

The Relationship Between Salivary Oxytocin and Intermittent Kangaroo Care

Presented by:
Jessica M. Gordon, PhD, ARNP
University of South Florida
College of Nursing



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

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

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

Research participants will be asked to:

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

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

All information is strictly confidential.

For further information please contact :
Jessica Gordon, USF College of Nursing
PhD Student at 813-469-3129
or
Attend an information session held in the NICU consult room

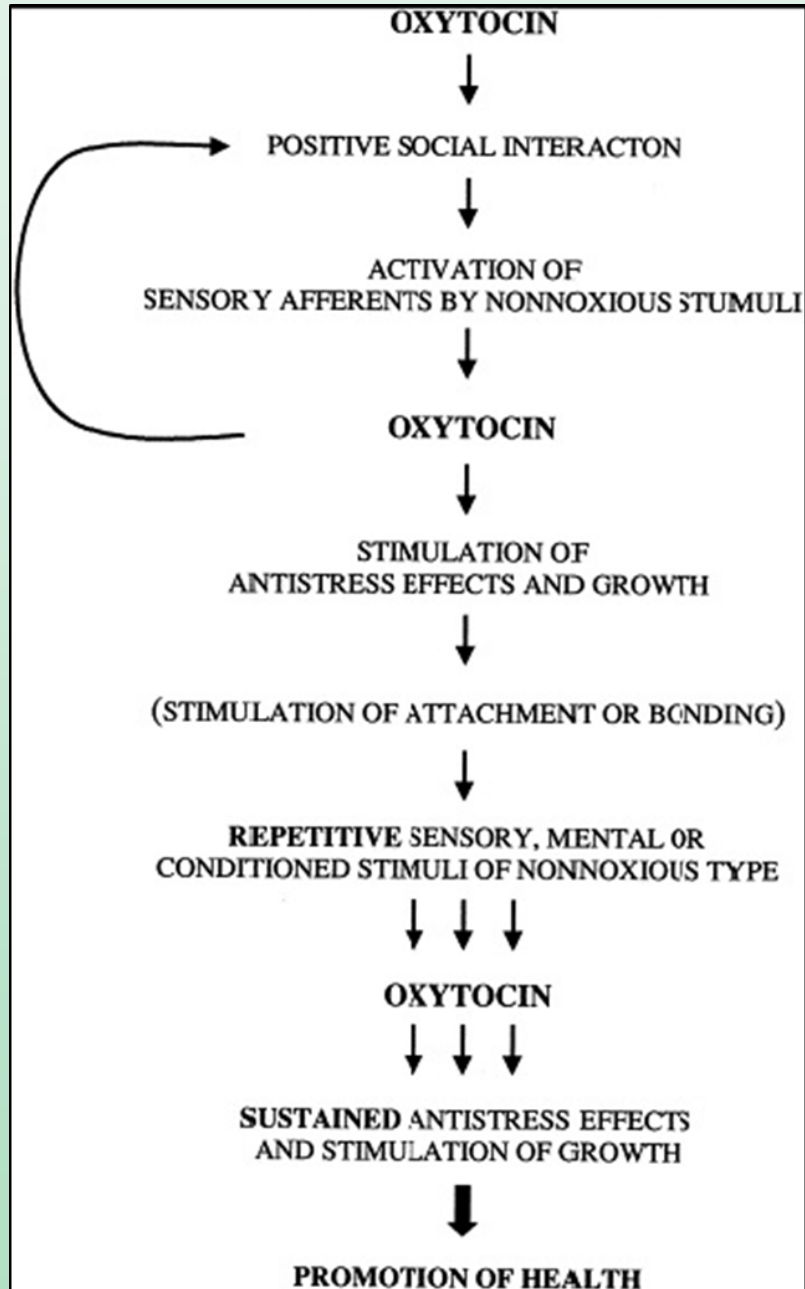
Date & Time TBA

MPMIRB# - 2014.001
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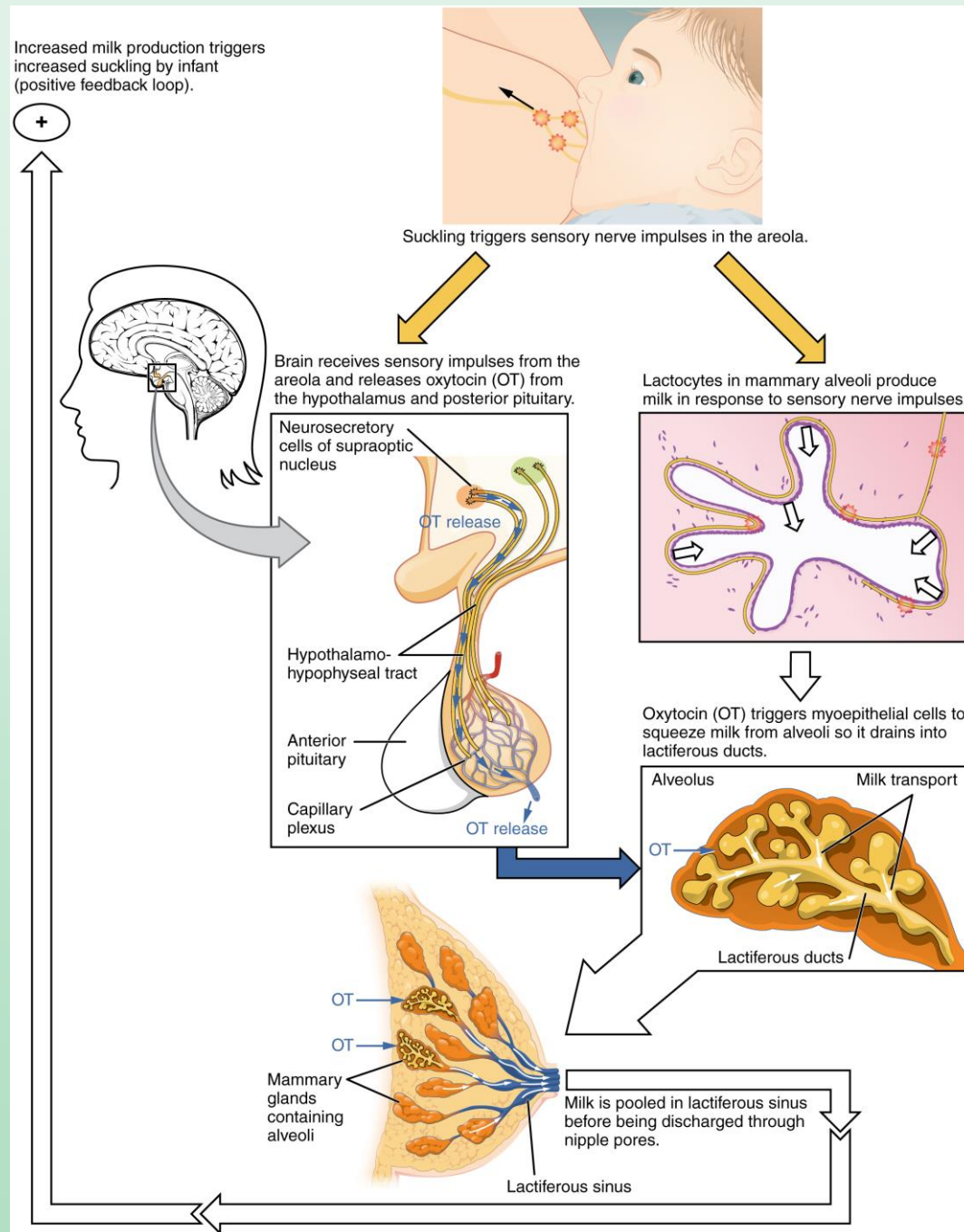
Background

- Prematurity is one of the leading causes of death among infants in the US annually. (Mathew & MacDorman, 2011)
- Immediate postnatal care with an emphasis on neonatal warmth and early initiation of breastfeeding are effective interventions that reduce infant mortality and prevent co-morbidities of prematurity (Robert et al, 2010; Ganapathy, Hay, & Kim, 2012; Meier, Engstrom, Patel, Jegier, & Bruns, 2010).
- Mothers of premature infants experience immediate maternal-infant separation, delayed onset of breastfeeding, poor suckling and physiologic effects of maternal stress and anxiety (Carvalho, Linhares, Padovani, & Martinez, 2009; Padovani, Linhares, Pinto, Duarte, & Martinez, 2008; Ukpog, Fatoye, Oseni, & Adewuya, 2003) .
- Compounding effects of these stressors inhibit the secretion of oxytocin and affect the ejection reflex (McNeilly et al., 1983)



The Possible Role of Oxytocin in Causing Long-term Benefits of Positive Social Interaction

(Uvnas-Moberg, 1998, pg 831)



- A poor ejection reflex perpetuates a cycle that negatively affects the amount of human milk expelled from the mammary glands.
- This in turn leads to altered human milk synthesis and insufficient human milk volume.
- Skin to skin contact (SSC) is expected to reduce maternal stress responses and improve the amount of oxytocin secreted to yield more milk ejected from the mammary gland.
- However, the true relationship between SSC and basal oxytocin levels have yet to be determined

Purpose

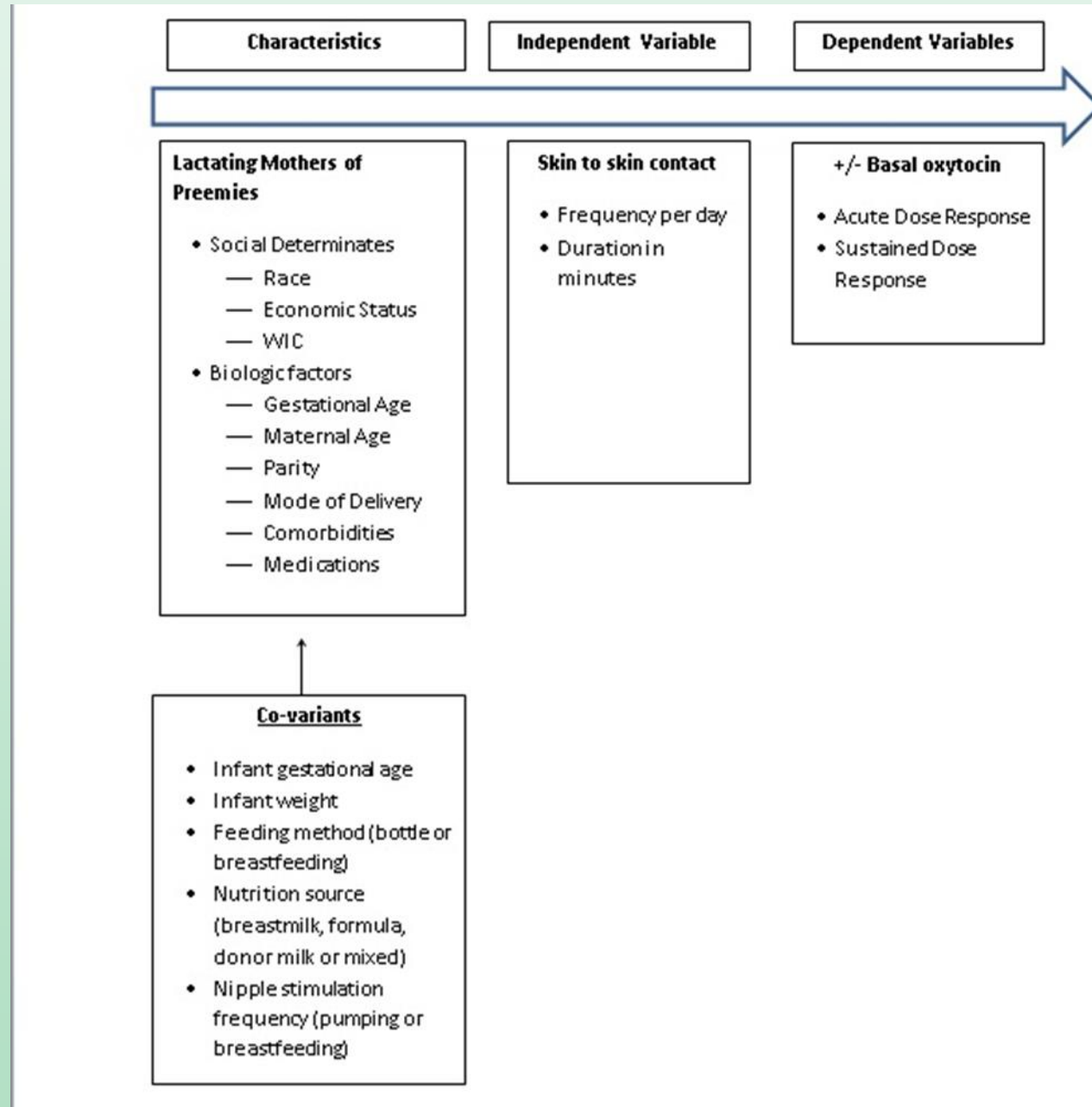
Specific Aim:

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

Research Questions:

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

Logic Model



Methods, Design and Sample



- One-group, repeated measures design
- Setting: BayCare Health Systems level 2&3 NICU
- Sample: 20 post-partum lactating mothers - premature infant dyads
- Inclusion Criteria:
 1. postpartum mothers of premature infants born 37 weeks and 0 days – 22 weeks and 0 days of gestational age
 2. mothers that had given birth within 0 -7 days & intended to breastfeed/pump
 3. initiated pumping within 12-24 hours after birth and
 4. mothers that implemented SSC with her neonate at least once since giving birth
- Exclusion Criteria
 - Contraindications in breastfeeding, saliva samples or SSC

Data Collection

Data collected over a 7 day time frame:

- baseline,
- session 1 and
- session 2

	Baseline	Session 1	Session2
Time Frame	0-7 days post delivery	0-3 days post consent	3 days post session 1
Data Collection	Informed consent	Saliva Sample	Saliva Sample
	Demographics	SSC tracking lod data verified	SSC tracking lod data
	Medical History	and transcribed	verified and transcribed
		Medical Hx update	Medical Hx update
# saliva samples	0	2	2

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

Tracking Log Day 1: 2 / 12 / 14

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

Activity Legend:

SSC – Skin-to-Skin Contact

BF – Breastfeed

BP – Breast pump

S – Saliva Collection

B – Bottle Feeding

* - nipple stimulation during SSC

Feeding Legend:

BF - Breastfed

PM – Pumped milk

DM – Donor milk

F – Formula

TB – Tube feeding

Salivary Oxytocin Collection & Specimen Handling Procedure

Participants were asked to arrive at the NICU at 9:00 am each morning of saliva sample collection



Salivary Oxytocin Assay Specimen Handling Procedure

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

Results

Preliminary Data Analysis

Sample Differences among Physiologic Factors

ID	Age	Gestational Age	Gravida	Para	Mode of Delivery	Comorbidities	Medications
1	19	29	2	2	Vaginal	-	-
2	31	34	2	1	Vaginal	Hypertension	PNV
3	23	34	1	1	Vaginal	Thyroid disease	-
4	19	34	2	2	Vaginal	-	-
5	27	34	6	5	Vaginal	-	-
6	30	32	1	2	Vaginal	Hypertension	PNV
7	31	33	1	1	Vaginal	-	-
8	35	33	2	2	Vaginal	-	PNV
9	28	33	1	1	Vaginal	-	Analgesic
10	19	34	1	2	Cesarean	-	Iron supplement
11	35	32	1	4	Cesarean	-	Analgesic
12	30	34	2	1	Cesarean	-	Analgesic
13	33	34	2	2	Vaginal	-	-
14	30	34	2	2	Vaginal	-	PNV
15	38	35	1	4	Cesarean	Thyroid disease	-
16	34	36	3	2	Cesarean	-	PNV
17	31	34	2	2	Vaginal	-	-
18	33	35	4	1	Cesarean	Hypertension	PNV
19	27	31	2	1	Cesarean	Hypertension	PNV
20	40	37	2	2	Cesarean	-	Iron supplement & Analgesic

- Determined via one-way ANOVA using SPSS software
- **Non-significant for comorbidities gravida and infant weight:**
 - (F(2,17)=0.119,p =0.888)
 - (F(1,18)=0.275,p = 0.660)
 - (F(1,18)=2.165,p=0.158)

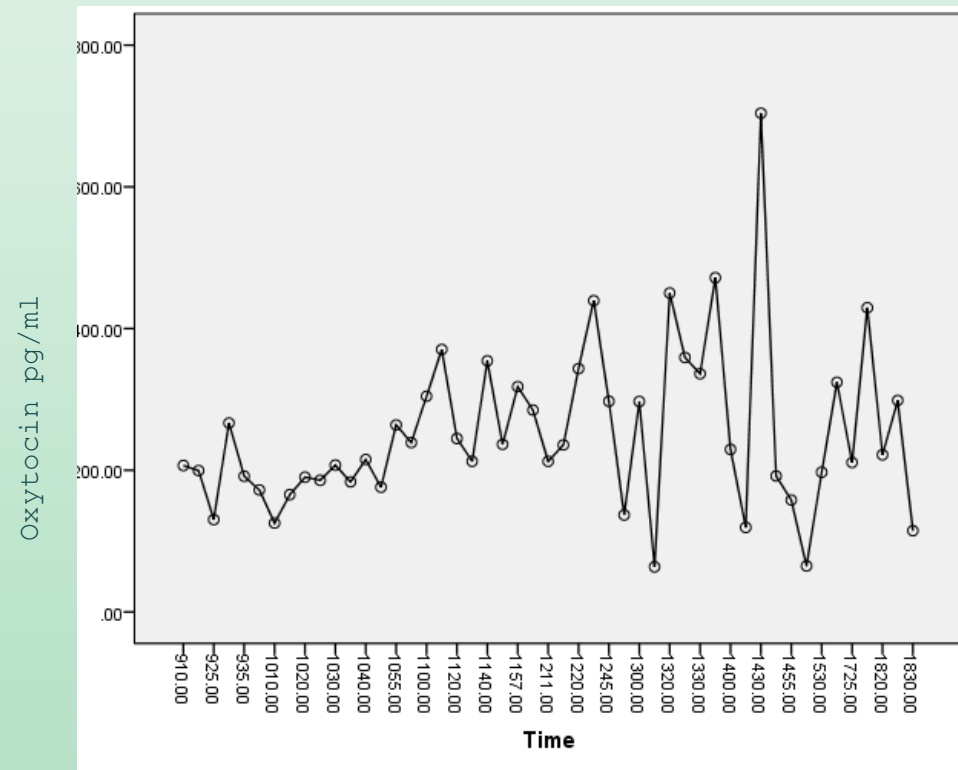
Sample Differences per Social Determinant Factors

ID	AGE	RACE	Ethnicity	Insurance	Marital Status	WIC Eligible
1	19	Black	Non-Hispanic	Medicaid	Single	Yes
2	31	White	Non-Hispanic	Medicaid	Single	Yes
3	23	Black	Non-Hispanic	Medicaid	Single	Yes
4	19	White	Hispanic	Self-pay	Single	Yes
5	27	White	Non-Hispanic	Medicaid	Married	Yes
6	30	White	Non-Hispanic	Private	Married	No
7	31	White	Non-Hispanic	Private	Married	No
8	35	White	Non-Hispanic	Private	Married	No
9	28	White	Non-Hispanic	Private	Married	No
10	19	Black	Hispanic	Medicaid	Single	Yes
11	35	White	Hispanic	Self-pay	Single	Yes
12	30	White	Hispanic	Medicaid	Single	Yes
13	33	White	Non-Hispanic	Private	Married	No
14	30	White	Non-Hispanic	Private	Married	No
15	38	White	Non-Hispanic	Private	Married	No
16	34	White	Non-Hispanic	Medicaid	Single	Yes
17	31	White	Non-Hispanic	Private	Married	No
18	33	Black	Non-Hispanic	Private	Single	Yes
19	27	White	Hispanic	Private	Single	Yes
20	40	Black	Non-Hispanic	Medicaid	Single	Yes

- One-way ANOVA per age group
- **Non-significant for marital status, race and insurance were**
 - (F(1,18)=0.184,p=0.673)
 - (F(1,18)= 0.275,p= 0.660)
 - (F(1,18)= 0.184,p= 0.673)

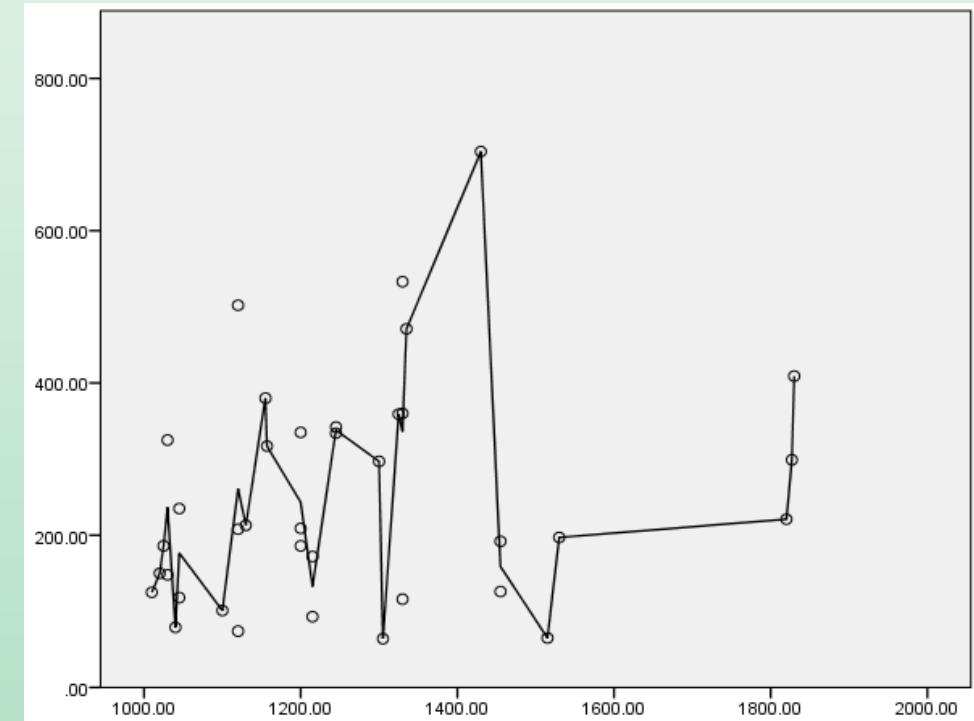
OT Comparison per Time of Day Collected

OT Mean per Time of Day (n=76)



Time of Collection in Military Time

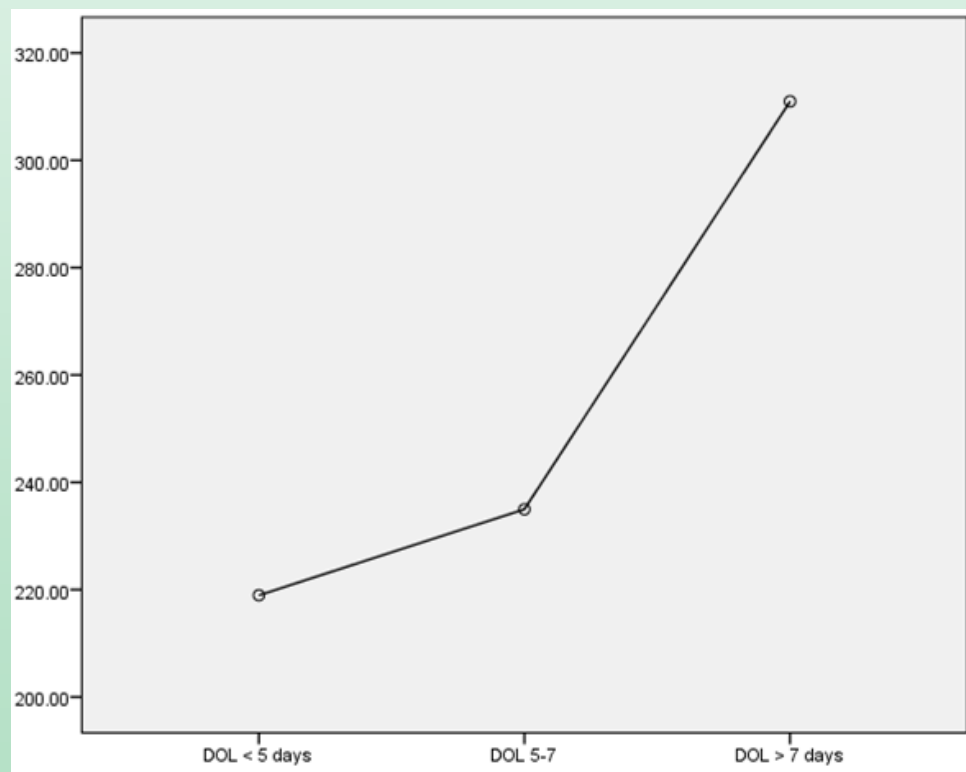
Basal OT Mean per Time of Day (n=37)



Time of Collection in Military Time

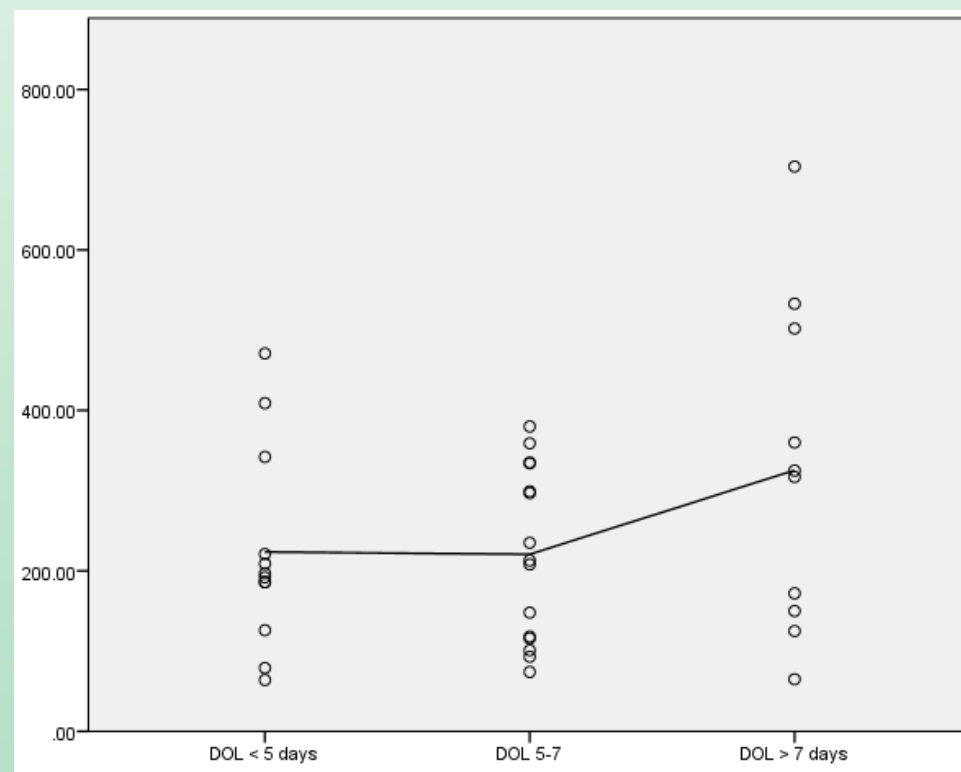
OT Comparison per Day of Life

OT Mean per DOL (n=76)*



(F (2, 73) = 0.3420, p = 0.038)

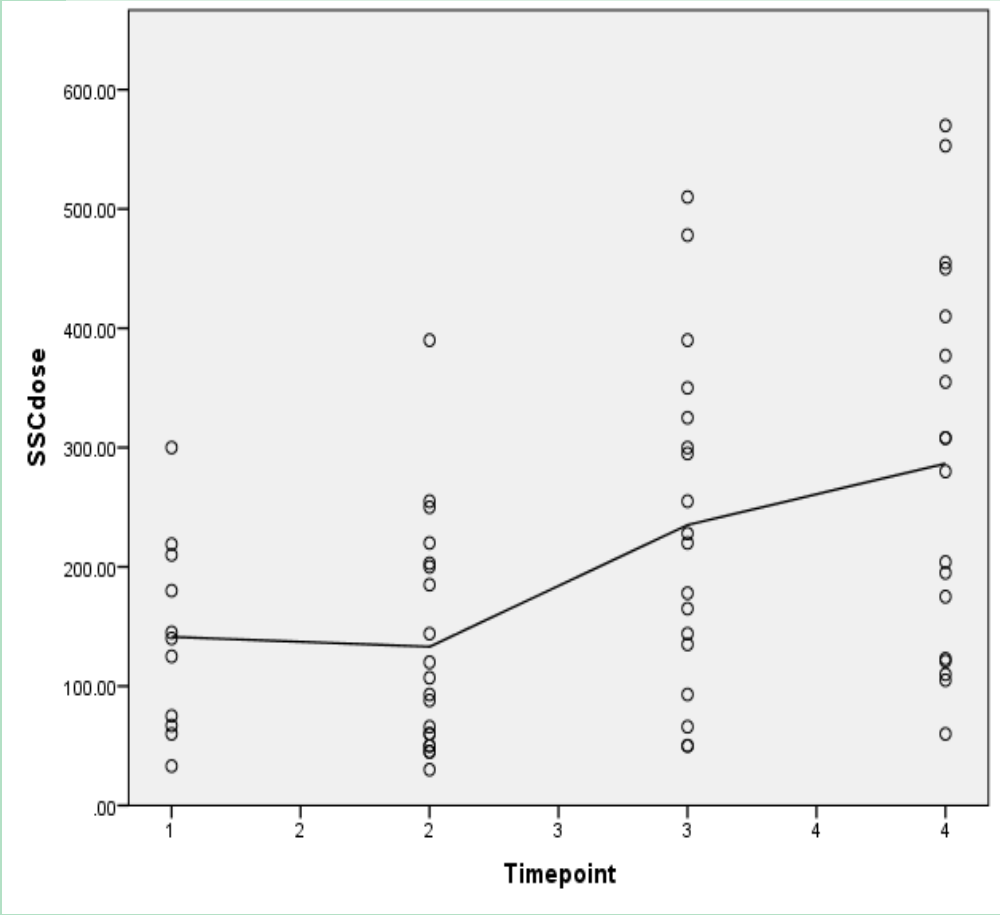
Basal OT mean per DOL (n=37)



(F (2, 36) = 1.860, p = 0.171)

Exposure of SSC on Oxytocin per Time Point

Descriptive Statistics



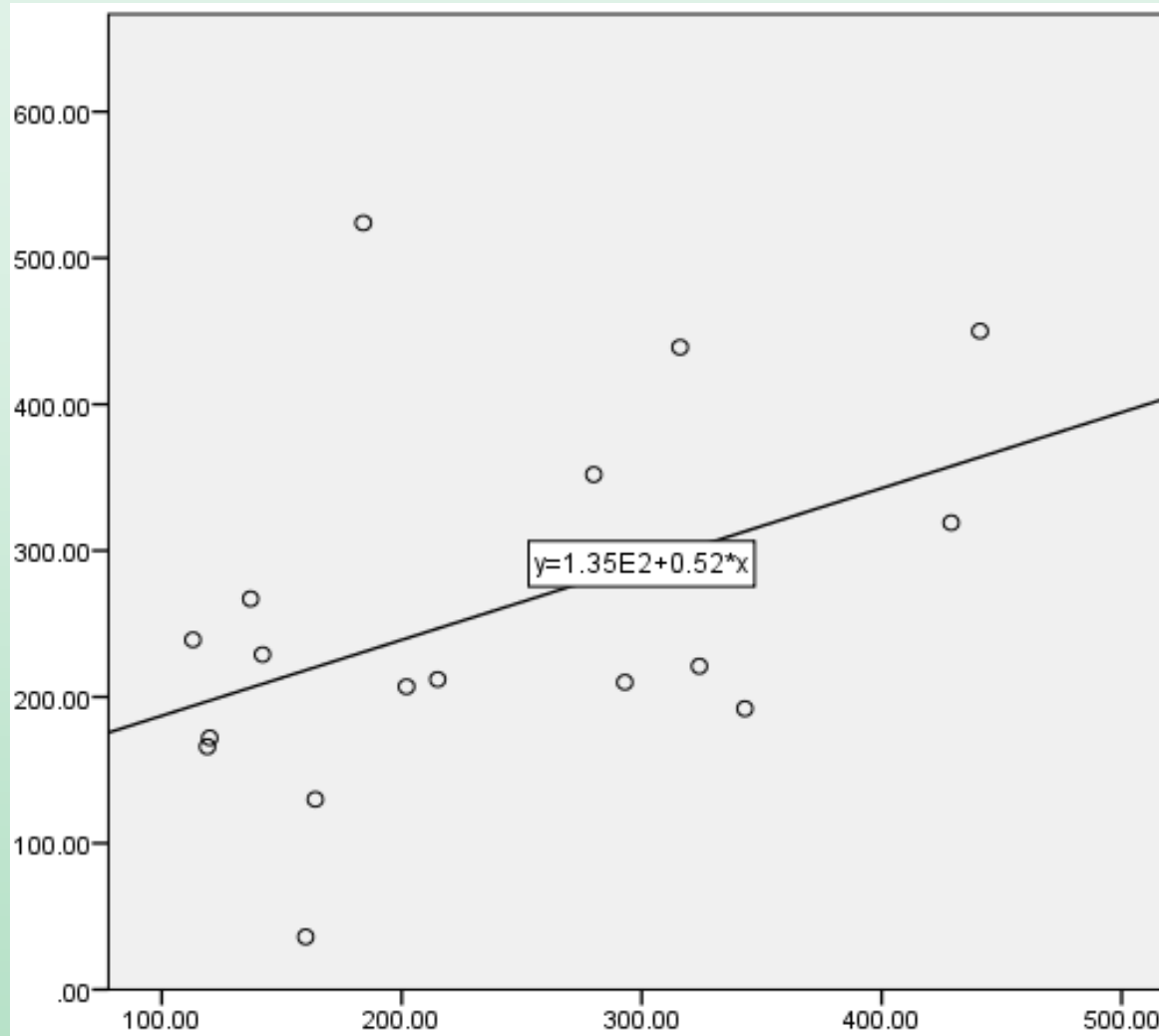
Time Point	Variable	N	Minimum	Maximum	Mean	SD
OT1	SSC minutes	11	33.00	300.00	141.27	81.22
	OT pg/ml	20	113.44	441.77	240.69	96.04
OT2	SSC minutes	20	30.00	390.00	133.05	95.66
	OT pg/ml	20	63.66	471.79	203.76	108.09
OT3	SSC minutes	18	50.00	510.00	235.11	139.77
	OT pg/ml	18	36.03	524.64	262.68	124.13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

Variable	N	Minimum	Maximum	Mean	SD
SSCTmin	20	281.80	294.50	291.2000	5.57033
SSCF	20	4.14	4.40	4.2500	.10075
SSCfmeanperday	20	63.75	78.80	67.9000	6.47523

Results

Statistical Data Analysis

Basal Oxytocin pg/ml at Time Point 3



Basal Oxytocin pg/ml at Time Point 1

Specific Aim 1: Question 1a

Does maternal basal salivary oxytocin differ across 7 days in mothers before SSC?

Paired sample t- test (n =17)

Time point 1 = **M 234 pg/ml**, SD 107 pg/ml

Time point 3 = **M 257 pg/ml**, SD 125 pg/ml

$t(16) = .756$, $p = .461$

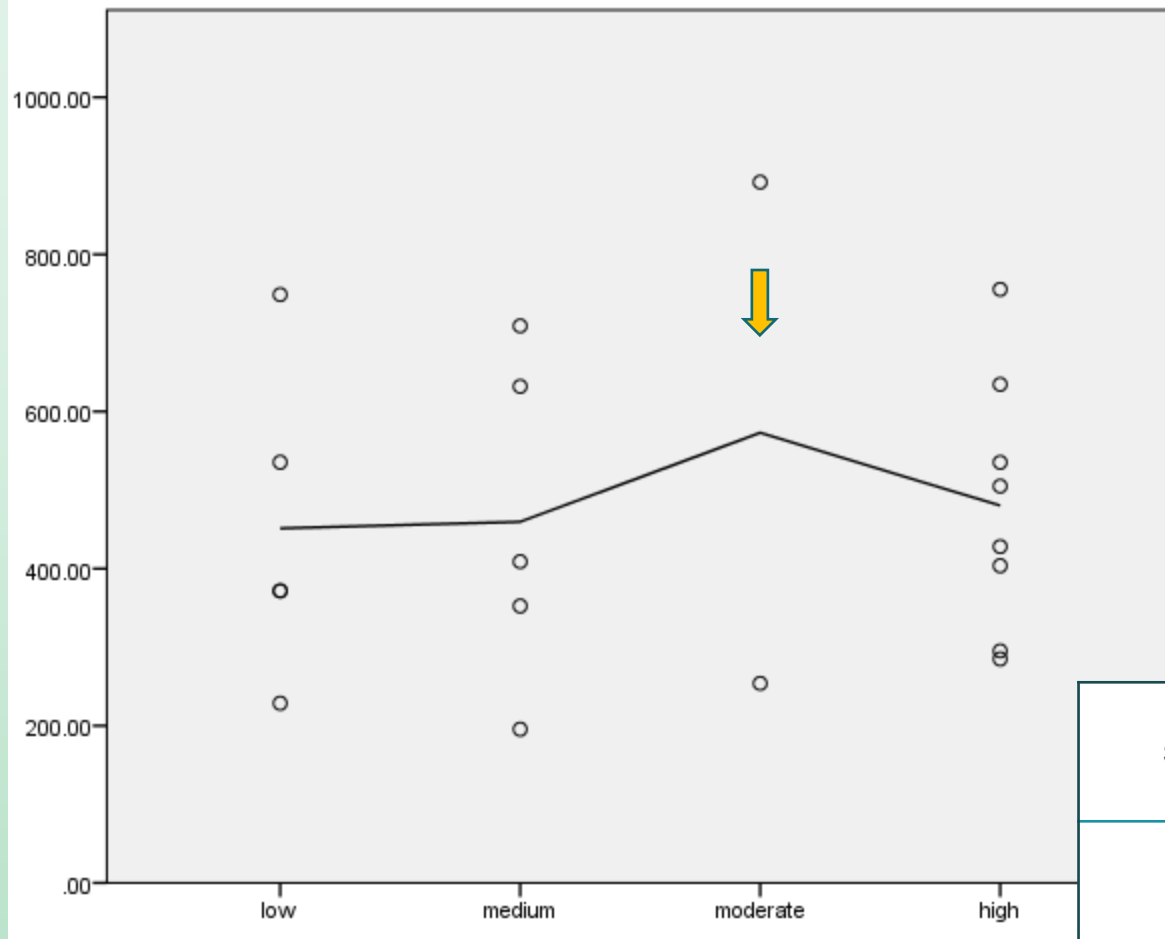
$r = .449$, $p = .071$

Findings:

Non-significant t test

No correlation

Basal Oxytocin pg/ml



Skin to Skin Frequency by Category

Specific Aim 1: Question 1b

**Does SSC frequency
affect basal salivary
oxytocin levels?**

- Descriptive statistics

SSC Frequency Category	N	Mean (pg/ml)	SD (pg/ml)
low	5	451.26	198.64
medium	5	459.59	209.56
moderate	2	572.92	451.15
high	8	480.26	162.20
Total	20	477.11	197.95

Note. low = < 50 minutes of SSC per day, medium = 50-75 minutes, moderate = 75-85 minutes and high = > 85 minutes of SSC per day

Question 1b

Does SSC frequency affect basal salivary oxytocin levels?

Mixed Effects Multilevel Model for SSC Frequency

Omnibus:

$F(1, 16) = 101, p = < 0.001$

Fixed Effects:

$t(16) = 6.389, p = < 0.001$

Coefficient estimates :

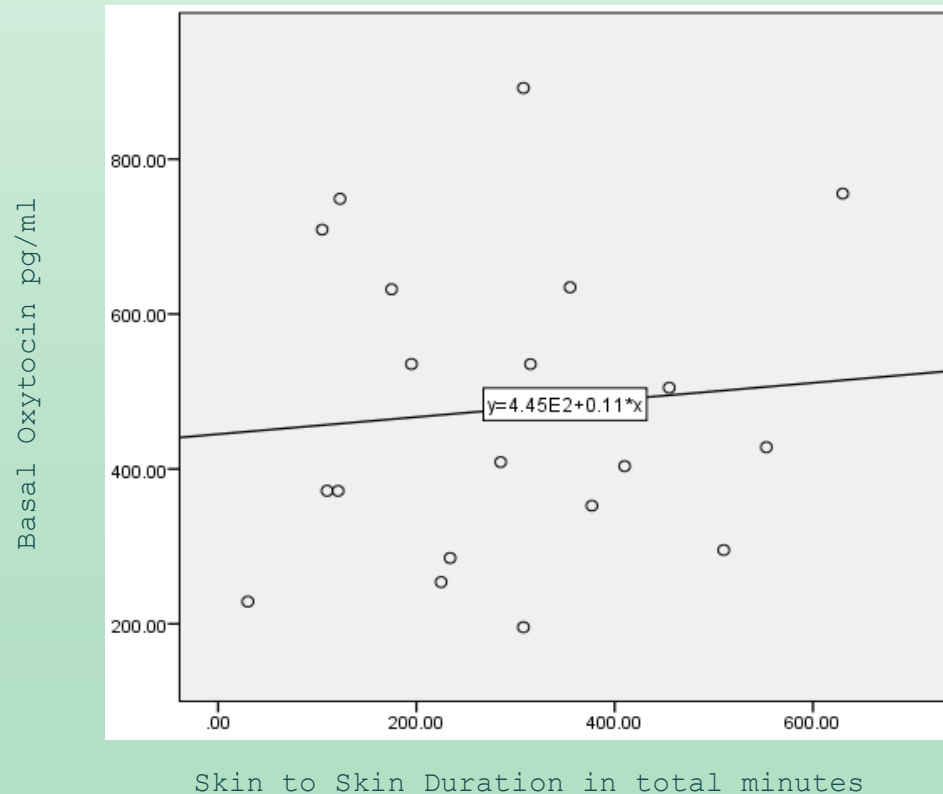
Basal OT exposed to moderate levels of SSC (75-85 minutes of SSC per day) were 92 pg/ml higher than the group mean of 480 pg/ml.

SSC Frequency Parameter	Coefficient (pg/ml)	Std. Error (pg/ml)	df	t	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	480.262562	75.06	16	6.398	.000*	321.137393	639.387732
low	-28.999942	121.03	16	-.240	.814	-285.581568	227.581683
medium	-20.671827	121.03	16	-.171	.867	-277.253452	235.909799
moderate	92.661087	167.84	16	.552	.589	-263.153608	448.475783
high	-	-

Note. * $p < .05$

SSC Duration Descriptive Statistics per Diary Data

Variable	N	Minimum	Maximum	Mean	SD
< 5 days	8	30.00	630.00	294.5000	214.43414
5 days	7	110.00	553.00	294.1429	163.87844
> 5 days	5	175.00	377.00	281.8000	78.33071



Question 1c

Does SSC duration affect basal salivary oxytocin levels?

Omnibus test:

$f(1, 17) = 109.11, p = < 0.001$

Fixed effects:

$t(17) = 6.867, p = < 0.001$

Coefficient estimates:

SSC group > 5 days (M 281 total minutes, SD 80 total minutes) indicate basal OT concentrations are 48 mg/ml higher than average mean basal OT (491 pg/ml)

		OTnoSSC	OTwithSSC	SSCTmin	SSCF	HMtotal
OTnoSSC	Pearson Correlation	1	.575**	.091	-.026	-.223
	Sig. (2-tailed)		.008	.702	.913	.344
	N	20	20	20	20	20
SSCTmin	Pearson Correlation	.091	.399	1	.842**	.585**
	Sig. (2-tailed)	.702	.081		.000	.007
	N	20	20	20	20	20
HM total	Pearson Correlation	-.223	.141	.585**	.750**	1
	Sig. (2-tailed)	.344	.554	.007	.000	
	N	20	20	20	20	20
Nipple stimulation	Pearson Correlation	.042	.326	.142	.101	-.020
	Sig. (2-tailed)	.860	.161	.551	.671	.935
	N	20	20	20	20	20
Feeding method	Pearson Correlation	-.258	.007	.162	.211	.088
	Sig. (2-tailed)	.272	.978	.494	.371	.713
	N	20	20	20	20	20

Additional Data Analysis

Pearson Correlations for OT and covariant variables

1. As SSC increases HM volume continues to increase over time.
2. Beyond this relationship HM volume did not directly predict or significantly affect OT collected with or without SSC.

Discussion

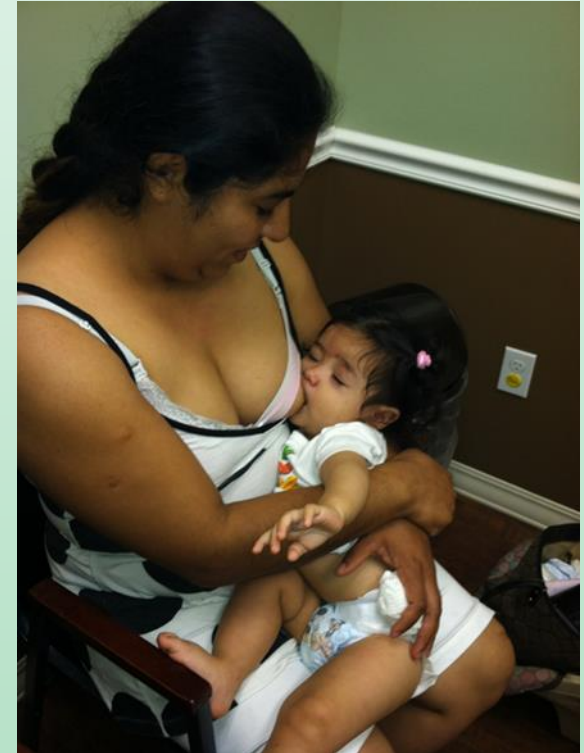
- Prior to this research salivary OT had never been measured during SSC in order to explore the relationship between basal OT and SSC among lactating mothers with hospitalized premature infants.
- Findings are unique and narrow the gap in the literature by satisfying three research questions
- Question 1a, maternal basal salivary oxytocin did differ across 7 days in mothers before SSC but the relationship was non-significant.
- Questions 1b and 1c, SSC frequency and duration did positively affected basal salivary oxytocin levels over the enrollment period.

Limitations

- Measurement Error
 - Means with large SD
 - Validity and reliability of OT as stable measure
 - Opposing views in the literature
- Sample size
 - Effect ($d=0.2$) and power analysis (20%)
 - Underpowered
 - Increase in Type II error – need to repeat these finding with larger sample size
- Design
 - No control group
 - Omission of maternal stress measure
 - Interval data not consistent
 - Self report data (SSC frequency and duration) not as reliable

Conclusions

- Preliminary findings of this study have a high level of significance for nursing science
- This new understanding adds to the importance of continued SSC intervention to promote sustained effects of SSC on the physiology of galactokinesis and maternal mental health
- Results suggest that SSC mediates human milk volume in response to an increase in OT over time.
- Immunologic benefits of breast milk, consumed by the premature infant offers a significant contribution to neonatal health care outcomes by reducing the incidences of neonatal complications associated with premature delivery such as sepsis, NEC, and infant mortality.



Recommendations for Future Research

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



Special Thanks

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 - Maureen Groer, PhD, Denise Maguire, PhD, Lance Wyble, MD, Melissa Shelton, PhD
- Funding via Southern Nurses' Research Society Dissertation Award
- Bay Care Health Systems – NICU and Laboratory Staff
- Family & colleagues
- CON staff and faculty



Questions?

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