Supplemental Table 2 – Summary of the Studies That Evaluated Biomarkers in Parents

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| Author, Year, Country, Purpose and Study Design | Sample Characteristics | Study Procedures and Measures | Main Findings | Strengths and Limitations |
| Castral et al. 2015Country: BrazilPurpose: Examine the concordance of SC reactivity between mothers and their stable preterm infants during routine infant HL while in mother kangaroo care (MKC) and to compare SC between groups of mothers with and without PPDA and their infants during HLDesign: comparative prospective design  | Sample size n = 42Age = 24.5 ± 5.9Ethnicity – Portuguese - 100%% Employed = NR Education (>4 years of college) =9.5%% Married/partnered = 71.4% | Procedure – Mothers SC was collected on days 5 and 30 of their infant’s post-natal life. On the day before the infant’s HL, mothers collected nocturnal (10 PM to 11PM) and morning (6 AM to 8 AM) saliva samples for SC.Mothers were asked not to eat or drink for one hour prior to sample collection. They were asked to bring the samples to the hospital.On the day of the infant’s testing, which occurred between 10 AM and 11AM, saliva was collected 10 minutes prior to the MKC procedure.Then following a standardized protocol, 10 minutes after SC collection, infants were positioned for MKC for a total of 30 mins (10 minutes before HL, during the HL procedure, and 10 mins after HL).Post MKC – SC was collected 20 mins after the infant’s HLSaliva was collected by having the mother chew on a cotton swab for 2 min and then spit the cotton swab into a tube. | No differences were found in SC levels between the pre- and post HL measures (p > 0.05).Nocturnal SC levels were significantly lower than morning levels (p < 0.001). No difference in SC levels between mothers who were and were not hospitalized during saliva collection (p > 0.05).No differences in SC reactivity response levels between mothers with or without PPDA (p > 0.05). | Strengths* Controlled the time of sample collection
* Independent and concurrent evaluation of the effects of MKC on both mothers and infants SC during routine infant HL

Limitations* Small sample size
* Lacked a control group of infants without MKC
* Convenience sample
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| Cong et al. 2015Country: USAPurpose: 1. Determine differences in levels of OT, SC, and self- reported anxiety before, during, and after M-SSC and P- SSC with preterm infants in the NICU.2. Establish relationships between maternal and paternal levels of OT, stress, and anxiety scale during the different phases of SSC. Design: Prospective randomized cross-over design over a two-day study period | Sample sizeMothers - n = 26Fathers - n = 19Age: Mothers - 31.5 ± 6.8Fathers - 35.6 ± 5.9EthnicityWhite:Mothers – 76.9%Fathers – 68.4%African American:Mothers – 19.2%Fathers – 21.0%Asian:Mothers – 3.8%Fathers – 10.5%Hispanic:Mothers – 26.9%Fathers – 26.3%% Employed = NREducation College or higherMothers – 61.5%Fathers – 78.9%High school Mothers – 38.5%Fathers – 21.1%% Married/partnered Mothers – 73.0%Fathers – 73.7% | Procedures – Three study phases were done between 1 and3 PM to be consistent in time and account for diurnal patterns of OT and SC. Parents were randomized to M-SSC or P-SSC on day 1 followed by P-SSC or M-SSC on day 2.**Pre-SSC phase –** Mother or father was asked to arrive at NICU at least 10 minutes before SSC and rest in a chair for several minutes. First, saliva was collected and then they were asked to self-rate their anxiety on a VAS. The infant remained in incubator during this time*.***During SSC duration phase: 30 mins**Infants were held naked with only diapers in a chest-to-chest position and placed upright in a prone position with their arms and legs flexed directly on the parent’s bare chest with their head directed sideways. Once the infant was placed properly, parent and baby were covered with a blanket and the parents’ gown.**Post SSC phase**The infant was transferred back to the incubator after 30 min. Mother/father was redressed and sat at the side of the incubator for 30 mins. During the last 5 minutes, saliva was collected and then parents were asked to self-rate their anxiety level.**Saliva collection procedure**: using the unstimulated passive drool method. No eating, drinking, alcohol or smoking 1 hour prior to data collection. On arrival to hospital parent was required to rinse their mouth thoroughly with water to remove any food particles.Number of SSC sessions = 2 (done over 2 consecutive days)Measures: SC and OT were collected prior to and following KMC or KFC. | Maternal OT were significantly increased from baseline (50.49 ± 20.05 pg/mL) to during-M-SSC (57.95 ± 25.07 pg/mL) and then dropped in post-M-SSC phase (51.50 ± 24.13 pg/mL), p < 0.05. Paternal OT levels were significantly increased from baseline (41.25 ± 25.74 pg/mL), to during-P-SSC (49.78 ± 25.39 pg/mL), and continued to maintain at a higher level during post-30 min of P-SSC (50.10 ± 31.50 pg/mL), p < 0.05.Maternal SC levels were significantly decreased from baseline (0.15 ± 0.12 μg/mL) to during-M-SSC (0.12 ± 0.10 μg/mL), and continuously dropped in post-M-SSC phase (0.10 ± 0.07 μg/mL), p < 0.05 Paternal SC levels were significantly decreased from baseline (0.30 ± 0.29 μg/mL) to during-P-SSC (0.24 ± 0.19 μg/mL), and then increased during post-P-SSC (0.45 ± 0.62 μg/mL), p < 0.05. | Strengths* Prospective comparative study with same infant acting as his or her own control
* Evaluated a dose response effect for KC in both mothers and fathers
* Longitudinal study 2 days
* Controlled for variations in SC by asking mothers and fathers not to smoke, exercise, or drink anything besides water for 60 minutes before and during KC

Limitations* Did not collect biomarkers from the infants
* The majority of parents were White and well educatedd with college or higher education
* 69% of the mothers and 53% of fathers had had prior experience with SSC

Very small sample size |
| Janevski et al. 2016Country: SerbiaPurpose: Measure SC in mothers and newborns before and after SSC in order to assess the effect of SSC on mothers’ and infants’ stress and to estimate the efficacy of collecting saliva samples in newbornsDesign: Prospective, descriptive study | Sample size – n = 35Mothers – n = 100%Age = NREthnicity – Serbian = 100%% Employed = NREducation = NR%Married/partnered = NR | Procedures – SSC was initiated once the infant was stable. SSC was performed daily for 5 consecutive days, for 2 hours between 10AM and 12 noon.SSC – not describeSCC duration – 2 hoursNumber of SSC sessions - 5MeasuresSC was measured using an eye sponge without any stimulationSC was collected prior to and following SSC at enrollment and before and after the first and fifth SSC sessions | SC levels in mothers decreased after SSC. The highest levels of SC were measured before the first and the lowest levels after the first SSC (*p* < 0.001)SC levels in mothers decreased after the fifth session of SSC (*p* < 0.001) | Strengths* Controlled the time for sample collection
* Demonstrated the feasibility of collecting SC samples in infants using an eye sponge under the tongue

Limitations* Range of gestational ages was wide

Small sample size |
| Morelius et al. 2005Country: SwedenPurpose: Primary aim was to investigate how SSC influences indicators of stress in the mother and her infant as measured by SC and mood changes. Secondary aim was to investigate whether there is a difference in the stress response between the 1st and 4th SSC, sessions.Design: Baseline response-paired  | Sample size n = 17First SSC – n = 17Fourth SSC – n = 14 AgeFirst SSC = 29 (± 5)Fourth SSC = 29 (± 4)Ethnicity - Swedish- 100%% Employed = NR Education (>4 years of college)First SSC = 29.4%Fourth SSC = 35.7%% Married/partnered - NR | Procedure – Prior to the infant being removed from the incubator, 30 minutes after beginning, and 30 minutes after completing the 60 minute SSC session, the mother’s saliva samples were collected during the 1st and 4th SSC sessions.Infant was gently removed from the incubator and placed on the mother’s chest in the upright position. Infant was wearing only a hat and a diaper and was placed inside the mother’s gown and covered with extra blankets.SSC duration = 1 hour Number of SSC sessions = 2SC was collected using cotton tip applicators | First SSC - Median post-SSC levels was 32% lower than the pre-SSC level (p = .002) and 27% lower during SSC (p = .01i)Fourth SSC: Median SC level post -SSC were 38% lower than the pre-SSC levels (p = .03) and 20% lower than during SSC (p = .02)No significant differences were found in SC levels between the 1st and 4th SSC sessions | Strengths* Stress response assessed using SC, changes in heart rate, and pain
* Longitudinal study design
* Changes in SC were assessed in both mothers and infants
* Food intake was controlled for prior to specimen collection

Limitations* Small sample size

Missing data associated with insufficient amount of saliva |
| Morelius et al. 2014Country: SwedenMultisite:2 different NICUsLevel-IIILevel-IIPurpose: Evaluate the effects of almost continuous skin-to-skin contact (SSC) after preterm birth on SC, parental stress, parental depression, and breast feedingDesign: Randomized controlled trial | Sample size SSC Mothers n = 18Fathers n = 18StCMothers n = 19Fathers = 19AgeSSCMothers – 31.0 ± 3.5Fathers – 33.5 ± 5.2StCMothers – 33.5 ± 5.3Fathers – 34.0 ± 5.1Ethnicity - Swedish- 100%% Employed = NR Education (>4 years of college)SSCMothers – 83.3%Fathers – 66.7%StCMothers – 78.9%Fathers – 57.9%% Married/partneredSSCMothers – 100%Fathers – 100%StCMothers – 89.5%Fathers – 89.5% | Procedure – Families were randomized to SSC or StC SSC group - Infant was placed in an upright position on the parent’s chest immediately after birth. Almost continuous beginning in the delivery room and continuing for almost 24 hours per day. Parents alternated until the infant’s discharge.StC group - both parents had the opportunity to provide SSC if they wanted to do so.SC samples were collected prior to (baseline) and 30 mins (response) after a videotaped diaper change performed by the mother in a home visit when the infant was one month corrected age.SC samples were collected prior to (baseline) and 30 mins (response) after a videotaped face-to-face session between mother and infant (i.e., 2 mins interaction, 2 mins still-face, and 2 mins interaction) during a home visit when the infant was 4 month corrected age. | No significant differences in the baseline levels of SC between the mothers in the SSC and StC groups at any time point.No significant between group differences in mothers’ SC reactivity at 4months (p = 0.58)Significant positive correlation between mothers’ and infants’ raw baseline SC levels at 4 months corrected age in the SSC group (ρ = 0.65, p = 0.005) but not in the StC group (ρ = 0.14, p = 0.63) | Strengths* First study to report SC in infants who experienced an average of 19 hours per day of SSC
* Controlled the time of sample collection

Limitations* Small sample size
* No saliva collected from fathers

StC group had an average of 7 hours of SSC |
| Neu et al. 2014Country: United StatesMultisite study: 5 NICUsPurpose: Determine whether kangaroo holding of healthy preterm infants over the first eight weeks of an infant’s life facilitates co-regulation of SC between mother and infant.Design: Randomized controlled trial | Sample size n = 79Groups:Kangaroo holding - n = 29Blanket holding - n = 26Control - n = 24Age = NREthnicity – NR% Employed = NR Education (>4 years of college) = NR% Married/partnered =NR | Procedure – Infants and mothers were randomly assigned to one of 3 groups:kangaroo holding, blanket holding, or controlKangaroo and blanket holding groups - mothers were encouraged to hold their infants at least one consecutive hour each day for 8 weeks.Kangaroo holding - mothers held their infants in SSC between their breasts with their blouse or gown or a blanket covering the infants’ backs and a cap or blanket covering their heads. Blanket holding – mothers wrapped their dressed infants in a blanketKangaroo and blanket holding groups – a study nurse visited these mothers weekly for 8 weeks. During visits mothers were encouraged to hold their infants in the assigned manner. Mothers were educated about early child development and infant cues. Nurse visits lasted ~60 mins.Control group – mothers were given no instructions or restrictions regarding holding time or style. Nurse visits to this group were for 10 to 20 minutes weekly for 8 weeks to collect holding diaries. In addition, general experiences with their infants were discussed.At each visit, SC were collected at the time the mother mother picked up her infant, 30 mins after holding began; and 60 mins after holding began.SC was collected using filter paper. The filter paper was placed on the mother’s tongue for 20 seconds to saturate at least the first 2 inches of the paper. | SC levels decreased over the course of each holding session (b = -.006, p < .01). Rate of decline in SC levels did not vary over the 8 weeks (b < .001, p = .09).No significant mother-infant cortisol co-regulation occurred in any group at any holding session or over time. | Strengths* Randomized controlled trial
* Multisite study
* Repeated measures of SC within and across time
* 8-week longitudinal study with serial measures
* Concurrent evaluation of the effects of two holding methods compared to a control group on mother-infant cortisol co-regulation

Limitations* Small sample size
* Sample characteristics were not reported
* Inadequate stressor to test HPA function
* Waiting 20 minutes after 60 mins of holding may have yielded significant differences in the groups

Only one physiologic measure of stress |
| Srinath et al. 2016Country: CanadaPurpose: Compare physiological and biochemical responses in stable preterm neonates and their parents following kangaroo mother care (KMC) and kangaroo father care (KFC)Design: Prospective randomized cross-over design | Sample sizeKMC - n = 26KFC – n = 26Age = NREthnicity - Canadian% Employed = NREducation - NR % Married/partnered - NR | Procedures – KC was provided by the mother or father in a random order based on availability.KC - Infants were held naked with only diapers and hats in a chest-to-chest position and placed upright in a prone position with their arms and legs flexed directly on the parent’s bare chest with their head directed sideways. Once the infant was placed properly, parent and baby were covered with a blanket and an extra sheetSCC duration – 60 minutesNumber of SSC sessions = 2 – the second session was done within 3 days of the first session.Measures: SC was collected prior to and following KMC or KFC. | No statistically significant difference between the change in SC levels in mothers and fathers before and after KC (p=0.70). | Strengths* Prospective comparative study with same infant acting as his or her own control
* Evaluated a dose response effect for KC in infants from both mothers and fathers
* Longitudinal study 3-4 days
* Controlled for variations in SC by asking mothers and fathers not to smoke, exercise, or drink anything besides water for 45 minutes before and during KC

Limitations* Mothers had experiences one to two sessions of KC

Very small sample size |
| Varela et al. 2018Country: CanadaPurpose: Explore the physiological stress responses of fathers during their first SSC with their premature babies Design: Descriptive, longitudinal study | Sample size n = 49Age = 31 ± 4.8Ethnicity – Canadian% Employed = 100 Education (>4 years of college) = 14.1 ± 3.7 years% Married/partnered =NR | Procedure Fathers were asked not to consume nicotine, caffeine, food or drugs for at least one hour before their arrival in the NICU.The bedside nurse in charge of baby prepared and placed the baby on the father’s chest.Six saliva samples and BP readings were taken at the same time (i.e., on arrival to the room, 15 to 30 mins later, immediately before starting SSC, 30 minutes after starting SCC (i.e., midpoint of the SSC), 60 minutes after starting SSC (i.e., end of SCC) and 15 to 30 minutes after the completion of SSCFathers rested in a comfortable chair during sample collection in the infants’ roomSaliva samples were collected using Salivette rolls that were held in the mouth for 40 seconds and stored in tubes at -20°C until processedAutomatic BP cuff was used to take BP readings from the nondominant arm | SC levels decreased over time (Wald Chi- square 39.23, p <0.001). Pairwise comparison of means showed that mean SC levels were significantly lower at the 5th (15 minutes after SCC finished) and 6th (30 minutes after SCC finished) timepoints compared to both the 2nd and 3rd timepoints (i.e., 15-30 minutes after arriving in the room and just before SCC started; p< .001) | Strengths* First study to measure father’s physiological stress responses before, during, and after their first experience of SSC
* Longitudinal assessment of SC
* Controlled for nicotine and caffeine consumption prior to starting the experiment

Limitations* Small sample size
* No control group

Did not control for diurnal variations in SC |
| Vittner et al. 2018Country: United StatesPurpose: Examine changes that occur in infant and parent salivary oxytocin (OT) and salivary cortisol (SC) levels during skin to skin contact (SSC) and whether SSC alleviates parental stress and anxiety while supporting mother-father-infant relationshipsDesign: Randomized cross over study  | Sample size - n = 56Mother - n = 28Father - n = 28Age = Mother – 32.0 ± 1.1 Father – 33.0 ± 1.3EthnicityMother FatherAsian – 3% 3%Black – 11% 14%Hispanic – 18% 14%White – 68% 68%% Employed = Mother - 69% Father - 88%Education (>4 years of college)Mother - 29%Father - 35%% Married/partneredMother - 82%Father - 82% | Procedure - Triads assigned to one of two sequences: Maternal-SSC (M-SSC) on Day 1 and paternal SSC (P-SSC) on Day 2 or P-SSC on Day 1 and M-SSC onDay 2 SCC duration = 60 minsNumber of SCC sessions = 2Pre-SSC (15 min) collected parental OT/SC salivaDuring-SSC (60 min) saliva samples collected during the last 10 minutes Post SSC (45 min) Infant returned to incubator and left undisturbed for 45 minutes then parents’ saliva was collectedMeasures:Salivary OT and SC using unstimulated passive droolVisual Anxiety Scale (VAS) at the same time points as OT and SC collectionsParent-infant interaction was examined prior to discharge on Day 3 via video using (5 min) Dyadic Mutuality Coding | OT levels: Maternal OT levels increased from pre-SSC to during and then decreased post SSC (*p =* <0.001)Paternal OT levels increased from Pre-SSC to during SSC and then decreased post-SSC (*p =* <0.001)SC levels: No significant differences in parental SC levelsParental anxiety:Maternal anxiety decreased from pre to during and post SSC (*p* = 0.001)Paternal anxiety decreased from pre to during SSC and then increased post SSC (*p* = 0.003)Parent-infant interaction: The majority of mothers (67%) and fathers (58%) scored as moderately responsive; 26% of both parents scored as highly responsive; only 7% of mothers and 16% of fathers scored as low responsiveness | Strengths* Established a consistent sampling time (1 to 3PM), approximately 1 hour after infant’s feeding to account for diurnal variations in biomarkers
* Evaluation of the effects of SCC both mothers’ and fathers levels of OT and SC

Limitations* Small sample size
* Primarily White and well educated parents
* Cross over design

Convenience sample |

Abbreviations: BP: blood pressure, Fr/WI: Friedman and Wilcoxon signed ranks test, g: grams, HL: heel lance, hr: hour, HPA: hypothalamic-pituitary adrenocortical axis, KC: kangaroo care, KMC: kangaroo mother care, KFC: kangaroo father care, mins: minutes, mL: milliliters, MKC: Mother kangaroo care, mmHg: millimeters of mercury M-SSC: maternal skin-to-skin contact, NICU: Neonatal Intensive Care Nursery, NR = not reported, OT: salivary oxytocin, pg: picograms, PPDA: post-partum depression and/or anxiety, P-SSC paternal skin-to-skin contact SC: salivary cortisol, SSC: skin-to-skin contact, StC: standard care, µg: micrograms VAS: Visual Analog Scale (measuring subjective emotions),

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