Phase 2 Study: Intranasal Oxytocin for Treatment of Infants and Children with Prader-Willi Syndrome in Nutritional Phase 1a

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BACKGROUND AND SIGNIFICANCE

Prader-Willi syndrome (PWS) is a rare, complex multisystem genetic disorder arising from the lack of expression of paternally inherited imprinted genes in the 15q11-q13 chromosomal region. The syndrome includes severe neonatal hypotonia with decreased appetite and impaired suck necessitating assisted feeding (nutritional phase 1a), followed by a period of improved appetite (nutritional phase 1b) (Miller et al, 2011). Weight gain without a change in appetite begins around age two years (nutritional phase 2a). Then an increased interest and awareness of food, with increased anxiety and behavioral problems typically begins around age 4-5 years (nutritional phase 2b) (Goldstone et al, 2012; Miller et al, 2011). This is followed by an insatiable appetite with worsening behavioural manifestations beginning around age 8 (nutritional phase 3) (Goldstone et al, 2012; Miller et al, 2011). These appetite issues are combined with other endocrine problems probably due to hypothalamic dysfunction. The pathophysiological mechanism of the occurrence of the various nutritional phases of PWS is unknown.

Nutritional phase 1a is characterized by the need for assisted feeding. The feeding can typically be accomplished using a special nipple/bottle, placement of a nasogastric tube, or placement of a gastrostomy tube. However, this is a dangerous time for infants with PWS, and has a high rate of morbidity and mortality due to aspiration and respiratory failure (Nagai et al, 2005). In addition to these intrinsic risks associated with the poor suck and swallow in infants with PWS, the delay in development of normal feeding patterns may be the cause of the nearly universal expressive speech delay seen in individuals with PWS (Miller and Plager, 2014). Normal sucking and swallowing, reflect the early developmental pathways that are the basis for later emergence of successful communication skills, so potentially correcting the sucking and swallowing abnormalities in infancy could change speech and language issues in the future. Feeding and swallowing skill development parallels psychosocial milestones of homeostasis, attachment, and separation/individuation. Mothers of infants with PWS often describe a feeling of lack of attachment or lack of bonding with their child due to the lack of suckling and responsiveness of the children.

Oxytocin nasal spray enhances face processing emotion recognition. There is growing evidence that oxytocin abnormalities may underlie repetitive stereotypic behaviors and that administering oxytocin may reduce restrictive repetitive behaviors. As they grow up, individuals with PWS have a clinical phenotype similar to autism. They exhibit disruptions in social engagement, including impairment in communication skills, restricted, repetitive and stereotyped patterns of behavior, and decreased ability for social recognition. Social recognition requires a complex set of processes: social approach and investigation, sensory processing and learning and memory. Oxytocin plays a very important role in social recognition, maternal behavior and maternal–infant bonding (Pedersen & Prange 1979; Kendrick *et al*. 1997; Ferguson *et al*. 2001).

Oxytocin is produced in the hypothalamus, including the supraoptic and paraventricular nucleus (Gainer, 1998). Magnocellular neurons in these nuclei send projections to the posterior pituitary, where oxytocin is released into the bloodstream and has effects on parturition and milk ejection during lactation. Swaab and colleagues (1995) reported a deficit in the oxytocin (OT)-producing neurons of the paraventricular nucleus (PVN) in the brain of these patients and Bittel and colleagues (2007) reported decreased oxytocin receptor gene function in PWS. In addition to decreasing appetite, OT is involved in establishing and maintaining social standards. Indeed, it has recently been shown in a double blind placebo study, that OT administration to adults with PWS significantly decreased depressive mood tendencies and tantrums while increasing trust in others, with data supporting a trend to decrease appetite with higher satiety (Tauber et al., 2011). Moreover, in a PWS

knock-out mouse model for the *Magel2* gene, a single OT injection at 5 hours of life prevented early death observed in 50% of new-born mice by recovering a normal suck (Schaller et al, 2010).

Proposed Mechanism of Oxytocin in Prader-Willi Syndrome – Neonatal Period:

Pathophysiology: Dysfunction of the oxytocin (OT) system has been implicated in the pathophysiology of PWS. Oxytocin (OT) is a nonapeptide synthesized in the paraventricular (PVN) and supraoptic nuclei (SON) of the hypothalamus. It has been proposed to play a role in social recognition, pair bonding, anxiety, maternal behaviors, metabolism, food motivation and hyperphagia. OT receptors are expressed in numerous brain regions including the amygdala, ventromedial hypothalamus, and the brainstem. The number and size of OT neurons in the PVN is significantly reduced in PWS (Swaab et al, 1995; Bittel et al, 2007). Although many investigators have attributed the poor suckling and failure to thrive in infants with PWS to their hypotonia, the poor feeding is accompanied by a lack of interest and/or avoidance of food. The great majority of infants with PWS will not wake to feed, will not open their mouth to accept the bottle, and do not cry for food.

Animal Models:

In animal models, oxytocin has been shown to play a critical role in social processing, recognition, and bonding, and also to influence stereotyped behaviors such as exaggerated grooming. In mammals, OTRs are expressed at higher levels in early development. OT knockout mice have been shown to maintain olfaction and cognitive performance, but suffer deficits in social recognition that were recovered by intraventricular OT but not by AVP administration. Compared to wild type, OTR knockout mice emit fewer ultrasonic vocalizations in response to social isolation, experience deficits in social discrimination, and demonstrate more aggressive behavior.

Human Data:

Synthetic OT (Pitocin/Syntocinon) is a 9 residue cyclic peptide; the hormone is prepared synthetically to avoid possible contamination with vasopressin and other small polypeptides with biologic activity. There have been multiple studies of IN-OT in lactating and non-lactating women which provide data regarding the safety of both the peptide and administration route. These studies have systematically reported none or only minimal side effects. In addition, there have now been at least a dozen published studies using IN-OT in healthy adults; here too, minimal side effects have been reported. Finally, data from a 6 week pilot treatment study in children with autism indicate that the medication was very well tolerated compared to placebo. Side effects of IN-OT may include nasal irritation, runny nose, or tearing of the eyes, shortness of breath, closing of the throat; hives; swelling of the lips, face, or tongue; rash; or fainting. Additional rare side effects reported in single cases and of unknown relationship with the medication include unusual bleeding, convulsions, nausea, drowsiness, headache, anxiety, and sad mood. Uterine contractions may occur in women and are more likely to occur in pregnant women especially at the end of pregnancy. Estrogen mediates Pitocin's effect on uterine muscle. Large doses of **intravenous** OT decrease both systolic and diastolic blood pressure through a transient relaxation of vascular smooth muscle. Any OT-induced decrease in blood pressure is followed by a mild but sustained increase. IN-OT, which will be used in the present study, **has not been found to substantially affect blood pressure.** Because few studies have reported on the use of IN-OT, there is a strong need for the collection of such side-effect data, as proposed in this project (Figure 1).

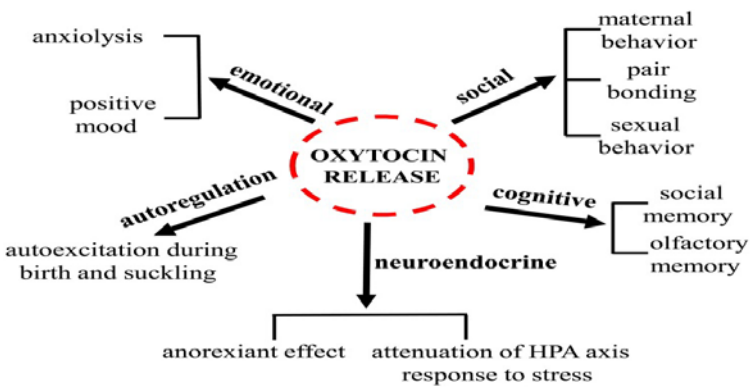

Figure 1: Oxytocin physiologic effects

Rationale for Intranasal Administration

OT is metabolized by chymotrypsin in the GI tract and therefore is not administered orally. Currently, the only form of OT that is available in Canada and the US is the intravenous form and although this formulation has been found to produce behavioral effects, it is invasive to administer and the extent to which this formulation crosses the blood-brain barrier is not known. However, a recent study by Ring and colleagues 2006, speaks to the issue of brain penetration. This study investigated the anxiolytic effects of centrally and peripherally administered OT in male mice. Although substantially larger doses of OT were required for peripheral administration to achieve comparable behavioral effects to those achieved by central administration (suggesting that OT exerts its anxiolytic effects through its action on the CNS), nonetheless, peripheral administration produced behavioral effects. These researchers argue, that "the anxiolytic-like effects of peripherally administered OT can be accounted for by the passage of relatively small, but sufficient, amounts of the peptide across the blood brain barrier (BBB) into the CNS" (Ring et al, 2006). The claim that peripherally administered OT penetrates the brain and exerts its behavioral effects by acting on the CNS was further supported by the finding in this study that a centrally administered OT antagonist that does not cross the BBB was able to fully reverse the anxiolytic-life effects of peripherally administered OT. Intravenous OT, however, is not practical for treatment studies.

One alternative is IN-OT; this form of administration is absorbed through the highly permeable nasal mucosa and has been shown to pass the BBB; it is also easy to self-administer. Withdrawal from the US and Canadian market by the manufacturer of the IN-OT (syntocinon Novartis) in 1997 and 1992 respectively, was not related to any safety issues but was at the request of the manufacturer, for poor market profits. Intranasal oxytocin has previously been approved for marketing in the United States from March 20, 1962 until its withdrawal from the market on August 7, 1997. At the time of its withdrawal from the market, the indication for IN-OT was for induction of labor in adults. The Federal Register notice issued on that date [Federal Register, Vol. 62, No. 152, Docket No. 97N-0326, pp. 42575 –42577], clearly states that this withdrawal was at the request of the manufacturer, because the drug was no longer being marketed. No safety reasons were cited in connection with the withdrawal. The intranasal form of OT remains on the market outside the US and Canada (e.g. Switzerland) and pitocin administered via intravenous infusion is still available in the US and Canada. The use of IN-OT in infants and young children is not anticipated to increase the risk over what is known for older children and adults.

Objective: The overall objective of this Phase 2 trial is to compare the change from baseline to day 5 of IN-OT on suck and swallow competency in infants/children with PWS who are in nutritional phase 1a. (The follow-up swallow study will occur the day after treatment visit day 5.) We have obtained an IND for the use of IN-OT for PWS (IND 120852) and will utilize IN-OT and matching placebo (Manufactured by Novartis as Syntocinon).

Study Hypothesis 1: We hypothesize that replacing OT in infants and children who are in nutritional phase 1a will improve their suck and swallow, potentially even eliminating the need for gastrostomy tubes and nasogastric tubes for feeding, and decreasing the risk of aspiration with oral feeding.

Study Hypothesis 2: We hypothesize that replacing OT in infants and children with PWS will result in improved eye contact, daytime alertness, and feelings of bonding between the parents and the infant.

Preliminary Data: A recent trial of IN-OT in infants with PWS enrolled both males and females, ages 1-6 months. Treatment with IN-OT resulted in improvements in suck and swallow in this population, with no safety concerns (personal communication: Tauber et al, submitted; Figure 2). IN-OT has been used safely in multiple human studies involving men and women and is well-tolerated in both adult and pediatric PWS populations and we expect a similar safety profile in our proposed study.

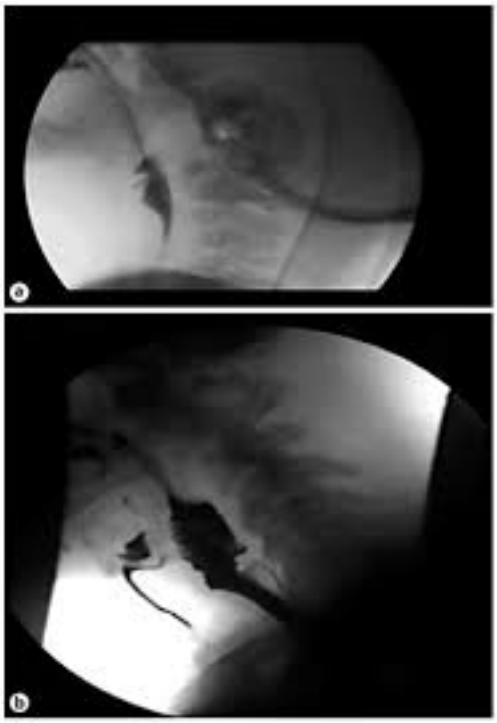

Figure 2: Effects of IN-OT on suck/swallow in PWS. (a) Poor suck/swallow competence in infant with PWS; (b) improved swallowing with decreased aspiration risk after 5 days of IN-OT

Summary: Given the fact that there has only been one previous study investigating the use of IN-OT in infants with PWS, it is necessary to confirm whether IN-OT can improve suckling and swallowing in these babies. If this medication helps, it could change the natural history of the syndrome by preventing the need for gastrostomy tubes or nasogastric tubes and reducing the risk of morbidity and mortality from aspiration and choking.

In selecting an orphan population, PWS, with a known specific genetic locus, and in selecting a treatment, IN-OT, that specifically matches the underlying deficit in the structure and function of OT neurons in PWS, we expect that this study will have substantial power to detect changes in these infants with administration of IN-OT.

Innovation:

This proposal investigates the use of IN-OT for feeding issues in infants with PWS, an orphan disease with well-described high morbidity and mortality in the infant period. PWS is characterized by a known genetic and neuropathological mechanism (disruption of chromosome 15q11-q13 and a decreased of number and size of OT-producing neurons in the paraventricular nuclei of the hypothalamus), and IN-OT in infants and children targets the specific pathophysiology of the condition (faulty OT signaling) to a specific risk factor in this disorder. This novel drug development approach provides a substantially greater chance of detecting a drug vs. placebo signal in a more targeted and homogeneous population.

Approach:

We will evaluate the effects of IN-OT compared to placebo on suck and swallow competency of infants with PWS using videofluoroscopy swallow study. Competency of suck/swallow will be determined by a board-certified speech and language pathologist who will be blinded to the treatment arm of each patient. Additionally, penetration and aspiration risk will be assessed during the videofluorscopic study to assess safety of oral feeding. Additionally, we will have the speech pathologist evaluate each infant and complete an Early-Feeding Skills Assessment (EFS) form prior to the first swallow study evaluation and on Day 5, one day before the final swallow study (Appendix 1).

Standard of care recommendation for infants with PWS is a videofluoroscopy swallow study prior to being discharged home on any oral feeding. This test is often repeated clinically up to 5 times per patient, depending on their degree of swallowing dysfunction. Therefore, this study will not be adding significant radiation risk beyond what is typically performed clinically.

The Mother-Infant Bonding Questionnaire (MIBQ) has been widely used to assess maternal emotional involvement with infants, and has been validated in the postpartum period (Klier, 2006; Ohara et al, 2016). This questionnaire will be administered before the first medication/placebo administration and at the completion of Day 5 of the study (Appendix 2).

The Tobii Eye Tracking system has been used extensively and validated in evaluating infant gaze (Jakobsen et al, 2016; Smith et al, 2013). Standardized assessments are available using this system with recording done during the assessment using a digital video camera. Areas of interest are defined around the eye and mouth region and infants are shown pictures of female faces displaying four different dynamic sequences, each lasting approximately 16 seconds (Figure 3).

Figure 3: Face presentation during eye tracking experiment

Dosing and Administration: The dose of OT to be used in this study was determined based on data from IN-OT trials in infants with PWS in Toulouse, France (Tauber et al, submitted). Doses administered in that study were: 4 IU/day of IN-OT; 4 IU twice a day; or 4 IU every other day.

Based on personal communication with the PI of that study, we will use a dose of 4 IU/day of IN-OT, as that was the lowest effective dose in that study (Personal communication, Tauber et al). We propose to administer 4 IU/day IN-OT in 66% of study population and 4 IU/day of placebo in 33% of the study population. Biasing the study towards intervention vs. placebo will allow for incentive for parents to have their children participate in the trial. IN-OT and placebo will be administered via a rhinal tube device each morning. This will be a double-blinded study, as no studies to date have compared this treatment in infants with placebo.

Research Strategy and Feasibility: We propose a 5 day intervention with IN-OT or placebo. Individuals will be brought into the Clinical Research Center (CRC) for initial evaluation by the PI and speech pathologist. A baseline evaluation, consisting of obtaining feeding history, physical examination, baseline laboratory evaluation, administration of the MIBQ, administration of the EFS, and videofluoroscopy swallow study will be performed. Participants will then be randomized to receive either IN-OT or IN-placebo via a computerized randomization program in the Investigational Pharmacy at the University of Florida (2:1 randomization drug: placebo). The participants will be seen daily for the next 5 days, during which they will receive intranasal medication or placebo once a day, and will have repeat EFS and videofluoroscopy swallow study on the day following Day 5 of the study.

Screening Day

Screening visit must take place within 10 days prior to study visit day 1. During the screening visit the following will events will occur:

- Parents will participate in the informed consent process
- Review of medical history
- Review of concomitant medications

- Physical exam by PI
- Vital signs (T,P,BP,R)
- Height and weight
- Blood draw for glucose, potassium, sodium
- Blood draw for study labs (OT, ghrelin, insulin, cortisol, orexin A),

Day 1

Subjects will present to clinic/CRC for their scheduled day 1 study visit. During this visit, the following will occur:

- Vital signs
- Weight
- Physical exam by PI
- Early-Feeding Skills Assessment (EFS) by speech pathologist
- Completion of Mother-Infant Bonding Questionnaire (MIBQ) by parent
- Eye Tracking measurement
- Videofluoroscopy swallow study
- Vital signs (T,P,BP,R) - pre and immediately post OT administration, then every 15 minutes X4
- Administration of first dose of 4 IU IN-OT or placebo

OT or placebo will be given by the PI, who has been trained in the method of administration of using a rhinal tube to administer the nasal spray, and who will give the drug or placebo to every patient enrolled in the study. The spray will be given as 1 dose (4 IU) measured squirt into one nostril (nostril will alternate daily) via rhinal tube device. Vital signs will be taken immediately after the administration is complete, and every 15 minutes times four.

Day 2

Subjects will present to the clinic/CRC for day 2 study procedures. During this visit the following will occur:

- Focused physical exam by PI
- Vital signs (T,P,BP,R) - pre and immediately post OT administration, then every 15 minutes X4
- Weight
- Blood draw for safety labs (glucose, potassium, sodium), prior to OT administration
- IN-OT or placebo administration

Day 3

Subjects will present to the clinic/CRC for day 3 study procedures. During this visit the following will occur:

- Focused physical exam by PI
- Vital signs (T,P,BP,R) - pre and immediately post OT administration, then every 15 minutes X4
- Weight
- Adverse event evaluation
- OT administration (see visit 1 for detailed instructions)

Day 4

Subjects will present to the clinic/CRC for day 4 study procedures. During this visit the following will occur:

- Focused physical exam by PI
- Vital signs (T,P,BP,R) - pre and immediately post OT administration, then every 15 minutes X4
- Weight
- Adverse event evaluation
- OT administration (see visit 1 for detailed instructions)

Day 5

Subjects will present to the clinic/CRC for day 5 study procedures. During this visit the following will occur:

- Focused physical exam by PI
- Vital signs (T,P,BP,R) - pre and immediately post OT administration, then every 15 minutes X4
- Weight
- Adverse event evaluation
- OT administration (see visit 1 for detailed instructions)
- Blood draw for safety labs (glucose, potassium, sodium), prior to OT administration
- Blood draw for study labs (OT, ghrelin, insulin, cortisol, orexin A), prior to OT administration
- Early-Feeding Skills Assessment (EFS) by speech pathologist
- Completion of MIBQ by parent

Day 6 – Videofluoroscopy swallow study; Eye Tracking measurement

Study Intervention: OT and placebo will be administered intranasally. There have been several trials of IN-OT in children, adolescents, and adults with PWS, and one successful trial of IN-OT in infants with PWS. To date, no trials of IN-OT in individuals with PWS have recorded any serious adverse effects and the treatment has been well tolerated.

Follow-Up: Parents will be contacted by phone one week and one month after their child receives his/her last dose of study medication/placebo. During this phone call parents will be asked about any side effects or concerns regarding the study treatment and/or study procedures. Parents will be reminded to contact the PI or Sub-I with any questions or concerns regarding the study.

Recruitment of Participants: Participants will be recruited from the clinic patients of the PI.

Outcome Measures: Project outcomes will be determined by changes in eye tracking and in aspiration and penetration on videofluoroscopy swallow study from baseline to the day following Day 5 (i.e., Day 6), and comparisons of Day 6 measures between active treatment and placebo groups.

Data and Safety Monitoring Plan

The study protocol will be reviewed and approved by the Food and Drug Administration (FDA) before submission to the UF-IRB for approval. Participant enrollment may only begin with IRB approved consent forms.

This is an interventional pilot study that meets the federal definition of moderate risk.

Safety measures in place to mitigate risk

The primary safety measures in place to mitigate risks are close monitoring of the participants during the study, 24 hour availability by pager for caregiver clinic concerns, abilities to schedule additional appointments whenever clinically required and close monitoring of laboratory parameters. If a subject requires emergency care, they will be advised to call 911 and preferable be taken to the nearest emergency room. They should also call the 24 hour beeper so that the investigator on call may contact the emergency room.

All risks will be reviewed in the consent form, and families will be informed of their right to refuse any procedure or withdraw from the study at any time. Trained and experienced staff will conduct all medical assessments. Individuals who are experiencing any worsening of symptoms identified by PI or speech pathologist will be discontinued from the trial and provided with appropriate referrals for follow-up care.

Lastly, the patients will be contacted by telephone one week and one month after study termination for each subject to assure that discontinuation of the medication has not caused any side effects or distress. The

subjects will be advised to call the PI or the 24 hour beeper for any concerns for the month past termination, and will always be able to set an appointment in addition to study visits, to address concerns regarding safety.

Study Oversight

The PI has primary oversight responsibility of this clinical trial. The PI will assemble a Data (Observational) Safety Monitoring Board (D/OSMB) which will have oversight responsibility of the Data Safety Monitoring Plan (DSMP) for this clinical trial. The D/OSMB will review accrual, patterns and frequencies of all adverse events, and protocol compliance every 6 months. The D/OSMB will make recommendations regarding the continuation status of the protocol.

The Principal Investigator and research team (co-Investigator, research nurses, clinical trial coordinator, and data manager) are responsible for identifying adverse events. Aggregate report- detailed by severity, attribution (expected or unexpected), and relationship to the study drug/study procedures – will be available from the DMCC for site review. Adverse events will be reviewed monthly by the research team. The research team will then evaluate whether the protocol or informed consent document requires revision based on the reports.

Definitions and Standards
The FDA defines an adverse event as: "…an unfavorable and unintended sign, symptom or disease associated with a participant's participation in a medication trial."

Serious adverse events include those events that: "result in death; are life-threatening; require inpatient hospitalization or prolongation of existing hospitalization; create persistent or significant disability/incapacity, or a congenital anomaly/birth defects."

An unexpected adverse event is defined as any adverse experience…the specificity or severity of which is not consistent with the risks of information described in the protocol.

Expected adverse events are those that are identified in the research protocol as having been previously associated with or having the potential to arise as a consequence of participation in the study

All reported adverse events will be classified using the current version of the Common Terminology Criteria for Adverse Events (CTCAE) developed and maintained by CTEP at National Cancer Institute.

Adverse Events and Risks:

Intervention

The most recent monograph for the intranasal form of the oxytocin medication only lists allergic rhinitis, and uterine contractions as side effects. Below we have listed other known side effects of oxytocin. Because very few studies have reported on the extended use of intranasal OT, there may be other unknown side effects. The PI (or a covering clinician) will be available at all times to study participants in the event of a clinical emergency; both this availability and how to reach the investigators in an emergency will be clearly communicated orally and in writing to study participants. All study interventions will be provided free of cost.

The following side effects are fairly common with intranasal oxytocin treatment and are usually mild:

- Dry or runny nose
- Headache
- Nausea
- Nasal irritation
- Tearing of eyes

Side effects that are less common, but may require medical attention include:

- Unusual bleeding (vaginal)
- Excessive drowsiness
- Lack of appetite

Side effects that are rare but serious include

- Alterations in blood pressure (high or low)
- Changes in level of alertness
- Convulsions

Medical monitoring

The medical monitoring for the study will be done by the PI and co-investigators. The PI has overall responsibility for monitoring the integrity of the study data and participant safety. This information, as well as any other unanticipated problems involving risks to subjects or others, will also be reported to the FDA.

Study Procedures

Venipuncture: The risks of drawing blood from a vein include discomfort at the site of puncture, possible bruising and swelling around the puncture site, rarely and infection, and uncommonly faintness from the procedure.

Swallow Study: The radiation exposure from the videofluoroscopy study is thought to be minor. However, the effects of radiation add up over a lifetime. Repeated exposures may increase risk of injury or disease. When deciding to enter this study, we will ask patients to consider previous and future potential exposures. Examples would include x-rays taken for a broken bone or radiation therapy treatments for cancer.

Reporting Timeline for adverse events

- Within **24 hours** (of learning of the event), investigators must report any reportable Serious Adverse Event (SAE) that:
  - Is considered life-threatening/disabling or results in death of subject

  -OR-
  - Is Unexpected/Unanticipated
- Investigators must report all other reportable SAEs within **5 working days** (of learning of the event).
- All other (suspected) reportable AEs must be reported to the RDCRN within **20 working days** of the notification of the event or of the site becoming aware of the event.

Local institutional reporting requirements to IRB, any GCRC oversight committee and the FDA, if appropriate, remain the responsibility of the treating physician and the Study Chair.

Serious adverse events: The PI and will determine causality (definitely not related, probably not related, possibly related, probably related, definitely related) of the adverse event. This will be reviewed with each event by the D/OSMB. The D/OSMB may request further information if necessary and possibly request changes to the protocol or consent form as a consequence of the adverse event. A back-up notification system is in place so that any delays in review by the D/OSMB beyond a specified period of time are forwarded to a secondary reviewer. The Adverse Event Data Management System (AEDAMS) maintains audit trails and stores data (and data updated) and communication related to any adverse event in the study.

Non-serious expected adverse events: Except those listed above as immediately reportable, non-serious expected adverse events that are reported to or observed by the investigator or a member of his/her research team will be submitted to the D/OSMB in a timely fashion (within 20 working days). The events will be presented in tabular form and given to the D/OSMB on a monthly basis. Local site investigators are also required to fulfill all reporting requirements of their local institutions.

Study Discontinuation
The FDA or IRB have the authority to stop or suspend this trial at any time. This study may be suspended or closed if:

- Early stopping rules have been met (evidence of clear benefit to individuals with PWS before accrual has been met)
- Accrual has been met
- The study objectives have been met
- The Study Chair / Study Investigators believe it is not safe for the study to continue
- The FDA suspends or closes the trial

5.7 Subject Discontinuation
An intent to treat approach will be used. All data acquired prior to termination for the reasons outlined below will be included in the primary analysis unless patient withdraws consent. Every effort will be made to conduct a final study visit with the participant and participants will be followed clinically until, if applicable, all adverse events resolve.

- Withdrawal of consent
- Withdrawal by the participant's legal guardians
- Withdrawal by the investigator
- Intercurrent illness or event that precludes further visits to the study site or ability to evaluate disease (e.g.-mental status change, large pleural effusion).

Inclusion Criteria:

1. Individuals with genetically confirmed PWS who are in nutritional phase 1a, as determined by PI
2. Physical exam and laboratory results that are within the normal range.
3. Presence of a parent/caregiver/guardian that is able to consent for their participation.

Exclusion Criteria:

1. Exposure to any investigational agent in the 30 days prior to randomization.
2. Prior chronic treatment with oxytocin.
3. A medical condition that might interfere with the conduct of the study, confound interpretation of study results or endanger the subject's well-being.

Statistical Analyses: Analyses will be based on the intent-to-treat approach in which subjects are analyzed in the group to which they were originally assigned regardless of treatment compliance. All variables will be initially screened for inconsistent or abnormal values, and continuous measures will be assessed for skew and outliers. Transformations to improve normality will be applied when necessary. The demographic characteristics of all randomized patients will first be summarized using standard descriptive statistics (means, medians, standard deviations, and ranges for continuous variables and frequencies for categorical variables). Although subjects will be randomized 2:1 to the two treatment groups, imbalances in patient characteristics may still occur by chance given the limited sample size of the trial. Between group differences in the baseline demographic and other prognostic variables will be assessed using the chi- square test for discrete variables and the T-test or Wilcoxon rank sum test for continuous variables. Baseline characteristics which are found to be significantly different will be considered for inclusions as potential confounders in secondary analyses of treatment effects.

Summary of Analytic Approach: The primary outcome measure is change in competency of suck and swallow as determined by videofluoroscopy.

Detailed Description of Analyses

1. Preliminary Analyses:
    a. Primary Analyses: Analyses for Specific Aim (1): To compare IN-OT vs. placebo with respect to improvement in the competency of suck and swallow.

b. Early Feeding Skills Assessment- Improvement
    i. All tests will be two-tailed with an alpha level of 0.05.
    ii. The relationship between feeding skills improvement and treatment will be assessed using swallowing competency on videofluoroscopy as well as scores on the EFS to evaluate if IN-OT significantly improves feeding, as rated by a clinician, when compared to placebo.
c. Eye contact, daytime alertness, and parent/infant bonding – Improvement
    i. Using the Tobii eye tracking device we will assess degree of eye contact of infant
    ii. Parent/infant bonding will be assessed using the Mother-Infant Bonding Questionnaire (MIBQ) to evaluate if IN-OT significantly improves eye contact and bonding as compared to placebo.
d. Safety Analyses
    i. Rates of specific side effects and other adverse events will be tabulated and compared between treatment groups using the chi-square test. Please see the section labeled "Safety Analyses" for further details on how adverse events will be monitored and rated.

Power Analysis: Sample size justification: The target sample size for this Phase 2 trial is 15 patients. The power for the study has been calculated to generate a detectable signal with the sample size of 14 subjects. With this sample size, the study will have 80% power to detect a minimum effect size of d = 0.8 standard deviations in the outcome measures between groups using a two-sample T-test and a two sided Type I error rate of 5%. The study is powered based on a conservative estimate from the infant population studied in France.

Data Management: The Biomedical Research Informatics Core (RIC) of the Clinical and Translational Research Institute (CTSI) at the University of Florida will be used to create and manage the database for the trial. The RIC supports the clinical data pipeline including supporting EDC tools, customized databases and a solution to securely link patient specimens to clinical and pathological data. Database programming will begin at the study start date, so that data can be entered prospectively as subjects move through the study. Data will be encoded using unique patient identifiers, and not patient names. IDs will be assigned sequentially, in a manner unrelated to name or other easily identifiable information. Data will be entered for both screened and randomized subjects to allow analyses to assess the degree to which randomized subjects may differ from other potential subjects. Periodic reports on recruitment, treatment, retention and follow up will be generated and reviewed by the study team. All subject files will be kept in a locked room at the study site. The biostatistics core of the CTSI will be responsible for the statistical support of the study, including the final data analysis. The PI will meet regularly with the study team to review the status of the study and devise any strategies needed to meet the study goals.

Ethical Considerations: Parents/caregivers of potential participants that are referred or indicate interest in the study will be phone screened by trained staff to determine eligibility. If there are no clear exclusionary criteria, a consent form will be sent and an appointment will be set up to discuss the benefits and risks of study participation, duration of study, alternative treatment options and to answer any questions about the study. When all questions are answered and the parent/caregiver and their child, when appropriate, indicate that they understand the study, written informed consent will be obtained. Consent procedures will be completed by a trained member of the study staff and will be reviewed by the Principal Investigator.

Subject Compensation: Each subject (family) will receive $2,000 compensation on Day 1 of the study to assist with the costs they incur for transportation to and from the study site, lodging during the study, and meals purchased during the study.

References:

Bittel DC, Kibiryeva N, Butler MG. Methylation-specific multiplex ligation-dependent probe amplification analysis of subjects with chromosome 15 abnormalities. Genet Test. 2007 Winter;11(4):467-75.

Dykens EM, Lee E, Roof E. Prader-Willi syndrome and autism spectrum disorders: an evolving story. J Neurodev Disord. 2011 Sep;3(3):225-37.

Ferguson JN, Aldag JM, Insel TR, Young LJ. Oxytocin in the medial amygdala is essential for social recognition in the mouse. J Neurosci. 2001 Oct 15;21(20):8278-85.

Gainer H. Cell-specific gene expression in oxytocin and vasopressin magnocellular neurons. Adv Exp Med Biol. 1998;449:15-27.

Goldstone AP, Holland AJ, Butler JV, Whittington JE : Appetite hormones and the transition to hyperphagia in children with Prader-Willi syndrome. Int J Obes (Lond). 2012, epub Jan 24.

Jakobsen KV, Umstead L, Simpson EA. Efficient human face detection in infancy. Dev Psychobiol. 2016 Jan;58(1):129-36.

Kendrick KM, Da Costa AP, Broad KD, Ohkura S, Guevara R, Lévy F, Keverne EB. Neural control of maternal behaviour and olfactory recognition of offspring. Brain Res Bull. 1997;44(4):383-95.

Klier CM. Mother-infant bonding disorders in patients with postnatal depression: The Postpartum Bonding Questionnaire in clinical practice. Arch Womens Ment Health. 2006 Sep;9(5):289-91.

Miller JL, Lynn CH, Driscoll DC, Goldstone AP, Gold JA, Kimonis V, Dykens E, Butler MG, Shuster JJ, Driscoll DJ : Nutritional phases in Prader-Willi syndrome. Am J Med Genet A. 2011, 155A(5):1040-9

Nagai T, Obata K, Tonoki H, Temma S, Murakami N, Katada Y, Yoshino A, Sakazume S, Takahashi E, Sakuta R, Niikawa N. Cause of sudden, unexpected death of Prader-Willi syndrome patients with or without growth hormone treatment. Am J Med Genet A. 2005 Jul 1;136(1):45-8.

Ohara M, Okada T, Kubota C, Nakamura Y, Shiino T, Aleksic B, Morikawa M, Yamauchi A, Uno Y, Murase S, Goto S, Kanai A, Masuda T, Ozaki N. Validation and factor analysis of mother-infant bonding questionnaire in pregnant and postpartum women in Japan. BMC Psychiatry. 2016 Jul 7;16(1):212.

Pedersen CA, Prange AJ Jr. Induction of maternal behavior in virgin rats after intracerebroventricular administration of oxytocin. Proc Natl Acad Sci U S A. 1979 Dec;76(12):6661-5.

Ring RH, Malberg JE, Potestio L, Ping J, Boikess S, Luo B, Schechter LE, Rizzo S, Rahman Z, Rosenzweig-Lipson S. Anxiolytic-like activity of oxytocin in male mice: behavioral and autonomic evidence, therapeutic implications. Psychopharmacology (Berl). 2006 Apr;185(2):218-25.

Schaller F, Watrin F, Sturny R, Massacrier A, Szepetowski P, Muscatelli F : A single postnatal injection of oxytocin rescues the lethal feeding behaviour in mouse newborns deficient for the imprinted Magel2 gene. Hum Mol Genet. 2010, 15;19(24):4895-905.

Skokauskas N, Sweeny E, Meehan J, Gallagher L. Mental health problems in children with Prader-Willi syndrome. J Can Acad Child Adolesc Psychiatry. 2012 Aug;21(3):194-203.

Smith NA, Gibilisco CR, Meisinger RE, Hankey M. Asymmetry in infants' selective attention to facial features during visual processing of infant-directed speech. Front Psychol. 2013 Sep 11;4:601

Swaab DF, Purba JS, Hofman MA : Alterations in the hypothalamic paraventricular nucleus and its oxytocin neurons (putative satiety cells) in Prader-Willi syndrome: a study of five cases. J Clin Endocrinol Metab. 1995, 80(2):573-9.

Tauber M, Mantoulan C, Copet P, Jauregui J, Demeer G, Diene G, Rogé B, Laurier V, Ehlinger V, Arnaud C, Molinas C, Thuilleaux D : Oxytocin may be useful to increase trust in others and decrease disruptive behaviours in patients with Prader-Willi syndrome: a randomised placebo-controlled trial in 24 patients. Orphanet J Rare Dis. 2011, 24;6:47.

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Appendix 1: Early Feeding Skills Assessment

EARLY FEEDING SKILLS ASSESSMENT (EFS)

Post-conceptional age today _____ Gestational age at birth _____ Birthweight _____ Weight today _____
Prescribed feeding volume_____ Caffeine/Theophylline: Yes / No NG tube in place during feeding: Yes / No
Amount of supplemental oxygen pre-feeding, if any _____
Prior to feeding, during a calm, inactive period when no demands are being placed on the infant and infant is not recovering from a recent change such as handling: Baseline oxygen saturation _____ Baseline respiratory rate _____ Baseline heart rate ____

Oral Feeding Readiness (Prior to Feeding)	
Able to hold body in a flexed position with arms/hands toward midline.	Yes No
Awake state.	Yes No
Demonstrates energy for feeding - maintains muscle tone and body flexion through assessment period.	Yes No
(Offering infant finger or pacifier) Attention is directed toward feeding - infant searches for nipple or opens mouth promptly when lips are stroked and tongue descends to receive the nipple.	Yes No
Baseline oxygen saturation > 95%.	Yes No

Examples of Feeding Readiness 1, 2, 3, 4

If all answers to the above are "YES" proceed to feed the infant and score feeding skill. If the infant does not score "YES" on all above items: re-alert the infant, swaddle/re-swaddle to better support; then, if the infant is interested, re-offer non-nutritive sucking for several minutes. Re-evaluate readiness. If all answers are now "YES" proceed to oral feed, otherwise gavage feed.

Oral Feeding Skill				Time Feeding Starts _____
Ability to Maintain Engagement in Feeding				
Length of optimal feeding state (Alert inactive or quiet awake state with eyes open or closed).	All of the Feeding	Most of the Feeding	Some of the Feeding	None of the Feeding
Demonstrates energy for feeding - maintains flexed body position with arms toward midline.	All of the Feeding	Most of the Feeding	Some of the Feeding	None of the Feeding
Ability to Organize Oral-Motor Functioning				
Opens mouth promptly when lips are stroked at feeding onsets. (*Example 2)*	All of the Onsets	Most of the Onsets	Some of the Onsets	None of the Onsets
Tongue descends to receive the nipple at feeding onsets.	All of the Onsets	Most of the Onsets	Some of the Onsets	None of the Onsets
Immediately after the nipple is introduced infant's sucking is organized, rhythmic, and smooth.	All of the Onsets	Most of the Onsets	Some of the Onsets	None of the Onsets
Once feeding is underway, maintains a smooth, rhythmical pattern of sucking.	All of the Feeding	Most of the Feeding	Some of the Feeding	None of the Feeding
Sucking pressure is steady and strong (i.e., sucks with steady and strong suction).	All of the Feeding	Most of the Feeding	Some of the Feeding	None of the Feeding
Able to engage in long sucking bursts (7-10 sucks) without behavioral stress signs or an adverse or negative cardiorespiratory response.	All of the Feeding	Most of the Feeding	Some of the Feeding	None of the Feeding
Tongue maintains steady contact on the nipple - does not slide off the nipple with sucking creating a clicking sound.	All of the Feeding	Most of the Feeding	Some of the Feeding	None of the Feeding
Ability to Coordinate Swallowing				
Manages fluid during swallow without loss of milk at lips (i.e., no drooling during...).	All of the Feeding	Most of the Feeding	Some of the Feeding	None of the Feeding
Pharyngeal sounds are clear - no gurgling sounds created by fluid in the pharynx.	All of the Feeding	Most of the Feeding	Some of the Feeding	None of the Feeding
Swallows are quiet - no gulping or hard swallows. (*Example 2, 3, 4)*	All of the Feeding	Most of the Feeding	Some of the Feeding	None of the Feeding
Airway opens fully after swallowing - no inspiratory stridor sounds (high-pitched crowing sounds, "yelping" sounds).	All of the Feeding	Most of the Feeding	Some of the Feeding	None of the Feeding
A single swallow clears the sucking bolus - multiple swallows are not required to clear fluid out of throat. (*Example 2, 3, 4)*	All of the Feeding	Most of the Feeding	Some of the Feeding	None of the Feeding
No overt evidence of potential for aspiration - no coughing or choking sounds.	All of the Feeding	Most of the Feeding	Some of the Feeding	None of the Feeding

 EFS_with_AudioVideo: 2/18/16

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Ability to Maintain Physiologic Stability				
In the first 30 seconds after a feeding onset, oxygen saturation is stable and there are no behavioral stress cues. *(Example 2, 3, 4)*	All of the Onsets	Most of the Onsets	Some of the Onsets	None of the Onsets
Stops sucking to breathe. Feeder does not need to provide a break for breathing. *(Example 2)*	All of the Feeding	Most of the Feeding	Some of the Feeding	None of the Feeding
When the infant stops sucking to breathe, a series of breaths is observed. Feeder does not need to provide a break for breathing because of a single, shallow, catch breath.	All of the Feeding	Most of the Feeding	Some of the Feeding	None of the Feeding
Infant stops to breathe before behavioral stress cues are evidenced.	All of the Feeding	Most of the Feeding	Some of the Feeding	None of the Feeding
Breath sounds are clear – no grunting breath sounds (prolonging the exhale, partially closing glottis on exhale). *(Example 2)*	All of the Feeding	Most of the Feeding	Some of the Feeding	None of the Feeding
Nasal flaring and/or blanching.	Never	Occasionally	Often	
Uses accessory breathing muscles (e.g., chin tugging/pulling head back, head bobbing, retracting, tracheal tugging).	Never	Occasionally	Often	
Color change during feeding (pallor, circum-oral or circum-orbital cyanosis).	Never	Occasionally	Often	
Oxygen saturation drops below 90%.	Never	Occasionally	Often	
Heart rate drops below 100 beats per minute.	Never	Occasionally	Often	
Heart rate rises 15 beats per minutes above the infant's baseline.	Never	Occasionally	Often	

Oral Feeding Tolerance (Post Feeding)						
	At End of Feeding	1 min	2 min	3 min	4 min	5 min
Muscle tone is strong /body is flexed with arms/hands toward midline.	Y N	Y N	Y N	Y N	Y N	Y N
Able to maintain an awake state.	Y N	Y N	Y N	Y N	Y N	Y N
Oxygen saturation (%)						
Heart rate (bpm)						

Feeding Descriptors:

Type of bottle/nipple used _____ Length of feeding (minutes) _____ Volume consumed _____ cc
Amount of supplemental oxygen during feeding, if any _____
Position during feeding (check all that apply): cradled _____ side-lying _____ semi-upright in front _____
Feeding skills: ☐ maintained across the feeding ☐ improve during the feeding ☐ decline during the feeding

Developmentally supportive actions used:
- ☐ no feeder actions required
- ☐ repositioned infant
- ☐ re-alerted infant
- ☐ rested infant
- ☐ provided steady pressure on the cheeks
- ☐ held/stabilized the jaw

Passive actions used which are NOT developmentally supportive:
- ☐ pulled nipple in and out to encourage sucking
- ☐ twisted/turned nipple to encourage sucking
- ☐ jiggled nipple to encourage sucking
- ☐ provided rhythmical squeezing action on the cheeks
- ☐ moved jaw up and down

Primary feeding concerns/recommendations for next feeding:

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Appendix 2: Mother-Infant Bonding Questionnaire

	Always	Very often	Quite often	Sometimes	Rarely	Never
I feel close to my baby						
I wish the old days when I had no baby would come back						
I feel distant from my baby						
I love to cuddle my baby						
I regret having this baby						
The baby does not seem to be mine						
My baby winds me up						
My baby irritates me						
I feel happy when my baby smiles or laughs						
I love my baby to bits						
I enjoy playing with my baby						
My baby cries too much						
I feel trapped as a mother						
I feel angry with my baby						
I resent my baby						
My baby is the most beautiful baby in the world						
I wish my baby would somehow go away						
I have done harmful things to my baby						
My baby makes me anxious						
I am afraid of my baby						
My baby annoys me						
I feel confident when changing my baby						
I feel the only solution is for someone else to look after my baby						
I feel like hurting my baby						
My baby is easily comforted						