there were fewer episodes of confirmed sepsis and no significant differences between the groups (4 infants with confirmed sepsis in the standard care (6.5%) and 3 (4%) in the co-bedding). One set of twins in the co-bedding group had a caregiver error related to mislabelling of blood work. The error was recognized immediately by the nursing staff, corrected without clinical consequence and did not exceed the normally expected incidence of mislabelling that is reported in non co-bedding twins.

Five infants in the co-bedding group compared to no infants in the standard care group required an additional heel lance to complete the blood collection, a difference of borderline significance, (P=0.06).

Response of co-twin.

In this section, the effect of co-bedding on the twin not receiving the heel lance (considered the observer co-twin) are presented systematically in a similar fashion to the findings previously reported for the twins undergoing the heel lance. To determine if there was any effect of co-bedding on the observer co-twin, mean PIPP scores were compared and reported across the procedure in 30 second epochs for the first 2 minutes of the co-twin's procedure contrasting the mean difference between groups using standardized t test, 95 percent confidence intervals and a P value. In addition, to determine if there is an interaction effect of co-bedding within pairs of twins, means for PIPP scores were compared between groups for all intervention and then all observer babies. Also the difference between the intervention and observation twin within each twin pair was computed and the groups compared on those differences. Lastly, observer twin response and twin interaction for the secondary outcomes, specifically time to recovery, salivary cortisol response, and heart rate variability were analyzed and are presented in a similar fashion.

PIPP Scores.

Mean PIPP scores across the first 2 minutes of the heel lance for the observer twins were lower than for twins undergoing a heel lance and not significantly different between the groups, mean 4.76 [95% CI 4.19 to 5.32] for the standard group and 5.08 (95% CI 4.55 to 5.61), F(1,90)=0.68, P=0.41 (Figure 16). Further analysis comparing the mean difference in PIPP scores or interaction within the twin sets showed no significant difference between the groups (Table 15).

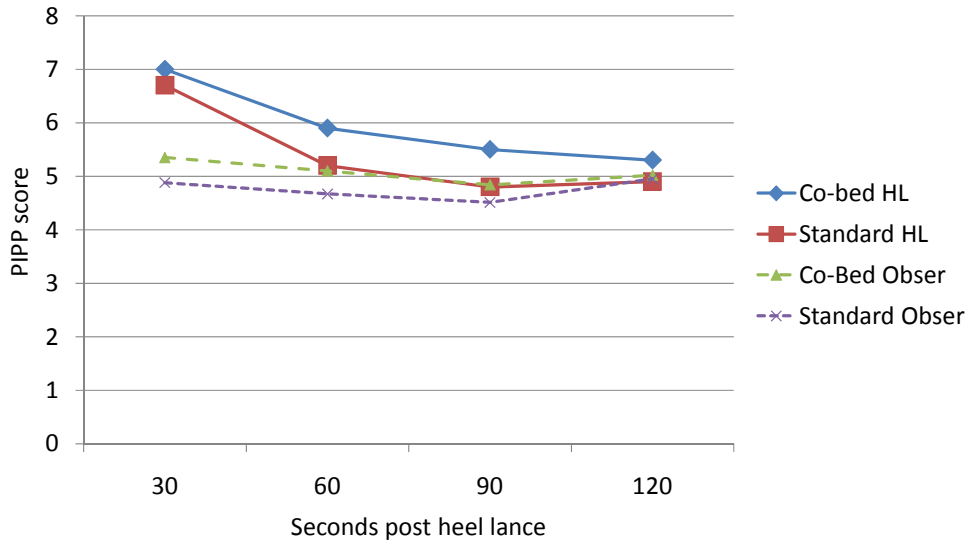


Figure 16: Comparison of mean PIPP scores at 30, 60, 90, and 120 seconds post lance for twin undergoing heel lance (HL) and co-twin observer (Observer)

Table 15: *Mean difference in PIPP scores between twin undergoing heel lance and co-twin observer.*

Difference in simultaneous PIPP score between twin having heel lance and observer twin	Group assignment	N	Mean	Std. Dev.	P Value	Mean Difference
30 seconds post lance	standard care	51	-2.6	4.2	0.20	-0.95
	co-bedding	56	-1.6	3.5		
60 seconds post lance	standard care	49	-0.6	2.9	0.47	0.52
	co-bedding	57	-1.1	4.3		
90 seconds post lance	standard care	44	-0.4	2.7	0.28	0.72
	co-bedding	54	-1.13	3.6		
120 seconds post lance	standard care	39	0.1	3.4	0.51	0.54
	co-bedding	44	-0.4	3.9		

There were no significant group differences in the change in maximum heart rate from baseline for any of the phases of the heel lance. The largest magnitude of change in maximum heart rate occurred during warm for both groups, mean increase of 8.2 (SD 16.1) during co-bedding and 5.0 (SD 14.7) during standard care.

No significant group differences were noted between groups for change from baseline for average heart rate or average oxygen saturation throughout heel lance. However, while oxygen saturation were 1.4 % lower from baseline in the co-bedding twins at 90 seconds post lance, they rose by 0.75% in the standard care group, P= 0.03 [95% CI -4.02 to 0.24].

Facial Actions of Observer twin.

There were no significant differences in combined facial response (percent time displaying eye squeeze, brow bulge and nasolabial furrow) between the 2 groups. For both groups, the highest percent time displaying total facial action for the observer twins was during

the 30 second epoch post heel lance, mean percent of 27.6% (SD 56.8) in the co-bedding group and 20.4% (SD 43.0) in the standard care group, mean difference of -7.2%, [95% CI -25.06 to 10.75].

Time to Recovery for observer twin.

The time to return to baseline for the twins not receiving the heel lance (observer twin) was not significantly different between the two groups, mean of 52.3 (SD 73.5) seconds in the co-bedding group and 62.8 (SD 42.4) seconds in the standard care group (Table 16). However, the mean difference in recovery time between the twin undergoing heel lance and being the observer was significantly different within the twin sets between groups, mean difference -25.3 seconds compared with -79.3 seconds, P=0.01 (Table 17). Figure 17 depicts the effect of role (twin undergoing heel lance compared to twin being an observer to the heel lance) on time to recover.

Table 16: *Comparison of mean time to recovery for observer twin*

	N	Mean	Std. Deviation	P value	Mean Diff	95% Confidence Interval of the Difference	
Standard care	61	62.8	73.5				
Co-bedding	68	52.3	42.4			Lower	Upper
				.328	10.54	-10.8	31.8

Table 17: *Comparison of mean difference in recovery time between twin undergoing heel lance and observer co-twin.*

	Group assignment	N	Mean	Std. Dev.	P value	Mean Diff	95% Confidence Interval of the Diff Lower	Upper
Difference in recovery time between twin undergoing heel lance and co-twin observer	Standard care	61	-79.2	147.0	0.007			
	Co-bedding	68	-25.3	63.8	0.01	-54.0	-94.44	-13.46

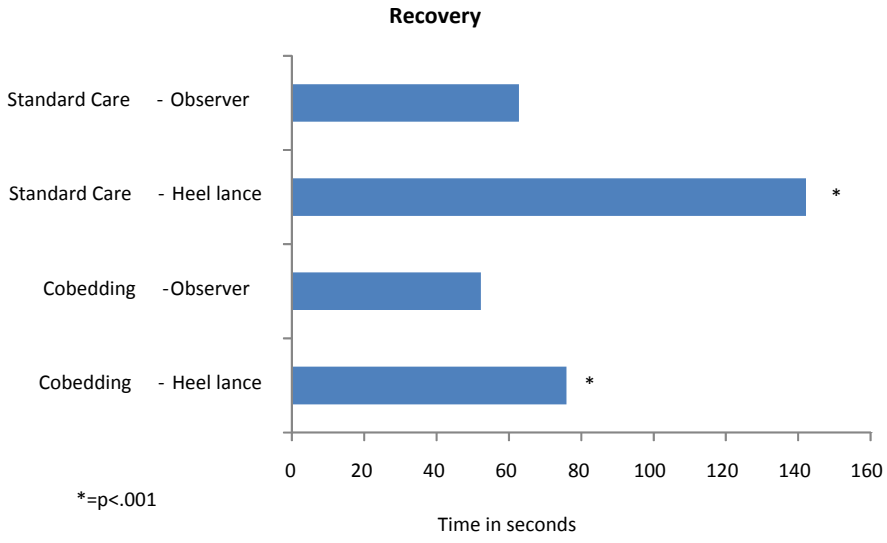


Figure 17: The effect of role (twin receiving heel lance vs. observer non heel lance) in time to recovery in seconds

Cortisol.

The baseline pre salivary cortisol levels of the observer twins (not receiving the heel lance) were not significantly different between the two groups, mean level of 0.31(SD 0.28) ug/dl in the co-bedding group and 0.37(SD 0.27) ug/dl in the standard care groups, P=0.70 (Table 18). In contrast to the twins who underwent the heel lance, no differences were found in the post cortisol levels of the observer twins regardless of group assignment to the co-bedding or standard care condition 0.34 (SD 0.35) ug/dl and 0.46 (SD 0.57) ug/dl respectively, P=0.12 (Table 19).

Table 18: *Comparison of Pre Salivary Cortisol levels between the groups for observer twins*

	N	Mean	Std. Deviation	P value	Mean Difference	95% Confidence Interval of the Difference	
Standard care	33	0.34	0.27				
Co-bedding	36	0.31	0.28			Lower	Upper
				0.70	0.03	-0.11	0.16

Table 19: *Comparison of Post Salivary Cortisol levels between the groups for observer twins*

	N	Mean	Std. Deviation	P value	Mean Difference	95% Confidence Interval of the Difference	
Standard care	39	0.46	0.57				
Co-bedding	47	0.34	0.35			Lower	Upper
				0.24	0.12	-0.08	0.32

No mean differences between the twin sets, taking into account the effect of role (i.e. whether the twin underwent the heel lance or was the observer), were noted between the two groups for pre or post cortisol levels (Table 20).

Table 20: *Mean differences in pre and post cortisol between twin undergoing heel lance and observer twin.*

Difference in simultaneous pre and post cortisol between twin undergoing heel lance and twin observer		N	Mean	Std. Deviation	P value	Mean Diff	95% Confidence Interval of the Difference Lower	Upper
Pre Cortisol	Standard care	26	-0.10	0.64				
	Co-bedding	33	-0.06	0.30	0.72	-0.05	-0.30	0.21
Post Cortisol	Standard care	38	-0.08	1.03				
	Co-bedding	44	0.06	0.41	0.44	-0.13	-0.47	0.21

Heart rate variability.

No significant group differences were noted at baseline, during lance or for the change from baseline to lance for LF, HF and the LF/HF Ratio for the twins not receiving the heel lance. See Appendix N comparison of HRV indices at baseline and lance between groups (Table 21).

Table 21: *Change in HRV (LF,HF,LF/HF ratio) at lance from baseline*

Mean change at lance from baseline	Group	N	Mean	Std. Deviation	P value	Mean Diff	95% Confidence Interval of the Difference Lower	Upper
Low Frequency (ms^2)	Standard care	41	18.0	325.3				
	Co-bedding	49	-51.4	546.8	0.48	69.3	-124.0	262.6
High Frequency(ms^2)	Standard care	37	-5.4	92.4				
	Co-bedding	49	10.2	96.1	0.45	-15.6	-56.6	25.3
LF/HF Ratio	Standard care	41	-0.7	14.3				
	Co-bedding	49	-1.0	6.8	0.90	0.3	-4.3	4.9

Chapter 5- Discussion

In this chapter, the findings from this study will be discussed, in an order that corresponds to the results section. Strengths and limitations will then be examined, and finally, theoretical contributions and implications for clinical practice and research will be presented.

To our knowledge this is the first study to examine the possible comforting effect of co-bedding for twins undergoing a tissue breaking procedure in the NICU. Our study showed that co-bedding did not lower PIPP scores, but did decrease time to recovery and salivary cortisol levels post heel lance substantially. PIPP scores at 90 seconds post lance were 1 point higher in the co-bedding group, however, since the scores were less than 6 (considered to be no or very mild pain), the clinical relevance of this finding is uncertain. The faster recovery and lower stress response is consistent with studies examining maternal skin-to-skin contact (Johnston et al., 2011). However, decreased recovery and stress response occurred without a concomitant decrease in behavioural pain scores for the initial minute post lance, or differences in heart rate variability; neither was there a decrease in provision of additional sucrose dosages, nor an increase in adverse events among the twins undergoing the heel lance or the co-twin. Heart rate was higher at baseline and across the phases of the heel lance in the co-bedding group although the magnitude of change following lance was lower in the co-bedding group. Twins in the co-bedding group were significantly less likely to receive additional non-pharmacologic interventions. Despite the slightly smaller than expected final sample, we have shown that co-bedding enhances physiological recovery and attenuates the stress response of preterm twins undergoing a heel lance in the NICU.

Pain response - Premature Infant Pain Profile

We hypothesized that co-bedding as a non-pharmacologic intervention would have an additive degree of comfort over the sucrose standard of care and would reduce pain scores in preterm twins who were co-bedding compared with non co-bedding twins. We compared pain scores using the validated Premature Infant Pain Profile (PIPP) calculated in 30 seconds epochs following the heel lance. As expected, overall pain scores varied across the phases of the procedure and twins, regardless of group assignment, had significantly higher scores within 30 second of the heel lance (Chimello, Gaspardp, Cugler, Martinez, & Linhares, 2009). We did not find that pain scores were lower in co-bedding twins. Pain scores were not significantly different between the groups when compared across the phases of the heel lance procedure for the initial minute or at 120 seconds. After accounting for potential non-independence of twins and differences in baseline characteristics, PIPP scores at 90 seconds were 1-point higher in the co-bedding twins. These findings were not in keeping with reports from other studies examining skin-to-skin contact provided by mothers which consistently show a lowering of pain scores for infants receiving maternal skin-to-skin during a tissue breaking procedure when compared to no treatment (Johnston et al., 2011). This difference may relate to the lack of full ventral skin contact associated with previously studied skin-to-skin contact which is not possible among co-bedding twins. Full ventral contact leads to a different tactile stimulation, additional warmth and containment and a upright versus vertical position. Or, it may simply support the premise that mothers provide something unique when compared with other providers, including another adult (Johnston, Campbell-Yeo & Filion 2011). Although fathers when compared to mothers providing skin-to-skin contact for a similar population of preterm singletons undergoing a heel lance were associated with lowered pain scores compared to historical controls, scores were

lower with mothers for the first minute post heel lance. We had theorized that familiar olfactory and auditory multisensory stimulation, perhaps more closely associated among twins and more similar to a mother than a father, may have accounted for these differences and thus the co-bedding of twins would have been shown to have added efficacy in reducing pain. The prolonged period of separation of the twins, mean of 18.7 days (range 2-87) may have diminished the familiar intrauterine scent and contributed to the lack of additive pain reduction found in the co-bedding group. Unfortunately, although we had anticipated that the immediate NICU stabilization would lead to separation of the twins for a short period following birth, we had expected that our relatively healthy sample would have been younger when deemed eligible for randomization and would have had much shorter separations. Interestingly, skin-to-skin contact does remain an effective pain relieving intervention postnatally even after prolonged maternal-infant separation. This is likely correlated to the fact that although amniotic scent is gone, the mother's familiar scent and the scent of her breast milk may still be recognizable to the infant, since the chemical profiles of amniotic fluid and colostrum are similar (Varendi & Porter, 2001). Infants have been shown to learn to recognize and have diminished pain response when exposed to a familiarized scent (Goubet, Rattaz, Pierrat, Bullinger & Lequien, 2003; Goubet, Strasbaugh, & Chesney, 2007). Since co-bedding should have provided opportunity for olfactory familiarization, it is also possible that the scent of a twin is not powerful enough to diminish behavioural pain response. Moreover, whether olfactory stimulation among co-bedding twins could be linked to self-regulatory or hormonal indicators of pain, remains uncertain.

Nevertheless, an important point to note in our findings is how low all PIPP scores were across the phases. The mean PIPP score at 30 seconds post lance, generally reported as the most painful epoch, were low, 7.1 (SD 2.8) in the co-bedding group and 7.2 (SD 3.4) during standard

care. Our mean PIPP scores at 30 seconds post lance were lower than previously reported, for infants with a gestational age of 32-36 weeks undergoing heel lance receiving maternal skin-to-skin contact, mean 10.1 (Johnston et al., 2003) and for age 28-32 weeks, mean 9.5 (Johnston et al., 2008). In a more recent study examining mothers versus fathers, mean pain scores at 30, 60, and 90 seconds post lance for fathers were 8.5, 8.6, and 7.6 and for mothers were 7.3, 7.4 and 6.9. Although a direct comparison cannot be made between the studies, it is worthwhile to note that all of these studies reported higher mean PIPP scores than those in our study.

It is possible that our lack of significant findings between the groups is related to these very low scores. It could be that the sucrose dose provided to all infants was so effective at lowering the pain that any additional intervention could not lower it further. A similar reason was cited for the lack of additional comfort demonstrated when enhanced kangaroo care consisting of maternal voice and rocking was added to maternal skin-to-skin contact (Johnston et al., 2009). Since all of the twins received sucrose, it is important that our PIPP scores are contrasted to previous studies comparing sucrose or sucrose plus pacifier. Three studies utilizing the PIPP as an outcome measure to compare 24% sucrose to incubator controls in preterm infants during heel lance showed that the mean scores at 30 seconds, 8.2 (SD 3.2) (Gibbons et al., 2002), 7.5 (SD 3.3) (Johnston et al., 1999) and 9.1(3.4) (Stevens et al., 1999) were all higher than our reported 30 second PIPP scores for either group. PIPP scores of < 6.0 have been reported to be indicative of no or little pain (Stevens, Johnston, Taddio, Gibbins, & Yamada, 2010) and our results are similar to those reported by Taddio (Taddio, Shah, & Katz, 2009) examining the stress associated with diaper change. In Taddio's study 40% of the infants exposed to prior sucrose (within 1 hour for venipuncture) and 62% in the placebo group had PIPP scores ≥ 6 during a diaper change. In our study, only 48.1% of the standard group and 51.9% of the co-bedding

group had peak PIPP scores ≥ 6 during the heel lance. This similarity in our study, in percentage of very low scores associated with a non-tissue breaking procedure such as diaper change, demonstrated that the pain associated with heel lance was considerably diminished regardless of group assignment. In fact, the mean PIPP scores that were reported at 90 seconds to be statically significantly higher in the co-bedding group were only 6.0 (SD 3.0) compared to 5.0 (SD 1.8) in the standard care group. Since both are considered to be no or little pain (Schiller, 1999; Stevens et al., 2010), the significance of the clinical implications is less clear.

Although it is plausible that the low scores were entirely linked with sucrose administration, most of the studies examining its efficacy report only a 16-28% reduction in behavioural pain response, similar to other strategies such as skin-to-skin contact and facilitated tucking (Axelin, Salantera, & Lehtonen, 2006; Johnston et al., 2011). This moderate degree of reduction, although clinically significant, raises the question whether the cumulative effect of the additional comfort measures provided to 95% of the standard care twins and 58% of the co-bedding infants likely contributed to our very low PIPP scores consistent with others examining multimodal combinations (Bellieni et al., 2001; Bellieni et al., 2002; Bellieni et al., 2007; Blass & Hoffmeyer, 1991; Gibbons, 2002) and sucrose plus pacifier (Gibbins et al., 2002). Sucrose plus pacifier compared to sucrose alone or sterile water and non-nutritive sucking resulted in the lowest PIPP scores for one hundred and ninety preterm and ill term neonates undergoing a heel lance (Gibbins et al., 2002). In another study the addition of sensorial saturation consisting of touch, containment and voice, to 33% oral glucose significantly diminished the amount of crying and essentially obliterated behavioural pain response associated with heel lance in full term newborns.

For the majority of infants, the need for additional interventions would be signalled to caregivers by behavioural and/or physiological changes. Therefore, non-signalling twins would be less likely to receive additional interventions. Whether the overall signalling in co-bedding twins was less and this contributed to the lower use of additional non-pharmacologic strategies in that group is unknown. We found that higher use of additional interventions by the nursing staff was associated with slightly higher pain scores. Clinically, this is not a surprising finding as the neonatal staff is educated to provide non-pharmacologic measures in response to infant signalling indicative of pain. The slightly higher display of facial action and heart rate in the co-bedding does question this explanation and differences may relate to the inability to blind staff to the co-bedding condition. The neonatal nurses may have been biased and withheld the use of additional strategies for co-bedding twins. However, similar to our PIPP scores, even at their highest, maximum heart rate and facial actions were remarkably low and subtle differences may have been difficult to discern by the care providers.

The neonatal staff in the participating NICU's, are educated to use non-pharmacologic strategies preemptively prior to a tissue breaking procedure (American Academy of Pediatrics Committee on Fetus and Newborn et al., 2006; Lago et al., 2009) and this may be another reason for the difference in non-pharmacologic interventions. Swaddling, an example of an effective preemptive strategy (Cignacco et al., 2007) was used four times more often in the standard care group. Staff may not have wanted to disturb both twins while co-bedding as they would have to swaddle them together. Nevertheless, all but one of the standard care twins who received swaddling had at least one other form of intervention in addition to swaddling. Furthermore, all twins regardless of condition were offered preemptive non nutritive sucking following administration of 24% sucrose and co-bedding infants were two times less likely to initiate

sucking. The reason for this lack of sucking is uncertain although it may be possible that co-bedding creates a degree of distraction for the twin. The use of facilitated tucking was seven times more likely to be offered to non co-bedding infants and may suggest that having a twin in close proximity simulates the human touch and containment provided by this intervention.

In summary, the very low PIPP scores reported in our study reflect the use of more than one non-pharmacologic intervention. More importantly, a better understanding of the exact mechanism of action and their effect on both pain reactivity and recovery would enable clinicians to determine which combinations would be most effective. The reason for the differences in the provision of additional non-pharmacologic strategies between the groups is uncertain and requires further examination.

Neurobehavioural state

We found no differences between the two groups with respect to sleep state although co-bedding has been previously associated with more stable respiratory patterns that contributed to better-regulated sleep when compared to non co-bedding infants (Touch, Epstein, Pohl, & Greenspan, 2002). Several studies have shown that one to three hours spent in maternal skin-to-skin contact resulted in increased frequency of quiet sleep, longer duration of quiet sleep and decreased crying in both preterm and full term infants (deLeeuw 1991; Erlandsson, Dsilna, Fagerberg, & Christensson, 2007; Feldman, Weller, Sirota, & Eidelman, 2002; Ferber & Makhoul, 2004; Kostandy et al., 2008; Ludington-Hoe, Thompson, Swinth, Hadeed, & Anderson, 1994; Michelsson, Christensson, Rothganger, & Winberg, 1996). The lack of differences between sleep state in our study may be a result of the high numbers of sleeping infants at baseline in both the groups, almost 85%. Or, it may reflect the more powerful nature of maternal skin-to-skin contact or full ventral contact that may provide different tactile stimulation or added warmth and containment.

Physiologic parameters

Heart rate

As expected, all of the twins in our study, regardless of group assignment responded to the heel lance with an increase in heart rate (Gibbins, Stevens, McGrath et al., 2008). Peak response in heart rate occurred at 30 and 60 seconds post lance and then fell over time, which is in keeping with others examining skin-to-skin contact (Johnston, et al., 2011) and sucrose (Stevens, Yamada, & Ohlsson, 2010). Our findings demonstrated higher baseline average and maximum heart rates in the co-bedding group compared to the standard care group. This is in contrast to an earlier study comparing a small convenient sample of similar aged twins, 16 who were co-bedding and 21 non co-bedding, which reported no differences in baseline heart rate (Byers, et al., 2003). Although transiently higher during the initial two days of the co-bedding period, overall mean high activity heart rate was found to be lower for the mean 5-day average in the co-bedding twins. The small non-random sample in their study may have accounted for the differences between our findings. Although inconsistent findings regarding heart rate have been reported from studies examining the effectiveness of maternal skin-to-skin contact compared to no treatment during heel lance, none of the studies reported an elevation in heart rate. Specifically, baseline heart rate (Castral, Warnock, Leite, Haas, & Scochi, 2008; Cong, Ludington-Hoe, McCain, & Fu, 2009; Johnston et al., 2003; Ludington-Hoe, Hosseini, & Torowicz, 2005) and maximum heart rate change from baseline (Cong et al., 2009; Ludington-Hoe et al., 2005) were not significantly different between groups of preterm infants while lower mean heart rates were reported in fullterms (Kashaninia, Sajedi, Rahgozar, & Noghabi, 2008) receiving SSC prior to heel lance compared to controls receiving no intervention. The main difference with this latter study is that the sample consisted of full term infants compared to

preterm infants and may be reflective of a more mature regulatory system or lack of NICU exposure, which was not the case for the twins in our study.

Although heart rate was higher in the co-bedding group, the magnitude of the change in response to the heel lance was not significantly different between groups. The baseline elevation in heart rate may have be associated with the added stimulation of being close to another person, a finding that has been reported in infants who were bed sharing with their mothers (Richard & Mosko, 2004). During bed sharing, as with co-bedding, infants would be lying flat with some skin contact with their twin or mother compared to skin-to-skin contact where infants are upright and have full ventral contact with their mothers.

Another possible reason for the higher heart rate in the co-bedding twins may be the lower incidence of initiating non-nutritive sucking shown to diminish baseline heart rate in non-pain conditions (Bahgat & Elsayed, 1999; DiPietro, Cusson, Caughy, & Fox, 1994; Pinelli & Symington, 2005; Woodson & Hamilton, 1988) and during heel lance (Corbo et al., 2000; Field & Goldson, 1984; Campos, 1994; Field & Goldson, 1984; Miller & Anderson, 1993). Others have postulated that non-nutritive sucking induces a feeling of calmness in an infant that contributes to lower metabolic needs, enhances self soothing behaviours and raises the pain threshold (Blass, 1994). That co-bedding may have induced a similar level of calmness that contributed to the lower initiation of sucking in this group is plausible. Regardless of the reason, even the highest mean maximum heart rates of 175 bpm [95% CI 171.1 to 179.0] in the co-bedding group and 169 bpm [95% CI 165.1-173.2] in the standard care group were similar to the average 5 day high activity heart rate reported in a similar group of co-bedding and control infants in a non pain condition, mean 178 and 186 bpm respectively (Byers, Yovaish, Lowman, & Francis, 2003).

Oxygen saturation

No differences were found at baseline and although variation occurred across the phases for oxygen saturation no group differences were found. This is in keeping with others examining co-bedding (Byers et al., 2003), maternal skin-to-skin contact (Johnston et al., 2011) and sucrose (Stevens, Craig, Johnston, Harrison, & Ohlsson, 2011). Although lower oxygen saturation have been reported in very young infants, this was not the case for our slightly older more stable infants and is consistent with findings for infants >32 weeks during and post heel lance (Gibbins, Stevens, McGrath et al., 2008b).

Facial Actions

The facial actions of brow bulge, eye squeeze and nasolabial furrow were significantly correlated (Pearson coefficient >0.94). We found that although the percent of time the twins displayed behavioural facial actions was almost two fold higher at baseline in the co-bedding infants compared to the standard care, mean scores in both groups were less than 9%. These percentages were very low, indicative of no or very little pain and the difference between groups were not statistically significant or considered clinically important.

Facial actions were not significantly different between the groups at any point post lance. In both groups, facial response was significantly higher only at 30 seconds post heel compared to baseline and all other phases consistent with anticipated response to a pain stimulus (Gibbins, Stevens, McGrath et al., 2008a). Interestingly, the twins in the co-bedding group did display more facial action at 60, 90 and 120 seconds post lance, mean difference less than -10 for each action at all phases. Facial actions are considered to be the most sensitive indicators of pain response and the findings of the current study are in contrast to others reporting a reduction in behavioural response associated with maternal skin-to-skin contact (Castral et al., 2008; Johnston et al., 2003; Johnston et al., 2008), sucrose (Gibbins et al., 2002) and sucrose plus pacifier (Blass

& Watt, 1999). Given the non significant findings, this trend may simply reflect that having another twin in the same incubator led to more stimulation and exaggerated or enhanced reactivity since immediate response to the heel lance at 30 seconds post lance was not different. Similarly, NNS has been shown to contribute to lower facial action and its lower use in the co-bedding group may have contributed to the higher scores (DiPietro, Cusson, Caughy, & Fox, 1994). The twins in the co-bedding group also had slightly higher postnatal age, which has been associated with more robust facial response (Gibbins, Stevens, McGrath et al., 2008b; Johnston et al., 1999).

Nevertheless, a display of facial action for 10-39% of a 30 epoch would be indicative of a minimum response to mild pain (Ballantyne, Stevens, McAllister, Dionne, & Jack, 1999; Schiller, Stevens, Sidani, Ballantyne, & McNair, 1999) An important point to emphasize in our findings is that despite the higher values, the maximum mean percent time displaying individual facial actions one-minute post lance did not exceed 15%. The clinical significance of this small display of reactivity is uncertain.

The slight differences in heart rate and facial actions at baseline also raise an important question about the optimal timing of the baseline data collection. In our study, to minimize the overall amount of time disturbing the infants, preparation for data collection that included salivary cortisol sampling and the placement of cardiac electrodes and saturation probe, was planned to be completed 15-20 minutes prior to the heel lance with adequate time to recover before baseline readings were obtained. What was not considered was that the study preparation for twins in the same incubator was much longer compared to the standard care group, thus increasing the amount of prior stimulation that the co-bedding twins received and possibly contributing to the differences in heart rate and facial scores at baseline between the groups.

Since this was an unanticipated factor, we did not collect data to confirm or refute this possibility; however, the possibility was acknowledged anecdotally by the research nurses and principal investigator.

Time to recovery

An important finding in our study was the significantly quicker time to recovery in the co-bedding twins. Specifically, how quickly they returned to their baseline pre-procedure state. The co-bedding twins recovered more than a minute faster than the standard care group. Pain reactivity or response is controlled by sympathetic activation of central nervous system while recovery appears to be more closely linked with mature parasympathetic control (Fitzgerald & Beggs, 2001). Both components play an integral part in the signalling and regulating of stress and pain response. The capacity to recover quickly, a sign of the ability to maintain homeostasis, is an important maturational task that preterm twins must accomplish in order to grow and develop (Als, 1986; Als, 1998; Charpak et al., 2005; R. V. Grunau & Craig, 1987).

Facilitation of homeostasis maintenance through co-bedding has been reported regarding short-term growth (Byers et al., 2003; Chin, Hope, & Christos, 2006), stability in respiratory patterns (Touch et al., 2002), less central apnea (Touch et al., 2002), and improved sleep (Touch et al., 2002) but not in the context of the additional stress of pain. A trend towards lower stress response and higher self-regulatory behaviours were reported in a small convenient study examining the response of 19 twin sets cared for in three conditions- co-bedding, separation and swaddled in a blanket. Although not statistically significant, the findings support our results (Stainton, Jozsa, & Fethney, 2005). Twin proximity and skin contact may support the acceleration or maturation of the automatic system and thus facilitate faster recovery. Our findings that mean recovery times for co-bedding twins were faster than those reported following 15 minutes of maternal SSC prior to heel lance (Johnston et al., 2008) (76 seconds versus 123

seconds) supports this hypothesis. The longer recovery time in the Johnston study may reflect the slightly younger infants being examined, mean age 30.5 versus 31.6 weeks and is consistent with our finding that gestational age <32 weeks was associated with prolonged recovery. Interestingly, lower gestational age did not contribute to the differences in recovery time reported when skin-to-skin contact provided by mothers or fathers of similarly aged infants (28-36 weeks) during heel lance was compared (Johnston et al., 2011). Recovery times although faster with mothers than fathers, were almost 2 minutes slower than in our study. The faster recovery observed in our study may be associated with the combination of sweet taste and co-bedding. Interestingly, the higher incidence of additional non-pharmacologic strategies used in the standard care group did not have a similar effect on recovery time. Thus, implicating something unique associated with co-bedding such as familiar proximity or skin contact with enhanced twin self-regulation.

Cortisol

We found that co-bedding of preterm twins in addition to sucrose when compared to non co-bedding twins receiving sucrose attenuated the release of cortisol for preterm infants undergoing a heel lance procedure. Mean salivary cortisol levels, similar at baseline, were almost two times lower in the co-bedding group 20 minutes post lance. Simultaneous cortisol samples collected from the co-twin, not undergoing the response and considered to be the observer twin, were not significantly different between groups at baseline or post procedure. Our results indicate a better-regulated stress response in preterm co-bedding twins.

To our knowledge there is no other study examining the effect of co-bedding on biochemical responses, thus allowing a direct comparison with other studies impossible. Nevertheless, our findings are consistent with others examining skin-to-skin contact without pain exposure (Gitau et al., 2002) and during heel lance (Cong, Ludington-Hoe, & Walsh, 2011).

Preterm infants receiving 20 minutes of maternal skin-to-skin contact had lower salivary cortisol levels when compared to infants receiving 20 minutes of massage, implicating maternal proximity rather than tactile stimulation as a mechanism of action (Gitau et al ., 2002). Lower post cortisol levels were also reported when 30 minutes of maternal skin-to-skin contact preceded a heel lance procedure for infants delivered at 30-32 weeks of gestation when compared to an incubator control condition (Cong et al., 2011). Familiar maternal odour has also been associated with lowered behavioural pain response and salivary cortisol following heel lance in healthy full term infants (Nishitani et al., 2009). Lower salivary cortisol levels following the stress of bathing were reported in 5 week old infants who were bed sharing with their mothers compared to solitary sleeping infants (Tollenaar, Beijers, Jansen, Riksen-Walraven, & de Weerth, 2011). Conversely, solitary sleep was not associated with stress response for older infants, aged 2 month undergoing intramuscular injections. Stabilization of the HPA axis has been associated with maternal grooming and feeding in rats although passive contact with littermates in the nest also appears to contribute (Levine, 2001). It may be that close proximity of a twin may diminish the stress of early maternal separation that has been associated with a less regulated stress response (Hofer, 1994; Serra, Pisu, Mostallino, Sanna, & Biggio, 2008). Or it may be that the ability for twins to freely touch and have skin contact with each one another modulates stress response as reported with maternal SSC (Cong et al., 2011), possibly through stimulation of C afferent skin fibres and activation of the limbic system (Olausson et al., 2001; Olausson et al., 2002).

We did not find a correlation between cortisol results and pain scores. This finding is in keeping with others who have reported dissociation between behavioural, autonomic and hormonal and HPA responses to pain (Fitzgerald & Walker, 2009; Slater, Boyd, Meek, &

Fitzgerald, 2006) and those reporting differences felt to be related to contextual factors such as the amount of prior pain exposure and lower gestational age at birth (Sellam, Cignacco, Craig, Engberg, 2011; Grunau et al., 2005; Herrington, Olomu, & Geller, 2004) but is in contrast to studies examining the effect of maternal skin-to-skin contact during heel lance. We did not find any significant correlation with prior pain exposure and gestational age at birth for any of the major outcomes. It may have been that the modulation effect of co-bedding was too strong especially in light of our very low pain scores. The reason for the difference noted with maternal skin-to skin contact may be that the maternal skin-to-skin contact was being compared to no treatment (untreated pain) while in our study both groups received 24% sucrose, a pain relieving intervention known to diminish pain response. Nevertheless, the effect of co-bedding on cortisol response appears to be unique when compared to the findings of others reporting the effect of sucrose or sucrose/pacifier compared to control (Greenberg, 2002; Joung & Cho, 2010) or across repeated painful procedures on cortisol response (Cignacco, Denhaerynck, Nelle, Bührer, & Engberg, 2009).

Sucrose was not associated with any differences in pre and post cortisol levels among infants undergoing heel lance (Greenberg, 2002; Joung & Cho, 2010); all painful procedures (Boyer, Johnston, Walker, Filion, & Sherrard, 2004) or circumcision (Stang, Gunnar, Snellman, Condon, & Kestenbaum, 1988). Our findings implicate co-bedding in the modulation of the stress response.

Interestingly, although the co-bedding group had higher mean average and maximum heart rate and higher facial responses, they had lower cortisol levels. This may reflect a more robust reactivity combined with an enhanced up-regulation of parasympathetic response and

modulation of hormonal stress response, a premise that would be supported by our finding of shorter time needed to recover.

Wide variances have been reported in infant cortisol levels and whether preterm infants have identifiable circadian rhythms affecting cortisol has yet to be shown (Ng, 2011). Despite this uncertainty, we did attempt to collect only morning samples. However, since the heel lances were clinically versus research driven, we were not always able to do this. Also since we had a random sample, and that that pre cortisol levels, similar at baseline for both intervention and observer babies in both groups, were collected on all babies and that significant differences were found only in the co-bedding group post heel lance, timing of cortisol collections was not related to our findings.

Heart rate variability

We did not find any correlation between HRV indices and composite pain scores, in keeping with others (Oberlander et al., 2005; Stevens et al., 2007). We did find a significant negative correlation between all facial actions (Brow bulge, eye squeeze and nasolabial furrow) at 30 seconds post lance and baseline LF/HF ratio (reflecting autonomic balance) and a positive correlation with LF during lance (reflecting sympathetic and parasympathetic influences). Our findings differed from those recently reported (Weissman, Aranovitch, Blazer, & Zimmer, 2009) that showed a negative correlation with NFCS scores and LF for full term infants undergoing heel lance. Sympathetic tone is dominant in preterm infants, whereas parasympathetic tone increases with higher gestational age and our findings may reflect differences in maturation (Chatow, Davidson, Reichman, & Akselrod, 1995; Smith, 2003).

As expected, HR increased from baseline to heel lance in both the co-bedding and standard care group indicating a clear response to the stimulus. There were significant differences noted between baseline and lance for all HRV indices (LF, HF, and LF/HF) that were

similar between groups. LF and HF were lower during lance than at baseline consistent with previous studies reporting lower overall HRV (Lindh, Wiklund, Sandman, & Hakansson, 1997) LF (Lindh et al., 1997) and HF (Oberlander, Grunau, Fitzgerald, & Whitfield, 2002) during heel lance. The exact control of LF remains uncertain, although it appears to be influenced by both sympathetic and parasympathetic systems. Conversely, HF is primarily associated with parasympathetic forces. Thus, our reported decrease of LF and HF and higher LF/HF ratio can be explained by parasympathetic withdrawal known to occur in response to a pain stimulus (Sweet &McGrath, 1998).

A fall in low and high frequency heart rate variability have also been reported in full term infants undergoing either heel lance or venipuncture (Padhye, Williams, Khattak, & Lasky, 2009) and postoperatively (Faye et al., 2010) suggesting HRV indices as a useful marker for prolonged as well as acute pain.

No studies have examined the effect of co-bedding on heart rate variability and the few studies that have evaluated the effect of pain relieving interventions during heel lance had inconsistent results (Gormally, 2001; Greenberg, 2002; Lindh, 2003; Cong, 2009, Weissman, 2010). HRV indices were not found to be different in older infants (age 3 months) undergoing immunization who received glucose and EMLA compared to placebo cream and water (Lindh, 2003). In the two studies examining full term infants receiving sucrose, Gormally (2001) reported no effect of sucrose on HRV indices whereas Greenberg (2002) reported lower vagal tone during lance in the group receiving a sugar coated pacifier. Interestingly, in the latter study, differences in vagal tone were not reported in infants receiving oral sucrose without pacifier. These findings suggest that pacifier/non-nutritive sucking alone or in combination with sweet taste may regulate vagal tone more than oral sucrose. Others have found that lower heart rate is

associated with non-nutritive sucking (Blass, 1994) and that in no pain conditions less parasympathetic withdrawal occurs following nipple feeding (McCain, Knupp, Fontaine, Pino, & Vasquez, 2010). Despite the lower amount of non-nutritive sucking in the co-bedding group we did not see group differences.

Maternal skin-to-skin contact was associated with less parasympathetic withdrawal when compared to incubator controls indicated by a rise rather than a fall from baseline in both LF and HF during heel lance, findings that were in contrast to our study (Cong et al., 2009). Their smaller sample size and variation in design may reflect the differences in the HRV indices. Or it may be associated with the larger range in the duration of the heel lance collection period in our study that may make the intervals less comparable (Oberlander & Saul, 2002). In our study, although our mean time was 3.35 minutes for the lance interval, our range was much broader (0.30 to 11.4 minutes), than the range reported by Cong (3.5 to 4.5 minutes) (Cong et al., 2009). Variation of interval times can alter HRV findings and make direct comparisons difficult (Oberlander & Saul, 2002). Additional reasons for the differences could also be attributable to the presence of the mother (Chatow et al., 1995; Longin, Schaible, Lenz, & Konig, 2005; Rosenstock, Cassuto, & Zmora, 1999), tilted KMC position (Schrod & Walter, 2002), or the greater stimulation of pressure points in the abdomen and chest of the infant with full ventral contact (Diego, Field, & Hernandez-Reif, 2005; Ireland & Olson, 2000) that may be more powerful regulator of heart rate variability than sucrose alone or in combination with co-bedding or other non-pharmacologic interventions.

Variation in heart rate variability has been linked with maturation of the autonomic system, and younger (Oberlander et al., 2000) and small-for-gestational age infants (Galland, Taylor, Bolton, & Sayers, 2006) appear to have less mature less regulated control generally

attributed to less robust sympathetic response, prolonged withdrawal and slower up-regulation of parasympathetic response. Although we did find an association with CGA <32 weeks and post natal age, we did not account for the possible influence of small-for-gestational age twins. Despite the faster recovery and modulated cortisol response in the co-bedding twins, both linked with more mature self-regulation, we did not find any differences for heart rate variability between the groups during heel lance. Given our very low pain scores and minimal differences in reactivity during heel lance between the groups, our lack of finding may reflect the timing of our HRV analysis. We did not measure HRV during or following recovery and it was during this point in the heel lance procedure where we demonstrated group differences associated with modulation of recovery and stress.

Although we did not have significant findings between groups, it is worthwhile to note that the diminution in parasympathetic dominance at lance compared to baseline was slightly less in the co-bedding group, -19.81 ms^2 versus -23.58 ms^2, compared to the standard care group consistent with the lowest decrease in parasympathetic tone being reported for breast feeding or bottle feeding full term infants undergoing heel lance compared to glucose alone (Weissman, Aranovitch, Blazer, & Zimmer, 2009). Whether this more robust parasympathetic response would have been more pronounced during recovery, in keeping with our faster return to physiologic baseline and lower post cortisol levels, is uncertain and warrants further study.

Frequency of sucrose dosages

We found no significant differences between the groups regarding the need for additional sucrose dosages and less than 16% of infants required more than one initial dose. This is not surprising given our very low pain scores. In addition the high use of additional strategies in almost all twins in the standard care group (95%) and 58 % of the co-bedding group may have mitigated the need for additional sucrose.

Response of the co-twin

We did not find any significant differences in the simultaneous response of the twin not undergoing the heel lance whether they were co-bedding with their twin or in a separate incubator with respect to PIPP scores, facial action, time to recovery, salivary cortisol or HRV. Our findings suggest that preterm twins do not appear to experience a heightened response to their twin's pain nor do they experience any adverse effects from being exposed to their twin undergoing a painful procedure. Our findings do not suggest that an empathetic response occurs between co-bedding twins when one twin sees another twin undergo a painful procedure in contrast to parents who report heightened empathetic reaction when observing their child undergo a painful procedure (Goubert et al. 2005). Our lack of co-twin response is consistent with others reporting that empathy is a learned process that develops over time (Kunyk & Olson 2001) with maturation of the pre frontal cortex (Miller & Cohen 2001) and the attainment of self-awareness (Keenan et al. 2003; Campbell-Yeo, Latimer, Johnston, 2007). Whether unconscious neuronal changes in response to being near their twin experiencing pain (Danziger et al. 2006; Decety and Jackson, 2004) occurs in the absence of biobehavioural or physiologic response remains unknown.

Adverse effects

No significant differences in adverse effects were reported between the groups with respect to incidence of infection, apnea, bradycardia, oxygen desaturation, and caregiver error. These findings are consistent with previous studies comparing co-bedding to a non co-bedding condition in the NICU (Chin et al., 2006; LaMar & Dowling, 2006; Longobucco, Bernstein, & Rossi, 2000; Lutes & Altimier, 2001; Touch et al., 2002). Six episodes of bradycardia and oxygen desaturation were recorded during our study. All of these episodes, 3 twins in each group, occurred immediately following sucrose administration. This constituted a 4.4%

occurrence in the co-bedding group and a 4.9% occurrence of adverse respiratory effects that is comparable to a 3% incidence reported by Gibbons (Gibbins et al., 2002). The twins' required minimal medical intervention following the episodes and recovered quickly. None of the twins had any sustained adverse effects. Temperature instability has been reported among co-bedding twins (Byers, 2002; Longobucco, 2000), however, this was not the case in our study. Although our study was not powered to definitively demonstrate safety, our results supports previous findings and provides further evidence regarding the safety of co-bedding twins in NICU settings.

Strengths and limitations

To our knowledge, this is the first study to examine the comforting effect of co-bedding for preterm twins undergoing a tissue breaking procedure in an NICU setting. There are several strengths to the study. We conducted a rigorous randomized trial that incorporated multiple strategies to diminish the risk of bias associated with intervention trials (Campbell-Yeo, et al, 2009). To ensure reliability, generalizability and uniformity of delivery, we clearly defined the co-bedding and standard care conditions. The use of an off-site web generated randomization site decreased possible research staff manipulation and ensured random sequence and allocation concealment of group assignment. Strict criteria for inclusion and exclusion led to a more homogenous sample among groups and completion of a daily intervention fidelity checklist provided important information regarding protocol adherence. Physiological data were derived directly from the infant recordings and could not be manipulated and salivary cortisol samples were analyzed off-site in a lab that was not involved with the study. Use of off-site coders, consistently trained to ensure intra and inter-rater reliability and kept blinded to the intervention and study objectives, diminished the risk of bias that can occur in single blind intervention trials.

In addition to the aforementioned strengths, other aspects of the study protocol were considered to enhance the quality of the study. Specifically, a well-established outcome measure was used which allowed indirect comparisons to be made to other studies. Including more than one site increased the generalizability of the results. Furthermore, digital and video recording of data increased precision of measurement and by controlling the number of personnel performing the heel lance, variation in technique that may have contributed to our outcomes was decreased.

Lastly, from a methodological perspective, since randomization of the twins as a unit of the dyad versus each twin separately led to a concern about potential correlation in outcomes of twin pairs, we selected statistical analysis (generalized estimating equation- GEE) that allowed us to address this issue.

Despite the numerous strengths, there were limitations in our study. As with many clinical intervention trials, blinding of the intervention being tested is not always possible. The possibility of investigator, participant, and observer bias and Hawthorne effect associated with direct recognition of group allocation represents other potential concerns. Limitations of this research include issues related to the unblinded nature of the co-bedding intervention, lack of a no-intervention control group, control of confounding variables, sample size and data collection. Although the facial coders and research assistants calculating physiological response and heart rate variability were blind to the intervention, neither the researcher nor the staff caring for the twins or performing the heel lance were blind to the intervention. Although this is a realistic issue in clinical interventions with visible physical or environmental attributes, it may have impacted the study findings, specifically use of additional non-pharmacologic strategies. Many of the nurses at all participating sites are in favour of co-bedding and believe that it is effective. This belief may have altered the amount of additional non-pharmacologic strategies that they

perceived the co-bedding twins needed. While this is possible, the very low PIPP scores and little clinically relevant difference in pain reactivity between the groups may simply have contributed to the caregivers response. It is also possible that the dedicated staff conducting the heel lance procedure tried to be gentler during the co-bedding condition even though the lack of difference in pain scores would not support this assumption. The higher need for repeat heel lance may have been associated with a gentler first attempt or may simply be related to variation in blood collection technique when twins share the same incubator.

The lack of a no intervention control group added to the complexity and interpretation of our findings and this could be considered a limitation to our study. However, since the provision of 24% sucrose prior to minor tissue breaking procedures is considered standard care in the study units, having a no control group was not possible for ethical reasons.

In addition to both groups receiving sucrose, our findings may have been confounded by the additional use of multiple non-pharmacologic strategies, specifically non-nutritive sucking, swaddling and facilitated tucking, initiated by the NICU staff not blind to the intervention. It is not possible to plan a study that withholds pain-relieving strategies routinely incorporated by staff in their daily practice. Furthermore, parental expectations that all available pain relieving strategies will be provided to their children may negatively impact recruitment rates if additional pain reducing strategies were restricted. Ethically, it is our belief that effective pain relieving measures should not be withheld from newborns; however, researchers should consider equivalence studies comparing more strictly controlled protocol driven combinations of non-pharmacologic interventions. Further studies should compare combination of strategies that use protocols to control initiation and order of pain relieving strategies to be tested making direct comparison less complex.

Our, slightly smaller than expected sample size may have limited the power of our analyses to attain statistically significant associations between the two groups in relation to the effect of co-bedding on pain scores, salivary cortisol, and heart rate variability. We had a slightly lower sample size than expected for the analysis of the PIPP, our primary outcome, because a few of the twins did not require blood work. For the most part, this occurred when one twin stabilized faster than their co-twin and no longer required a medically indicated blood collection by the time their twin was stable enough to begin co-bedding. Although every attempt was made to ensure all data were collected, we also had some loss of data secondary to equipment failure and movement artefact.

Consistent with others examining salivary cortisol in preterm infants (Cignacco, 2009; Cong et al., 2011), we encountered some difficulty in collecting adequate amounts of salivary cortisol (31.5% of the pre samples and 13.5% of the post samples). Thus, sample size for cortisol analysis was decreased to 89 (n=49 co-bedding, n=40 standard care) for pre levels and 113 for post samples (n=58 co-bedding, n=55 standard care). When the differences from pre to post levels were calculated, sample size was further lowered to 82, (n=45 co-bedding, n=37 standard care). Nevertheless, our findings are important and contribute to the small body of growing evidence regarding salivary cortisol as a biomarker for pain response in preterm infants.

Another limitation in our study that may have contributed to the lack of significant results for heart rate variability may have been associated with our loss of complete data for HRV due to the inability of our analysis software (Compumedics) to analyze some of the shorter baseline and collection phases that lasted less than 2 minutes in duration. This was particularly evident when the total heel lance collection period was of short duration. Sample size was lowest when differences in baseline and stick HRV indices were calculated, (n=62 co-bedding; n=64 standard

care). We also analyzed the entire baseline and heel lance phase regardless of the length, thus comparing different time intervals that may have impacted our results. Lastly, we did not analyze HRV for the recovery phase. Given our other findings, HRV analysis for this period may have added valuable information and possible group differences. However, since many of our recovery times are very short, analysis would not have been possible. Another option might have been to record another 2-3 minute epoch just prior to collection of the post cortisol 20 minutes following lance.

The scheduling of blood work for clinical purposes led to some variation in collection times for the cortisol sample. However, the majority (92.5% co-bedding, 94.7% standard care group) of the samples were morning collections and no differences in the amount of collections outside the morning time frame were found between the groups.

Contributions

The findings of this dissertation have a number of important clinical, research and theoretical contributions for the management of pain in preterm twins, who are at risk for a multiple procedural pain exposure during their NICU stay.

Implications.

Practice.

Our study provides further evidence that the pain associated with heel lance can be significantly lowered when 24% sucrose in combination with co-bedding or other non-pharmacologic strategies in particular non-nutritive sucking, swaddling or facilitated tucking are provided to preterm infants. However our results do not explain what combination of strategies is most beneficial. Our results implicate co-bedding in optimizing self-regulation following heel lance but does not demonstrate that co-bedding provides additional pain relieving properties. Until further studies are conducted, co-bedding of twins should be implemented to stabilize

recovery and diminish stress response from heel lance, and used in combination with known pain relieving strategies.

Every effort should be made in NICU settings to implement pain prevention programs that facilitate practice change to alleviate repeated pain exposure in preterm infants. Although these pain-reducing strategies are primarily nurse driven (Fernandes, Campbell-Yeo, Johnston, 2011), many effective pain relieving strategies are also mother or family driven (Campbell-Yeo, Fernandes & Johnston, 2011). Mothers and parents should be encouraged and supported to become active participants and advocates for pain management for their children.

In addition to staff and family education, administrators should pay close attention to the unit context to ensure that measures to enhance facilitation of sustainable practice change are taken (Stevens et al., 2010) and that studies examining the feasibility of novel pain relieving approaches are supported (Cignacco, Axelin, Stoffel, Sellam, Anand, Engberg, 2010).

In keeping with studies examining skin-to-skin contact, our findings implicate the practice of co-bedding in the enhancement of self-regulation and attenuation of the stress response associated with heel lance without related adverse risk. In addition to enhanced self-regulation of the pain response, the faster achievement of baseline physiologic stability may impact other neonatal outcomes such as bronchopulmonary dysplasia and retinopathy of prematurity which have been linked with fluctuations in physiological parameter (Legendre, Burtner, Martinez, & Crowe, 2011).

To date, co-bedding has not been associated with any increased risk related to temperature instability, respiratory compromise, infection or misidentification (Tomashek, 2007) and our findings add to the current body of evidence related to the safety of co-bedding of preterm twins in the NICU setting. Our study is the first to examine the effect of co-bedding

during a tissue breaking procedure and no adverse effects were reported for the twin undergoing the heel lance. In addition, the twin not undergoing the heel lance but exposed to their co-twin's procedure did not experience any adverse effects of co-bedding.

Theoretical considerations and future research.

Theoretically, our study is important because there is an increasing realization that alleviation of pain and promotion of self regulation among infants is critical for healthy growth and long-term development. This doctoral research provides valuable information to help us better understand the mechanisms contributing to increased comfort within a multisensorial context and initial information related to the role of co-bedding and the possible importance of twin proximity and self regulatory processes. Our findings provide the first information regarding co-bedding and the associated impact on preterm twins exposed to pain and stress in the NICU and stimulates future research related to the effect of continued skin contact and presence of a twin on pain reactivity and self regulation.

Co-bedding provides twins with multiple sensory inputs (closeness, tactile, auditory, olfactory), and the neuromatrix theory of pain provides a strong conceptual model to further investigate what aspect of a twin's proximity contributed to the enhancement of self regulatory processes found in our study. It has been postulated that pain reactivity and recovery are modulated separately (Fitzgerald & Walker, 2009). The significantly faster recovery time following heel lance and lower post cortisol associated with co-bedding despite the lack of differences in pain scores and heart rate variability raises numerous questions regarding the significance of reactivity and recovery during a painful stimulus. Our findings implicate that co-bedding and other forms of non-pharmacologic strategies may work through more than one mechanism of action (Figure 18).

Although we did not find a dose response of the duration of co-bedding, it is important to note that we collected data during the first heel lance only after the twins were placed together, it is uncertain whether a more prolonged exposure of co-bedding may have led to lower pain response across repeated painful procedures over time or for a different type of procedure (Sellam, et al., 2011). Given the reported variability in longitudinal pain response in preterm, further studies are needed.

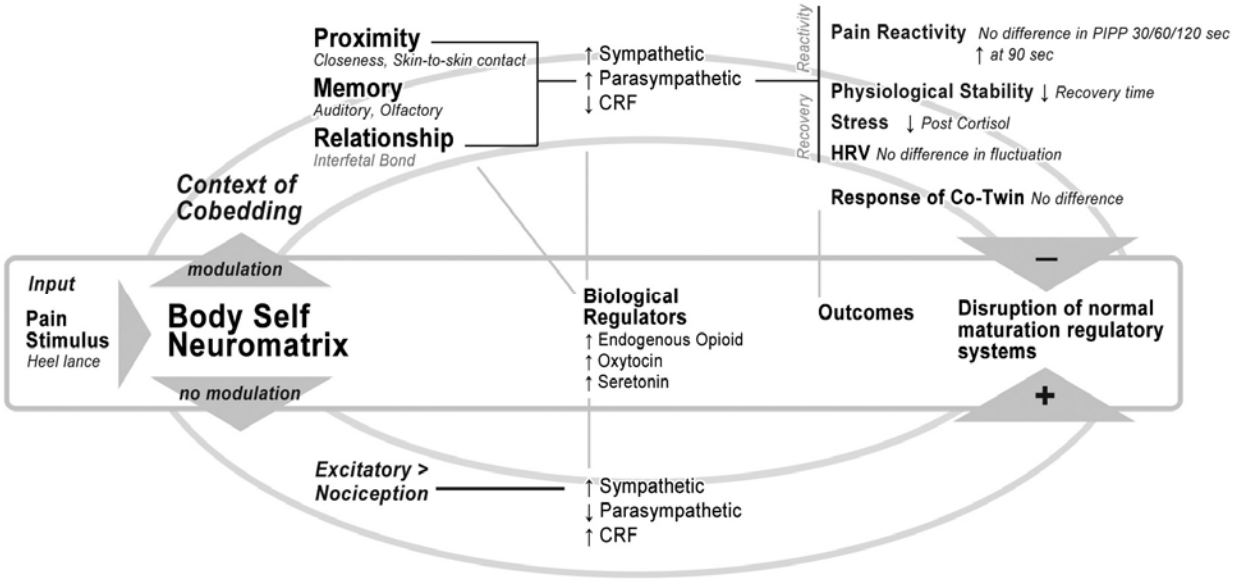


Figure 18: Comfort of Co-Bedding modulated via the conceptual model of the Body-self Neuromatrix

The heel lance procedure led to an excitatory nociceptive pain stimulus that triggered autonomic hormonal and biobehavioural responses in the twin. Co-bedding likely via the continued proximity of the twins, stimulation of auditory and olfactory memory and continued twin relationship modulated recovery from the heel lance as demonstrated by a faster physiologic recovery and lower salivary cortisol release. Pain reactivity was diminished in both groups but immediate response in the first minute was not different and at 90 seconds, co-bedding twins had higher reactivity in response to the heel lance. There was no effect on the need for additional

24% sucrose. Whether the modulating effect of co-bedding enhances maturation of self-regulatory systems requires further elucidation.

CRF = corticotrophin releasing factor; PIPP = premature infant pain profile; # sucrose = number of 24% sucrose doses administered during heel lance procedure; HR = heart rate; RR= respiratory rate; SaO2-oxygen saturation; HRV= heart rate variability; ΔHPA= alteration in hypothalamic pituitary adrenal axis.

Despite the important contribution of studies examining pain reactivity in infants, few studies have examined the relationship between reactivity and recovery following procedural pain in newborns. The ability to recover from a stressful experience is a measure of infant self-regulation ability and repeated untreated pain exposure has been linked with mal adaptive changes in regulatory systems that have long lasting effects (Grunau, Holsti, & Peters, 2006). Further investigation is needed to address the significance of reactivity and recovery in the context of treated pain and the impact of various strategies related to attenuating long-term consequences and enhancing infant self-regulation ability. Future research is needed to tease out the efficacy of various combinations of non-pharmacologic strategies. Given our findings, examination of the possible mechanisms of action and regulatory contributions of co-bedding preterm twins is also warranted.

Lastly, we believe that our study also raises questions related to the optimal balance between reactivity and recovery. Should the goal of multimodal pain relieving strategies be to eliminate all reactivity, thus removing all pain signally, or should we aim to diminish pain reactivity and promote an immature infant's ability to engage regulatory systems to recover from these experiences. This question in no way implies that any form of painful procedure should go untreated in the NICU but as with many aspects of neonatal care (for example continuous analgesia for all preterm infants while ventilated regardless of pain score), we may be challenged

to ask ourselves– if we repeatedly remove all reactivity are we altering an infant's ability to react to and signal pain and learn to regulate and recover from stress later in life?

In light of recent reports that sucrose may be more sedating than analgesic (Slater et al., 2010), further study examining the neuronal impact of co-bedding and combination of additional non-pharmacologic strategies on pain response and recovery are warranted. Combining neuroimaging techniques and neuro-electrical testing with future studies examining the contribution of co-bedding and other non-pharmacologic measures could help elucidate these questions and provide valuable insights into the possible protective aspects of these strategies in relation to long term sequellae and optimal neurodevelopmental outcomes.

Conclusion

Our study has shown that co-bedding enhances physiologic recovery and diminishes stress response of preterm twins undergoing heel lance in the NICU but does not lead to lower pain scores when added to 24% sucrose. Co-bedding does not diminish the frequency of additional sucrose doses. Nor does co-bedding contribute to higher adverse effects for the twin undergoing heel lance or his/her co-twin.

The lowered incidence of associated non-pharmacologic pain strategies specifically non nutritive sucking, bundling and facilitated tucking in the co-bedding compared to standard care group with respect to the cumulative impact on pain scores requires additional study. In addition, further investigation regarding the exact mechanisms of action of the various forms of non-pharmacologic strategies with respect to reactivity and self-regulation are warranted.

Our findings raise important questions regarding the significance of the balance between reactivity to pain and recovery from it with respect to longer-term outcome especially those associated with self-regulation and maturation of pain processes.

Dissemination Plan

This study provides data that are applicable to like populations within Canada and elsewhere. Moreover, although the results are the first to be presented examining this intervention and thus should be interpreted with some caution, the findings are generalizable to similar age preterm twins, considered to be medically stable being cared for in NICUs in Canadian and similar managed neonatal centres. The findings help in the formulation of evidence-based recommendations for vulnerable twin infants experiencing procedural pain and the role of developmental care practices. This program of research has significant implications for the health of hospitalized at-risk infants, both short and long-term. Ongoing research is aimed at identifying optimal developmental strategies that may lessen the adverse effects of procedural pain. Findings will be communicated locally to front line care providers from multiple disciplines, families and administrators to facilitate uptake of knowledge and generate policy change directly affecting patient outcomes. Practice considerations and outcomes will be communicated via best practice networks to stimulate uptake nationally. Our research also has worldwide relevance in the area of neonatal care and every effort will be made to communicate the findings nationally and internationally in peer-reviewed journals, conference proceedings and via consultation with community interest groups (Parents of multiple birth association (POMBA) and related networks (worldwide twin neonatal group-Vermont Oxford).

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Appendix A Co-bedding protocol

For infants who are randomized to receive Co-bedding care:

Place infants in Giraffe Incubator or crib lying side-by-side. One side of the incubator/crib will be for twin 'a' and one side for twin 'b'.

Centre the incubator between the two care sites.

All wires and tubes for twin 'a' will exit on one side of the incubator while all wires and tubes for twin 'b' will exit on the other side of the incubator.

A spare incubator will be kept at the site for procedures if necessary.

Assign a colour code to each infant. Attach colour code tape to charts, monitors and cable, crib I.D. cards, soothers, creams and medications, temperature probe covers, suction and bagging lines, stethoscopes, and all individual equipment.

Ensure that colour-coded identification bands are securely attached to each infant (colour code identification insert before placing in plastic sleeve). Check ID bands as per NICU identification policy.

Maintain infants in a single nest. Position them close to each other, permitting them to touch each other, alternate face-to-face, back-to-back, and spooning positions.

Document on kardex designated colour code for each infant.

Coordinate schedules and routines to maximize clustering of care.

Maintain good hand washing technique between individual diaper changes, feedings and all procedures.

While co-bedding infants must remain on heart and saturation monitoring.

Once assigned to this group, co-bedding may continue until 48 hours prior to discharge at which time monitors were discontinued and infants separated.

If an infant has an IV, place a mitt on the twin's closest hand to prevent accidental dislodgement.

If one or both infants is receiving oxygen therapy, use nasal prongs to deliver oxygen so that only the appropriate twin receives oxygen therapy.

If there are any clinical signs of sepsis, the twins will be separated. They may return to co-bedding care after the concern of sepsis has subsided.

A research nurse will monitor and video-tape the twins during baseline, warming, heel lance procedure and recovery during the ~20 minute monitoring session the twins will be further monitored using the Somté system.

A research assistant will be monitoring the charts to accumulate ongoing data.

Assign the same nurse to care for both twins each shift.

For additional questions please contact the investigator Marsha Campbell-Yeo 470-8888, pager # 2086 or research nurse Kim Caddell, 470-8888, pager #1901.

We are co-bedding twins in the NICU for the purposes of research only. We do not intend for this research to indicate support of co-bedding after discharge. Since there is currently little research completed to support the benefits or risks of co-bedding, this will remain a parental decision. We do recommend that parents follow the back-to-sleep program and refrain from smoking regardless of which ever sleep arrangements they choose.

Appendix B Standard Care

For infants who are randomized to receive standard care:

Remain in separate incubators as per current NICU policy.

Assign the same nurse to care for both twins each shift.

Assign a colour code to each infant. Attach colour code tape to charts and crib I.D.

Ensure that colour-coded identification bands are securely attached to each infant. (Colour code identification insert before placing in plastic sleeve). Check ID bands as per NICU identification policy.

A research nurse will monitor (using the Somté and Massimo oxygen saturation systems) and video-tape the twins for a baseline period (5-10 minutes prior to heel lance, warming (3 minutes) heel lance (2-5 minutes) and recovery phase (~10 minutes).

A research assistant will review the charts to accumulate ongoing data.

For additional questions please contact the investigator Marsha Campbell-Yeo 470-8888, pager # 2086 or research nurse Kim Caddell, 470-8888, pager #1901.

We are co-bedding twins in the NICU for the purposes of research only related to twin comfort during heel lance. We do not intend for this research to indicate support of co-bedding after discharge. Since there is currently little research completed to refute or support the benefits or risks of co-bedding, this will remain a parental decision. We do recommend that parents follow the back-to-sleep program and refrain from smoking regardless of the sleep arrangement they choose post discharge.

Appendix C PIPP Scoring Sheet

PROCESS	INDICATOR	0	1	2	3	SCORE
Chart	Gestational Age (at time of observation)	36 weeks and more	32 weeks to 35 weeks, 6 days	28 weeks to 31 weeks, 6 days	less than 28 weeks	
Observe Infant 15 sec *Observe baseline:* *Heart Rate*____ *Oxygen saturation* ____	Behavioral State	active/awake eyes open facial movements crying (with eyes open or closed)	quiet/awake eyes open no facial movements	active/sleep eyes closed facial movements	quiet/sleep eyes closed no facial movements	
Observe Infant 30 sec	Heart Rate Max. ____	0 to 4 beats/ minute increase	5 to 14 beats/ minute increase	15 to 24 beats/ minute increase	25 beats/minute or more increase	
	Oxygen Saturation Min. ____	0 to 2.4% decrease	2.5 to 4.9% decrease	5.0 to 7.4% decrease	7.5% or more decrease	
	Brow Bulge	None 0-9% of time	Minimum 10-39% of time	Moderate 40-69% of time	Maximum 70% of time or more	
	Eye Squeeze	None 0-9% of time	Minimum 10-39% of time	Moderate 40-69% of time	Maximum 70% of time or more	
	Nasolabial Furrow	None 0-9% of time	Minimum 10-39% of time	Moderate 40-69% of time	Maximum 70% of time or more	
					TOTAL SCORE:	

Appendix D Neonatal Facial Coding System

Action	Description
I. Brow bulge:	Bulging, creasing and vertical furrows above and between brows occurring as a result of lowering and drawing together of the eyebrows.
II. Eye Squeeze:	Identified by the squeezing or bulging of the eyelids. Bulging of the fatty pads about the infant's eyes are pronounced.
III. Naso-Labial Furrow:	Primarily manifested by the pulling upwards and deepening of the naso-labial furrow (a line or wrinkle which bends adjacent to the nostril wings and runs down and outwards beyond the lip corners.

Appendix E Recording data on Somte

Turn power (left key ⏻) on Somte. Orange light will blink.

Figure 1-2: Somté Recorder

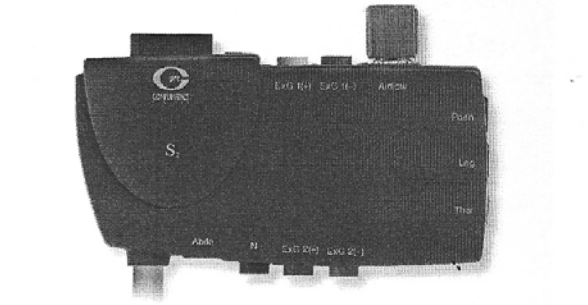
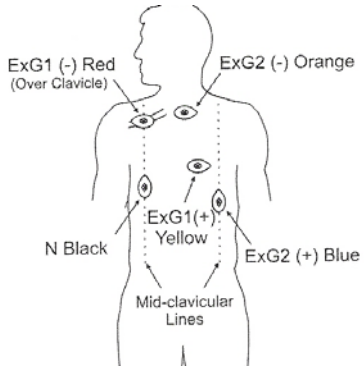


Figure 1-3: Patient Input Box

Channel 1- ECG

Channel 2- off

Attach leads and sat probe to baby. Follow illustration below using 3 leads (red, yellow and black)



Check status of ECG, pleth and saturation read out by scrolling thru screens using right key ()

To start recording:

Return to status screen () You can only record from this screen.

Press middle key (▶/■) to start recording. Screen will ask you to confirm start of recording. Press middle key a 2nd time (within 2-3 sec) to confirm. Light will now flash green. The Somte is now locked (padlock symbol appears) and recording cannot stop until the system is unlocked.

End of Recording:

Once 3 hours is up you can stop recording. You first must unlock the system. Press the 2 outside keys (⏻ and ) at the same time to unlock. An unlocked padlock with a question mark will appear.

Press middle button (▶/■) to confirm. Now that the system is unlocked, you may stop recording by pressing the middle button again. “Stop recording?” will appear. Press middle button to confirm. The blinking light were orange again.

Turn off Somte by pressing left key (⏻) until screen darkens and unit shuts off.

Appendix F Reliability & Validity of PIPP and NFCS Measures

Measure	Reliability	Validity
PIPP	Interrater reliability (0.93-0.96) Intrarater reliability (0.94-0.98) (Ballantyne, Stevens, McAllister, Dionne, & Jack, 1999)	Construct validity & Internal consistency (alpha 0.59-0.76) (Stevens et al., 1996)
NFCS	Interrater reliability (<0.85) Intrarater reliability (<0.85) (Grunau, Oberlander, Holsti, & Whitfield, 1998)	Construct & convergent validities (*r* = 0.89) (Grunau et al., 1998)

Appendix G Heart Rate Variability Responses in Newborns

AUTHOR	POPULATION	OUTCOMES	PAINFUL CONDITION	FINDINGS
Oberlander 2010	FT newborns (n = 14) heavily exposed to alcohol during pregnancy (exposed), born to abstainers (n = 14) or light (<0.5 oz absolute alcohol/d) drinkers (controls).	Heart rate and respiratory sinus arrhythmia (RSA), HRV indices Baseline Heel lance Recovery	Heel lance	In both groups, HR ↑during heel lance ↓ ↓during the post lance period. alcohol-exposed group ↓ mean HR than controls throughout, and showed no change in RSA over time.
Faye 2010	Fullterm newborns n=28	EDIN (neonatal pain and discomfort scale) pain scores	Post operative	HFVI was ↓ in "High EDIN" than "Low EDIN" (0.7+/-0.2 vs. 1.2+/-0.3, respectively; P<0.01). An HFVI <0.9 was able to predict an EDIN score >=5, with a sensitivity of 90%, and a specificity of 75%.
Weissman 2009	180 term newborns 1) control (no pain relief intervention); (2) nonnutritive sucking; (3) holding by mother; (4) oral glucose solution; (5) oral formula feeding; or (6) breastfeeding.		Heel lance	↓ HR increase in breastfed or oral formula feeding groups compared to control (21 and 23 beats per minute, respectively, vs 36; P < .01), ↓decrease in parasympathetic tone (-2 and -2.4, respectively, vs 1.2; P < .02) compared with the other groups.
Padhye et al., 2009	Very low birth weight infants (<1,500 g) from 23 to 38 weeks post-menstrual age (PMA)		Heel lance Venipuncture	Venipuncture and mechanical ventilation associated with ↓ HRV Baseline HRV ↑ with advanced post-menstrual age.
Cong et al, 2009	30-32 weeks gestational age less than 9 days postnatal age.	Baseline Lance Recovery	Heel lance	LF ↑in KC at Baseline (p<.01) and at Heel Stick (p<.001), HF ↑in KC at Baseline than in the IC condition (p< .05). The LF/HF ratio ↓ fluctuation across the periods in KC than in IC condition and ↓ during Recovery in KC than in IC (p< .001).
Schaffer et. al., 2008	Full term, 2 groups 72–96 h postpartum 27 small for gestational age (SGA) 27 appropriate for gestational age (AGA)	Baseline Lance	Heel lance	No significant differences between groups
Grunau, et al,, 2005	Preterm, 2 groups 30 infants = 22-28 wks GA 57 infants = 29-32 wks GA	Baseline 2.2 minutes, Lance 2.2 minute values	Heel lance	HR. LF and HF No correlation to pain nor morphine exposure during heel lance for both groups
Lindh, et al. 2003	Full-term, 3 month old 2 groups: EMLA + glucose 60 minutes before warming = 45 infants Placebo cream + water = 45 infants	Baseline 3 minutes, treatment administration, injection values	Immunization	Mean HR ↑; Total HRV↑; LF ↑; from baseline to injection in both groups but no change in HF No sig. diff. between EMLA-glucose and placebo-water groups Biphasic transient HR with a deceleration followed by aacceleration ↑ in placebo group
Oberlander, Grunau, Fitzgerald, & Ellwood,	Full-term, 3 groups: 22 = Prenatal exposure to SSRIs (SE) 16 = SSRI	Baseline 2.2 minutes, lance 2.2 minutes, recovery 2.2	Heel lance	Mean HR ↑ with stick in all groups. LF ↓ with lance and ↑ in recovery. HF↓ with lance and ↑ in recovery LF/HF no significant change.

et al. (2002)	+clomazepan exposure (SE+) 23 Control = No exposure	minutes values		SE and SE+ groups had greater parasympathetic return in recovery than controls.
Oberlander, Grunau, Fitzgerald, & Whitfield. (2002)	Preterms, 2 groups: 32 wks PCA n = 12 neurologically impaired control: n = 12 no impairment	baseline 2.2 m minutes, blood collection 2.2 minutes, recovery 2.2 minutes values	Heel lance	Mean HR ↑ from baseline to heel lance. LF ↓ from baseline to heel lance. HF↓ from baseline to heel lance LF:HF ratio ↓ from baseline to heel lance No group differences Infants with proven brain injury had more tongue protrusion at lance than non-impaired.
Morrison, et al. (2001)	Preterm, 3 groups: 48 infants (23- 26 wks GA) 52 infants (27 - 29 wks GA) 36 infants (30 - 32 wks GA)	Baseline 200 secs, blood collection 200 secs, recovery 200 secs values	Heel lance	Mean HR moderately correlated with facial and state response No correlation LF to behavioural responses all groups No correlation with HF to behavioural responses all groups No correlation LF/HF to behavioural responses in all groups
Oberlander, et al. (2000)	4 month old preterms, 2 groups: 21= ELBW 24= Full-term	Baseline 2.2 mins, stick 2.2 mins, recovery 2.2 mins values	Heel lance	Mean HR ↑ baseline to heel lance LF ↓ from baseline, ↑ in recovery HF ↓ from baseline, ↑ in recovery LF/HF no changes No sig. diff. between groups ELBW group: less parasympathetic withdrawal during stick; more sympathetic response in recovery
Lindh, et al. (2000)	Full-term, 2 groups: 28 = EMLA 28 = Placebo	Baseline 5 min, warming 2 min, stick 80 seconds values	Venipuncture	HR ↑ in placebo than EMLA Group Total HRV ↓ in placebo than EMLA group LF ↓ in placebo than EMLA Group
Lindh, et al. (1999)	23 Full-term, 1 group 40 Cross over sham vs. heel lance (no actual stick, just the motions and touch), sharp heel lance	Baseline 5 minutes, warming 2 minutes, 40 secs, and squeezing values	Heel lance	Mean HR ↑ during heel lancing and Squeezing Total HRV↑ in heel lancing and ↓ in squeezing LF ↑ in heel lancing and ↓ in Squeezing HF ↓ in squeezing
Lindh, et al. (1997)	10 Preterm, 1 group 24-33 GA 27-35 wks PCA	Baseline 1 min, Von Frey 1 min, rest 3 minutes, warming 1 min, stick 1 min	Heel lance	Mean HR ↑ during heel lance Total HRV↓ during heel lance LF↓ during heel lance HF no changes LF/HF ratio no changes

Appendix H Salivary Cortisol Protocol

SALIMETRICS LLC

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SALIVA COLLECTION INSTRUCTIONS

(Part No. 5029, Sorbette, Part No. 5002-1, Cryovial, 2mL)

For use only with the following salivary analytes: **Cortisol, alpha-amylase, & cotinine**
Recommended for use in collecting samples from infants.

Supplies Needed: Sorbette, scissors, 2 mL cryovials, sample ID labels.

1. Remove one Sorbette from envelope. Close envelope immediately to protect the remaining Sorbettes from contact with moisture.
2. Put Sorbette under the tongue for 45 seconds to 1 minute. The Sorbette can be moved around in the mouth to take advantage of saliva pooling under the tongue. This may help increase collection volume.
3. Sampling for 1 minute should result in approximately 200ul of saliva. If a greater volume is needed, use a second Sorbette.
4. **Sample collection from infants:** The Sorbette can be placed in the mouth under the tongue for 15 to 30 seconds at a time, then re-introduced until the Sorbette begins to expand (at least one minute total time). The Sorbette can also be moved around in the mouth and lip area to collect saliva that may be drooling down the cheek or pooling in the mouth.
5. Cut off half of the plastic shaft and insert the Sorbette(s) into a labeled 2mL cryovial with the **absorbent end up** (facing the cap).
6. If processing samples in-house, centrifuge the cryovial for 20 minutes at 1500 Xg to extract the saliva.

Notes:

1. Sorbettes are currently offered for research use only.
2. Sorbettes are packaged in a sterile bag under a biological safety cabinet [vent hood] to avoid contamination.
3. Data from Sorbettes frozen for 48 hours at -60 degrees C, stored at 4 degrees C, and room temperature for 48 hours, show no statistically significant difference in mean or CV (expressed as a proportion) from samples collected with the Salivette cotton device.
4. Customers who plan to send adult samples to the Salimetrics testing service may need to use three Sorbettes per collection event to assure sufficient sample volume due to the use of automated sample handlers.

Update: 4/8/05

Appendix I Example of 24% Sucrose Standing Orders

ADMITTING ORDERS TO NICU

The following orders were carried out by a nurse ONLY ON THE AUTHORITY OF A PHYSICIAN. OR NEONATAL NURSE PRACTITIONER.

Complete all orders in sequence. Where choice occurs, check as appropriate.

1. Admit to NICU.

2. Give vitamin K_1 intramuscularly, if not given prior to admission. Dose must be given within 6 hours of birth.

- ❑ 1 mg IM for infants with birth weight of ≥1500 grams
- ❑ 0.5 mg IM for infants with birth weight < 1500 grams.

3. If not already given, administer erythromycin ophthalmic ointment 0.5% 1-2 cm strip in each eye unless eyes are fused.

4. Sucrose 24% PO administered to the anterior tip of the tongue 2 minutes prior to painful skin breaking procedures:

- ❑ < 1500 grams - 0.1 mL
- ❑ 1500 - 2500 grams - 0.3 mL
- ❑ > 2500 grams - 0.5 mL

- May be repeated q 2 minutes for 2 additional doses during the procedure as needed.
- MAXIMUM 3 doses per procedure. MAXIMUM 4 procedures in 24 hours.
- Consult physician/NNP if sucrose is required for more than 4 procedures in 24 hours.

5. Other:

Appendix J Infant and maternal data collection form

ID#_____
Gestational age at birth: _____ Sex: _____ Birth order:_____
Birth date: _____ Birth weight: _____
Type of delivery_____ Placentation _____
Born at study centre Y/N
Apgar at 1_____ Apgar at 5_____ Apgar at 10_____
Comments _____
Date entered into study: _____ Date of randomization_____
Days at randomization: _____ Gest. age on randomization_____
Days at procedure_____ Days since randomization_____
History of Apnea/Bradycardia: Y/N Date of last apnea: _____
Caffeine Y/N: Other medications: _____
Number of times septic workup completed: (Positive blood, urine, radiological or CSF findings, misc.)
Prior to study enrolment _____following study enrolment _____

Number of episodes of confirmed sepsis: (Positive blood, urine, radiological or CSF findings, misc.)
Prior to study enrolment: _____ # days treated with antibiotics: _____
Following study enrolment _____ # days treated with antibiotics: _____

Date when weaned from Ventilator: _____
Gestational age: _____ Corrected age: _____ Post natal days_____

Date when weaned from oxygen: _____
Gestational age: _____ Corrected age: _____ Post natal days_____

Severity of IVH at time of procedure _____None, Grade 1, 2, 3 or 4
Post natal steroids Y/N

Intervention Fidelity checklist:
1. Were twin pairs cared for by the same nurse each shift during study period (time of enrolment until completion of heel lance procedure and data collection)
1. No 2. Yes

2. Did the twin pair remain allocated to their randomization group until heel lance procedure
1. No 2. Yes 3. NA

3. Did proper adherence to the study protocol occur
1. No 2. Yes

If no, please indicate exact nature of non compliance
_____

Appendix K Maternal Demographics

Comfort of Co-bedding
ID#_____

B1. Mom's age in years as a number _____
B2 A Gravida. _____ (1=1 etc.)
B2 B Para _____
B2 C Abortus _____
B2 D Neonatal death _____
B3A. Previous experience with SSC with this twin set:
1. No
2. Yes
B3B If yes :
1.Separately
2. Together
B4A Previous experience with co-bedding or co-sleeping
1. No
2. Yes
3. N/A
B4B If yes:
1. This twin set
2. Previous child
3. Previous HOM
B5 Maternal ethnicity
1 Caucasian
2 Afro-American or African
3 Asian
4 Other, specify: _____

B6. Family arrangement (at time of delivery)
1. Single- parent family
2. Two-parent family
B7 Mother's education (check highest level obtained)
1 Primary
2 Some secondary
3 Secondary graduation
4 Some college or university
5 College or university graduation
6 Post-graduate training
B8. Occupation at time of delivery
1 Self-employed
2 Employed
3 Unemployed
4 Student
5 Other
MATERNAL MANAGEMENT

B9. Were antenatal steroids given
1 No
2 Yes

B9A If YES, date last course started
___ / ___ / _____ Date
mm dd yyyy

B9B Total number of courses
0. incomplete
1
2

B10. Were any medications given for tocolysis?
1. No
2. Yes

B10A If YES, specify (*check all that apply*)
1 Indomethacin
2 Magnesium Sulfate
3 Nifedipine
4 Ritodrine
5 Salbutamol
6 Terbutaline
7 Other,specify:

B10B Other, specify: 1, 2.3.etc.

B11. Was magnesium sulfate given for any indication other than tocolysis?
1 No
2 Yes

B12. Maternal smoking?
1. No
2. Yes

Appendix L Prior Painful Procedures

Procedure	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10 etc	Total
Heel lance												
IV Start												
IM Injection												
Lumbar Puncture												
Arterial/Venous Puncture												
Peripheral Art. Line insertion												
Peripheral Art. Line removal												
Long line insertion												
Long line removal												
Chest tube insertion												
Chest tube removal												
Gavage tube insertion												
Suction												
ET/NT tube insertion												
ET/NT tube removal												
Tape removal												
Surfactant administration												
TCPO2 probe change												
Other												

Appendix M Information and Authorization Form

Research Title CO-BEDDING AS A COMFORT MEASURE FOR TWINS UNDERGOING PAINFUL PROCEDURES

Researcher(s)

Marsha Campbell-Yeo, Neonatal Nurse Practitioner, IWK Health Centre
Doctoral Student, McGill University (Principal Investigator)
Céleste Johnston, James McGill Professor, McGill University (PhD Supervisor)
KS Joseph, Dept. of Obstetrics & Gynaecology and Paediatrics, IWK Health Centre and Dalhousie University
Christine Chambers, Dept. of Psychology and Paediatric Pain Centre, IWK Health Centre and Dalhousie University
Nancy Feeley, Assistant Professor, McGill University
Keith Barrington, Department of Neonatology and Professor, McGill University
Funding: Canadian Institutes of Health Research,
Research Nurse: Kim Caddell, RN, MScN

Introduction

You are being invited to take part in the research study named above. This form provides information about the study. Before you decide if you want to take part, it is important that you understand the purpose of the study, the risks and benefits and what you will be asked to do. You do not have to take part in this study. Taking part is entirely voluntary (your choice). Informed consent starts with the initial contact about the study and continues until the end of the study. A staff member of the research team will be available to answer any questions you have. You may decide not to take part or you may withdraw from the study at any time. This will not affect the care you or your family members will receive from the IWK Health Centre in any way.

Why are the researchers doing the study?

Co-bedding is a developmental care practice in which twins are cared for together in one incubator instead of caring for each twin in separate incubators. Swaddled together they are able to freely touch each other. The purpose of this study is to test if during the practice of co-bedding, twins could provide comfort to each other while undergoing an uncomfortable even such as a heel lance for medically needed blood work.
Skin-to-skin contact done with mothers has been found to be comforting and has reduced pain for babies born preterm (newborns between 28 to 36 weeks postmenstrual age). No study has been done to verify the effect of twin contact and pain response. It would be helpful to study if twins could provide similar comfort to each other as part of our management of procedural pain in preterm neonates. If you choose to take part in this study, your twin pair will have an equal chance of being assigned to a co-bedding

condition or a traditional standard care condition. Twins assigned to co-bed will be placed close together in a large incubator. Twins assigned to the traditional standard group will receive care separately within their own incubator. Twins will be assigned to a group as a pair but each twin will be considered a participant. Each participant will have a medically required heel lance done in a separate isolate/crib (traditional care) or in the same isolate/crib as the other twin (co-bedding).

How will the researchers do the study?

This research study will be conducted at the NICU at the IWK Health Centre in Nova Scotia. This study will enrol 64 sets of twin newborns born at greater than or equal to 28-weeks gestational age.

What will I be asked to do?

If you choose to allow us to include your twins in this study, they will be observed during a medically necessary heel lance. All twins will be closely watched before, during and following the heel lance according to the routine care in the Neonatal Intensive Care Unit.

If your twins are in the study, each will have their face videotaped and have normal body functions (heart rate, oxygen saturation and ECG tracing) measured before (5-10 minutes), during (2-3 minutes) and after (5-15 minutes) the required heel lance. The heart rate, oxygen saturation and ECG tracing are taken from monitors that the twins are routinely connected to. If your twins are not connected to a monitor, it is possible that we may need to put up to 5 leads on your twins to capture these measures. These extra leads will not hurt your twins because they are made especially for preterm newborns. Since saliva contains hormones that show stress, a small sample of saliva will be collected from each twin before and after the heel lance procedure. By placing a "Sorbette", the size of a Q-Tip and made especially to collect saliva from infants, into the space between the inner cheek and gum for no more than one minute, we will gather saliva for the test. The principle investigator or a research nurse will always supervise this sampling and will hold the "Sorbette". We will videotape each twins face separately during the heel lance procedure. The picture will be a close up of each baby. There will be a monitor that you may watch to see what is being recorded.

Finally, the twins IWK Health records will be reviewed by the Principle Investigator or a research nurse after each videotaped session to gather information about the baby's birth history, normal body functioning and the number of procedures he/she has experienced during his/her hospital stay. The majority of the data being collected will be taken from

the medical chart; some questions may require that parents are asked directly by the Principle Investigator or a research nurse. You may decline to answer any of the questions if they make you feel uncomfortable.

If your twins are in the study, no changes to routine standard care other than co-bedding (if enrolled in co-bedding group) were made. Non-pharmacologic measure used to diminish pain response and promote physiological stability routinely used in the NICU such as sucking on a pacifier, containment and sweet tasting 24% sucrose will be given regardless of which group your twins are assigned to.

You will receive a copy of the consent form to keep.

What are the burdens, harms, and potential harms?

There are no known risks for babies participating in this study. Each twin will have continuous monitoring of his/her heart rate, breathing patterns and oxygen saturation as per NICU routine. Each twin will have his/her own equipment assigned specifically to them. If your one or both of the twins develop any unforeseen side effects, such as significant change in oxygen level or change in their heart rate or breathing which is considered unusual for them, the attending physician or delegate notified immediately and the study session will be stopped. Although no research study has shown that co-bedding increases the spread of infection between twins, it still may be possible. Therefore, infection rates will be monitored on an ongoing basis by an independent data monitoring committee.

What are the possible benefits?

Taking part in this study may be of no help to you personally. It is hoped that what is learned will be of benefit other twins undergoing painful procedures.

What alternatives to participation do I have?

The practice of co-bedding twins during a heel lance is not considered part of routine care in the Neonatal Intensive Care. You may choose not to participate in the study and this choice will not affect the level of care that your twins receive at the IWK. If your babies do not participate in the study, the heel lance will be carried out in the standard manner. This includes performing the heel lance with the baby on his/her back or side in his/her isolette or crib. Non-pharmacologic measures used to diminish pain response and promote physiological stability routinely used in the NICU such as sucking on a pacifier, containment and sweet tasting 24% sucrose will be given.

Can I withdraw from the study?

The participation of you and your twins in the study is voluntary. Withdrawing from the study will not affect the care your twins receive at the IWK. Consent may be withdrawn at any time. All data and videotapes would be destroyed and twins who had been enrolled in the in the co-bedding group would be separated.

Will the study cost me anything and, if so, how will I be reimbursed?

Participation in this study will not result in any expenses to you or your babies.

Are there any conflicts of interest?

The research investigators state that they have no conflict of interest or have anything to disclose. This study is conducted in partial fulfillment of the requirements for a PhD for the Principle Investigator.

What about possible proft from commercialization of the study results?

There is no potential profit from commercialization of the study results.

How will I be informed of study results?

Overall research study results were made available to you at the completion of the study. Please indicate in the box following your signature if you wish to have a copy of the results.

How will my privacy be protected?

Your twins will not be identified as a study participant in any reports or publications of this research. Your twins study data and videotapes were kept in a locked file cabinet at the IWK Health Centre. Any data sent to the McGill School of Nursing pain lab for analysis will have your baby's name removed and sent identified only by a code. The code will be kept in a locked file cabinet at the IWK Health Centre. Study records were kept for 5 years past the age of majority. Only the staff involved in the research will see them. Members of the IWK Research Ethics Board may look at the records to make sure that they are correct. Your baby's confidentiality will be maintained.

Saliva samples were coded with a number and sent to McGill University for analysis of cortisol (an indicator of stress). Cortisol samples will not be used for any other purpose. In order to verify the ethical management of the research project, it is possible that a delegate of the Research Ethics Committee or a delegate from the Canadian Institutes of Health Research may review the research data and your twin's charts.

What are my Research Rights?

If your twins become ill or injured as a direct result of participating in this study, necessary medical treatment will be available at no additional cost to you. You signature on this form only indicates that you have understood to your satisfaction the information regarding the participation of your twin infants in the study and agree to their participation as a subject. In no way does this waive you or your twin's legal rights nor release the investigator, the researcher, the study sponsor or involved institutions from their legal and professional responsibilities.

If you have any questions at any time during or after the study about research in general you may contact the Research Office of the IWK Health Centre at (902) 470-8765, Monday to Friday between 9a.m. and 5p.m.

Contact Person

The Principal Investigator carries a study pager at all times. Her name is Marsha Campbell-Yeo. She can be reached at (902) 470-8888, pager 2086. Additionally, a study nurse, Kim Caddell will also be available to answer any questions or concerns following your enrolment, you may call the IWK Health Centre at 470-8888 and ask for her to be paged at pager number 1901 or call her at (902) 470-6704.

Future contact/future research/other use

The video recordings were kept in the hard drive of the principal investigators' computer at the IWK Health Centre. Copies sent to Dr Johnston's lab at the McGill School of Nursing for coding were kept for an undetermined period of time. Both computers have an access code kept confidential and are available only to the research team. These recordings may be used for research and teaching of health professionals and students beyond the scope of this study. There will be no identifying information about you or your twins in these recordings.

Please indicate on the signature page if you consent to the use of your twin's video recordings for research and teaching of health professionals and students beyond the scope of this study.

The twin's medical record may be reviewed by the Research Ethics Committee for assurance of compliance to ethical guidelines. Group results may be presented at a later time, but twins will not be individually identified. These group results were made available upon request.

AUTHORIZATION AND CONSENT FORM

Research title: CO-BEDDING AS A COMFORT MEASURE FOR TWINS UNDERGOING PAINFUL PROCEDURES
PARTICIPANT ID: _____
PARTICIPANT INITIALS: _____

PARTICIPANT CONSENT

I have read or had read to me this information and authorization form and have had the chance to ask questions which have been answered to my satisfaction before signing my name. I understand the nature of the study and I understand the potential risks of reactions. I understand that I have the right to withdraw my children from this study at any time without affecting my children's care in any way. I have received a copy of the Information and Authorization form for future reference. I freely agree to have my children participate in this research study.

Name of Participant: (Print)_____
Name of Participant (Print)_____
Name of Parent (Guardian) _____
Signature of Parent (Guardian) _____
Date:_____ Time: _____
I wish to have a copy of the results sent to me: Yes_____ No_____
Mailing address
_____
I allow the video recordings to be used for teaching of health professionals Yes ___
No___

STATEMENT BY PERSON PROVIDING INFORMATION ON STUDY

I have explained the nature and the demands of the research study and judge that the person authorized named above understands the nature and demands of the study.
Name: (Print)_____

Signature:_____Position:_____

Date: _____ Time: _____

STATEMENT BY PERSON OBTAINING CONSENT

I have explained the nature of the consent process to the participant and judge that they understand that participation is voluntary and that they may withdraw at any time from participating.
Name: (Print)_____

Signature:_____Position: _____

Date: _____ Time: _____

Appendix N Comparison of Facial Actions at Baseline between Groups

Facial Action		N	Mean	Std. Deviation	Sig.	t	df	Sig.	Mean Diff	95% Confidence Interval of the Difference	
Brow Bulge	Standard	61	3.56	10.90							
	Co-bedding	68	8.08	15.72						Lower	Upper
					.005	-1.91	119.68	.059	-4.51	-9.19	.17
Eye Squeeze	Standard	61	3.71	10.37							
	Co-bedding	68	7.66	14.74							
					.016	-1.77	120.40	.079	-3.95	-8.35	.46
Naso Labial Furrow	Standard	61	3.88	10.74							
	Co-bedding	68	6.59	13.89							
					.106	-1.22	126	.224	-2.71	-7.10	1.68

Appendix O Comparison of baseline and Stick LF, HF and LF/HF Ratio

	Group_assignment	N	Mean	Std. Deviation	Std. Error Mean
Baseline_LowF	standard care	45	245.37	500.96	74.67
	co-bedding	56	189.50	484.05	64.68
Baseline_HighF	standard care	45	63.06	133.70	19.93
	co-bedding	56	32.58	55.32	7.39
Baseline_Ratio	standard care	45	8.81	11.13	1.65
	co-bedding	56	7.78	5.62	.75
Stick_LowF	standard care	54	210.96	286.33	38.96
	co-bedding	56	144.41	202.07	27.00
Stick_HighF	standard care	49	45.61	48.39	6.91
	co-bedding	56	46.39	98.29	13.13
Stick_ratio	standard care	54	8.0761	7.60	1.04
	co-bedding	56	6.9852	5.214	.69

		Levene's Test for Equality of Variances		t test for Equality of Means						
	Equal variances	F	Sig.	t	df	Sig. (2-tailed)	Mean Diff	Std. Error Diff	95% CI	
									Lower	Upper
Baseline LowF	assumed	1.012	.317	.568	99	.572	55.87	98.42	-139.42	251.176
	not assumed			.566	92.9	.573	55.87	98.79	-140.31	252.07
Baseline HighF	assumed	8.726	.004	1.550	99	.124	30.47	19.66	-8.53	69.49
	not assumed			1.434	56.090	.157	30.47	21.25	-12.10	73.06
Baseline Ratio	assumed	2.144	.146	.608	99	.545	1.03	1.70	-2.34	4.42
	assumed			.569	61.81	.571	1.03	1.82	-2.60	4.67
Stick LowF	assumed	3.096	.081	1.412	108	.161	66.55	47.11	-26.84	159.94
	not assumed			1.404	95.01	.164	66.55	47.40	-27.56	160.66
Stick HighF	assumed	.994	.321	-.050	103	.960	-.780	15.46	-31.45	29.89
	not assumed			-.053	82.45	.958	-.780	14.84	-30.30	28.74
Stick ratio	assumed	2.341	.129	.880	108	.381	1.09	1.23	-1.36	3.54
	not assumed			.874	93.426	.384	1.09	1.24	-1.38	3.56