Consequences of Synthetic Oxytocin in the Maternal Brain

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Dedications

This dissertation is first dedicated to my Lord and Savior, Jesus Christ, for providing vision and purpose for this work. This is also dedicated to all who have supported me throughout my nursing education.

To my mother, Brenda McDonald

for her continued prayers and encouragement,

To my twin sister, Marissa McDonald

who has walked beside me in every stage of life

and

in loving memory of my father

Morris McDonald

who now cheers me on from Heaven.

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CHAPTER ONE: Introduction

Postpartum depression (PPD) is considered the most common complication of pregnancy and childbearing affecting approximately 10-20% of women within the first year after delivery (Ko et al., 2017; Gavin et al., 2005; Wang et al., 2021). PPD is defined as the onset of depression within the first 4 weeks postpartum and is associated with serious consequences for both the mother and child (Mughal et al., 2022). Numerous studies have described the adverse sequelae of PPD including poor maternal health habits, decreased breastfeeding initiation, poor maternal-infant bonding, decreased parental safety practices, and increased maternal suicidal ideation (Davis et al., 2019; Kingston et al., 2012; Minkovitz et al., 2005; Wouk et al., 2017). Mental health conditions, including maternal death by suicide, account for approximately 9% of pregnancy-related deaths and are often preventable by changes to patient, provider, community, and system-level factors (Davis et al., 2019).

Understanding modifiable risk factors for PPD is crucial to mitigate poor maternal and child health outcomes. One factor that has gained attention in the literature as a risk factor for PPD is maternal exposure to synthetic oxytocin (i.e., Pitocin) during parturition. Synthetic oxytocin has become one of the most widely used medications during parturition with rates of labor induction in the United States tripling from 9.6% in 1990 to 32.1% in 2021 (Osterman, et al., 2023, Simpson, 2022). In addition, synthetic oxytocin is widely administered in the hospital setting for indications such as slow labor progress, high-risk births, and the prevention and/or treatment of postpartum hemorrhage (Oscilla & Sharma, 2022). In many ways, the use of synthetic oxytocin has demonstrated life-saving benefits; however, its administration during labor and delivery is not without risk. The Food and Drug Administration (FDA) (2014) recognizes several important adverse effects, particularly associated with high dose regimens of

oxytocin, including uterine hypertonicity, uterine spasms, tetanic contractions, postpartum hemorrhage, and rupture of the uterus. In addition, there is growing concern for adverse effects on maternal mental health.

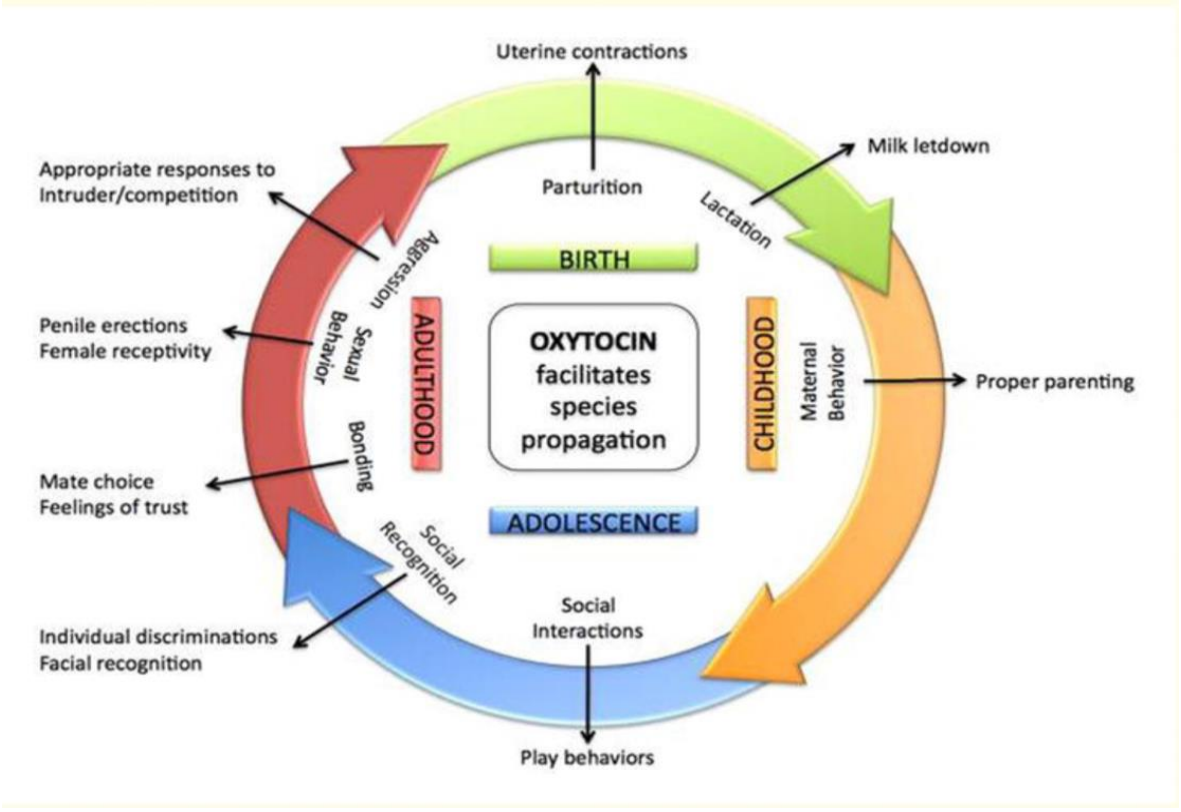
A mother's mental health can be impacted by a combination of various social, environmental, and biological risk factors. Environmental risk factors for maternal PPD have been well-established in the literature including a history of intimate partner violence, an unintended or unwanted pregnancy, a lack of social support, and stressful life events (Alhusen et al., 2014; Kornfeld et al., 2012; Lancaster et al., 2010; Yim et al., 2015). Evidence suggests there is also a higher incidence of maternal PPD when mothers are exposed to birth interventions such as synthetic oxytocin; however, the biological mechanisms underlying this relationship have not been fully elucidated (Bell et al., 2015; Gu et al., 2015; Kroll-Desrosiers et al., 2017; Thul et al., 2020; Tichelman et al., 2021). More research is needed to understand the long-term effects of synthetic oxytocin on maternal mental health and the biological mechanisms by which poor maternal mental health outcomes may occur.

Background

Oxytocin is an endogenous polypeptide with complex actions across many body systems. Throughout the lifespan, oxytocin demonstrates a key role in promoting uterine contractions during parturition, facilitating milk letdown during breastfeeding, and regulating social interactions and bonding behaviors (see Figure 1) (Erbas & Altuntas, 2020). Oxytocin is predominately released from the posterior pituitary gland, however, peripheral tissues (including the uterus, placenta, corpus luteum and amnion) may also synthesize and release oxytocin throughout the body (Gimpl & Fahrenholz, 2001). At various times, circulating oxytocin levels can differ within and between individuals making it challenging to assess associations with

outcomes such as maternal PPD (McCullough et al., 2013). Oxytocin relies on binding to its major receptor, *OXTR*, which is a G-protein coupled receptor produced by the cell and positioned in the cell membrane for hormone binding. When oxytocin binds to *OXTR* it results in a conformational change in the structure of the receptor, increased Ca^{2+} release, and subsequent effects such as vasodilation, gene transcription and smooth muscle contraction (Baribeau & Anagnostou, 2015). Examining factors that would decrease the availability of *OXTR* may provide important insight regarding the downstream actions of oxytocin in its target tissues. In recent years, researchers have examined genetic and epigenetic modifications to the *OXTR* gene that would, presumably, affect the presence of *OXTR* within target tissues and hinder the function of oxytocin (Bell et al., 2015; Kimmel et al., 2016; King et al., 2017; Toepfer et al., 2019).

Figure 1. Illustration of the Role of Oxytocin Across Birth and the Lifespan

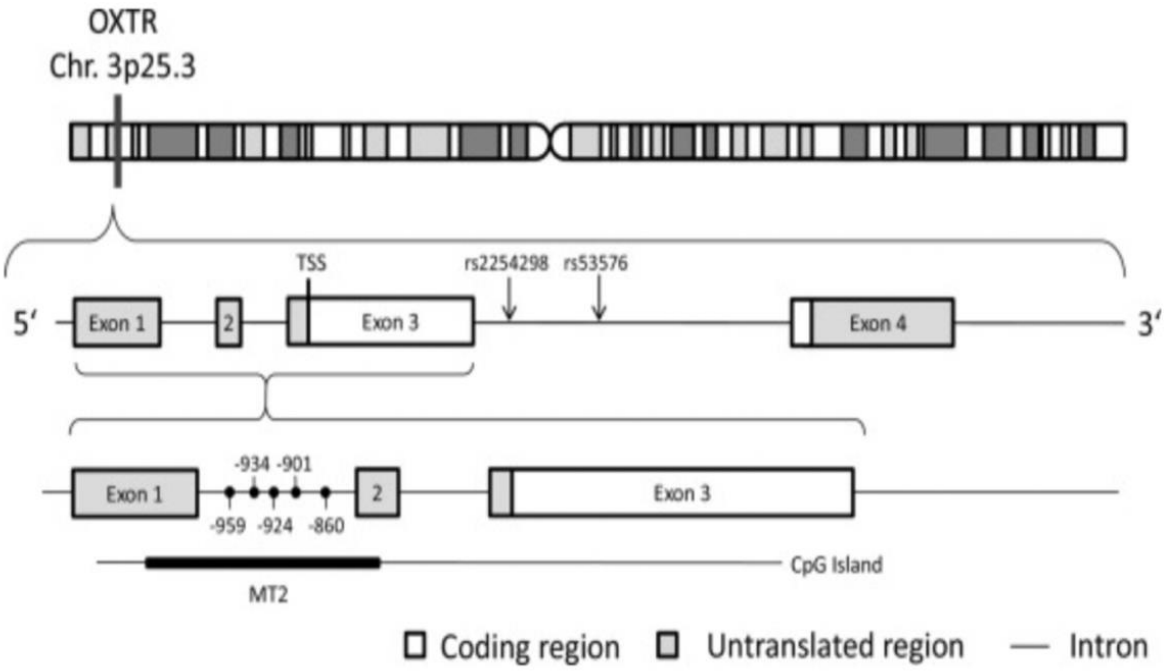


Note. This figure is retrieved from Erbas, O., & Altuntas, I. (2020). *Oxytocin and Health.* IntechOpen.

The *OXTR* gene spans 19,206 base pairs with four exons and three introns. A region within the *OXTR* gene promoter (denoted as MT2) covers much of exon 1 and the first intron and is suggested to be particularly vulnerable to epigenetic control via DNA methylation (see Figure 2) (Kusui et al., 2001; Tops et al., 2019). DNA methylation in the MT2 region is found to have a downregulating effect on *OXTR* such that higher DNA methylation reduces (or silences) *OXTR* gene expression (Kusui et al., 2001). In animal studies, reduced expression of the *Oxtr* gene within central tissues has been associated with variations in typical social and maternal behaviors (Keebaugh & Young, 2011; Danoff et al., 2021; Olazabal & Young, 2006; Perkeybile et al., 2019). Importantly, prolonged synthetic oxytocin exposure in human mothers has been implicated in the downregulation of *OXTR* gene expression in the uterus, mimicking the downregulating effect of DNA methylation (Phaneuf et al., 2000). It is thought that higher DNA methylation and lower *OXTR* gene expression likely underlie the relationship between maternal exposure to synthetic oxytocin during parturition and adverse outcomes such as uterine atony and subsequent postpartum hemorrhage (Erickson et al., 2023; Robinson et al., 2003).

In the present dissertation study, the impact of synthetic oxytocin on epigenetic regulatory factors in the maternal brain (including *Oxtr* DNA methylation and *Oxtr* gene expression) is further assessed to examine a potential pathway for variations in maternal postpartum mood and behaviors. In addition, the present study examines the process of active DNA demethylation via hydroxymethylation which may be important for changing the maternal brain in the context of synthetic oxytocin exposure. Evidence suggests DNA methylation and demethylation mechanisms are dynamic in response to environmental events and, each process may have important implications for disordered health outcomes (Turnpin & Salbert, 2022).

Figure 2. The structure of *OXTR* including the MT2 region and pertinent CpG sites studied in relation to PPD.

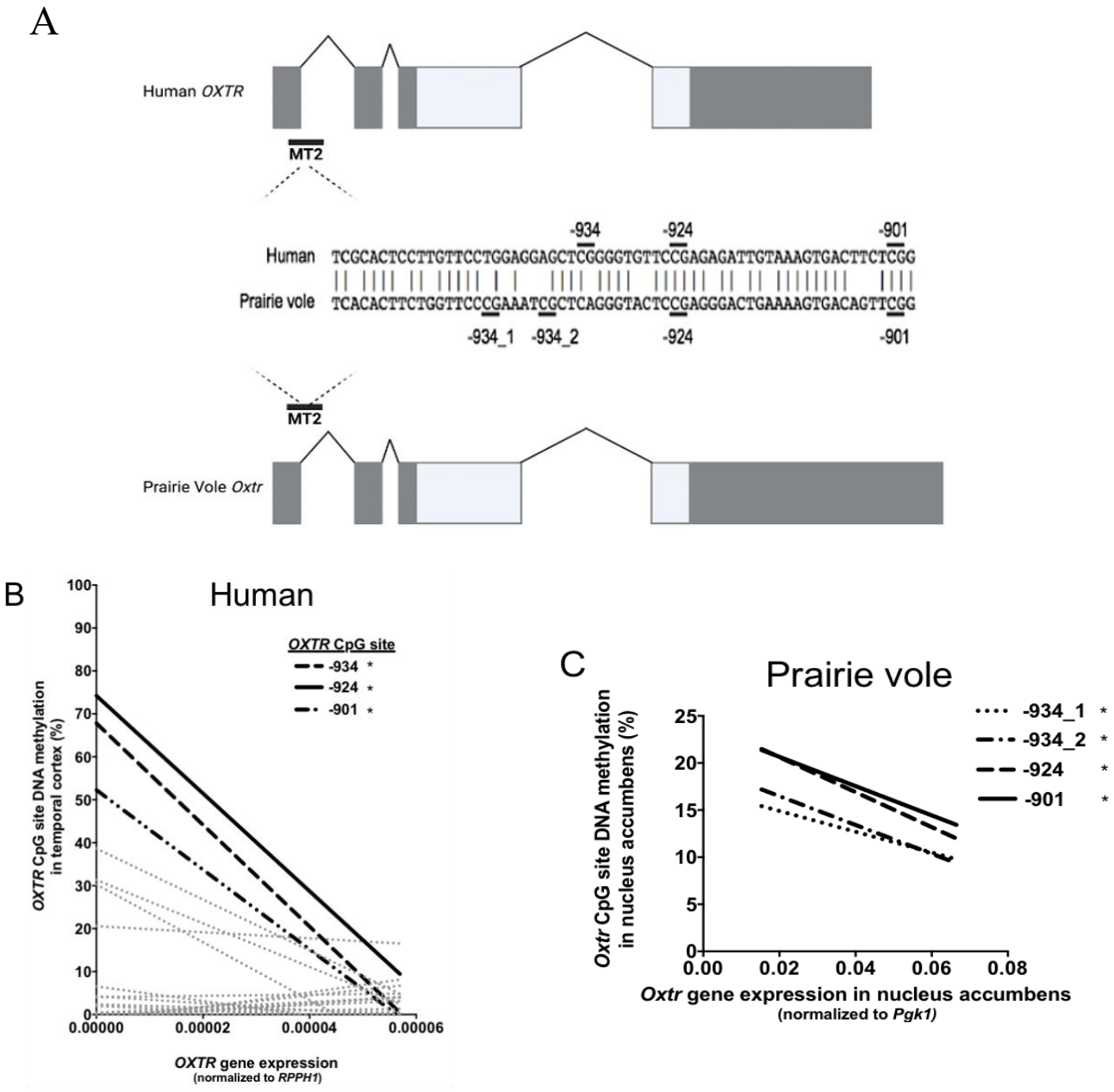


Note. This figure is retrieved from Tops et al. (2019). Genetic and epigenetic regulatory mechanisms of the oxytocin receptor gene *(OXTR)* and the (clinical) implications for social behavior. *Hormones and Behavior, 108*, 84-93. https://doi.org/10.1016/j.yhbeh.2018.03.002

Preliminary Data

The Connelly Lab focuses on transcription and epigenetic regulatory factors that contribute to outcomes of complex psychological disorders in human and animal models. Using the prairie vole as a model organism, researchers in the lab have studied the role of *Oxtr* in regulating social behaviors including variations in parental care (Danoff et al., 2021; Perkeybile et al., 2019). Previous RNA sequencing of the oxytocin receptor in the nucleus accumbens has shown that the *Oxtr* gene in prairie voles has four exons and three introns much like the *OXTR* gene in humans (see Figure 3) (Danoff et al., 2021). Unlike traditional laboratory species (e.g., rats and mice), the MT2 region in the *Oxtr* promoter (CpG sites -934, -924, -901) is homologous in humans and in prairie voles with 64.3% shared identity (Danoff et al., 2021). Moreover, increased DNA methylation in this conserved region has been found to decrease gene expression in both prairie voles and in humans (see Figure 3B and 3C) (Danoff et al., 2021; Perkeybile et al., 2019; Gregory et al., 2009). Taken together, this data demonstrates the important utility of the prairie vole as a model organism for future translational research in humans.

Figure 3. The oxytocin receptor gene (OXTR) is homologous in humans and in prairie voles with conserved CpG sites -934, -924, and -901 (A). Higher DNA methylation at the conserved CpG sites shows a downregulating effect of the oxytocin receptor gene in brain regions of humans and prairie voles (B and C). *p < 0.05



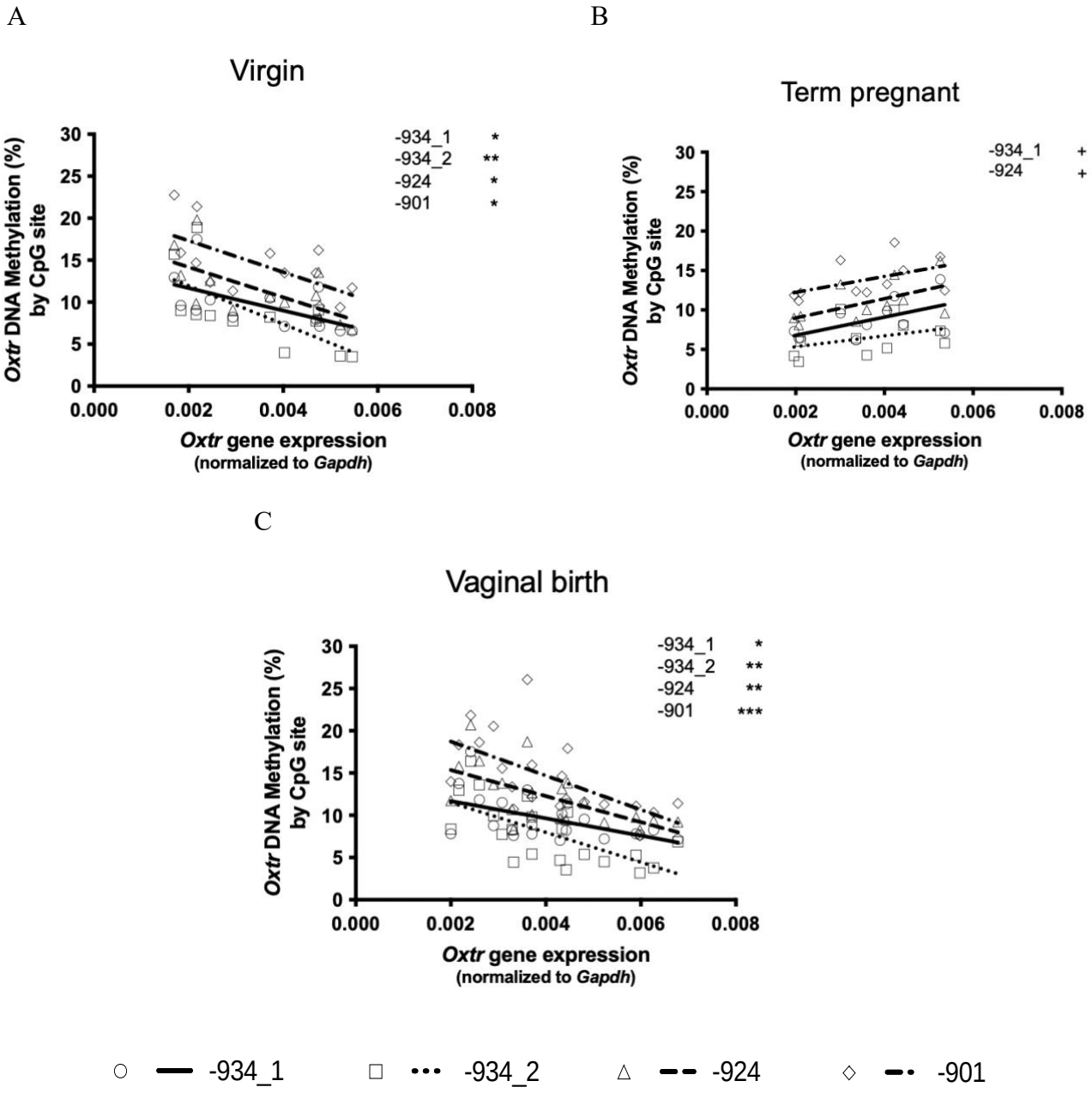
Note. Figure 3A and B are retrieved from Danoff et al. (2021). Genetic, epigenetic, and environmental factors controlling oxytocin receptor gene expression. *Clinical Epigenetics, 13*(23). https://doi.org/10.1186/s13148-021-01017-5

The impact of synthetic oxytocin on *Oxtr* DNA methylation and gene expression

In assessment of baseline epigenetic mechanisms in term pregnancy, researchers in the Connelly Lab examined the relationship between *Oxtr* DNA methylation and *Oxtr* gene expression in the nucleus accumbens of virgin, term pregnant, and postpartum female prairie voles after a vaginal delivery (see Figures 4A, B, and C) (Perkeybile et al., *in prep*). Findings from this study confirmed a negative correlation between *Oxtr* DNA methylation and *Oxtr* gene expression in the adult virgin females (see Figure 4A). At term pregnancy, however, they observed no significant relationship between *Oxtr* DNA methylation and *Oxtr* gene expression just prior to giving birth (see Figure 4B). Within 90 minutes of vaginal delivery, a negative correlation was again observed, demonstrating a rapid return of this relationship to the pre-pregnancy state (see Figure 4C). This pattern demonstrates a change in the relationship between *Oxtr* DNA methylation and *Oxtr* gene expression in term pregnant females that is likely important for the process of birth and an adaptation to motherhood.

Additional findings in this study demonstrate a strong correlation between the methylation state in the nucleus accumbens and in peripheral whole blood for all female subjects (Perkeybile et al., *in prep*). These correlations were confirmed across four homologous CpG sites (-934_1, -934_2, -924, -901) in MT2 and were replicated in other brain regions including the hypothalamus, amygdala, medial preoptic area, and the insular cortex (Perkeybile et al., *in prep*). A strong correlation between the methylation state within various brain regions and in whole blood has important implications for translational research in humans as whole blood may serve as a proxy for examining changes in central neurobiology in human mothers.

Figure 4. DNA methylation and gene expression in virgin females (A), term pregnancy (B), and birth (C). *p < 0.05, $^{+}$p < 0.1.



Note. This figure was retrieved from Perkeybile, A., Kenkel, W., Yee, J., Lillard, T., Ferris, C., Carter, S., & Connelly, J. Pregnancy and birth epigenetically shape the maternal oxytocin receptor. *(In prep).*

Specific Aims

Given the impact of the oxytocin system on social and maternal behaviors in human and animal studies, the goal of this dissertation study is to assess the impact of synthetic oxytocin administration on *Oxtr* DNA methylation and *Oxtr* gene expression in the maternal brain. This study builds upon preliminary data in the Connelly Lab by examining the impact of synthetic oxytocin on *Oxtr* DNA methylation and *Oxtr* gene expression in the maternal nucleus accumbens using a prairie vole as a model organism. Dose-dependent effects of synthetic oxytocin are examined using a low, medium, and high dose which may mimic the clinical use of low-dose and high-dose oxytocin regimens during labor induction. Based on previous work showing a downregulating effect of synthetic oxytocin on *OXTR* in the human uterus, we hypothesized that high doses of peripherally administered synthetic oxytocin in term pregnant females would have a similar downregulating effect on *Oxtr* gene expression in the maternal brain (Phaneuf, 2000). We further differentiate DNA methylation and hydroxymethylation to examine specific effects of each mechanism on *Oxtr* gene expression in the context of maternal exposure to synthetic oxytocin.

The study consists of 75 term pregnant female prairie voles divided into 5 treatment groups (low-dose oxytocin (OXT) [0.125mg/kg], medium-dose OXT [0.25mg/kg], high-dose OXT [0.5mg/kg], saline vehicle, and no treatment) and examines tissues including the nucleus accumbens and whole blood for all subjects. Homologous CpG sites between humans and prairie voles in MT2 are assessed including CpG sites -934_1, -934_2, -924, and -901, for potential future translation of this work to human participants. In addition, correlations between the methylation state in the nucleus accumbens and whole blood are also examined.

The specific aims of this study are as follows:

(1) Examine the relationship between synthetic oxytocin dose (low-dose OXT, medium-dose OXT, high-dose OXT, saline vehicle, and no treatment), DNA methylation in the 3' MT2 region of *Oxtr* (CpG sites -934_1, -934_2, -924, and -901) and *Oxtr* gene expression in the nucleus accumbens of term pregnant female prairie voles.

(2) Analyze correlations between *Oxtr* DNA methylation in the nucleus accumbens and in whole blood of term pregnant prairie voles.

Innovation and Significance of the Study

This study has several innovative features. It is a multidisciplinary study that explores clinically derived questions and underlying biological pathways that effect the processes of childbirth and maternal postpartum mental health. Key innovative features of this work include 1) examining the dose-dependent impact of synthetic oxytocin on epigenetic regulation of *Oxtr* in the maternal brain and 2) utilizing a prairie vole labor induction model to analyze correlations between epigenetic regulation in central and peripheral tissues in the context of maternal exposure to synthetic oxytocin during parturition. Assessing correlations between DNA methylation in the brain and whole blood provides a foundation for future research in human participants as whole blood may serve as a proxy for epigenetic changes in the maternal brain. The current study will be important for the future translation of this work to human mothers and may have implications for progression in personalized health care regarding the administration of synthetic oxytocin during parturition.

Theoretical Framework

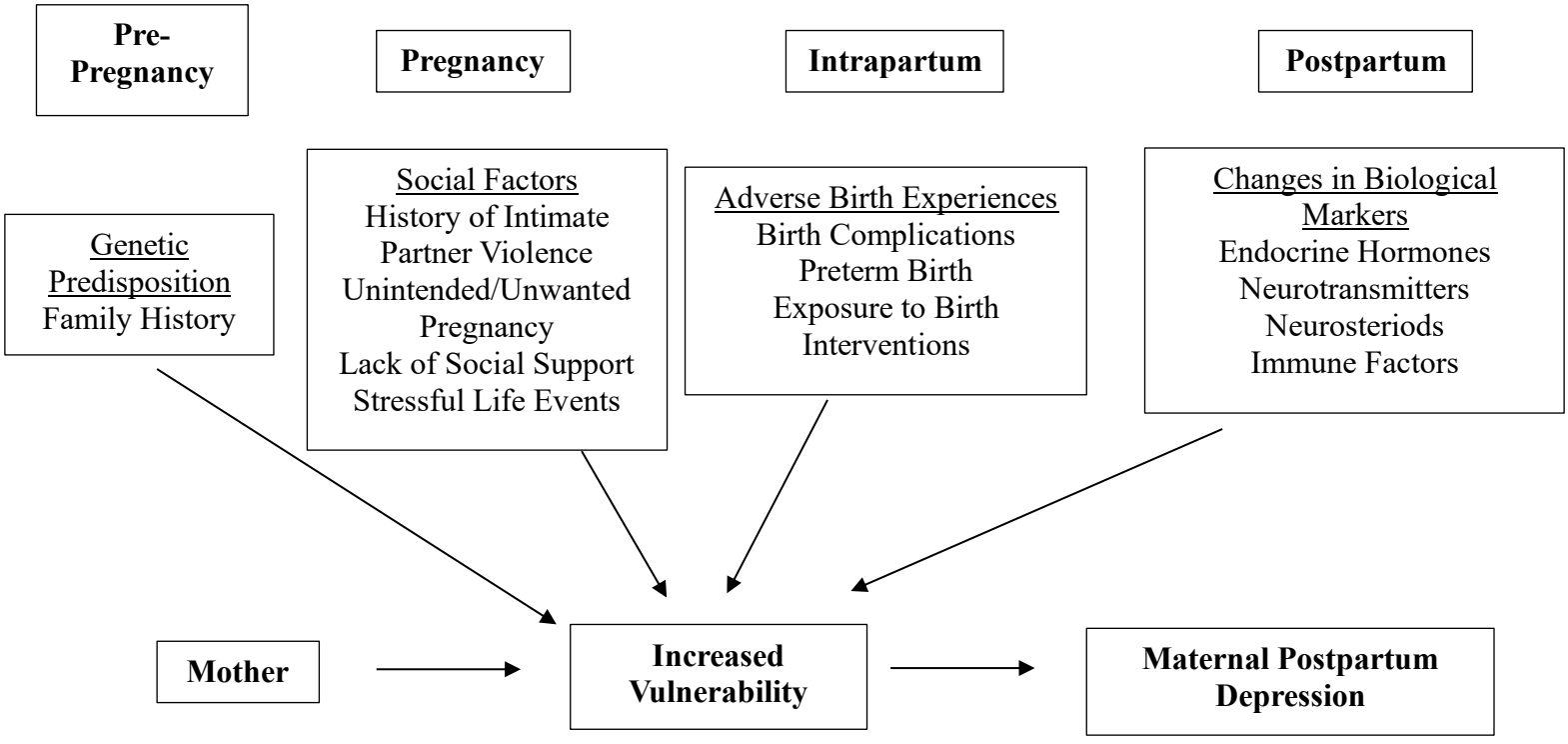
The Diathesis-Stress model provides an important theoretical framework for this research and has been applied to the study of many psychological disorders (Bleuler, 1963; Mann et al., 1999; Monroe & Simons, 1991; Vogel, 1991; Yim et al., 2015). The term "diathesis" is derived

from the Greek word meaning disposition. The model of diathesis-stress denotes an increased vulnerability to behavioral or psychological disorders in the context of genetic susceptibility and exposure to environmental stressors (Zuckerman, 1999). Environmental stress (including both chemical and physical stressors) can impact gene function in various ways and the study of these gene-environment interactions is known as epigenetics. In psychology, the diathesis-stress model was originally developed to understand the origins of schizophrenia and was later adapted in the 1980s to understand the complexities of depression (Bleuler, 1963; Yim et al., 2015). Over time, the model has been widely recognized in the health and social sciences as a framework to investigate interactions between environment, biology, and affective outcomes (Yim et al., 2015).

Complementary theories describing environmental and biological influences in psychology also include 1) the civilization byproduct perspective; 2) the medical perspective; and 3) the fetal programming theory. In 2014, Hahn-Holbrook and Haselton proposed that PPD was a byproduct of changes in civilization citing an increasing prevalence of early weaning, vitamin D deficiency, low consumption of omega-3 fatty acids, and sedentary lifestyle. Per Hahn-Holbrook and Haselton (2014), these poor lifestyle changes (related to evolving cultural norms) can lead to biological consequences (such as elevated inflammation) and an increased risk for PPD. The biological component of this perspective is somewhat aligned with the medical model which has been a dominant theoretical perspective in clinical practice settings for centuries (Beck, 2002). From this viewpoint, a diagnosis of PPD would be considered a medical condition with a distinct pathological process. Although social and environmental factors are considered, greater emphasis is placed on biological factors and underlying pathophysiological mechanisms (Beck, 2002).

The theoretical perspective of fetal programming provides an additional framework to investigate potential pathways for offspring psychological disorders. Fetal programming is based on the hypothesis that stressful conditions incurred during pregnancy pose an important risk for poor fetal development. Studies have further analyzed psychopathological outcomes during childhood that occurred following exposure to stressful prenatal conditions (LeWinn et al., 2015; Rouse & Goodman, 2014; Swales et al., 2017). Although offspring outcomes will not be assessed in this current study, it is important to consider the potential impact of synthetic oxytocin use on both maternal and child psychology.

Figure 5. A hypothesized conceptual framework for the development of maternal postpartum depression based on the Diathesis-Stress Model



Important Concepts

Postpartum Depression

Leading organizations, such as The United States Preventive Services Task Force (USPSTF) and the American College of Obstetricians and Gynecologists (ACOG), have highlighted the importance of provider screening for maternal depressive symptoms and risk factors for PPD (American College of Obstetricians and Gynecologists [ACOG], 2015; U.S. Preventive Services Task Force [USPSTF], 2020). In the clinical setting, it is important to first exclude other common postpartum conditions such as "baby blues" which involves maternal mood fluctuations during the first two weeks after delivery and occurs in approximately 50 to 80% of new mothers (Henshaw, 2003). The fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) provides diagnostic characteristics for Major Depressive Disorder (MDD) that are also used to define symptoms of PPD (Halverson, 2019). Criteria for diagnosis of MDD consist of at least 5 depressive symptoms nearly every day during the same two-week period (Halverson, 2019). PPD is further defined as the onset of a depressive episode within the first 4 weeks postpartum though symptoms are often assessed up to one year after delivery (ACOG, 2015). Nine distinct symptoms of MDD and PPD include: (1) depressed mood; (2) loss of interest or pleasure; (3) weight loss or weight gain; (4) insomnia or hypersomnia; (5) psychomotor agitation; (6) fatigue; (7) feeling worthless or excessive/inappropriate guilt; (8) decreased concentration; and (9) thoughts of death/suicide (Halverson, 2019). In addition, mothers with PPD often report difficulty in their relationships and an interference in maternal functioning (Alderdice & Kelly, 2019; Forder et al., 2020; Recto & Champion, 2018).

Although leading healthcare organizations recommend screening for maternal PPD up to 1 year after delivery, evidence suggests symptoms of PPD may persist for several years after

birth (Putnick et al., 2020). PPD can disrupt the maternal and child relationship with mothers having more negative thoughts about themselves and their newborns (Pedro et al., 2019). Dangerous consequences of untreated maternal PPD can include maternal suicide and psychosis with higher rates of infanticide (Brockington, 2017). Though treatment is often necessary to mitigate adverse maternal and child events, treating maternal PPD, particularly with pharmacological agents, can be challenging. Providers must often weigh the risks and benefits of treatment with consideration for the safety and preferences of the pregnant or postpartum mother (McDonald & Alhusen, 2022).

Due to significant challenges in treating maternal PPD, it is critical to identify and mitigate maternal risk factors. In a review of pathophysiological mechanisms involved in maternal PPD, Payne and Maguire (2019) highlight numerous pathways including a combination of biological and environmental factors. The authors describe the complex interplay of biochemical factors including the HPA pathway, reproductive hormones, neurotransmitters, neurosteroids, and inflammatory mechanisms that can be impacted by maternal experiences during pregnancy and parturition (Payne & Maguire, 2019). Environmental factors such as low social support, perceived stress, low socioeconomic status, maternal history of abuse, and adverse birth experiences involving birth interventions are known contributors to outcomes of maternal PPD; however, biological mechanisms linking adverse environmental experiences and outcomes of maternal PPD are less understood (Mughal et al., 2022).

Oxytocin and the Stages of Labor

Oxytocin was first discovered in 1906 by Sir Henry Dale who found that a pituitary extract was important for stimulating uterine contractility in cats (Dale, 1909). Since its initial discovery, oxytocin has been further characterized as a polypeptide with nine amino acids and is

synthesized in the magnocellular neurosecretory cells in the supraoptic nucleus and the paraventricular nucleus in the hypothalamus. Oxytocin is then transferred to the posterior lobe of the pituitary gland where it is stored prior to release into the circulating vasculature. Once released into circulation, oxytocin binds to its receptor, *OXTR,* and has important functions including producing and maintaining uterine contractions during parturition (Bell et al., 2014).

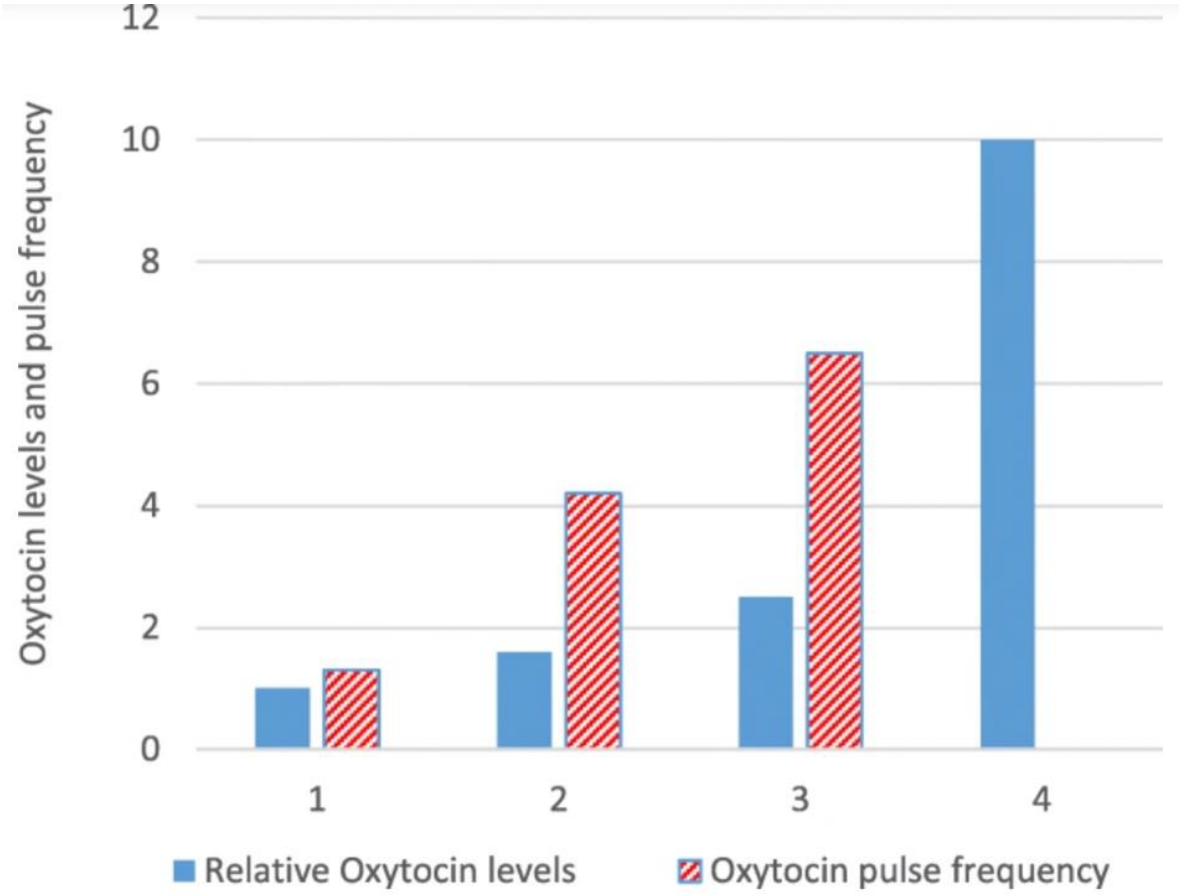
The process of human parturition can be divided into three stages. The first stage of labor (including the latent and active phase) begins with labor onset and ends when the cervix is fully dilated to 10 centimeters. During this stage, labor may be spontaneous or medically induced. A normal latent period can last up to 20 hours in nulliparous women and evidence suggests that when hospital admissions occur in the latent phase there is a higher risk for labor arrest, increased use of synthetic oxytocin, and cesarean birth (ACOG, 2019). During the active phase of labor, a lack of cervical change across 4 hours with appropriate contractions or a lack of adequate contractions for 6 hours is considered a labor arrest and typically warrants additional birth interventions (Hutchison et al., 2023).

During the second stage of labor, pulses of oxytocin increase in frequency and amplitude as the neonate is delivered (Uvnas-Morber et al, 2019). This stage is usually less than 3 hours in nulliparous women and less than 2 hours in multiparous women (Hutchison et al., 2023). If labor persists beyond this timeframe, it is often considered prolonged, and birth interventions may be necessary. Third-stage labor begins once the neonate is delivered and ends with the delivery of the placenta. Expulsion of the placenta make take between 5 to 30 minutes and is usually associated with some blood loss as the placenta is detached from the uterine wall. A longer third-stage labor may result in significant blood loss or postpartum hemorrhage which is defined as the cumulative blood loss greater than or equal to 500mL in a vaginal delivery or 1,000 mL in a

cesarean delivery (Hutchison et al., 2023). Among other techniques (e.g., traction on the umbilical cord, fundal pressure), synthetic oxytocin is often the first-line mechanism for preventing and treating postpartum hemorrhage (ACOG, 2017; WHO, 2012).

The release of oxytocin during parturition demonstrates a positive feedback mechanism in which greater amounts of oxytocin are released from the posterior pituitary gland as labor progresses (see Figure 6) (Uvnas-Morber et al., 2019). Importantly, once released into the circulating vasculature, oxytocin is not thought to reenter the brain because of the blood-brain barrier. Instead, across the maternal and general population, oxytocin's impact on mood and social behavior is thought to reflect the secretion of oxytocin from neurons that project to various brain regions and the spinal cord (Veening et al., 2010). Oxytocin receptors are found in several brain regions (including the paraventricular nucleus and the supraoptic nucleus of the hypothalamus, the amygdala, the nucleus accumbens, and the olfactory bulb) and numerous studies examining the role of oxytocin in the central nervous system have reported positive effects on sexual arousal, social bonding, maternal behavior, trust, learning and memory formation (Argiolas & Gessa, 1991; Baribeau & Anagnostou, 2015; Jones, 2017; Trifu et al., 2019). Specifically, evidence in human and animal studies suggests endogenous oxytocin facilitates maternal care behaviors including bonding with the offspring and maternal protective behaviors (Bosch, 2013; Scatliffe et al., 2019).

Figure 6. Illustration of relative oxytocin and oxytocin pulse frequency in (1) term pregnancy, (2) first stage labor, (3) second stage labor, and (4) birth.



Note. This figure was retrieved from Uvnas-Morber et al. (2019). Maternal plasma levels of oxytocin during physiological childbirth- A systematic review with implications for uterine contractions and central actions of oxytocin. *BMC Pregnancy and Childbirth, 19*(285). https://doi.org/10.1186/s12884-019-2365-9

Synthetic Oxytocin and Labor Induction

A method to synthesize oxytocin was first discovered in the mid 1950's by a chemist named Vincent du Vigneud. Since the 1960's, clinicians have refined techniques to provide synthetic oxytocin (i.e., Pitocin) when medically indicated. Synthetic oxytocin has important clinical indications including inducing and augmenting human labor when medically necessary. If administered properly, synthetic oxytocin will stimulate uterine contractions comparable to contractions experienced in normal labor; however, overstimulation of the uterus due to high doses of synthetic oxytocin and increased maternal sensitivity to oxytocin can lead to detrimental effects for both the mother and fetus (Budden et al., 2014; Robinson et al., 2003). Guidelines for proper use of synthetic oxytocin have varied globally with continued debate regarding the risks and benefits of high-dose versus low-dose oxytocin induction and augmentation regimens (Daly et al., 2020).

In contrast to the pulsatile manner of endogenous oxytocin release from the posterior pituitary gland in spontaneous labor, intravenous synthetic oxytocin is often provided continuously across several hours (Daly et al., 2020; Uvnas-Morber et al., 2019). In the clinical setting, an oxytocin induction regimen may begin with a low-dose infusion rate of 1-3 mU/min and increase incrementally every 15-40 minutes to a maximum dose of 32 mU/min (Kruit et al., 2022; Uvnas-Morber et al., 2019). A high-dose oxytocin regimen for labor induction may begin with 2-6 mU/min and will also increase every 15-40 minutes until adequate contractions are achieved (Budden et al., 2014). A steady state of oxytocin in circulation is generally achieved after 40 minutes of infusion (Seitchick et al., 1985). One study found that when oxytocin doses reached 10-16 mU/min this was roughly double that of endogenous circulating oxytocin typically found in physiological labor (Fuchs et al., 1983). Globally, the total amount of synthetic oxytocin

administered during labor induction and augmentation can range from roughly 2.8 IU to 27 IU across eight hours of infusion (Daly et al., 2020). In addition, during third-stage labor, a bolus dose of synthetic oxytocin (approximately 10 international units [IU] delivered intramuscularly or 20 IU delivered intravenously) is often provided for the prevention of postpartum hemorrhage (WHO, 2020).

Due to oxytocin's positive actions in social bonding and maternal behavior, questions have been raised about the clinical utility of synthetic oxytocin for improvements in maternal mood in addition to support for the labor process (Argiolas & Gessa, 1991; Baribeau & Anagnostou, 2015; Jones, 2017; Trifu et al., 2019). Conversely, there have been concerns about the overuse of synthetic oxytocin across parturition and potential neurobiological consequences. The half-life of synthetic oxytocin is approximately 10-12 minutes and was not previously thought to enter the central nervous system in significant amounts or have a lasting impact on maternal neurobiology (Arias, 2000; Buckley et al., 2023). However, recent evidence demonstrates the ability of synthetic oxytocin to reach the cerebrospinal fluid up to 60 minutes after peripheral administration via the intranasal and intravenous route (Freeman et al., 2016; Lee et al., 2016). Findings regarding the benefit of synthetic oxytocin have been inconsistent and, studies showing adverse effects on maternal mental health have raised important concerns (Bell et al., 2014; Tackacs et al., 2019).

Epigenetics and DNA Methylation

Epigenetics refers to the study of how behaviors and environment influence gene expression without changing the underlying DNA sequence. Epigenetic regulatory factors, including DNA methylation, histone modification, and non-coding RNA, can impact the expression of genes and subsequently impact health outcomes (Turnpin & Salbert, 2022). DNA

methylation is one of the most widely studied and well-characterized epigenetic mechanisms. In eukaryotic cells, a methyl group is transferred from the S'Adenosyl methionine (SAM) to the fifth position of a cytosine forming a 5-methyl cytosine (5mC). This process is catalyzed by DNA methyltransferases (DNMTs) and has important functions in gene regulation and cell differentiation.

Modifications of a cytosine occur especially when the cytosine is positioned next to a guanine nucleotide called a CpG site. Approximately 70-80% of CpGs are methylated across the genome regulating gene transcription in a cell-specific manner (Turnpin & Salbert, 2022). In the mammalian genome, regions called CpG islands are areas enriched with CpGs and are often associated with gene promoters and transcription start sites. Although most CpGs are methylated in the genome, CpG islands are seemingly protected from the process of DNA methylation showing that DNA methylation is location-dependent as well as cell-type specific (Turnpin & Salbert, 2022).

When methylation occurs in CpG islands within or nearby promoter regions and transcription start sites, this is often associated with gene repression or reduced gene transcription (Kusui et al., 2001; Liu et al., 2018). It is suggested that reduced transcription of the gene is due to a direct interference with the binding ability of transcription factors and an indirect effect on the organization of chromatin such that methyl-CpG binding proteins (e.g., MeCP2) are recruited and cause the chromatin structure to become condensed (Turnpin & Salbert, 2022). DNA methylation within gene promoter regions and near transcription start sites have been associated with adverse health outcomes such as autism, schizoaffective disorders, attachment disorders, anxiety, and depression and can be modulated by environmental factors (Lui et al., 2018).

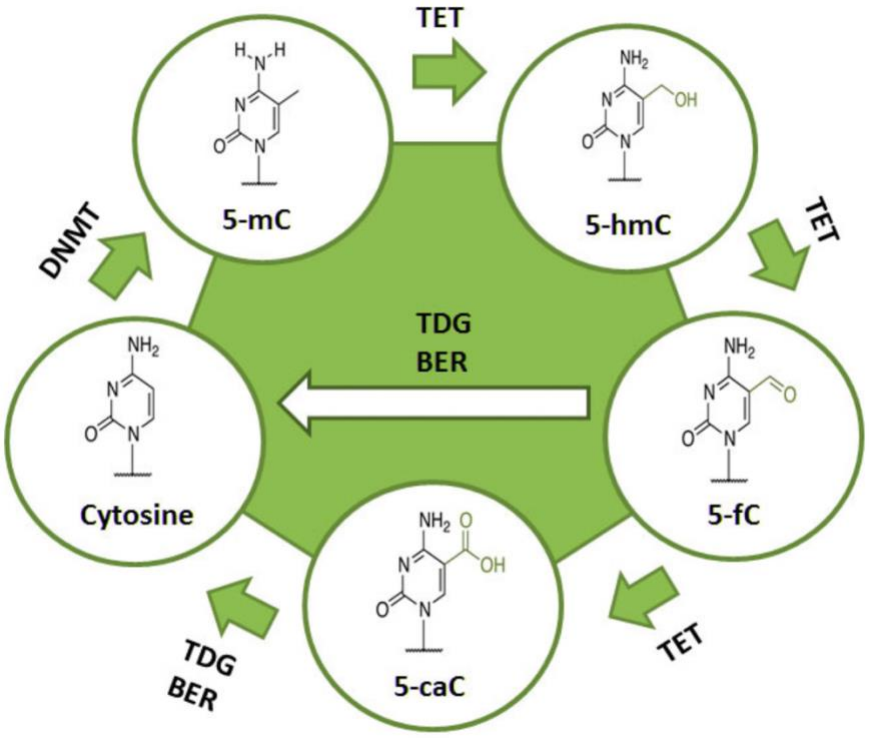
Regarding the development of maternal PPD, systematic reviews have discussed various genes that likely underpin outcomes of maternal depression including *5-HTT, TPH 1 and 2, COMT, MAO, BDNF, CYP2D6, MTHFR, Per2, OXTR, and GR/CHR1* (Castro e Couto et al., 2015; Payne & Maguire, 2019). The authors of these reviews suggest that outcomes of maternal PPD likely involve a combination of genetic and epigenetic factors (Castro e Couto et al., 2015; Payne & Maguire, 2019). Previous work has demonstrated an increased risk for maternal PPD considering genetic and epigenetic interactions in whole blood. In a case-control study of 545 women with and without postpartum depression (n= 269 cases, n= 276 controls), Bell and colleagues (2015) found a significant interaction between genotype rs53576, *OXTR* DNA methylation at CpG site -934 in MT2, and maternal PPD in a subgroup of 257 women without symptoms of depression during pregnancy ($p = 0.026$, adjusted for psychosocial covariates). Specifically, women with the GG genotype at rs53676 showed 2.63 greater odds of PPD (95% CI: 1.37, 5.03) for every 10% increase in methylation (Bell et al., 2015). Though the authors did not find significant interactions for those with PPD and depression during pregnancy, the data demonstrated an epigenetic modification that is associated with the development of depression after delivery (Bell et al., 2015).

DNA Hydroxymethylation

For decades, researchers thought DNA methylation was a static epigenetic marker but, in recent years, evidence suggests methylation is a dynamic process counteracted by demethylation mechanisms (Hackett et al., 2012; Ito et al., 2011; Shen et al., 2013). DNA hydroxymethylation is the process by which a methyl group located at the C5- position of the cytosine is replaced by a hydroxymethyl group and becomes 5-hydroxymethylcytosine (5hmC). 5hmC is an initial step in the active DNA demethylation pathway and plays a key role in plasticity during important

stages of development (Richa & Sinha, 2014). In continuation of this active demethylation pathway, TET proteins oxidize 5hmC to form 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) before returning to an unmethylated cytosine (see Figure 7). Evidence suggests 5hmC is sensitive to the environment, dynamic, and, although it is found within all tissues, it is most highly enriched in the mammalian brain (Branco et al., 2011; Globisch et al., 2010; Hackett et al., 2013; Madrid et al., 2016; Szulwach et al., 2011; Turnpin & Salbert, 2022; Wang et al., 2012). Whereas the presence of 5mC is associated with heterochromatin (or condensed chromatin) and inactive transcription, 5hmC is often associated with euchromatin (or loose chromatin) and active transcription (Branco et al., 2011; Turnpin & Salbert, 2022). 5hmC has important functions in epigenetic regulation and reprogramming of tissue-specific gene expression which may have important implications for outcomes of health and disorder (Turnpin & Salbert, 2022).

Figure 7. The process of active demethylation.



Note. This figure was retrieved from https://www.whatisepigenetics.com/dna-methylation/

Dissertation Organization

This dissertation consists of 5 chapters. Chapter one includes an overview of the dissertation study with a focus on the purpose, specific aims, the theoretical framework which guided the study, and a brief review of key concepts.

Chapter two includes a literature review examining relationships between maternal exposure to synthetic oxytocin during parturition and outcomes of maternal PPD. Further the review explores epigenetic regulation of the oxytocin receptor gene in the maternal brain as a potential underlying biological mechanism for the development of maternal PPD.

Chapter 3 outlines the methodology used to complete the current study including the study design, data collection procedures, and tissue processing procedures.

Chapter Four describes the major findings of the dissertation study including the dose-dependent effects of synthetic oxytocin on epigenetic regulation of the *Oxtr* gene in the nucleus accumbens and whole blood of term pregnant female prairie voles. The results of this study demonstrate an impact of synthetic oxytocin dose on the relationship between *Oxtr* DNA methylation and *Oxtr* gene expression in the nucleus accumbens. Correlations are further observed between the nucleus accumbens and whole blood indicating the translational potential of this work to human mothers. Chapter 4 is written in the form of a manuscript intended for researchers in clinical psychology to examine the neurobiological effects of synthetic oxytocin administration during parturition using the prairie vole as a model organism.

Chapter 5 provides a summary of the dissertation work and future clinical implications. The chapter concludes with a discussion regarding the contribution of this work to the advancement of maternal mental health research and offers suggestions for further translational study.

References

Alderdice, F., & Kelly, L. (2019). Stigma and maternity care. *Journal of Reproductive and Infant Psychology, 37*(2), 105-107. https://doi.org/10.1080/02646838.2019.1589758

Alhusen, J., Bullock, L., Sharps, P., Schminkey, D., Comstock, E., & Campbell, J. (2014). Intimate partner violence during pregnancy and adverse neonatal outcomes in low-income women. *J Womens Health (Larchmt), 23*(11), 920-926. https://doi.org/10.1089%2Fjwh.2014.4862

The American College of Obstetricians and Gynecologists (ACOG). (2015). *Screening for Perinatal Depression.* https://www.acog.org/clinical/clinical-guidance/committee-opinion/articles/2018/11/screening-for-perinatal-depression

ACOG. (2017). Postpartum hemorrhage. Retrieved from https://www.acog.org/clinical/clinical-guidance/practice-bulletin/articles/2017/10/postpartum-hemorrhage

ACOG. (2019). Approaches to limit intervention during labor and birth. Retrieved from https://www.acog.org/clinical/clinical-guidance/committee-opinion/articles/2019/02/approaches-to-limit-intervention-during-labor-and-birth

Argiolas, A., & Gessa, G. (1991). Central functions of oxytocin. *Neuroscience & Biobehavioral Reviews, 15*(2), 217-231. https://doi.org/10.1016/S0149-7634(05)80002-8

Arias, F. (2000). Pharmacology of oxytocin and prostaglandins. *Clin Obstet Gynecol, 43*(3), 455-468. https://doi.org/10.1097/00003081-200009000-00006

Baribeau, D., & Anagnostou, E. (2015). Oxytocin and vasopressin: Linking pituitary neuropeptides and their receptors to social neurociruits. *Frontiers in Neuroscience, 9.* https://doi.org/10.3389/fnins.2015.00335

Beck, C. (2002). Theoretical perspectives of postpartum depression and their treatment implications. *Am J Matern Child Nurs, 27*(5), 282-287. https://doi.org/10.1097/00005721-200209000-00008

Bell, A., Carter, S., Steer, C., Golding, J., Davis, J., Steffan, A., Rubin, L., Lillard, T., Gregory, S., Harris, J., & Connelly, J. (2015). Interactions between oxytocin receptor DNA methylation and genotype is associated with risk of postpartum depression in women without depression in pregnancy. *Frontiers in Genetics, 6*(243). https://doi.org/10.3389/fgene.2015.00243

Bell, A., Erickson, E., & Carter, S. (2014). Beyond labor: The role of natural and synthetic oxytocin in the transition to motherhood. *Journal of Midwifery & Women's Health, 59*(1), 35-42. https://doi.org/10.1111%2Fjmwh.12101

Bleuler, M. (1963). Conception of schizophrenia within the last fifty years and today. *Proc R Soc Med, 56*(10), 945-952. https://pubmed.ncbi.nlm.nih.gov/19994296

Bosch, O. (2013). Maternal aggression in rodents: Brain oxytocin and vasopressin mediate pup defense. *Philos Trans R Soc Lond B Biol Sci, 368*(1631). https://doi.org/10.1098%2Frstb.2013.0085

Branco, M., Ficz, G., & Reik, W. (2011). Uncovering the role of 5-hydroxymethylcytosine in the epigenome. *Nature Reviews Genetics, 13,* 7-13. https://doi.org/10.1038/nrg3080

Brockington, I. (2017). Suicide and filicide in postpartum psychosis. *Archives of Womens Mental Health, 20*(1), 63-69. https://doi.org/10.1007%2Fs00737-016-0675-8

Buckley, S., Uvnas-Morberg, K., Pajalic, Z., Luegmair, K., Ekstrom-Berstrom, A., Dencker, A., Massarotti, C., Kotlowska, A., Callaway, L., Morano, S., Olza, I., & Magistretti, C. (2023). Maternal and newborn plasma oxytocin levels in response to maternal synthetic

oxytocin administration during labor, birth and postpartum- A systematic review with implications for the function of the oxytocinergic system. *BMC Pregnancy and Childbirth, 23*(137). https://doi.org/10.1186/s12884-022-05221-w

Budden, A., Chen, L., & Henry, A. (2014). High-does versus low-dose oxytocin infusion regimens for induction of labor at term. *Cochrane Database Syst Rev, 2014*(10), https://doi.org/10.1002%2F14651858.CD009701.pub2

Dale, H. (1909). The action of extracts of the pituitary body. *Biochemical Journal,* 4(9), 427-447. https://doi.org/10.1042/bj0040427

Daly D, Minnie K, Blignaut A, Blix, E., Nilsen, A., Dencker, A., Beeckman. K., Gross, M., Pehlke-Milde, J., Grylka-Baeschlin, S., Koenig-Bachmann, M., Clausen, J., Hadjigeorgiou, E., Morano, S., Iannuzzi, L., Baranowska, B., Kiersnowska, I., & Uvnas-Moberg, K. (2020). How much synthetic oxytocin is infused during labour? A review and analysis of regimens used in 12 countries. *PLOS ONE,* 15(7). https://doi.org/10.1371/journal.pone.0227941

Danoff, J., Wroblewski, K., Graves, A., Quinn, G., Perkeybile, A., Kenkel, W., Lillard, T., Parikh, H., Golino, H., Gregory, S., Carter, S., Bales, K., & Connelly, J. (2021). Genetic, epigenetic, and environmental factors controlling oxytocin receptor gene expression. *Clinical Epigenetics, 13*(23). https://doi.org/10.1186/s13148-021-01017-5

Davis, N., Smoots, A., & Goodman, D. (2019). Pregnancy-related deaths: Data from 14 U.S. maternal mortality review committees, 2008-2017. Retrieved from https://www.cdc.gov/reproductivehealth/maternal-mortality/erase-mm/mmr-data-brief.html

Erbas, O., & Altuntas, I. (2020). *Oxytocin and Health.* IntechOpen.

Erickson, E., Myatt, L., Danoff, J., Krol, K., & Connelly, J. (2023). Oxytocin receptor DNA methylation is associated with exogenous oxytocin needs during parturition and postpartum hemorrhage. *Communications Medicine, 3*(11). https://doi.org/10.1038/s43856-023-00244-6

Food and Drug Administration (FDA). (2014). Pitocin. Retrieved from https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/018261s031lbl.pdf

Forder, P., Rich, J., Harris, S., Chojenta, C., Reilly, N., Austin, M., & Loxton, D. (2020). Honesty and comfort levels in mothers when screened for perinatal depression and anxiety. *Women and Birth, 33*(2), 142-150. https://doi.org/10.1016/j.wombi.2019.04.001

Freeman, S., Samineni, S., Allen, P., Stockinger, D., Bales, K., Hwa, G., & Roberts, J. (2016). Plasma and CSF oxytocin levels after intranasal and intravenous oxytocin in awake macaques. *Psychoneuroendocrinology, 66,* 185-194. https://doi.org/10.1016/j.psyneuen.2016.01.014

Fuchs, A., Goeschen, K., Husslein, P., Rasmussen, A., & Fuchs, F. (1983). Oxytocin and the initiation of human parturition: III. Plasma concentration of oxytocin and 13,14-dihydro-15-keto-prostaglandin F_{2a} in spontaneous and oxytocin-induced labor at term. *American Journal of Obstetrics and Gynecology, 147*(5), 497-502. https://doi.org/10.1016/0002-9378(83)90005-4

Gavin, N., Gaynes, B., Lohr, K., Meltzer-Brody, S., Gartlehner, G., & Swinson, T. (2005). Perinatal depression: A systematic review of prevalence and incidence. *Obstetrics and Gynecology, 106*(5), 1071-1083. https://doi.org/10.1097/01.aog.0000183597.31630.db

Gimpl, G., & Fahrenholz, F. (2001). The oxytocin receptor system: Structure, function and regulation. *Physiol Rev, 81*(2), 629-683. https://doi.org/10.1152/physrev.2001.81.2.629

Globisch, D., Munzel, M., Muller, M., Michalakis, S., Wagner, M., Koch, S., Bruckl, T., Biel, M., & Carell, T. (2010). Tissue distribution of 5-hydroxymethylcytosine and search for active demethylation intermediates. *PLOS ONE, 5*(12). https://pubmed.ncbi.nlm.nih.gov/21203455

Gregory, S., Connelly, J., Towers, A., Johnson, J., Biscocho, D., Markunas, C., Lintas, C., Abramson, R., Wright, H., Ellis, P., Langford, C., Worley, G., Delong, G., Murphym, S., Cuccaro, M., Perisco, A, & Pericak-Vance, M. (2009). Genomic and epigenetic evidence for oxytocin receptor deficiency in autism. *BMC Medicine, 7*(62). https://doi.org/10.1186/1741-7015-7-62

Gu, V., Feeley, N., Gold, I., Hayton, B., Robins, S., Mackinnon, A., Samuel, S., Carter, S., & Zelkowitz, P. (2015). Intrapartum synthetic oxytocin and its effects on maternal well-being at 2 months postpartum. *Birth, 43*(1), 28-35. https://doi.org/10.1111/birt.12198

Hackett, J., Sengupta, R., Zylickz, J., Murakami, K., Lee, C., Down, T., & Surani, M. (2012). Germline DNA demethylation dynamics and imprint erasure through 5-hydroxymethylcytosine. *Science, 339*(6118), 448-452. https://doi.org/10.1126/science.1229277

Hahn-Holbrook, J., & Haselton, M. (2014). Is postpartum depression a disease of modern civilization? *Curr Dir Psychol Sci, 23*(6), 395-400. https://doi.org/10.1177%2F0963721414547736

Halvereson, J. (2019). What are the DSM-5 criteria for diagnosis of major depressive disorder (clinical depression)? *Medscape.* https://www.medscape.com/answers/286759-14692/what-are-the-dsm-5-criteria-for-diagnosis-of-major-depressive-disorder-clinical-depression

Henshaw, C. (2003). Mood disturbance in the early puerperium: A review. *Archives of Women's Mental Health, 6*(2), 33-42. https://doi.org/10.1007/s00737-003-0004-x

Hutchison, J., Mahdy, H., & Hutchison, J. (2023). Stages of labor. *StatPearls.* Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK544290/

Ito, S., Shen, L., Dai, Q., Wu, S., Collins, L., Swenberg, J., He, C., & Zhang, Y. (2011). Tet proteins can convert 5-methylcytosine to 5-formlycytosine and 5-carboxylcytosine. *Science, 333*(6047), 1300-1303. https://doi.org/10.1126/science.1210597

Jones, C. (2017). Oxytocin and social functioning. *Dialogues in Clinical Neuroscience, 19*(2), 193-201. https://doi.org/10.31887%2FDCNS.2017.19.2%2Fcjones

Keebaugh, A., & Young, L. (2011). Increasing oxytocin receptor expression in the nucleus accumbens of pre-pubertal female prairie voles enhances alloparental responsiveness and partner preference formation as adults. *Hormones and Behavior, 60*(5), 498-504. https://doi.org/10.1016/j.yhbeh.2011.07.018

Kimmel, M., Clive, M., Gispen, F., Guintivano, J., Brown, T., Cox, O., Beckmann, M., Kornhuber, J., Fasching, P., Osbourne, L., Binder, E., Payne, J., & Kaminsky, Z. (2016). Oxytocin receptor methylation in postpartum depression. *Psychoneuroendocrinology, 69,* 150-160. https://doi.org/10.1016/j.psyneuen.2016.04.008

King, L., Robins, S., Chen, G., Yerko, V., Zhou, Y., Nagy, C., Feely, N., Gold, I., Hayton, B., Turecki, G., & Zelkowitz, P. (2017). Perinatal depression and DNA methylation of oxytocin-related genes: A study of mothers and their children. *Hormones and Behavior, 96,* 84-94. https://doi.org/10.1016/j.yhbeh.2017.09.006

Kingston, D., Tough, S., & Whitfield, H. (2012). Prenatal and postpartum maternal psychological distress and infant development: A systematic review. *Child Psychiatry and Human Development, 43*(5), 683-714. https://doi.org/10.1007/s10578-012-0291-4

Ko, J., Rockhill, K., Tong, V., Morrow, B., & Farr, S. (2017). Trends in postpartum depressive symptoms – 27 states, 2004, 2008, 2012. *MMWR.* Retrieved from https://www.cdc.gov/mmwr/volumes/66/wr/pdfs/mm6606a1.pdf

Kornfeld, B., Bair-Merritt, M., Frosch, E., & Solomon, B. (2012). Postpartum depression and intimate partner violence in urban mothers: Co-occurrence and child healthcare utilization. *J Pediatr, 161*(2), 348-353. https://doi.org/10.1016/j.jpeds.2012.01.047

Kroll-Desrosiers, A., Nephew, B., Babb, J., Guilarte-Walker, Y., Simas, T., & Deligaiannidis, K. (2017). Association of peripartum synthetic oxytocin administration and depressive and anxiety disorders within the first postpartum year. *Depress Anxiety, 34*(2), 137-146. https://doi.org/10.1002%2Fda.22599

Kruit, H., Nupponen, I., Heinonen, S., & Rahkonen, L. (2022). Comparison of delivery outcomes in low-dose and high-dose oxytocin regimens for induction of labor following cervical ripening with a balloon catheter: A retrospective observational cohort study. *PLOS ONE.* https://doi.org/10.1371/journal.pone.0267400

Kusui, C., Kimura, T., Ogita, K., Nakamura, H,. Matsumara, Y., Koyama, M., Azuma, C., Murata, Y. (2001). DNA methylation of the human oxytocin receptor gene promoter regulates tissue-specific gene suppression. *Biochemical and Biophysical Research Communications, 289*(3), 681-686. https://doi.org/10.1006/bbrc.2001.6024

Lancaster, C., Gold, K., Flynn, H., Yoo, H., Marcus, S., & Davis, M. (2010). Risk factors for depressive symptoms during pregnancy: A systematic review. *Am J Obstet Gynecol, 202*(1), 5-14. https://doi.org/10.1016/j.ajog.2009.09.007

Lee, M., Scheidweiler, K., Diao, X., Akhlaghi, F., Cummins, A., Huestis, M., Leggio, L., & Averbeck, B. (2018). Oxytocin by intranasal and intravenous route reaches the cerebrospinal fluid in rhesus macaques: Determination using a novel oxytocin assay. *Molecular Psychiatry, 23*(1), 115-122. https://doi.org/10.1038/mp.2017.27

LeWinn, K., Stroud, L., Molnar, B., Ware, J., Koenen, K., & Buka, S. (2009). Elevated maternal cortisol levels during pregnancy are associated with reduced childhood IQ. *International Journal of Epidemiology, 38*(6), 1700-1710. https://dx.doi.org/10.1093%2Fije%2Fdyp200

Liu, C., Jiao, C., Wang, K., & Yuan, N. (2018). DNA methylation and psychiatric disorders. *Progress in Molecular Biology and Translational Science, 157*, 175-232. https://doi.org/10.1016/bs.pmbts.2018.01.006

Madrid, A., Papale, L., & Alisch, R. (2016). New hope: The emerging role of 5-hydroxymethylcytosine in mental health and disease. *Future Medicine*. https://doi.org/10.2217/epi-2016-0020

Mann, J., Waternaux, C., Haas, G., & Malone, K. (1999). Toward a clinical model of suicidal behavior in psychiatric patients. *American Journal of Psychiatry, 156*(2), 181-189. https://doi.org/10.1176/ajp.156.2.181

McCullough, M., Churchland, P., & Mendez, A. (2013). Problems with measuring peripheral oxytocin: Can the data on oxytocin and human behavior be trusted. *Neuroscience and*

Biobehavioral Reviews, 37(2013), 1485-1492. https://doi.org/10.1016/j.neubiorev.2013.04.018

McDonald, M., & Alhusen, J. (2022). A review of treatments and clinical guidelines for perinatal depression. *Journal of Perinatal and Neonatal Nursing, 36*(3), 233-242. https://doi.org/10.1097/jpn.0000000000000661

Minkovitz, C., Strobino, D., Hou, W., Miller, T., Mistry, K., & Swartz, K. (2005). Maternal depressive symptoms and children's receipt of health care in the first 3 years of life. *Pediatrics, 115*(2), 306-314. https://doi.org/10.1542/peds.2004-0341

Monroe, S., & Simons, A. (1991). Diathesis-stress theories in the context of life stress research: Implications for the depressive disorders. *Psychol Bull, 110*(3), 406-425. https://doi.org/10.1037/0033-2909.110.3.406

Mughal, S., Azhar, Y., Siddiqui, W. (2022). Postpartum depression. *StatPearls.* Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK519070/

Olazabal, D., & Young, L. (2006). Oxytocin receptors in the nucleus accumbens facilitate "spontaneous" maternal behavior in adult female prairie voles. *Neuroscience, 141*(2), 559-568. https://doi.org/10.1016/j.neuroscience.2006.04.017

Oscilla, E., & Sharma, S. (2022). Oxytocin. *StatPearls.* Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK507848/

Osterman, M., Hamilton, B., Martin. J., Discroll, A., & Valenzuela, C. (2023). Births: Final data for 2021. *National Vital Statistics Reports.* Retrieved from https://www.cdc.gov/nchs/data/nvsr/nvsr72/nvsr72-01.pdf

Payne, J., & Maguire, J. (2019). Pathophysiological mechanisms implicated in postpartum depression. *Frontiers in Neuroendocrinology, 52*, 165-180. https://doi.org/10.1016/j.yfrne.2018.12.001

Pedro, L., Branquinho, M., Canavarro, M., & Fonseca, A. (2019). Self-criticism, negative automatic thoughts and postpartum depressive symptoms: The buffering effect of self-compassion. *Journal of Reproductive and Infant Psychology.* https://doi.org/10.1080/02646838.2019.1597969

Perkeybile, A., Carter, S., Wroblewski, K., Puglia, M., Kenkel, W., Lillard, T., Karaoli, T., Gregory, S., Mohammadi, N., Epstein, L., Bales, K., & Connelly, J. (2019). Early nurture epigenetically tunes the oxytocin receptor. *Psychoneuroendocrinology, 99,* 128-136. https://doi.org/10.1016/j.psyneuen.2018.08.037

Perkeybile, A., Kenkel, W., Yee, J., Lillard, T., Ferris, C., Carter, S., & Connelly, J. Pregnancy and birth epigenetically shape the maternal oxytocin receptor. *(In prep).*

Phaneuf, S., Rodríguez Liñares, B., TambyRaja, R., MacKenzie, I., & Bernal, A. (2000). Loss of myometrial oxytocin receptors during oxytocin-induced and oxytocin-augmented labour. *Journal of Reproduction and Fertility,* 120(1), 91-97. https://doi.org/10.1530/jrf.0.1200091

Putnick, D., Sundaram, R., Bell, E., Ghassabian, A., Goldstein, R., Robinson, S., Vafai, Y., Gilman, S., & Yeung, E. (2020). Trajectories of maternal postpartum depressive symptoms. *Pediatrics, 146*(5). https://doi.org/10.1542/peds.2020-0857

Recto, P., & Champion, J. (2018). "We don't want to be judged": Perceptions about professional help and attitudes towards help-seeking among pregnant and postpartum Mexican

American Adolescents. *Journal of Pediatric Nursing, 42,* 111-117. https://doi.org/10.1016/j.pedn.2018.04.010

Richa, R., & Sinha, R. (2014). Hydroxymethylation of DNA: An epigenetic marker. *EXLI Journal, 13,* 592-610. https://pubmed.ncbi.nlm.nih.gov/26417286

Robinson, C., Schumann R., Zhang, P., & Young, R. (2003). Oxytocin-induced desensitization of the oxytocin receptor. *American Journal of Obstetrics & Gynecology, 188*(2), 497-502. https://doi.org/10.1067/mob.2003.22

Rouse, M., & Goodman, S. (2014). Perinatal depression influences on infant negative affectivity; timing, severity, and co-morbid anxiety. *Infant Behavior & Development, 37,* 739-751. https://psycnet.apa.org/doi/10.1016/j.infbeh.2014.09.001

Scatliffe, N., Casavant, S., Vittner, D., & Cong, X. (2019). Oxytocin and early parent-infant interactions : A systematic review. *International Journal of Nursing Sciences, 6*(4), 445-453. https://doi.org/10.1016%2Fj.ijnss.2019.09.009

Seitchik, J., Amico, J., & Castillo, M. (1985). Oxytocin augmentation of dysfunctional labor. V. An alternative oxytocin regimen. *Am J Obstet Gynecol, 151*(6), 757-761. https://doi.org/10.1016/0002-9378(85)90514-9

Shen, L., Wu, H., Diep, D., Yamaguchi, S., D'Alessio, A., Fung, H., Zhang, K., & Zhang, Y. (2013). Genome-wide analysis reveals TET- and TDG- dependent 5-Methylcytosine oxidation dynamics. *Cell, 153*, 692-706. http://dx.doi.org/10.1016/j.cell.2013.04.002

Simpson, K. (2022). Trends in labor induction in the United States, 1989 to 2020. *The American Journal of Maternal Child Nursing, 47*(4), 235. https://doi.org/10.1097/NMC.0000000000000824

Swales, D., Winiarski, D., Smith, A., Stowe, Z., Newport, D., & Brennan, P. (2017). Maternal depression and cortisol in pregnancy predict offspring emotional reactivity in the preschool period. *Developmental Psychobiology, 60,* 557-566. https://doi.org/10.1002/dev.21631

Szulwach, K., Li, X., Li, Y., Song, C., Wu, H., Dai, Q., Irier, H., Upadhyay, A., Gearing, M., Levey, A., Vasanthakumar, A., Godley, L., Chang, Q., Cheng, X., He, C., & Jin, P. (2012). 5-hmC-mediated epigenetic dynamics during postnatal neurodevelopment and aging. *Nat Neurosci, 14*(12), 1607-1616. https://doi.org/10.1038%2Fnn.2959

Tackacs, L., Seidlerova, J., Sterbova, Z., Cepicky, P., & Havlicek, J. (2019). The effects of intrapartum synthetic oxytocin on maternal postpartum mood: Findings from a prospective observational study. *Archives of Women's Mental Health, 22*(4), 485-491. https://doi.org/10.1007%2Fs00737-018-0913-3

Thul, T., Corwin, E., Carlson, N., Brennan, P., & Young, L. (2020). Oxytocin and postpartum depression: A systematic review. *Psychoneuroendocrinology, 120.* https://doi.org/10.1016/j.psyneuen.2020.104793

Tichelman, E., Warmink-Perdijk, W., Henrichs, J., Peters, L., Schellevis, F., Berger, M., & Burger, H. (2021). Intrapartum synthetic oxytocin, behavioral and emotional problems in children and the role of postnatal depressive symptoms, postnatal anxiety and mother-to-infant bonding: A Dutch prospective cohort study. *Midwifery, 100.* https://doi.org/10.1016/j.midw.2021.103045

Toepfer, P., O'Donnell, K., Entringer, S., Garg, E., Heim, C., Lin, D., MacIsaac, J., Kobor, M., Meaney, M., Provencal, N., Binder, E., Wadhwa, P., & Buss, C. (2019). Dynamic DNA methylation changes in maternal oxytocin gene locus (OXT) during pregnancy predict

postpartum maternal intrusiveness. *Psychoneuroendocrinology, 103,* 156-162. https://doi.org/10.1016/j.psyneuen.2019.01.013

Tops, S., Hable, U., & Radke, S. (2019). Genetic and epigenetic regulatory mechanisms of the oxytocin receptor gene *(OXTR)* and the (clinical) implications for social behavior. *Hormones and Behavior, 108*, 84-93. https://doi.org/10.1016/j.yhbeh.2018.03.002

Trifu, S., Vladuit, A., & Popescu, A. (2019). The neuroendocrinological aspects of pregnancy and postpartum depression. *Acta Endocrinology, 15*(3), 410-415. https://doi.org/10.4183%2Faeb.2019.410

Turnpin, M., & Salbert, G. (2022). 5-methylcytosine turnover: Mechanisms and therapeutic implications in cancer. *Frontiers in Molecular Biosciences, 9.* https://doi.org/10.3389/fmolb.2022.976862

U.S Preventive Services Task Force. (2020). *Perinatal depression: Preventive Interventions*. https://www.uspreventiveservicestaskforce.org/uspstf/document/RecommendationStatementFinal/perinatal-depression-preventive-interventions

Uvnas-Morber, K., Ekstrom-Berstrom, A., Berg, M., Buckley, S., Pajalic, Z., Hadjigeorgiou, E., Kotlowska, A., Lengler, L., Kielbratowska, B., Leon-Larios, F., Magistrettie, C., Downe, S., Lindstrom, B., & Dencker, A. (2019). Maternal plasma levels of oxytocin during physiological childbirth- A systematic review with implications for uterine contractions and central actions of oxytocin. *BMC Pregnancy and Childbirth, 19*(285). https://doi.org/10.1186/s12884-019-2365-9

Veening, J., de Jong, T., & Barendregt, H. (2010). Oxytocin-messages via the cerebrospinal fluid: behavioral effects; a review. *Physiol Behav, 101*(2), 193-210. https://doi.org/10.1016/j.physbeh.2010.05.004

Vogel, F. (1991). Schizophrenia genesis: The origins of madness. *Am. J Human Genet, 48,* 1218.

Wang, Z., Liu, J., Shuai, H., Cai, Z., Fu, X., Liu, Y., Xiao, Y., Zhang, W., Krabbendam, E., Liu, S., Liu, Z., Li, Z., & Yang, B. (2021). Mapping global prevalence of depression among postpartum women. *Translational Psychiatry, 11.* https://doi.org/10.1038/s41398-021-01663-6

Wouk, K., Stuebe, A., & Meltzer-Brody, S. (2017). Postpartum mental health and breastfeeding practices: An analysis using the 2010-2011 Pregnancy Risk Assessment Monitoring System. *Maternal Child Health Journal, 21*(3), 636-647. https://doi.org/10.1007/s10995-016-2150-6

World Health Organization (WHO). (2012). WHO recommendations for the prevention and treatment of postpartum hemorrhage. https://apps.who.int/iris/bitstream/handle/10665/75411/97?sequence=1

Yim, I., Stapleton, L., Guardino, C., Hahn-Holbrook, J., & Schetter, C. (2015). Biological and psychosocial predictors of postpartum depression: Systematic review and call for integration. *Annual Review of Clinical Psychology, 11,* 99-137. https://doi.org/10.1146/annurev-clinpsy-101414-020426

Zuckerman, M. (1999). *Vulnerability to psychopathology: A biosocial model.* American Psychological Association

CHAPTER TWO

A Review of Synthetic Oxytocin and Maternal Postpartum Depression

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Abstract

Synthetic oxytocin is widely administered in hospital settings to induce and augment uterine contractions when medically indicated and to prevent and treat maternal postpartum hemorrhage. Though there are important benefits of synthetic oxytocin administration during parturition, studies have reported a potential risk for adverse maternal psychological outcomes. Specifically, a link between maternal exposure to synthetic oxytocin during parturition and postpartum depression (PPD) is reported in the literature and raises important concerns. This review synthesizes the current evidence regarding the relationship between maternal exposure to synthetic oxytocin and outcomes of maternal PPD. In addition, epigenetic regulation of the oxytocin receptor gene in the maternal brain is explored as a potential underlying biological mechanism for the development of maternal PPD. The findings in this review are mixed with some studies reporting an increased risk for maternal PPD, while others report either no relationship or a decreased risk for maternal PPD when mothers are exposed. Recommendations for future studies are to consider factors such as dose-dependent effects of synthetic oxytocin, variations in the mother's endogenous oxytocin level and differentiation of PPD from other common postpartum conditions, such as postpartum blues. There were no findings regarding the impact of synthetic oxytocin as a birth intervention on epigenetic regulation of the oxytocin receptor gene in the maternal brain, though two animal studies analyzed effects in the offspring. More research is needed to understand the effects of synthetic oxytocin on maternal postpartum mental health and the biological mechanisms that may underlie outcomes of maternal PPD.

Keywords: Oxytocin, synthetic oxytocin, epigenetics, *OXTR,* maternal brain, parturition, postpartum depression

A Review of Synthetic Oxytocin and Maternal Postpartum Depression

Synthetic oxytocin is widely administered in hospital settings to induce and augment uterine contractions when medically necessary and to prevent and treat maternal postpartum hemorrhage. In the mid-1950s, chemist Vincent du Vigneaud discovered a method to synthesize oxytocin to improve uterine contractions and the process of childbirth. Since the 1960's, obstetricians have worked to refine techniques to use synthetic oxytocin (i.e., Pitocin) for indications such as maternal hypertension, toxemia, fetal distress, and prolonged pregnancy (Food and Drug Administration [FDA], 2014). The World Health Organization has further recommended the use of synthetic oxytocin as a first-line agent during third-stage labor for the prevention and treatment of maternal postpartum hemorrhage (World Health Organization [WHO], 2012).

Although there are important benefits of synthetic oxytocin administration during parturition, studies have reported adverse maternal health outcomes particularly associated with high dose oxytocin regimens during labor induction and augmentation (Erickson et al., 2023; Phaneuf et al., 2000; Robinson et al., 2003). Specifically, excessive dosing of synthetic oxytocin can lead to adverse reproductive outcomes such as uterine hypertonicity, uterine spasms, tetanic contractions, uterine atony, and uterine rupture (FDA, 2014). In 2000, Phaneuf and colleagues found that prolonged use of synthetic oxytocin during labor induction and augmentation resulted in decreased expression of the oxytocin receptor *(OXTR)* gene in the maternal uterus. Importantly, downregulation of *OXTR* in the maternal uterus can underlie outcomes of uterine atony and subsequent postpartum hemorrhage (Erickson et al., 2023; Robinson et al., 2003). Phaneuf and colleagues (2000) found that augmentation of labor for an average of 4.7 hours resulted in decreased *OXTR* mRNA in the uterus by 60-fold (Phaneuf et al., 2000). Moreover,

the use of synthetic oxytocin for an average of 7.7 hours during labor induction demonstrated a 300-fold decrease in *OXTR* mRNA in the maternal uterus (Phaneuf et al., 2000). In a recent population-based study, Ahmadzia and colleagues (2020) found that rates of postpartum hemorrhage caused by uterine atony have continued to increase over time despite increases in the use of synthetic oxytocin. Importantly, the incidence of postpartum hemorrhage caused by uterine atony increased by 14.1% from the year 2001 to 2012 in vaginal deliveries that were induced with synthetic oxytocin while an 18.3% decrease was observed in vaginal deliveries that were not induced during this same period (Ahmadzia et al., 2020).

In addition to adverse maternal reproductive risks, researchers have highlighted the potential negative effects of synthetic oxytocin on maternal mental health (Thul et al., 2021). As an endogenous polypeptide, oxytocin has important and complex functions across various body systems. In addition to its crucial role in promoting uterine contractions during parturition, studies have found an increase in basal levels of oxytocin within cerebrospinal fluid which is thought to facilitate maternal-infant bonding and an adaptation to motherhood (Bell et al., 2014; Uvnas-Morber et al., 2019). Alterations to the maternal oxytocinergic system during parturition with use of synthetic oxytocin may have important implications for subsequent variations in maternal postpartum mood. Several studies have examined relationships between endogenous oxytocin, epigenetic regulation of *OXTR,* and outcomes of maternal postpartum mood; however, less is understood regarding the impact of synthetic oxytocin on maternal neurobiology and subsequent mental health outcomes (Mah, 2016; Moura et al., 2016; Thul et al., 2021).

For decades, there has been significant controversy regarding the ability of synthetic oxytocin to cross the blood-brain barrier and impact neurobiology. Previously, oxytocin administered peripherally was not thought to cross the blood-brain barrier due to its molecular

size and hydrophilic nature; however, recent evidence using animal models suggests that synthetic oxytocin can be found in the cerebral spinal fluid (CSF) after peripheral administration (Freeman et al., 2016; Lee et al., 2016). Moreover, the degradation of oxytocin in the central nervous system is found to be much slower than degradation in the peripheral bloodstream, which may contribute to lasting behavioral and psychological effects (Amico et al., 1983; Gu et al., 2015; Uvnas-Morber et al., 2019).

A mother's mental health can have important implications for long-term maternal and child health outcomes. Specifically, extant research describes adverse effects of maternal postpartum depression (PPD) on early childhood development including a decline in cognitive function, an increase in internalizing and externalizing problems, and an increase in irritability and emotional negativity toward stressors (LeWinn et al., 2009; Rouse & Goodman, 2014; Swales et al., 2017). Thus, it is important to understand factors that may increase the risk for poor maternal postpartum mental health. The purpose of this review is to examine the current evidence regarding the relationship between synthetic oxytocin administration during parturition and outcomes of maternal PPD. In addition, epigenetic regulation of the oxytocin receptor gene in the maternal brain is explored as a potential underlying biological mechanism for the development of maternal PPD.

Maternal Effects of Synthetic Oxytocin in Human Research

Five studies are found in the literature involving the relationship between synthetic oxytocin administration during parturition and outcomes of maternal PPD (Gu et al., 2016; Hinshaw et al., 2008; Kroll-Desrosiers et al., 2017; Takacs et al., 2019; Tichelman et al., 2021, see Table 1). In the studies reviewed, symptoms of maternal depression were identified by the mother's score on a validated depression scale (e.g., the Edinburgh Postnatal Depression scale

(EPDS)), a diagnosis of PPD reported in the electronic medical record, or by use of an antidepressant or anxiolytic medication. In addition, some studies examined outcomes of comorbid maternal anxiety (Gu et al., 2016; Kroll-Desrosiers et al., 2017; Tichelman et al., 2021), posttraumatic stress (Gu et al., 2016), somatization symptoms (Gu et al., 2016), mother-infant bonding (Tichelman et al., 2021) and child internalizing and externalizing behaviors (Tichelman et al., 2021). Various timepoints were used to examine maternal psychological outcomes across studies ranging from 48 hours after delivery to one year postpartum.

Most of the studies (n= 4) identified maternal exposure to synthetic oxytocin via the electronic medical record (Gu et al., 2016; Kroll-Desrosiers et al., 2017; Takacs et al., 2019; Tichelman et al., 2021). Three studies examined the total dose of synthetic oxytocin including uses for labor induction, augmentation and prevention and treatment of postpartum hemorrhage (Gu et al., 2016; Kroll-Desrosiers et al., 2017; Takacs et al., 2019). One study examined the effects of synthetic oxytocin provided only for labor induction and augmentation (Tichelman et al., 2021) and one study assessed the effects of immediate or delayed use of synthetic oxytocin during labor augmentation on outcomes of maternal depression after delivery (Hinshaw et al., 2008).

Three of the five studies reported a higher incidence of maternal PPD after exposure to synthetic oxytocin during parturition (Gu et al., 2016; Kroll-Desrosiers et al., 2017; Tichelman et al., 2021). Gu and colleagues (2016) examined maternal outcomes for 386 mothers in Montreal Canada from 2009 to 2013. At two months postpartum, the mothers completed psychological distress questionnaires assessing symptoms of depression, anxiety, posttraumatic stress, and somatization. In addition, all maternal participants provided blood samples for the authors to assess endogenous oxytocin measurements at two months postpartum. The authors found that

maternal exposure to synthetic oxytocin was significantly correlated with symptoms of depression ($r = 0.15$, $p < 0.01$), anxiety ($r= 0.11$, $p < 0.05$) and somatic symptoms ($r = 0.18$, $p < 0.01$) at two months postpartum. Further, a significant positive relationship was found between synthetic oxytocin dosing and endogenous plasma oxytocin levels at two months postpartum ($r= 0.16$, $p < 0.01$), indicating a sustained response to synthetic oxytocin exposure during parturition.

In 2017, Kroll-Desrosiers and colleagues reported outcomes of a large population-based study including 9,684 mothers exposed to synthetic oxytocin during parturition and 37,048 mothers that were not exposed. Maternal PPD up to one year postpartum was identified by a record of diagnosis or the use of an antidepressant or anxiolytic medication after delivery. The results of a risk comparison model demonstrated a 32% increased risk of maternal PPD for women exposed to synthetic oxytocin during parturition and without a previous history of depressive or anxiety disorders (RR: 1.32; CI: 1.23–1.42). In women with a history of pre-pregnancy depression or anxiety, exposure to synthetic oxytocin increased the risk of maternal PPD by 36% (RR: 1.36; 95% CI: 1.20-1.55).

In 2021, Tichelman and colleagues reported results of a prospective cohort study involving 1528 mothers. A total of 607 mothers received synthetic oxytocin for labor induction or augmentation purposes and were assessed in the study. The authors examined maternal PPD symptoms via an EPDS score at six months postpartum and assessed additional measures including postpartum anxiety, maternal-infant bonding and child internalizing and externalizing problems. The authors determined a small significant effect of maternal exposure to synthetic oxytocin on outcomes of maternal PPD ($\beta = 0.17$, 95% CI of 0.03 to 0.30). They did not find any significant associations with outcomes of postpartum anxiety or mother-infant bonding; however,

the authors noted that maternal PPD symptoms, maternal anxiety and mother-infant bonding were associated with subsequent internalizing and externalizing problems of the child.

One study analyzing the relationship between maternal synthetic oxytocin exposure and maternal PPD found no association between these two variables (Hinshaw et al., 2008). Hinshaw and colleagues (2008) conducted a randomized controlled trial in which synthetic oxytocin was administered immediately in an active labor augmentation protocol or was delayed for eight hours in conservative management. In the delayed group of 204 mothers, a positive PPD screen (as evidenced by a EPDS score > 12) within the first 48 hours of delivery occurred 15 % of the time as compared to 20 % in the group with longer exposure (n= 208 mothers). The difference between groups was not statistically significant; thus, the authors determined there were no clear maternal psychological benefits or harms associated with immediate or delayed oxytocin administration (Hinshaw et al., 2008).

In 2019, Takacs and colleagues reported findings from a longitudinal perspective study of 601 mothers analyzing symptoms of maternal depression across four time points (T1: third trimester; T2: 1-7 days post-delivery; T3: 6 weeks postpartum; T4: 9 months postpartum). When assessing outcomes of PPD specifically (as evidenced by EPDS score > 12 at 9 months postpartum), the authors reported a protective effect of synthetic oxytocin administration during parturition (HR = 0.65, 95% CI 0.45 – 0.95, p = 0.025). In addition, the authors noted important confounding risk factors for maternal PPD including a history of depression (HR = 3.20, 95% CI 2.33-4.40, p < 0.001) and a negative childbirth experience (HR = 1.39, 95% CI 1.01-1.90, p= 0.04).

Maternal Effects of Synthetic Oxytocin in Animal Research

Animal models can be beneficial for studying biological mechanisms that underlie psychological outcomes. In the context of maternal psychosocial outcomes, studies involving animal subjects have explored relationships between the oxytocinergic system in the maternal brain and subsequent maternal behaviors (Bosch, 2013; Olazabal & Young, 2008; Pedersen et al., 1994). For example, Olazabal and Young (2008) found that female prairie voles demonstrating maternal behaviors (including licking, grooming, and/or huddling over prairie vole pups) had higher oxytocin receptor density, specifically in the nucleus accumbens (shell subregion), than those that did not show maternal responses or attacked the pups ($p < 0.05$). In addition, an infusion of an oxytocin antagonist directly into the nucleus accumbens temporarily blocked spontaneous maternal behaviors in adult female prairie voles (Olazabal & Young, 2008). In rats, the delivery of an oxytocin antagonist into the ventral tegmental area (VTA) or the medial preoptic area (MPOA) has also been shown to block maternal behaviors including pup retrieval and typical nursing postures (Pedersen et al., 1994).

Several animal studies have reported positive effects of synthetic oxytocin (e.g., anti-depressant effects and increased sexual arousal) when administered via a direct injection in the brain or via the intranasal route (Artletti & Bertolini, 1987; Lui et al., 2019; Melis et al., 2007; Wang et al., 2018); however, there are few studies examining the effects of synthetic oxytocin as a birth intervention in term pregnant female subjects (see Table 1). Two studies are found in the literature using the prairie vole as a model organism to assess the effects of synthetic oxytocin administration at term pregnancy (Kenkel et al., 2019; Kenkel et al., 2023). Neither of these studies assessed neurobiological or behavioral outcomes in the mothers but instead focused on offspring outcomes.

In 2019, Kenkel and colleagues report findings from four experimental studies in which offspring outcomes were measured after exposure to various doses of synthetic oxytocin in utero. The results of experiment 1 demonstrate an increase in fetal oxytocin plasma levels and an increase in neuronal activity after an intraperitoneal injection of synthetic oxytocin in the mother at term pregnancy. In experiment 2, the authors found bradycardic responses in fetal heart rate after direct and indirect exposure to synthetic oxytocin demonstrating immediate physiological effects in the offspring. In the third experiment, findings reveal an impact of synthetic oxytocin and fetal weight on epigenetic regulation of the *Oxtr* gene such that higher *Oxtr* DNA methylation was observed among heavier pups in the oxytocin treated groups. Interestingly, fetal weight also led to an increase in *Oxtr* gene expression, though increased DNA methylation is generally associated with reduced gene expression (Kusui et al., 2001; Turnpin & Salbert, 2022). In the fourth experiment, the authors found an effect of synthetic oxytocin on offspring behavior such that offspring exposed to oxytocin in utero emitted more vocalizations at postnatal day 1 and 4 than offspring exposed to a saline vehicle ($p = 0.013$). Offspring in the oxytocin-treated group also demonstrated more alloparental caregiving at postnatal day 50 to 55 ([$F(5,55) = 4.43$, $p = 0.002$], and spent more time huddling side-by-side with a partner [$F(1,90) = 12.86$, $p = 0.001$) and with a stranger ($p = 0.056$).

In 2023, Kenkel and colleagues describe a subsequent study analyzing the neuroanatomical consequences of synthetic oxytocin treatment at birth in prairie vole offspring. The authors found increased functional connectivity, particularly in adult male offspring, with increased closeness and degree of centrality. Importantly, these neuroanatomical changes were observed after one maternally administered dose of synthetic oxytocin. Given that the clinical

administration of synthetic oxytocin in human mothers often occurs over a period and in varying amounts, these findings provide an intriguing basis for future research (Daly et al., 2020).

Table 1

Studies examining relationships between the maternal oxytocinergic system and maternal postpartum mental health.

Consequences of Synthetic Oxytocin Reported in Human Research					
Authors/Date	Participants	Methods	Results	Conclusion	Study Limitations
Hinshaw et al., 2008	N = 412 nulliparous women (n = 208 in active arm; n = 204 in conservative arm)	Participants randomized to active management (oxytocin infusion within 20 minutes of randomization) or conservative management (oxytocin infusion withheld for 8 hours unless medically indicated) Psychological assessments (EPDS, Labor Agentry Scale, Attitudes Towards the Pregnancy and Baby Scale) completed within 48 hours of delivery	No significant differences seen in depression symptoms between the active or conservative group (EPDS > 12; p = 0.22) No significant difference in outcomes of required c-section between groups (p > 0.05)	Early versus delayed synthetic oxytocin administration does not predict differences in depression or c-section outcomes	Obstetricians and patients were not blinded to treatment group Small sample size
Gu et al., 2016	N = 386 mothers in Montreal Canada (2009-2013)	Data collected at recruitment and 2 mo postpartum At 2 months postpartum: completed psychological distress	SynOT significantly and positively correlated with endogenous OXT levels at 2 months postpartum SynOT exposure significantly	Synthetic oxytocin is positively associated with postpartum endogenous oxytocin and long-term adverse	Correlational design cannot determine causation Did not control for prenatal mental health, labor duration

		questionnaires (assessed depression, anxiety, posttraumatic stress, and somatization); reported breastfeeding; blood samples collected for circulating oxytocin measurement at least 30 minutes after breastfeeding	predicted endogenous OXT levels Significant positive relationship between SynOT dose and depressive symptoms, anxiety and somatic symptoms at 2 months postpartum	maternal mental health outcomes	or endogenous oxytocin levels prior to administration of synthetic oxytocin
Kroll-Desrosiers et al., 2017	N= 9,684 mothers exposed to peripartum synthetic oxytocin N= 37,048 mothers not exposed to peripartum synthetic oxytocin	Depressive or anxiety disorders classified by diagnosis or antidepressant or anxiolytic medication use	Women with history of depression or anxiety prepregnancy had 36% higher risk of postpartum depression and anxiety after exposure to synthetic oxytocin (RR: 1.36; 95% CI: 1.20-1.55) Women without history of depression or anxiety prepregnancy had 32% increased risk of postpartum depression and anxiety compared to those not exposed (RR: 1.32; 95% CI: 1.23-1.42)	Women exposed to peripartum synthetic oxytocin have a higher risk of postpartum depression and anxiety	Did not assess effects of synthetic oxytocin on endogenous oxytocin Could not determine causal effects Could not differentiate risk based on higher or lower amounts of synthetic oxytocin Data missing mode of delivery, and potential confounding variables (family history of PPD or anxiety, maternal BMI, maternal gestational

					diabetes, Pitocin dosing)
Takacs et al., 2019	N= 601 mothers	Depressive symptoms reported at T1 (last trimester of pregnancy), T2 (1-7 days after delivery), T3 (6 weeks postpartum) and T4 (9 months postpartum)	25.3% of participants received SynOT SynOT exposed group had less frequent depressive symptoms at 9 months postpartum SynOT found to be a protective factor against PPD (HR = 0.65, 95% CI 0.45 – 0.95, p = 0.025) Confounding risk factors include history of depression (HR = 3.20, 95% CI 2.33-4.40, p < 0.001) and negative childbirth experience (HR = 1.39, 95% CI 1.01-1.90, p= 0.04)	Synthetic oxytocin may decrease the risk of PPD symptoms weeks after childbirth	Did not assess timing or dose of synthetic oxytocin administration Did not control for endogenous oxytocin
Tichelman et al., 2021	N = 1528 mothers n= 921 mothers without intrapartum oxytocin exposure n= 607 mothers with intrapartum oxytocin exposure	Prospective cohort study Intrapartum oxytocin exposure identified by medical record; administration defined as yes/no Child behavioral checklist assessed up to 60 months postpartum	Small effect of synthetic oxytocin on maternal depression at 6 months postpartum (β=0.17, 95%CI of 0.03 to 0.30) No association between intrapartum oxytocin, maternal anxiety, mother-infant bonding or child internalizing	Intrapartum oxytocin weakly associated with maternal PPD, but not with maternal postpartum anxiety, mother-infant bonding or child behavioral or emotional problems	Overall, low response rate on surveys (34%) Dose of synthetic oxytocin was not assessed Did not distinguish between induction or labor augmentation

		Maternal symptoms measured by EPDS, Postnatal Depression Scale, State Trait Anxiety Inventory and Mother-to-Infant Bonding scale 6 months postpartum	and externalizing problems		
Consequences of Synthetic Oxytocin Reported in Animal Research					
Authors/Date	Subjects	Methods	Results	Conclusion	Study Limitations
Kenkel et al., 2019	*Experiment 1:* n= 8 OXT group (term pregnant female prairie voles) n= 6 SAL group (term pregnant female prairie voles) *Experiment 2:* n=7 prairie vole fetuses injected directly with OXT n= 7 prairie vole fetuses receiving maternal circulation with OXT n= 5 prairie vole fetuses pre-treated with OXT antagonist *Experiment 3:* n= 15-22 term pregnant female prairie voles per treatment group (low-OXT, medium-OXT,	*Experiment 1:* term pregnant females injected with saline or OXT (0.03mg/kg); fetal plasma pooled across litter for OXT assay; fetal brains processed for c-fos immunochemistry *Experiment 2:* Fetal heart rate monitoring with direct injection of OXT or maternal circulation via umbilical cord *Experiment 3:* pregnant females assigned to 1 of 5 treatment groups; fetal brains sectioned and analyzed for OXTRm across 4 CpG sites (-901, -924, -934_1, -934_2)	*Experiment 1:* significantly increased fetal plasma OXT level after maternally administered OXT; increased c-fos activity in PVN and SON of hypothalamus *Experiment 2:* acute bradycardic response 5 min after direct fetal injection with OXT; no change in fetal heart rate when pre-treated with OXT antagonist Experiment 3: OXTRm negatively predicted OXTR mRNA levels in saline and no treatment group; fetal weight positively correlated with OXTR mRNA in all brain regions assessed; dose-	Prairie vole fetuses acutely sensitive to maternally administered OXT Long-term changes in offspring following a single dose of OXT to pregnant female voles Fetal heart rate declines noted after maternal OXT administration not likely due to uterine contractions Dose-dependent increased in OXTRm in fetal brain with increases in OXTR expression for a given	Doses below threshold to induce labor Potential indirect effects with uterine contractions Provides evidence for single dose of synthetic oxytocin, but human mothers often receive a Pitocin drip over time; effects may differ in translation

	high-OXT, sal, no treatment) *Experiment 4:* n= 101 term pregnant female prairie voles	*Experiment 4:* pregnant females received saline or medium dose; offspring cross-fostered; offspring behavior (PND 1&4: ultrasonic vocalizations; PND 20: open field test; PND 50: alloparental test; PND 60: cohabitation; PND 61: partner preference test)	dependent increase in OXTRm in fetal forebrain, midbrain and hindbrain *Experiment 4:* pups born to OXT-treated dams had more vocalizations and longer vocalizations at PND 1&4; male pups vocalized more than female pups; males more alloparental than females at PND 50-55; OXT treated group showed more alloparental caregiving; more time spent side-by-side huddling in OXT-treated group	methylation level OXT-treated dams had offspring with more vocalizations, more alloparental care; more side-by-side contact and less time alone	
Kenkel et al., 2023	N= 7 control female prairie voles N= 12 control male prairie voles N= 13 OXT-exposed female prairie voles N= 13 OXT-exposed male prairie voels	Pregnant females injected intraperitoneally with OXT (0.25mg/kg) or untreated; offspring (PND 60-70) underwent three neuroimaging scans (aT1-weighted anatomical scan for voxel based morphometry [VBM], resting state functional scan [rs-fMRI], diffusion-weighted imaging scan [DWI]).	Males more neuroanatomically sensitive to maternally administered oxytocin treatment at birth Exposed males had smaller volumes in 8 of 17 assessed cortical regions Larger volumes seen in 11 brainstem/cerebellar regions in exposed females Males showed wider pattern of functional connectivity when	Overall, neuroanatomical effects in male and female offspring were small but larger effects seen in functional connectivity of oxytocin exposed males	Untreated group did not receive any interventions; stress of the injection may have consequences that were not measured Could not determine direct versus indirect effects of synthetic oxytocin (i.e., effects of uterine contractions, maternal stress)

			exposed to synthetic oxytocin		

Discussion

Studies have examined associations between the maternal oxytocinergic system and outcomes of maternal PPD (Bell et al., 2015; Kimmel et al., 2016; King et al., 2017; Toepfer et al., 2019); however, research examining the impact of synthetic oxytocin administration during parturition is limited. Five studies involving human participants were found in the literature and provided mixed results (Gu et al., 2016; Hinshaw et al., 2008; Kroll-Desrosiers et al., 2017; Takacs et al., 2019; Tichelman et al., 2021). Three studies reported a positive association between maternal exposure to synthetic oxytocin during parturition and outcomes of maternal PPD (Gu et al., 2016; Kroll-Desrosiers et al., 2017; Tichelman et al., 2021), one study found no association (Hinshaw et al., 2008), and another study reported a potential protective effect of synthetic oxytocin administration during parturition (Takacs et al., 2019).

Variations in these outcomes may be due to a few important factors. First, measurements of synthetic oxytocin were often dichotomous involving a yes or no response and did not account for dose-dependent effects (Kroll-Desrosiers et al., 2017; Takacs et al., 2019; Tichelman et al., 2021). Further, two studies examined the effects of synthetic oxytocin during labor augmentation but did not describe whether the mothers were exposed to synthetic oxytocin for the prevention or treatment of postpartum hemorrhage (Hinshaw et al., 2016; Tichelman et al., 2021). Takacs and colleagues (2019) highlight that suboptimal doses of synthetic oxytocin across parturition may contribute to a negative birth experience which the authors found to be an important risk factor for maternal PPD. Moreover, high doses of synthetic oxytocin across parturition can lead to adverse reproductive outcomes and may also have important implications for maternal mental

health (Erickson et al., 2023; Phaneuf et al., 2000; Robinson et al., 2003). More research is needed to examine the dose-dependent effects of synthetic oxytocin on adverse maternal psychological outcomes.

A second factor that was not observed across many of the studies was the mother's level of endogenous oxytocin during parturition and postpartum. Only one study examined the effect of synthetic oxytocin on endogenous oxytocin and subsequent maternal PPD outcomes (Gu et al., 2016). Across gestation, basal levels of oxytocin increase 3 to 4-fold in preparation for delivery (Uvnas-Morber et al., 2019). During spontaneous physiological labor, pulses of oxytocin are released with increasing frequency, duration, and amplitude to produce uterine contractions required for birth (Uvnas-Morber et al., 2019). Variations in maternal endogenous oxytocin may be further impacted by the administration of synthetic oxytocin during parturition and should be further assessed in future studies.

Another influential factor may be the time at which maternal PPD was assessed across the reviewed studies. Only one study differentiated maternal PPD and postpartum blues which can be common in the early postpartum period (Henshaw, 2003; Takacs et al., 2019). Hinshaw and colleagues (2008) analyzed the effects of synthetic oxytocin during labor augmentation on outcomes of maternal PPD only 48 hours after delivery and found no significant effects. Maternal PPD can be identified across the first 4 weeks postpartum with symptoms often lingering for years if left untreated (American College of Obstetricians and Gynecologists [ACOG], 2015; Putnick et al., 2020). Studies examining the effects of synthetic oxytocin during parturition should differentiate maternal PPD and common transient conditions, such as postpartum blues, to understand potential long-term effects on maternal postpartum mood.

The animal studies in this review further highlight an important gap in the literature. Only two studies were found involving the effects of synthetic oxytocin as a birth intervention and the studies focused on variations in offspring outcomes rather than maternal neurobiological or behavioral outcomes (Kenkel et al., 2019; Kenkel et al., 2023). Important offspring effects included physiological changes (e.g., changes in heart rate), epigenetic alterations of *OXTR,* and sex specific effects on offspring neurodevelopment (Kenkel et al., 2019; Kenkel et al., 2023). These findings indicate that peripherally administered synthetic oxytocin in term pregnancy can impact neurobiology and subsequent behavioral outcomes in offspring; however, more research is needed to understand if these effects also extend to maternal neurobiological and behavioral outcomes.

Conclusion

Synthetic oxytocin has important indications such as to improve the process of parturition and reduce maternal complications such as postpartum hemorrhage. Excessive use of synthetic oxytocin, however, raises important concerns for adverse effects on maternal reproductive and psychological health. Specifically, evidence in human studies demonstrates an increased risk for PPD after maternal exposure to synthetic oxytocin during parturition, though studies are limited, and conflicting evidence suggests either no association or a protective effect of synthetic oxytocin administration during parturition. Further research is needed to elucidate these relationships and to understand the neurobiological mechanisms that may underlie variations in maternal postpartum mood. Recommendations for future studies are to consider additional factors such as dose-dependent effects of synthetic oxytocin, variations in maternal endogenous oxytocin levels, and differentiation of PPD and other common postpartum conditions, such as postpartum blues.

References

Ahmadzia, H., Grotegut, C., & James, A. (2020). A national update on rates of postpartum haemorrhage and related interventions. *Blood Transfusion, 18*(4), 247-253. https://doi.org/10.2450%2F2020.0319-19

The American College of Obstetricians and Gynecologists (ACOG). (2015). *Screening for Perinatal Depression.* https://www.acog.org/clinical/clinical-guidance/committee-opinion/articles/2018/11/screening-for-perinatal-depression

Amico, J., Tenicela, R., Johnston, J., & Robinson, A. (1983). A time-dependent peak of oxytocin exists in cerebrospinal fluid but not in plasma of humans. *J Clin Endocrinol Metab, 57*(5), 947-951. https://doi.org/10.1210/jcem-57-5-947

Arletti, R., & Bertolini, A. (1987). Oxytocin acts as an antidepressant in two animal models of depression. *Life Sciences, 41*(14), 1725-1730. https://doi.org/10.1016/0024-3205(87)90600-x

Bell, A., Carter, S., Steer, C., Golding, J., Davis, J., Steffan, A., Rubin, L., Lillard, T., Gregory, S., Harris, J., & Connelly, J. (2015). Interactions between oxytocin receptor DNA methylation and genotype is associated with risk of postpartum depression in women without depression in pregnancy. *Frontiers in Genetics, 6*(243). https://doi.org/10.3389/fgene.2015.00243

Bell, A., Erickson, E., & Carter, S. (2014). Beyond labor: The role of natural and synthetic oxytocin in the transition to motherhood. *Journal of Midwifery & Women's Health, 59*(1), 35-42. https://doi.org/10.1111%2Fjmwh.12101

Bosch, O. (2013). Maternal aggression in rodents: Brain oxytocin and vasopressin mediate pup defense. *Philos Trans R Soc Lond B Biol Sci, 368*(1631). https://doi.org/10.1098%2Frstb.2013.0085

Daly D, Minnie K, Blignaut A, Blix, E., Nilsen, A., Dencker, A., Beeckman. K., Gross, M., Pehlke-Milde, J., Grylka-Baeschlin, S., Koenig-Bachmann, M., Clausen, J., Hadjigeorgiou, E., Morano, S., Iannuzzi, L., Baranowska, B., Kiersnowska, I., & Uvnas-Moberg, K. (2020). How much synthetic oxytocin is infused during labour? A review and analysis of regimens used in 12 countries. *PLOS ONE,* 15(7). https://doi.org/10.1371/journal.pone.0227941

Erickson, E., Myatt, L., Danoff, J., Krol, K., & Connelly, J. (2023). Oxytocin receptor DNA methylation is associated with exogenous oxytocin needs during parturition and postpartum hemorrhage. *Communications Medicine, 3*(11). https://doi.org/10.1038/s43856-023-00244-6

Food and Drug Administration (FDA). (2014). Pitocin. Retrieved from https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/018261s031lbl.pdf

Freeman, S., Samineni, S., Allen, P., Stockinger, D., Bales, K., Hwa, G., & Roberts, J. (2016). Plasma and CSF oxytocin levels after intranasal and intravenous oxytocin in awake macaques. *Psychoneuroendocrinology, 66,* 185-194. https://doi.org/10.1016/j.psyneuen.2016.01.014

Gu, V., Feeley, N., Gold, I., Hayton, B., Robins, S., Mackinnon, A., Samuel, S., Carter, S., & Zelkowitz, P. (2015). Intrapartum synthetic oxytocin and its effects on maternal well-being at 2 months postpartum. *Birth, 43*(1), 28-35. https://doi.org/10.1111/birt.12198

Hinshaw, K., Simpson, S., Cummings, S., Hildreth, A., & Thornton, J. (2008). A randomized controlled trial of early versus delayed oxytocin augmentation to treat primary dysfunctional labor in nulliparous women. *BJOG: An International Journal of Obstetrics & Gynaecology, 115*(10), 1289-1296. https://doi.org/10.1111/j.1471-0528.2008.01819.x

Kimmel, M., Clive, M., Gispen, F., Guintivano, J., Brown, T., Cox, O., Beckmann, M., Kornhuber, J., Fasching, P., Osbourne, L., Binder, E., Payne, J., & Kaminsky, Z. (2016). Oxytocin receptor methylation in postpartum depression. *Psychoneuroendocrinology, 69,* 150-160. https://doi.org/10.1016/j.psyneuen.2016.04.008

King, L., Robins, S., Chen, G., Yerko, V., Zhou, Y., Nagy, C., Feely, N., Gold, I., Hayton, B., Turecki, G., & Zelkowitz, P. (2017). Perinatal depression and DNA methylation of oxytocin-related genes: A study of mothers and their children. *Hormones and Behavior, 96,* 84-94. https://doi.org/10.1016/j.yhbeh.2017.09.006

Kenkel, W., Ortiz, R., Yee, J., Perkeybile, A., Kulkarni, P., Carter, S., Cushing, B., & Ferris, C. (2023). Neuroanatomical and functional consequences of oxytocin treatment at birth in prairie voles. *Psychoneuroendocrinology, 150*. https://doi.org/10.1016/j.psyneuen.2023.106025

Kenkel, W., Perkeybile, A., Yee, J., Pournajafi-Nazarloo, H., Lillard, T., Ferguson, E., Wroblewski, K., Ferris, C., Carter, S., & Connelly, J. (2019). Behavioral and epigenetic consequences of oxytocin treatment at birth. *Science Advances, 5*(5). https://doi.org/10.1126/sciadv.aav2244

Kroll-Desrosiers, A., Nephew, B., Babb, J., Guilarte-Walker, Y., Simas, T., & Deligaiannidis, K. (2017). Association of peripartum synthetic oxytocin administration and depressive and

anxiety disorders within the first postpartum year. *Depress Anxiety, 34*(2), 137-146. https://doi.org/10.1002%2Fda.22599

Kusui, C., Kimura, T., Ogita, K., Nakamura, H,. Matsumara, Y., Koyama, M., Azuma, C., Murata, Y. (2001). DNA methylation of the human oxytocin receptor gene promoter regulates tissue-specific gene suppression. *Biochemical and Biophysical Research Communications, 289*(3), 681-686. https://doi.org/10.1006/bbrc.2001.6024

Lee, M., Scheidweiler, K., Diao, X., Akhlaghi, F., Cummins, A., Huestis, M., Leggio, L., & Averbeck, B. (2018). Oxytocin by intranasal and intravenous route reaches the cerebrospinal fluid in rhesus macaques: Determination using a novel oxytocin assay. *Molecular Psychiatry, 23*(1), 115-122. https://doi.org/10.1038/mp.2017.27

LeWinn, K., Stroud, L., Molnar, B., Ware, J., Koenen, K., & Buka, S. (2009). Elevated maternal cortisol levels during pregnancy are associated with reduced childhood IQ. *International Journal of Epidemiology, 38*(6), 1700-1710. https://dx.doi.org/10.1093%2Fije%2Fdyp200

Lui, X, Li, D., Li, T., Lui, H., Cui, D., Liu, Y., Jia, S., Wang, X., Jaio, R., Zhu, H., Zhang, F., Qin, D., & Wang, Y. (2019). Effects of intranasal oxytocin on pup deprivation-evoked aberrant maternal behavior and hypogalactia in rat dams and the underlying mechanisms. *Frontiers in Neuroscience, 26.* https://doi.org/10.3389/fnins.2019.00122

Mah, B. (2016). Oxytocin, postnatal depression, and parenting: A systematic review. *Harvard Review of Psychiatry, 24*(1), 1-13. https://doi.org/10.1097/HRP.0000000000000093

Melis, M., Melis, T., Cocco, C., Succu, S., Sanna, F., Pillolla, G., Boi, A., Ferri, G., & Argiolas, A. (2007). Oxytocin injected into the ventral tegmental area induces penile erection and increases extracellular dopamine in the nucleus accumbens and paraventricular nucleus of

the hypothalamus of male rats. *The European Journal of Neuroscience, 26*(4), 1026-1035. https://doi.org/10.1111/j.1460-9568.2007.05721.x

Moura, D., Canavarro, M., & Figueiredo-Braga, M. (2016). Oxytocin and depression in the perinatal period- A systematic review. *Archives of Women's Mental Health, 19,* 561-570. https://doi.org/10.1007/s00737-016-0643-3

Olazabal, D., & Young, L. (2006). Oxytocin receptors in the nucleus accumbens facilitate "spontaneous" maternal behavior in adult female prairie voles. *Neuroscience, 141*(2), 559-568. https://doi.org/10.1016/j.neuroscience.2006.04.017

Pedersen, C., Caldwell, J., Walker, C., Ayers, G., & Mason, G. (1994). Oxytocin activates the postpartum onset of rat maternal behavior in the ventral tegmental and medial preoptic areas. *Behavioral Neuroscience, 108*(6), 1163–1171. https://doi.org/10.1037/0735-7044.108.6.1163

Phaneuf, S., Rodríguez Liñares, B., TambyRaja, R., MacKenzie, I., & Bernal, A. (2000). Loss of myometrial oxytocin receptors during oxytocin-induced and oxytocin-augmented labour. *Journal of Reproduction and Fertility,* 120(1), 91-97. https://doi.org/10.1530/jrf.0.1200091

Putnick, D., Sundaram, R., Bell, E., Ghassabian, A., Goldstein, R., Robinson, S., Vafai, Y., Gilman, S., & Yeung, E. (2020). Trajectories of maternal postpartum depressive symptoms. *Pediatrics, 146*(5). https://doi.org/10.1542/peds.2020-0857

Robinson, C., Schumann R., Zhang, P., & Young, R. (2003). Oxytocin-induced desensitization of the oxytocin receptor. *American Journal of Obstetrics & Gynecology, 188*(2), 497-502. https://doi.org/10.1067/mob.2003.22

Rouse, M., & Goodman, S. (2014). Perinatal depression influences on infant negative affectivity; timing, severity, and co-morbid anxiety. *Infant Behavior & Development, 37,* 739-751. https://psycnet.apa.org/doi/10.1016/j.infbeh.2014.09.001

Swales, D., Winiarski, D., Smith, A., Stowe, Z., Newport, D., & Brennan, P. (2017). Maternal depression and cortisol in pregnancy predict offspring emotional reactivity in the preschool period. *Developmental Psychobiology, 60,* 557-566. https://doi.org/10.1002/dev.21631

Tackacs, L., Seidlerova, J., Sterbova, Z., Cepicky, P., & Havlicek, J. (2019). The effects of intrapartum synthetic oxytocin on maternal postpartum mood: Findings from a prospective observational study. *Archives of Women's Mental Health, 22*(4), 485-491. https://doi.org/10.1007%2Fs00737-018-0913-3

Thul, T., Corwin, E., Carlson, N., Brennan, P., & Young, L. (2020). Oxytocin and postpartum depression: A systematic review. *Psychoneuroendocrinology, 120.* https://doi.org/10.1016/j.psyneuen.2020.104793

Tichelman, E., Warmink-Perdijk, W., Henrichs, J., Peters, L., Schellevis, F., Berger, M., & Burger, H. (2021). Intrapartum synthetic oxytocin, behavioral and emotional problems in children and the role of postnatal depressive symptoms, postnatal anxiety and mother-to-infant bonding: A Dutch prospective cohort study. *Midwifery, 100.* https://doi.org/10.1016/j.midw.2021.103045

Toepfer, P., O'Donnell, K., Entringer, S., Garg, E., Heim, C., Lin, D., MacIsaac, J., Kobor, M., Meaney, M., Provencal, N., Binder, E., Wadhwa, P., & Buss, C. (2019). Dynamic DNA methylation changes in maternal oxytocin gene locus (OXT) during pregnancy predict

postpartum maternal intrusiveness. *Psychoneuroendocrinology, 103,* 156-162. https://doi.org/10.1016/j.psyneuen.2019.01.013

Turnpin, M., & Salbert, G. (2022). 5-methylcytosine turnover: Mechanisms and therapeutic implications in cancer. *Frontiers in Molecular Biosciences, 9.* https://doi.org/10.3389/fmolb.2022.976862

Uvnas-Morber, K., Ekstrom-Berstrom, A., Berg, M., Buckley, S., Pajalic, Z., Hadjigeorgiou, E., Kotlowska, A., Lengler, L., Kielbratowska, B., Leon-Larios, F., Magistrettie, C., Downe, S., Lindstrom, B., & Dencker, A. (2019). Maternal plasma levels of oxytocin during physiological childbirth- A systematic review with implications for uterine contractions and central actions of oxytocin. *BMC Pregnancy and Childbirth, 19*(285). https://doi.org/10.1186/s12884-019-2365-9

Wang, T., Shi, C., Li, X., Zhang, P., Lui, B., Wang, H., Wang, Y., Yang, Y., Wu, Y., Li, H., & Xu, Z. (2018). Injection of oxytocin into paraventricular nucleus reverses depressive-like behaviors in the postpartum depression rat model. *Behavioral Brain Research, 336*(15), 236-243. https://doi.org/10.1016/j.bbr.2017.09.012

World Health Organization (WHO). (2012). WHO recommendations for the prevention and treatment of postpartum hemorrhage. https://apps.who.int/iris/bitstream/handle/10665/75411/97?sequence=1

CHAPTER THREE

Methodology

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Abstract

The socially monogamous prairie vole (*Microtus ochrogaster)* has emerged as a beneficial model for examining variations in maternal reproductive and social behavioral outcomes. Female prairie voles demonstrate an important use in reproductive research as they require exposure to a male for the induction of estrus and subsequent pregnancy. In addition, prairie voles display unique social behaviors that mimic human characteristics making them an exceptional model for translational research. This chapter describes a prairie vole labor induction model using a timed-mating paradigm to examine the effects of synthetic oxytocin during partition on maternal neurobiology. Moreover, we describe procedures for future studies to assess dose-dependent effects of synthetic oxytocin across various maternal and fetal tissues.

Methodology

The socially monogamous prairie vole (*Microtus ochrogaster)* has emerged as a beneficial model for examining variations in maternal reproductive and social behavioral outcomes. Regarding maternal reproductive processes, female prairie voles do not have a spontaneous estrus induction and require stimuli from a male prairie vole to induce the estrus cycle. Per Carter and colleagues (1987), female estrus is most effectively induced after the female has been in physical contact with an unfamiliar male and has been housed for at least 24 to 48 hours in a cage that has been soiled by a male. This process is crucial for researchers examining reproductive outcomes as it allows for timed mating and observation of subsequent pregnancy and birth.

Prairie voles are also exceptionally useful in the social sciences due to their display of social and parental behaviors. Unlike other traditional laboratory species (i.e., mice and rats), prairie voles engage in pair bonding, biparental caregiving, and alloparenting behaviors which closely mimics human characteristics (Tabbaa et al., 2017). A pair bond in a socially monogamous species is identified by the preferential selection of sexually mature adults to be in contact and mate with their partner over a stranger (Young et al., 2011). Animals in a monogamous pair bond will often show selective affiliation or attachment with their partner, aggression toward potentially competitive strangers, and biparental care of their offspring (Potretzke & Ryabinin, 2019).

Alloparental caregiving refers to the care of an offspring that is not one's own biological offspring and is not observed in all animal species (Kenkel et al., 2016). Importantly, social and parental behaviors, including pair bonding, biparental caregiving and alloparental behaviors, are observed in only 3-5% of mammalian species (Johnson & Young, 2015; Rogers & Bales, 2019).

Other vole species, including the meadow vole (*M. pennsylvanicus*) and the montane vole (*M. montanus*), generally do not form these social bonds demonstrating the unique utility of the prairie vole in psychosocial research. In addition, unlike mice and rats, prairie voles display natural variations in behavior within and between populations which provides an interesting phenomenon for further genetic and epigenetic research (Tabbaa et al., 2017). Studies involving laboratory-reared prairie voles are often systematically outbred to assess biological processes underlying variations in social-behavioral outcomes.

The onset of parenting behaviors in prairie voles also makes them uniquely suited as a model organism for assessing the effects of birth interventions in late gestation. Both male and female prairie voles typically participate in the care of their offspring; however, the initiation of parental care differs among males and females. While adult males are spontaneously paternal, female responses to pups often depend on their age and reproductive status (Lonstein & de Vries, 2001). Most adult nulliparous females are infanticidal and will only demonstrate maternal behaviors after the vaginal delivery of offspring (Hayes & de Vries, 2007; Lonstein & de Vries, 2001). Evidence suggests that this shift in female behavior is likely linked to mechanisms involved in the process of vaginal birth and may be impacted by exposure to birth interventions in term pregnancy (Hayes & de Vries, 2007).

A Prairie Vole Labor Induction Model

Chapter 4 further describes the present dissertation study with the primary aim to examine associations between maternal exposure to synthetic oxytocin during parturition and outcomes of DNA methylation and oxytocin receptor *(Oxtr)* gene expression in the maternal brain. In addition to social and parental behaviors that are commonly displayed in both prairie voles and in humans, RNA sequencing of the *Oxtr* gene in the nucleus accumbens of prairie

voles has revealed a similar gene structure to that of the *OXTR* gene in humans (Danoff et al., 2021). Unlike traditional laboratory species (including mice and rats) the conserved MT2 region in the *Oxtr* gene promoter (including CpG sites -934, -924, -901) is homologous in humans and in prairie voles with 64.3% shared identity (Danoff et al., 2021). Importantly, increased DNA methylation in this conserved region has been found to decrease gene expression in both prairie voles and in humans (Danoff et al., 2021; Perkeybile et al., 2019; Gregory et al., 2009). Taken together, this data supports the use of the prairie vole in the current dissertation study and as a beneficial model for future translational research in human participants.

In this chapter, methods for the dissertation study will be discussed including a timed mating paradigm (also previously described in Kenkel et al., 2019), observation of pregnancy, administration of synthetic oxytocin and the collection of accessible and relevant tissues. Additional tissue processing procedures for Aim 1 and Aim 2 are reported in Chapter 4 along with statistical analyses and the results of the present study. The following procedures in this chapter were completed in accordance with approved guidelines by the Institutional Animal Care and Use Committee (IACUC) of the University of Virginia.

Subjects

The subjects used in this study were systematically outbred male and virgin female prairie voles descended from a wild prairie vole population captured near Champaign, Illinois. Male and female voles were weaned on postnatal day (PND) 20 and housed in standard cages in sibling pairs containing wood chips and nestlet bedding until pairing. None of voles used in this study had a previous pregnancy or prior exposure to pups. All subjects were given high-fiber Purina rabbit chow and water *ad libitum*. Cotton nestlets were provided for nesting material in each standard cage and voles were maintained on a 14:10 light/dark cycle.

Male and Female Pairing

Each virgin female prairie vole (PND 60-90) was weighed and paired with an experienced breeding male in a standard breeder cage (polycarbonate cages: 44 cm x 22 cm x 16 cm) for 24 hours to initiate female estrus. 24 hours after pairing, the cages were divided by a perforated Plexiglas barrier with the virgin female on one side and the male on the other to allow for the process of estrus induction. The divider was removed after three days, and mating behavior was observed. After observed mating, the male and female were allowed to remain in the same cage throughout gestation (approximately 21.5 days).

Observation of Pregnancy

On the expected day of birth, each female was weighed, and her abdomen was palpated to check for term pregnancy. Females determined to be term pregnant (based on factors including observed mating, weight gain of at least 18 grams across gestation, the presence of pups during palpation, and prominent nipple development) were injected in the ventral abdominal area with one of three doses of oxytocin (0.0125mg/kg, 0.25mg/kg, 0.5mg/kg; oxytocin acetate salt dissolved in sterile saline), a saline vehicle or were allocated to the no-injection control group.

Preparation of Synthetic Oxytocin

Synthetic oxytocin was previously purchased in powder form and stored in a -20°C freezer. Thirty minutes prior to administration, the synthetic oxytocin was removed from the freezer and suspended in normal saline. The suspended oxytocin was further diluted into a low (0.125 mg/kg), medium (0.25 mg/kg) and high (0.5 mg/kg) dose. Since the synthetic oxytocin was mixed in normal saline prior to the injection, we used a saline control group to determine the specific effect of synthetic oxytocin. Term pregnant female prairie voles in the oxytocin and saline-treated groups were injected into the ventral abdominal area on the expected day of birth.

All doses of synthetic oxytocin in the present study were beneath the threshold for labor induction in the prairie vole (Kenkel et al., 2019; Kenkel et al., 2023); however, the medium dose of 0.25mg/kg is equivalent to 5 IU that is common for labor induction in the clinical setting (Grotegut et al., 2017; Zhang et al., 2011).

Tissue Collection

Ninety minutes after maternal administration of oxytocin, saline, or no treatment, the term pregnant females were euthanized via cervical dislocation and rapid decapitation under deep isoflurane anesthesia prior to tissue collection. Maternal whole brains were first collected and immediately frozen on dry ice. Maternal whole blood was then collected in 1.5ml tubes lined with EDTA and was also immediately frozen on dry ice. After maternal tissues were collected, pups were excised from the maternal uterus and weighed for confirmation of term pregnancy. Only mothers with pups weighing at least 2g each were included in the present study. In addition to maternal whole brain and whole blood tissue analyzed in the present study, the procedure allowed for additional maternal tissues to be collected including uterine tissue, mammary glands, and plasma. 200 ul of maternal whole blood in a 1.5ml microtube was placed in a centrifuge to spin at 2500rpm for 10 minutes to separate plasma. Approximately 100 ul of plasma was collected in a separate 1.5ml microtube and stored at -80°C until further processing. Additional postmortem fetal tissues were collected including fetal whole brain, pooled litter whole blood (collected in 1.5ml microtubes lined with EDTA), and placental tissue. All maternal and fetal tissue samples were stored in a -80°C freezer until further processing.

Maternal Whole Brain Dissection

For the present study, maternal whole brains were stored at -80°C and then thawed to -20°C for 1 hour prior to regional sectioning. During the dissection process, a coronal cut was

made to first remove the olfactory bulb. This was followed by a second coronal cut 2mm caudal to the frontal pole to collect bilateral punches (1mm in diameter and 2mm in depth) of the nucleus accumbens. Nucleus accumbens tissue from each subject was placed in a separate DNase/RNase free microcentrifuge tube and stored at -80°C until further processing. The present study selected the nucleus accumbens for further analysis due to previous data demonstrating variations in social and maternal behaviors associated with *Oxtr* DNA methylation and *Oxtr* gene expression in the nucleus accumbens (Danoff et al., 2021; Olazabal & Young, 2006; Perkeybile et al., 2019). However, assessing the effects of synthetic oxytocin across various maternal brain regions may provide additional insight in future studies.

Discussion

Extracting central tissue to examine maternal neurobiological effects of synthetic oxytocin administration during parturition in human mothers is not ethical or practical; thus, it is important to identify a model organism that can be used to further understand these effects. Previous studies using the prairie vole as a model species have examine epigenetic regulation of the *Oxtr* gene in the context of early life adverse events (Danoff et al., 2021; Perkeybile et al., 2019). Findings revealed a strong correlation between *Oxtr* DNA methylation in the nucleus accumbens and whole blood at four CpG sites (-934_1, -934_2, -924, -901) in MT2 that are homologous to the human *OXTR* gene. This demonstrates the utility of whole blood to serve as a proxy for assessing epigenetic regulatory factors in the brain and has important implications for future translational research. Additional studies are needed to understand the relationship between synthetic oxytocin administration during parturition and regulation of the *Oxtr* gene in the nucleus accumbens of maternal subjects. Further, it is important to assess whether

correlations exist between epigenetic factors in the nucleus accumbens and in whole blood for future translation of this research in human participants.

Several animal studies have demonstrated positive effects of synthetic oxytocin administration when delivered via a local injection in the brain or the intranasal route (Arletti & Bertolini, 1987; Lui et al., 2019; Melis et al., 2007; Wang et al., 2019); however, research examining the maternal neurobiological effects of synthetic oxytocin during parturition is limited. Two studies have highlighted the effects of synthetic oxytocin administration in term pregnant female prairie voles on offspring outcomes; however, the authors do not report maternal neurobiological or behavioral outcomes (Kenkel et al., 2019; Kenkel et al., 2023). Here, we describe the utility of the prairie vole as a model for assessing the neurobiological effects of synthetic oxytocin in the maternal brain. We further describe our methods to analyze the effects of various doses of synthetic oxytocin which mimics the experience of mothers in the clinical setting.

In addition to the maternal whole brains and whole blood processed in the present study, we collected maternal and fetal tissues including, maternal plasma, uterus, mammary glands, fetal whole brains, and placenta. Though the current study did not examine outcomes across these various maternal and fetal tissues, there are important benefits in analyzing these tissues in future studies. Specifically, in humans, synthetic oxytocin is found to have important downregulating effects on *OXTR* in the maternal uterus which demonstrates subsequent adverse outcomes such as uterine atony and postpartum hemorrhage (Erickson et al., 2023; Phaneuf et al., 2000; Robinson et al., 2003). Using the current labor induction method, it is possible to examine dose-dependent effects of synthetic on epigenetic regulation of *Oxtr* in the female uterus of the prairie vole for future translational research. In addition, previous studies have

demonstrated neurobiological and neuroanatomical effects of synthetic oxytocin in offspring which may be further examined using the current labor induction model (Kenkel et al., 2019; Kenkel et al., 2023).

Conclusion

Previous studies have demonstrated the benefits of the prairie vole as a model organism for examining variations in typical social and maternal behaviors. In addition, a timed estrus induction in female voles provides an important advantage for researchers examining pregnancy and birth related outcomes. In the context of motherhood, the prairie vole can be useful model for assessing the impact of birth interventions such as the administration of synthetic oxytocin in in term pregnancy. In the present study, we describe our methods using a prairie vole labor induction model to examine the impact of synthetic oxytocin in the maternal brain.

References

Arletti, R., & Bertolini, A. (1987). Oxytocin acts as an antidepressant in two animal models of depression. *Life Sciences, 41*(14), 1725-1730. https://doi.org/10.1016/0024-3205(87)90600-x

Carter, S., Witt, M., Schneider, J., Harris, L., & Volkening, D. (1987). Male stimuli are necessary for female sexual behavior and uterine growth in prairie voles (Microtus ochrogaster). *Hormones and Behavior, 21*(1), 74-82. https://doi.org/10.1016/0018-506x(87)90032-8

Danoff, J., Wroblewski, K., Graves, A., Quinn, G., Perkeybile, A., Kenkel, W., Lillard, T., Parikh, H., Golino, H., Gregory, S., Carter, S., Bales, K., & Connelly, J. (2021). Genetic, epigenetic, and environmental factors controlling oxytocin receptor gene expression. *Clinical Epigenetics, 13*(23). https://doi.org/10.1186/s13148-021-01017-5

Erickson, E., Myatt, L., Danoff, J., Krol, K., & Connelly, J. (2023). Oxytocin receptor DNA methylation is associated with exogenous oxytocin needs during parturition and postpartum hemorrhage. *Communications Medicine, 3*(11). https://doi.org/10.1038/s43856-023-00244-6

Gregory, S., Connelly, J., Towers, A., Johnson, J., Biscocho, D., Markunas, C., Lintas, C., Abramson, R., Wright, H., Ellis, P., Langford, C., Worley, G., Delong, G., Murphy, S., Cuccaro, M., Perisco, A., & Pericak-Vance, M. (2009). Genomic and epigenetic evidence for oxytocin receptor deficiency in autism. *BMC Medicine, 7*(62). https://doi.org/10.1186/1741-7015-7-62

Grotegut, C., Lewis, L., Manuck, T., Allen, T., James, A., Seco, A., Deneux-Tharaux, C. (2017). The oxytocin product correlates with total oxytocin received during labor: A research

methods study. *American Journal of Perinatology, 35*(1), 78-83. https://doi.org/10.1055/s-0037-1606119

Hayes, U., & de Vries, G. (2007). Role of pregnancy and parturition in induction of maternal behavior in prairie voles *(Microtus ochrogaster). Hormones and Behavior, 51*(2). https://doi.org/10.1016%2Fj.yhbeh.2006.10.011

Johnson, Z., & Young, L. (2015). Neurobiological mechanisms of social attachment and pair bonding. *Current Opinion in Behavioral Sciences, 3,* 38-44. https://doi.org/10.1016/j.cobeha.2015.01.009

Kenkel, W., Ortiz, R., Yee, J., Perkeybile, A., Kulkarni, P., Carter, S., Cushing, B., & Ferris, C. (2023). Neuroanatomical and functional consequences of oxytocin treatment at birth in prairie voles. *Psychoneuroendocrinology, 150*. https://doi.org/10.1016/j.psyneuen.2023.106025

Kenkel, W., Perkeybile, A., & Carter, S. (2016). The neurobiological causes and effects of alloparenting. *Dev Neurobiol, 77*(2), 214-232. https://doi.org/10.1002%2Fdneu.22465

Kenkel, W., Perkeybile, A., Yee, J., Pournajafi-Nazarloo, H., Lillard, T., Ferguson, E., Wroblewski, K., Ferris, C., Carter, S., & Connelly, J. (2019). Behavioral and epigenetic consequences of oxytocin treatment at birth. *Science Advances, 5*(5). https://doi.org/10.1126/sciadv.aav2244

Lonstein, L, & de Vries, G. (2001). Sex differences in the parental behaviour of adult virgin prairie voles: Independence from gonadal hormones and vasopressin. *Journal of Neuroendocrinology.* https://doi.org/10.1046/j.1365-2826.1999.00361.x

Lui, X, Li, D., Li, T., Lui, H., Cui, D., Liu, Y., Jia, S., Wang, X., Jaio, R., Zhu, H., Zhang, F., Qin, D., & Wang, Y. (2019). Effects of intranasal oxytocin on pup deprivation-evoked aberrant

maternal behavior and hypogalactia in rat dams and the underlying mechanisms. *Frontiers in Neuroscience, 26.* https://doi.org/10.3389/fnins.2019.00122

Melis, M., Melis, T., Cocco, C., Succu, S., Sanna, F., Pillolla, G., Boi, A., Ferri, G., & Argiolas, A. (2007). Oxytocin injected into the ventral tegmental area induces penile erection and increases extracellular dopamine in the nucleus accumbens and paraventricular nucleus of the hypothalamus of male rats. *The European Journal of Neuroscience, 26*(4), 1026-1035. https://doi.org/10.1111/j.1460-9568.2007.05721.x

Olazabal, D., & Young, L. (2006). Oxytocin receptors in the nucleus accumbens facilitate "spontaneous" maternal behavior in adult female prairie voles. *Neuroscience, 141*(2), 559-568. https://doi.org/10.1016/j.neuroscience.2006.04.017

Perkeybile, A., Carter, S., Wroblewski, K., Puglia, M., Kenkel, W., Lillard, T., Karaoli, T., Gregory, S., Mohammadi, N., Epstein, L., Bales, K., & Connelly, J. (2019). Early nurture epigenetically tunes the oxytocin receptor. *Psychoneuroendocrinology, 99,* 128-136. https://doi.org/10.1016/j.psyneuen.2018.08.037

Phaneuf, S., Rodríguez Liñares, B., TambyRaja, R., MacKenzie, I., & Bernal, A. (2000). Loss of myometrial oxytocin receptors during oxytocin-induced and oxytocin-augmented labour. *Journal of Reproduction and Fertility,* 120(1), 91-97. https://doi.org/10.1530/jrf.0.1200091

Potretzke, S., & Ryabinin, A. (2019). The prairie vole model of pair-bonding and its sensitivity to addictive substances. *Frontiers in Psychology, 10.* https://doi.org/10.3389/fpsyg.2019.02477

Robinson, C., Schumann R., Zhang, P., & Young, R. (2003). Oxytocin-induced desensitization of the oxytocin receptor. *American Journal of Obstetrics & Gynecology, 188*(2), 497-502. https://doi.org/10.1067/mob.2003.22

Rogers, F., & Bales, K. (2019). Mothers, fathers, and others: Neural substrates of parental care. *Trends in Neuroscience, 42*(8), 552-562. https://doi.org/10.1016/j.tins.2019.05.008

Tabbaa, M., Paedae, B., Liu, Y., & Wang, Z. (2017). Neuropeptide regulation of social attachment: The prairie vole model. *Compr Physiol, 7*(1), 81-104. https://doi.org/10.1002%2Fcphy.c150055

Wang, T., Shi, C., Li, X., Zhang, P., Lui, B., Wang, H., Wang, Y., Yang, Y., Wu, Y., Li, H., & Xu, Z. (2018). Injection of oxytocin into paraventricular nucleus reverses depressive-like behaviors in the postpartum depression rat model. *Behavioral Brain Research, 336*(15), 236-243. https://doi.org/10.1016/j.bbr.2017.09.012

Young, K., Gobrogge, K., Liu, Y., & Wang, Z. (2011). The neurobiology of pair bonding: Insights from a socially monogamous rodent. *Frontiers in Neuroendocrinology, 32*(1), 53-69. https://doi.org/10.1016/j.yfrne.2010.07.006

Zhang, J., Branch, D., Ramirez, M., Laughon, S., Reddy, U., Hoffman, M., Bailit, J., Kominiarek, M., & Chen, Z. (2011). Oxytocin regimens or labor augmentation, labor progression, perinatal outcomes. *Obstetrics and Gynecology, 118*(201), 249-256. https://doi.org/10.1097%2FAOG.0b013e3182220192

CHAPTER FOUR

Consequences of Synthetic Oxytocin in the Maternal Brain

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Abstract

Oxytocin has complex actions throughout the body and is commonly known for its key role in promoting uterine contractions during parturition. Synthetic oxytocin is widely administered in hospital-based settings to improve uterine contractions when medically indicated. Although there are important benefits of synthetic oxytocin administration during parturition, less is understood regarding potential adverse effects on the mother long-term. Importantly, evidence suggests there is a link between maternal exposure to high doses of synthetic oxytocin and outcomes of maternal postpartum depression (PPD); however, the biological mechanisms underlying this relationship have not been fully elucidated. Examining epigenetic regulatory factors that impact transcription of the oxytocin receptor (*OXTR*) gene can be useful for understanding how synthetic oxytocin might change maternal neurobiology. Here, we analyze dose-dependent effects of synthetic oxytocin on *Oxtr* gene expression in the maternal brain using the prairie vole as a model organism. Specifically, our findings demonstrate a change in the relationship between *Oxtr* DNA methylation and *Oxtr* gene expression in the nucleus accumbens of term pregnant female prairie voles exposed to a high dose of synthetic oxytocin. We further demonstrate correlations between the DNA methylation state of the *Oxtr* gene in the nucleus accumbens and in whole blood for future translational research in human mothers.

Keywords: Synthetic oxytocin, *OXTR,* birth, postpartum depression, maternal brain, prairie vole

Consequences of Synthetic Oxytocin in the Maternal Brain

Oxytocin has complex actions across many body systems and is commonly known for its key role in promoting uterine contractions during parturition. Adequate uterine contractions are vital for the process of childbirth as well as to fully expel the placenta and reduce the risk for excessive maternal bleeding. Data from the Centers for Disease Control (CDC) highlights excessive maternal bleeding (i.e., postpartum hemorrhage) as one of the leading causes of pregnancy-related death, and, for decades, researchers and medical professionals have sought to develop effective mechanisms to mitigate this risk (Trost et al., 2022). For the prevention and treatment of postpartum hemorrhage, the World Health Organization recommends use of synthetic oxytocin as a first line agent during third stage labor (World Health Organization [WHO], 2012). In addition, synthetic oxytocin is indicated for induction and/or augmentation of labor when gestation is prolonged or for concerns of maternal-fetal health (WHO, 2012).

Synthetic oxytocin has become one of the most widely used medications during parturition with rates of labor induction in the United States tripling from 9.6% in 1990 to 32.1% in 2021 (Osterman et al., 2023; Simpson, 2022). Although there are important benefits associated with the use of synthetic oxytocin, guidelines for administration vary globally with continued debates regarding the benefits and risks of high-dose versus low-dose oxytocin regimens (Daly et al., 2020). Importantly, higher doses of synthetic oxytocin can result in overstimulation of the uterus leading to detrimental effects such as uterine atony and subsequent postpartum hemorrhage (Erickson et al., 2023; Food and Drug Administration [FDA], 2014; Phaneuf et al., 2000). Recent studies have further highlighted long-term maternal psychological effects such as postpartum depression (PPD); however, the biological mechanisms underlying these

relationships are not fully understood (Gu et al., 2016; Kroll-Desrosiers et al., 2017; Tichelman et al., 2021).

Studies examining associations between maternal oxytocin and PPD have provided mixed results largely due to individual variability in circulating oxytocin peptide levels (McCullough et al., 2012; Thul et al., 2020). Instead, researchers have focused on epigenetic modifications of the oxytocin receptor (*OXTR*) gene as a more consistent predictor for maternal psychological outcomes (Bell et al., 2015; Keebaugh & Young, 2011; Kimmel et al., 2016; King et al., 2017; Toepfer et al., 2019). The oxytocin peptide functions by binding to *OXTR* within target tissues. Thus, examining epigenetic changes to the *OXTR* gene can provide important insight regarding the ability of circulating oxytocin to function within target tissues.

The *OXTR* gene spans 19,206 base pairs with four exons and three introns. A region denoted as MT2 covers much of exon 1 and the first intron and is suggested to be particularly vulnerable to epigenetic regulation via DNA methylation (see Figure S1 in supplement) (Kusui et al., 2001). DNA methylation of 5'- Cytosine-phosphate-Guanine-3' (CpG) dinucleotide pairs is one type of epigenetic modification that has been associated with decreased gene expression and subsequent variations in behavioral phenotypes such as autism, schizoaffective disorders, attachment disorders, anxiety, and depression (Bleuler, 1963; Costa et al., 2009; Gregory et al., 2009; Jack et al., 2012; King et al., 2017; Puglia et al., 2015).

In the context of motherhood, women who have a higher percentage of DNA methylation in the MT2 region of the *OXTR* gene in peripheral whole blood have demonstrated a higher risk for PPD (Bell et al., 2015). Specifically, Bell and colleagues (2015) found a significant effect of *OXTR* DNA methylation at CpG site -934 in whole blood and genotype rs53576 (G/G) on outcomes of PPD in women without symptoms of depression during pregnancy ($p = 0.026$,

adjusted for psychosocial covariates). Evidence in the prairie vole model has further identified a downregulating effect of DNA methylation in the MT2 region on *Oxtr* gene expression in the nucleus accumbens which is shown to regulate social and maternal behaviors (Danoff et al., 2021; Olazabal & Young, 2006; Perkeybile et al., 2019). Interestingly, the use of synthetic oxytocin during parturition has been implicated in the downregulation of *OXTR* gene expression in the human uterus, mimicking the effect of DNA methylation (Phaneuf et al., 2000). More research is needed to examine the relationship between maternal administration of synthetic oxytocin, *OXTR* DNA methylation and *OXTR* gene expression in the maternal brain which may have long-term implications for maternal postpartum mood and behavior.

The goal of the present study is to examine associations between maternal exposure to synthetic oxytocin during parturition and outcomes of *Oxtr* DNA methylation and *Oxtr* gene expression in the maternal brain using the prairie vole as a model organism. Here, we explore dose-dependent effects of synthetic oxytocin (including a low, medium, and high dose), mimicking the different doses of oxytocin provided during labor induction in the clinical setting. CpG sites in the MT2 region that are homologous in humans and prairie voles (including CpG sites -934_1, -934_2, -924, -901) are assessed for future translation of this work to human participants (see Figure S1 in supplement). We further examine correlations between the methylation state of CpG sites in the nucleus accumbens and in whole blood to determine the utility of whole blood as a proxy for examining changes in central neurobiology.

The study consists of 75 term pregnant female prairie voles divided into 5 treatment groups (low-dose oxytocin (OXT), medium-dose OXT, high-dose OXT, saline vehicle, and no treatment). The specific aims are as follows:

(1) Examine the relationship between synthetic oxytocin dose (low-dose OXT, medium-dose OXT, high-dose OXT, saline vehicle, no treatment), DNA methylation in the 3' MT2 region of *Oxtr* (CpG sites -934_1, -934_2, -924, -901) and *Oxtr* gene expression in the nucleus accumbens of term pregnant female prairie voles

(2) Analyze correlations between *Oxtr* total DNA methylation in the nucleus accumbens and in whole blood of term pregnant female prairie voles

Methodology

The study procedures consist of a timed mating paradigm previously described in Kenkel et al., 2019, using the prairie vole as a model organism. Procedures included male and female vole pairing, observation of pregnancy, administration of synthetic oxytocin treatment, and collection of accessible and relevant tissues. Specific tissue processing methods for aim 1 and aim 2 are discussed below as well as the statistical processes used in each analysis. All procedures were completed in accordance with approved guidelines by the Institutional Animal Care and Use Committee (IACUC) of the University of Virginia.

Subjects

All subjects in the present study were outbred prairie voles (*Microtus ochrogaster)* descended from a wild-caught prairie vole population captured near Champaign, Illinois. Male and female voles were weaned on postnatal day (PND) 20 and housed in standard cages in sibling pairs containing wood chips and nestlet bedding until pairing. They were all provided access to water *ad libitum* and high-fiber Purina rabbit chow. The vivarium housing the subjects was maintained at room temperature with a 14:10 light-dark controlled cycle. Polycarbonate breeder cages (44 cm x 22 cm x 16 cm) were used to pair the male and female voles as described

in the breeding strategy below. The male voles in this study were used only for breeding purposes.

Breeding Strategy and Tissue Collection

Each virgin female prairie vole (PND 60-90) was weighed and paired with an experienced breeding male in a standard breeder cage for 24 hours to initiate female estrus. Pairs were then divided by a perforated Plexiglas barrier to continue female estrus induction, with a virgin female on one side and a male on the other. Per Carter and colleagues (1987), female estrus is most effectively induced after the female vole has been in physical contact with an unfamiliar male and has been housed for at least 24 to 48 hours in a cage that has been soiled by a male. After three days of producing the estrous condition, the dividers were removed, and mating was observed. After observed mating, the male and female were allowed to remain in the same cage throughout gestation (approximately 21.5 days). On the expected day of birth, pregnant females were weighed, and their abdomen was palpated to check for a term pregnancy. Mothers determined to be term pregnant (based on factors including observed mating, adequate weight gain across gestation, the presence of pups during palpation, and prominent nipple development) were injected in the ventral abdominal area with one of three doses of oxytocin (0.0125mg/kg, 0.25mg/kg, 0.5mg/kg; oxytocin acetate salt dissolved in sterile saline), a saline vehicle or were allocated to the no-injection control group. Ninety minutes after treatment, the term pregnant females were euthanized via cervical dislocation and rapid decapitation under deep isoflurane anesthesia. Maternal whole brains were collected and immediately frozen on dry ice. Maternal whole blood was collected in 1.5ml tubes lined with EDTA and was also immediately frozen on dry ice. After maternal tissues were collected, pups were excised from the maternal uterus and weighed for confirmation of term pregnancy. Only mothers with pups

weighing at least 2g each were included in the present study. All tissue samples were stored in a -80°C freezer until further processing.

Synthetic Oxytocin Formulation

The synthetic oxytocin was purchased in a powder form and stored in a -20°C freezer. Thirty minutes prior to injection, the synthetic oxytocin was removed from the freezer and suspended in normal saline. The suspended oxytocin was further diluted into a low (0.125 mg/kg), medium (0.25 mg/kg) and high (0.5 mg/kg) dose. Since the oxytocin was mixed in normal saline prior to injection, a saline vehicle group was included to determine the specific effects of synthetic oxytocin. All doses of synthetic oxytocin in the present study were beneath the threshold for labor induction in the prairie vole (Kenkel et al., 2019; Kenkel et al., 2023); however, the medium dose of 0.25mg/kg is equivalent to 5 IU that is common for labor induction in the clinical setting (Grotegut et al., 2017; Zhang et al., 2011).

Whole Brain Dissection

Maternal whole brains were stored at -80°C and then thawed to -20°C for 1 hour prior to regional sectioning. During the dissection process, a coronal cut was made to first remove the olfactory bulb. This was followed by a second coronal cut 2mm caudal to the frontal pole to collect bilateral punches (1mm in diameter and 2mm in depth) of the nucleus accumbens. Nucleus accumbens tissue from each subject was placed in a separate DNase/RNase free microcentrifuge tube and stored at -80°C until further processing.

Tissue Processing Procedures for Aim 1

Oxtr DNA Methylation Procedure

Extraction of DNA from the nucleus accumbens was completed using the Qiagen AllPrep DNA/RNA Mini Kit (Qiagen, Valencia, CA). 200 nanograms (ng) of genomic DNA from each

subject underwent bisulfite treatment (Kit MECOV50, Invitrogen, Carlsbad, CA) per the manufacturer's instructions. Twelve nanograms of bisulfite converted DNA was included in the PCR analysis using a Pyromark PCR kit (Qiagen, Valencia, CA) with 0.2 u M of primers TSL201_F 5'- GGGGATAGGATGGTTAGTTAGTATT- 3' and TSL201_R 5'-[biotin]CCAACAACCTCAAAACTCTACT-3'. Samples were amplified in triplicate using three identical PCR machines (S1000 Thermal Cycler, Bio-Rad, Hercules, CA.) and the following cycling conditions: Step 1: (95 °C/15 min)/1 cycle; Step 2: (94 °C/30 s, 58 °C/30 s, 72 °C/30 s)/50 cycles; Step 3: (72 °C/10 min)/1 cycle, Step 4: 4 °C hold. This allowed for the amplification of the target regions in *Oxtr* including CpG sites -934_1, -934_2, -924 and -901. Standard controls including 0% and 100% methylated DNA, a no DNA control, and a positive control were used on each PCR plate. Pyrosequencing was performed using sequencing primer TSL201_S1: 5'-GAGGGAAGGTTTTGGAGTTTTTTATAT-3' on a Pyromark Q24 using PyroMark Gold Q24 Reagents (Qiagen, Valencia, CA). The epigenotypes were identified as an average of the three replicates with a mean standard deviation below 2%.

RNA Processing Procedure

The extraction of RNA from the nucleus accumbens was completed using the Qiagen AllPrep DNA/RNA Mini Kit (Qiagen, Valencia, CA). 500ng of RNA from each subject was processed using the cDNA Synthesis kit (Bio-Rad, Hercules, CA) and Power SYBR Green. Real-time PCR technology (Applied Biosystems) was used for amplification. The primer sequences used for *Oxtr* were TSL401_F 5′-GCCTTTCTTCTTCGTGCAGATG-3′ and TSL401_R 5′-ATGTAGATCCAGGGGTTGCAG-3′. Amplification occurred under the following cycling conditions: Step 1: (95 °C/10 min) 1 cycle; Step 2: (95 °C/15 s, 63.4 °C/60 s)/35 cycles. All reactions were run in triplicate and their specificity was verified by a melting curve analysis and

separation on a 2% agarose gel. A comparative C_t method included analysis of target expression to *Gapdh* on the same real-time PCR machine.

Hydroxymethylation Procedure

100ng of genomic DNA from the nucleus accumbens for each subject underwent oxidative bisulfite sequencing (oxBS-Seq) per the manufacturer's protocol (TrueMethyl oxBS Module, Tecan Genomics, Inc, Redwood City, CA). Samples were amplified in triplicate using primer sequences TSL201_F 5'- GGGGATAGGATGGTTAGTTAGTATT- 3' and TSL201_R 5'-[biotin]CCAACAACCTCAAAACTCTACT-3'. Three identical PCR machines (S1000 Thermal Cycler, Bio-Rad, Hercules, CA.) were used with the following cycling conditions: Step 1: (95 °C/15 min)/1 cycle; Step 2: (94 °C/30 s, 58 °C/30 s, 72 °C/30 s)/45 cycles; Step 3: (72 °C/10 min)/1 cycle, Step 4: 4 °C hold. This process amplified the target regions in the *Oxtr* gene including CpG sites -934_1, -934_2, -924 and -901. Pyrosequencing was performed using sequencing primer OXTRv924LOWSnewA: 5'-GTTTTTTATATTTTTGGTT- 3' on a Pyromark Q48 using PyroMark Gold Q48 Reagents (Qiagen, Valencia, CA). The epigenotypes were identified as an average of the three replicates with a mean standard deviation below 2%.

Tissue Processing Procedures for Aim 2

DNA from the nucleus accumbens was extracted and processed as previously mentioned in Aim 1. Additional procedures to complete Aim 2 included DNA isolation from maternal whole blood using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) per the manufacturer's instructions. DNA methylation analysis for nucleus accumbens and whole blood from each subject was completed using the same bisulfite conversion treatment and pyrosequencing methods described in Aim 1.

Statistical Analysis

For aim 1, we assessed comparisons of *Oxtr* total DNA methylation across treatment groups at each CpG site (-934_1, -934_2, -924, -901) using a two-way analysis of variance (ANOVA). A one-way ANOVA was used to assess differences in *Oxtr* gene expression across treatment groups. All values used in the ANOVA tests are represented as an average for each group and the P-values were generated using the *ggpubr* R package. A Spearman correlation coefficient was used to assess the relationship between *Oxtr* DNA Methylation and *Oxtr* gene expression in each treatment group in aim 1. Normality of the data was assessed via a Shapiro-wilk test in R. To assess the relationship between *Oxtr* DNA methylation in the nucleus accumbens and whole blood in aim 2, we again used a Spearman correlation coefficient due to non-normal data. Correlation values and figures were all generated using the *ggpubr* R package.

Results

Synthetic oxytocin changes the relationship between *Oxtr* DNA methylation and *Oxtr* gene expression in the maternal nucleus accumbens 90 minutes post-injection

The purpose of aim 1 was to examine the impact of synthetic oxytocin dose (low dose-OT, medium dose-OT, high dose-OT, saline control, and no treatment) on DNA methylation in the 3' MT2 region of *Oxtr* (CpG sites -934_1, -934_2, -924, and -901) and *Oxtr* gene expression in the nucleus accumbens of term pregnant female prairie voles. N= 62 term pregnant female subjects (n= 11 no treatment; n = 12 saline; n= 14 low-dose OXT; n= 12 medium-dose OXT; n = 13 high-dose OXT) were included in this experiment. N=5 samples were determined to be outliers in this analysis and were removed. N= 7 samples were removed due to low initial RNA concentrations and an unreliable gene expression output. N= 1 sample was removed due to missing methylation values after pyrosequencing.

First, we examined differences in the average methylation state of all subjects by CpG site (-934_1, -934_2, -924, -901) and treatment group. We conducted a two-way ANOVA and observed no significant differences in the average methylation state across treatment groups and CpG sites (934_1: $F_{(4,57)} = 0.404$, $p = 0.805$; 934_2: $F_{(4,57)} = 0.138$, $p = 0.968$; 924: $F_{(4,57)} = 0.209$, $p = 0.932$; 901: $F_{(4,57)} = 0.288$, $p = 0.885$, see Figure S2 in supplement). We then examined whether there were differences in the average gene expression level across each treatment group and found no significant differences using a one-way ANOVA analysis ($F_{(4,57)} = 0.334$, $p = 0.854$, see Figure S3 in supplement).

As a next step, we used a spearman correlation coefficient to examine the relationship between *Oxtr* total DNA methylation and *Oxtr* gene expression in each treatment group. Previous studies in nonpregnant populations have described a negative relationship between *Oxtr* total DNA methylation and *Oxtr* gene expression due to gene suppressing effects of DNA methylation (Danoff et al., 2022; Kusui et al, 2001; Liu et al., 2018; Perkeybile et al., 2019; Turnpin & Salbert, 2022). However, previous findings in our laboratory have demonstrated no relationship between *Oxtr* DNA methylation and *Oxtr* gene expression in the nucleus accumbens of term pregnant females likely demonstrating an underlying epigenetic mechanism that is needed for the mother to prepare for birth. Due to our previous findings, we hypothesized that we would observe no relationship in term pregnant females in the no treatment and saline vehicle group (Perkeybile et al., *in prep*).

In the no treatment group, we found no significant relationship between *Oxtr* total DNA methylation and *Oxtr* gene expression in the nucleus accumbens at CpG sites -934_1, -934_2, and -924 (-934_1: $r(9) = -0.12$, $p = 0.73$; -934_2: $r(9) = -0.21$, $p = 0.54$, -924: $r(9) = 0.16$, $p = 0.63$, see Figure 1A); however, a trending negative relationship was observed at CpG site -901

(r(9) = -0.56, p = 0.076, see Figure 1A). The saline vehicle group demonstrated no significant relationship at any of the four CpG sites (-934_1: r(10) = 0.23, p= 0.47; -934_2: r(10) = 0.07, p= 0.83; -924: r(10) = 0.17, p= 0.6; -901: r(10) = -0.021, p= 0.96, see Figure 1B).

When examining the impact of synthetic oxytocin treatment, we found no relationship between *Oxtr* total DNA methylation and *Oxtr* gene expression at any of the four CpG sites in the low-dose OXT group (-934_1: r(12)= 0.046, p= 0.88; -934_2: r(12)= 0.2, p= 0.49; -924: r(12) = -0.059, p= 0.84; -901: r(12) = 0.022, p = 0.95, see Figure 2A) and in the medium-dose OXT group (-934_1: r(10)= 0.2, p = 0.53; -934_2: r(10) = -0.056, p= 0.87; -924: r(10) = 0.17, p= 0.59; -901: r(10) = 0.16, p= 0.63, see Figure 2B). In the high-dose OXT group, however, we observed a trending positive relationship at CpG site -934_1 (r(11) = 0.57, p= 0.071, see Figure 2C). While the relationships at CpG sites -934_2 (r(11) = 0.46, p= 0.15), -924 (r(11) = 0.51, p= 0.11), and -901 (r(11) = 0.14, p= 0.69) were not significant, trend lines indicated a shift toward a positive relationship between methylation and expression at these sites (see Figure 2C).

Hydroxymethylation (5hmC) upregulates *Oxtr* gene expression in the high-dose OXT group

In a further analysis, we used refined techniques to differentiate true DNA methylation (5mC) and hydroxymethylation (5hmC) in relation to *Oxtr* gene expression in the nucleus accumbens of the term pregnant female subjects. DNA hydroxymethylation denotes the process by which a methyl group located at the C5- position of the cytosine is replaced by a hydroxymethyl group, becoming a 5-hydroxymethylcytosine (5hmC), and is an initial step in the active DNA demethylation pathway (Richa & Sinha, 2014). Because we identified a positive relationship between *Oxtr* total DNA methylation and *Oxtr* gene expression specifically in the high-dose OXT group, we examined whether this positive relationship was due to the mechanism

of 5mC or 5hmC. We found no significant relationship between 5mC and *Oxtr* gene expression in the high-dose OXT group at any of the four CpG sites (-934_1: r(11)= 0.12, p= 0.76; -934_2: r(11) = 0.26, p= 0.47; -924:(r(11) = 0.091, p = 0.81; -901: r(11) = -0.5, p= 0.14, see Figure 3). Conversely, we found a significant positive relationship between 5hmC and *Oxtr* gene expression at CpG site -901 (r(11)= 0.73, p= 0.021) in the high-dose OXT group (see Figure 4). We did not observe a significant relationship at CpG sites -934_1 (r(11)= 0.25, p = 0.48), -934_2 (r(11) = 0.53, p= 0.12) and -924 (r(11) = 0.39, p= 0.26), though trend lines indicate a positive relationship between hydroxymethylation and expression (see Figure 4).

Oxtr total DNA methylation in peripheral whole blood is correlated with the methylation state of CpG sites in the nucleus accumbens

The purpose of aim 2 was to analyze correlations between *Oxtr* total DNA methylation at CpG sites -934_1, -934_2, -924, -901 in whole blood and in the nucleus accumbens of term pregnant female prairie voles 90 minutes post-injection with a dose of synthetic oxytocin, a saline vehicle or no treatment. Previous work in our laboratory identified a significant positive relationship between central and peripheral levels of *Oxtr* total DNA methylation in term pregnant females receiving no treatment (Perkeybile et al., *in prep*). In the present study, we sought to assess whether whole blood may serve as a proxy for epigenetic changes occurring in the nucleus accumbens in the context of maternal exposure to synthetic oxytocin at term pregnancy. We hypothesized that *Oxtr* total DNA methylation in whole blood and in the nucleus accumbens would correlate at each CpG site regardless of the treatment group.

For aim 2, we included a total of 69 term pregnant female voles (n= 12 no treatment; n= 13 saline, n = 15 low-dose OXT, n = 15 medium-dose OXT, n= 14 high-dose OXT). N = 5

samples that were determined to be outliers in in aim 1 were also removed in this analysis. N= 1 sample was removed due to a lack of methylation data during pyrosequencing. Across all treatment groups, we found a significant positive correlation between *Oxtr* total DNA methylation in whole blood and *Oxtr* total DNA methylation in the nucleus accumbens at CpG sites 934_1 ($r(67) = 0.41$, $p < 0.001$), -934_2 ($r(67) = 0.49$, $p < 0.001$), and -924 ($r(67) = 0.47$, $p < 0.001$) (see Figure 5A, B and C); however, we found no relationship at CpG site -901 ($r(67) = 0.11$, $p = 0.39$) (see Figure 5D).

Oxtr total DNA methylation in whole blood is correlated with _Oxtr_ gene expression in the nucleus accumbens at CpG site -901

We further examined whether a relationship exists among *Oxtr* total DNA methylation in whole blood and *Oxtr* gene expression in the nucleus accumbens 90 minutes after treatment with synthetic oxytocin, a saline vehicle, or no treatment. In this analysis, we included a total of N= 62 term pregnant females (n= 11 no treatment; n = 12 saline; n= 14 low-dose OXT; n= 12 medium-dose OXT; n = 13 high-dose OXT). N = 5 samples that were previously determined to be outliers were removed in this analysis. N = 7 samples were removed due to low initial RNA concentrations as noted in aim 1. N= 1 sample was removed due to a lack of methylation data during pyrosequencing. In the low-dose OXT group, we found a significant negative relationship between *Oxtr* total DNA methylation in whole blood and *Oxtr* gene expression in the nucleus accumbens at CpG site -901 ($r(12)= -0.57$, $p = 0.035$) (see Figure 6C). There was no significant relationship at CpG site -934_1 ($r(12)$: -0.35, $p = 0.22$), -934_2 ($r(12) = -0.24$, $p = 0.42$) or -924 ($r(12) = -0.4$, $p= 0.16$) in the low-dose OXT group (see Figure 6C). Conversely, in the high-dose OXT group, we found a trending positive relationship between *Oxtr* total DNA methylation in

whole blood and *Oxtr* gene expression in the nucleus accumbens at CpG site -901 (r(11) = 0.48, p= 0.097). No significant correlations were observed at CpG site -934_1 (r(11) = 0.43, p = 0.14), 934_2 (r(11) = 0.35, p = 0.25), or -924 (r(11) = 0.4, p= 0.18) in the high-dose OXT group (see Figure 6E). We found no relationship between *Oxtr* total DNA methylation in whole blood and *Oxtr* gene expression in the nucleus accumbens across all CpG sites in the no treatment group (-934_1: r(9) = 0.18, p = 0.6; -934_2: r(9) = -0.027, p = 0.95; -924: r(9) = -0.17, p = 0.61; -901: r(9) = -0.0091, p= 0.99), the saline vehicle group (-934_1: r(10)= 0.077, p= 0.82; -934_2: r(10) = -0.13, p = 0.7; -924: r(10) = -0.098, p= 0.77; -901: r(10) = 0.007, p= 0.99), and the medium-dose OXT group (-934_1: r(10) = -0.32, p = 0.31; -934_2: r(10) = -0.007, p= 0.99; -924: r(10) = -0.063, p= 0.85; -901: r(10) = -0.29, p= 0.37) (see Figure 6A, B and D).

Discussion

The purpose of this study was to examine the impact of synthetic oxytocin on epigenetic regulation of the *Oxtr* gene in the maternal brain. To accomplish this goal, we examined the effects of three doses of synthetic oxytocin (low-dose OXT, medium-dose OXT, high-dose OXT), a saline vehicle and no treatment on *Oxtr* DNA methylation and *Oxtr* gene expression in the nucleus accumbens of term pregnant female prairie voles. In aim 1, we assessed the methylation state of four CpG sites in MT2 (CpG -934_1, -934_2, -924, and -901) that are homologous to the human *OXTR* gene and have been shown to regulate *Oxtr* gene expression in previous literature (Danoff et al., 2022; Kusui et al., 2001; Perkybile et al., 2019). We further examined how a high dose of synthetic oxytocin changes the relationship between methylation and expression by distinguishing true methylation (5mC) from hydroxymethylation (5hmC) across four CpG sites.

In the present study, we did not observe differences in *Oxtr* total DNA methylation or *Oxtr* gene expression average across treatment groups; however, we observed dose-dependent differences in the relationship between *Oxtr* DNA methylation and *Oxtr* gene expression in the nucleus accumbens of the term pregnant female subjects. In studies involving nonpregnant populations, findings support a negative relationship between *Oxtr* total DNA methylation and *Oxtr* gene expression due to gene suppressing mechanisms of DNA methylation (Danoff et al., 2022; Kusui et al, 2001; Liu et al., 2018; Perkeybile et al., 2019; Turnpin & Salbert, 2022). During term pregnancy, however, our lab previously found a change in the relationship between *Oxtr* DNA methylation and *Oxtr* gene expression such that there is no relationship in the nucleus accumbens of female prairie voles receiving no treatment (Perkeybile et al., *in prep*). This lack of relationship then rapidly shifts back to a negative relationship during the early postpartum period demonstrating the likely effect of the birth process (Perkeybile et al., *in prep*).

In the present study, we examined how synthetic oxytocin might further impact the relationship between *Oxtr* DNA methylation and *Oxtr* gene expression in the nucleus accumbens at term pregnancy. Previous work examining the effect of synthetic oxytocin in human mothers demonstrates the downregulation of *OXTR* gene expression in the maternal uterus (Phaneuf et al., 2000). Per Phaneuf and colleagues (2000), increased duration of synthetic oxytocin exposure during parturition was associated with decreased *OXTR* mRNA and oxytocin binding in the uterus. Based on previous findings regarding the relationship between methylation and expression in the nucleus accumbens of term pregnant female voles and the downregulating effect of synthetic oxytocin in the maternal uterus, we hypothesized that the relationship between *Oxtr* DNA methylation and *Oxtr* gene expression would become more negative as the dose of synthetic oxytocin increased.

Conversely, we observed trend lines in the high-dose OXT group that indicated a shift toward a positive relationship between *Oxtr* DNA methylation and *Oxtr* gene expression in the nucleus accumbens 90 minutes post-injection. In this analysis, we assessed total DNA methylation which includes a combination of true methylation (5mC) and hydroxymethylation (5hmC). Whereas the presence of 5mC is associated with heterochromatin (or dense chromatin) and inactive transcription, 5hmC is associated with euchromatin (or loose chromatin) and active transcription and would likely demonstrate a positive relationship with gene expression (Branco et al., 2011; Turnpin & Salbert, 2022). In the following analysis, we expected to observe a positive relationship between 5hmc and *Oxtr* gene expression such that a higher level of 5hmc at each CpG site in the high-dose OXT group would correlate with higher *Oxtr* gene expression in the nucleus accumbens.

Specifically, at CpG -901, we observed a significant positive relationship between 5hmC and *Oxtr* gene expression in the nucleus accumbens 90 minutes post-injection with a high dose of synthetic oxytocin. We did not observe a significant relationship at CpG site -934_1, -934_2, or -924 though trend lines indicated a positive relationship at these sites. A significant association between 5hmC and *Oxtr* gene expression at only one CpG site suggests that the relationship between hydroxymethylation and gene expression may be location and time dependent. Moving from the 5' to the 3' end of the *Oxtr* gene, CpG site -901 is the most downstream site in MT2 examined in this study and is positioned nearest to the translation start site in the canonical model of the *Oxtr* gene (see Figure S4 in supplement). It is possible that we captured a significant positive relationship between 5hmC and gene expression at CpG site -901 due to the 90-minute post-injection timepoint at which the tissue was collected. Additional analyses will be needed to test whether this effect is time dependent across CpG sites.

The purpose of aim 2 was to analyze correlations between *Oxtr* DNA methylation in the nucleus accumbens and in whole blood of term pregnant prairie voles for future translation of this work to human mothers. Research focused on the neurobiological effects of synthetic oxytocin as a birth intervention has been limited. Though several studies report a link between maternal exposure to synthetic oxytocin during childbirth and outcomes of maternal psychological disorders such as PPD, studies in human mothers are often limited to examining accessible tissues such as peripheral whole blood (Gu et al., 2016; Kroll-Desrosiers et al., 2017; Tichelman et al., 2021).

Epigenetic regulatory factors like DNA methylation and hydroxymethylation are often cell-type specific with a unique balance of methylation and demethylation processes across tissues (Turnpin & Salbert, 2022). This cell-type specificity can make it challenging to draw conclusions about changes in central tissues when only peripheral tissues are accessible. Using animal models, researchers have sought to identify associations between the methylation state of the *Oxtr* gene in whole blood and central tissues to determine the potential utility of whole blood as a proxy for examining neurobiological mechanisms. In previous studies assessing methylation of conserved CpG sites in the *Oxtr* gene (-934_1, -934_2, -924, and -901), our lab has demonstrated strong positive correlations between DNA methylation in whole blood and in the nucleus accumbens in the context of adverse early life experiences and in term pregnant female prairie voles (Danoff et al., 2021; Perkeybile et al., *in prep;* Perkeybile et al., 2019).

In the present study, we show that a correlation between central and peripheral tissues also exists in the context of maternal administration of synthetic oxytocin at term pregnancy. We specifically observed a significant positive correlation between *Oxtr* total DNA methylation in the nucleus accumbens and *Oxtr* total DNA methylation in whole blood at CpG sites -934_1, -

934_2 and -924; however, this relationship was not observed at CpG site -901. We found no relationship between total DNA methylation in whole blood and total DNA methylation in the nucleus accumbens across all treatment groups at CpG site -901. In aim 1, we found variability in the relationship between methylation and gene expression at CpG site -901 in the nucleus accumbens. However, it appears that methylation in whole blood may not reflect the same variability at the examined time point.

Interestingly, upon further analysis, we found a significant negative relationship between *Oxtr* total DNA methylation at CpG site -901 in whole blood and *Oxtr* gene expression in the nucleus accumbens in the low-dose OXT group. We did not observe this significant relationship in the nucleus accumbens which may be due to differences in the methylation state of CpG sites across tissue types. In the context of a low dose of synthetic oxytocin, higher methylation in whole blood is correlated with lower gene expression in the nucleus accumbens which is similar to the negative relationship observed in the nucleus accumbens of virgin and postpartum female prairie voles (Perkeybile et al., *in prep)*. A negative relationship at CpG site -901 may indicate an early onset of the postpartum state particularly for female voles exposed to a low dose of synthetic oxytocin during parturition.

Conversely, in the high-dose OXT group, we observed a trending positive relationship between *Oxtr* total DNA methylation at CpG site -901 in whole blood and *Oxtr* gene expression in the nucleus accumbens. A shift toward a positive relationship in the high-dose OXT group may be reflecting the process of hydroxymethylation that was previously identified in the nucleus accumbens in aim 1. Hydroxymethylation is a mechanism involved in increasing gene expression and would be important for producing the receptors needed to meet the demand of excess circulating oxytocin. Importantly, subjects with a higher level of methylation have a

greater capacity to become hydroxymethylated with subsequent increases in gene expression. For females with high levels of methylation at CpG site -901 in whole blood, we observed an association with higher gene expression in the nucleus accumbens 90 minutes post-injection. Observing a positive relationship at this timepoint may be important for the transition to motherhood. Whereas an early onset of the postpartum state may be occurring in the low-dose OXT group, a positive relationship in the high-dose OXT group may mean that the shift toward a negative relationship in the maternal postpartum state is prolonged. Like methylation, hydroxymethylation is cell-type specific and can demonstrate unique relationships with gene expression across tissue types. In the current study, we did not differentiate 5mC and 5hmC in whole blood which may further explain the relationships observed in the low-dose and high-dose OXT group. Additional research differentiating 5mC and 5hmC in whole blood at CpG site -901 is needed to understand the specific effects of synthetic oxytocin on the relationship between methylation in peripheral tissues and gene expression in central tissues.

Strengths and Limitations

There were two important limitations in this study. First, we had to exclude several samples due to a lack of methylation data and low-quality gene expression data. This was a result of low DNA and RNA yield during the isolation procedure. Future studies should account for this potential loss by incorporating larger sample sizes during data collection. Secondly, we found five subjects in our study with a unique variation in their genetic sequence which altered their level of *Oxtr* total DNA methylation and *Oxtr* gene expression across CpG sites and treatment groups. Previous evidence shows an important interaction between genetic and epigenetic alterations of the *OXTR* gene and outcomes of maternal postpartum mood disorders (Bell et al.,

2015). Thus, future studies assessing the effects of synthetic oxytocin in mothers should consider the likely effect of genetic underpinnings.

A major strength of this study was the use of a prairie vole labor induction model to mimic the effects of synthetic oxytocin administration during parturition in the maternal brain. Previous studies have used this labor induction model to assess physiological and neuroanatomical effects in offspring; however, to our knowledge, this is the first study to assess neurobiological effects in mothers considering mechanisms of methylation and hydroxymethylation (Kenkel et al., 2019; Kenkel et al., 2023). In addition, we examined dose-dependent effects of synthetic oxytocin in both central and peripheral tissues which has been an important gap in the literature. Here, we describe differences in the epigenetic regulation of *Oxtr* in the maternal brain across multiple doses of synthetic oxytocin. We also demonstrate the utility of whole blood as proxy for changes in central tissue across multiple CpG sites in the *Oxtr* gene. Future research is needed to assess whether these neurobiological changes at term pregnancy may be linked to variations in maternal postpartum behavior and adverse outcomes such as maternal PPD in human mothers.

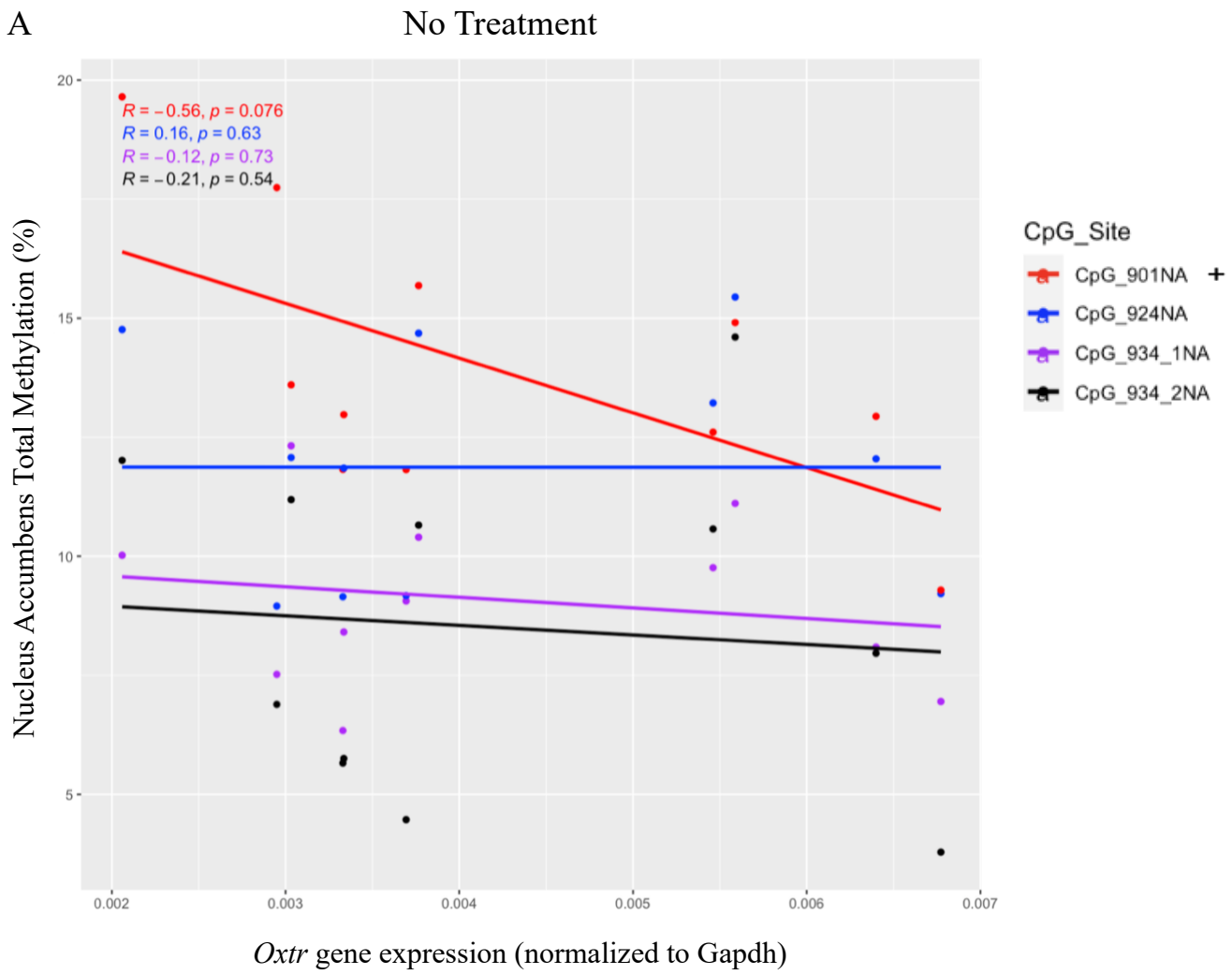
Conclusion

Synthetic oxytocin is widely administered across hospital settings with important indications for use. When administered properly, synthetic oxytocin (i.e., Pitocin) stimulates uterine contractions that are comparable to contractions required for birth and has demonstrated life-saving benefits. Conversely, maternal exposure to high doses of synthetic oxytocin during parturition is associated with important short-term reproductive risks and long-term adverse psychological outcomes for the mother (Erickson et al., 2023; Gu et al., 2016). Considering the long-term adverse effects on maternal mental health, we sought to investigate dose-dependent

effects of synthetic oxytocin on neurobiological mechanisms in the maternal brain. In the present study, we show a dose-dependent impact of synthetic oxytocin on the relationship between *Oxtr* DNA methylation and *Oxtr* gene expression in the nucleus accumbens of term pregnant female prairie voles. Furthermore, we demonstrate the utility of whole blood to serve as a proxy for examining the *Oxtr* DNA methylation state in the nucleus accumbens at specific CpG sites. We show that total DNA methylation in whole blood reflects *Oxtr* gene expression changes in the nucleus accumbens, specifically at CpG site -901, though additional studies are needed to confirm this relationship and to differentiate 5mC and 5hmC in whole blood. This work expands the current knowledge regarding neurobiological effects of synthetic oxytocin in the maternal brain. Our findings further demonstrate dose-dependent outcomes that may underlie the link between maternal exposure to synthetic oxytocin during parturition and variations in maternal postpartum mood.

Figures

Figure 1. A trending negative relationship between *Oxtr* total DNA methylation and *Oxtr* gene expression is observed in the no treatment group at CpG site -901 (A). No significant relationships are observed in the no treatment group at CpG sites -934_1, _934_2, or _924 (A) or in the saline vehicle group at any of the four CpG sites (B). Spearman's correlation: + $p < 0.1$.



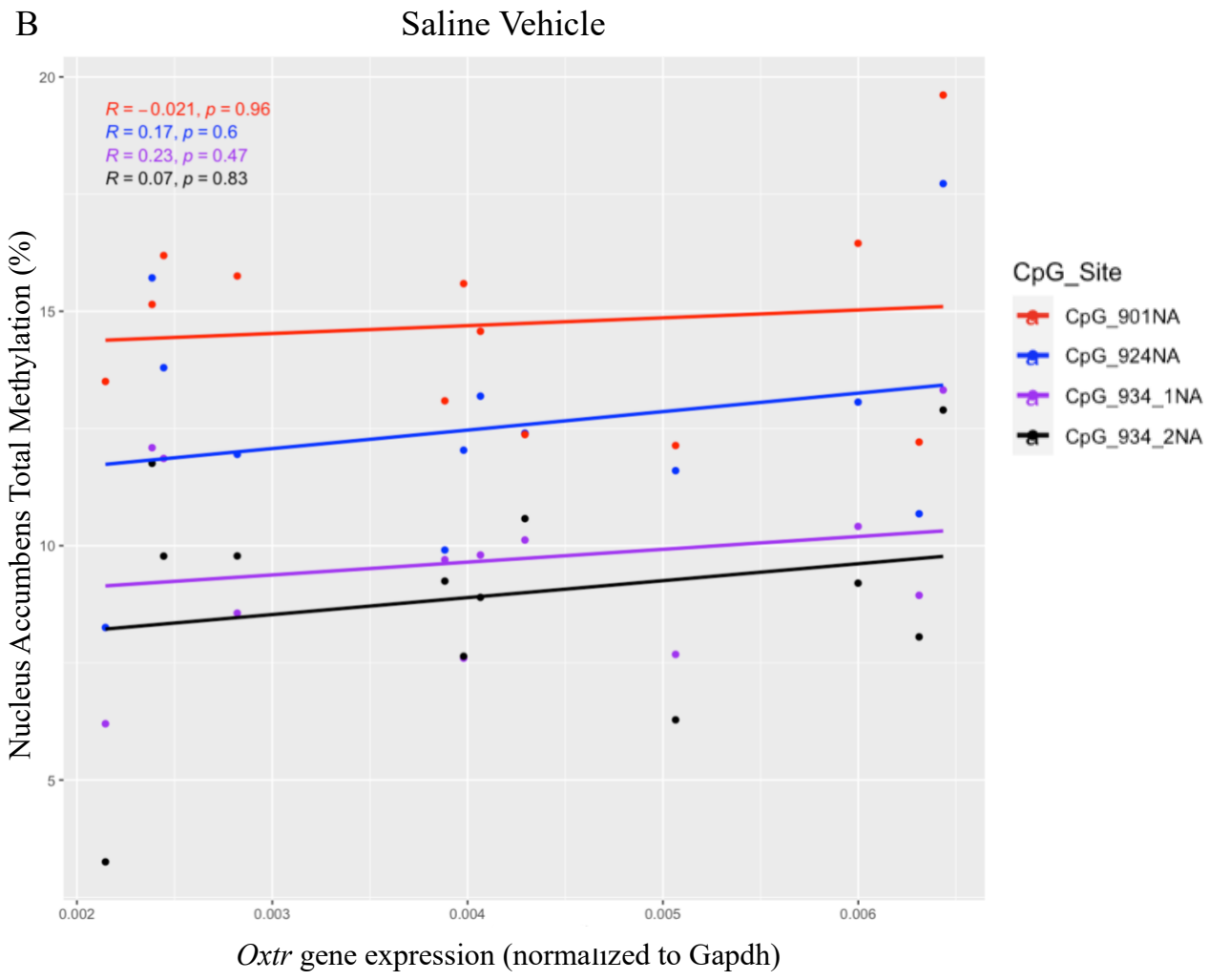
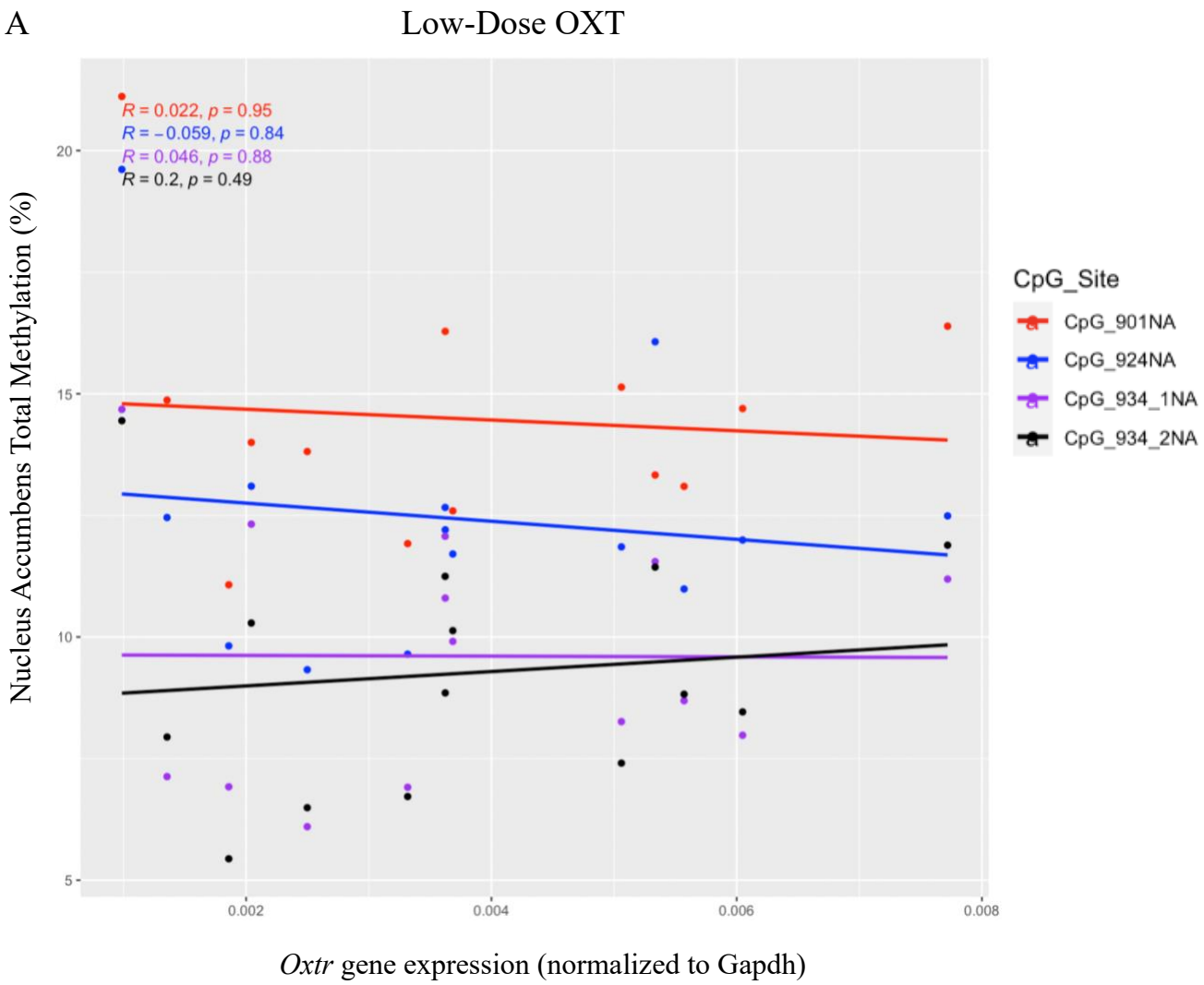
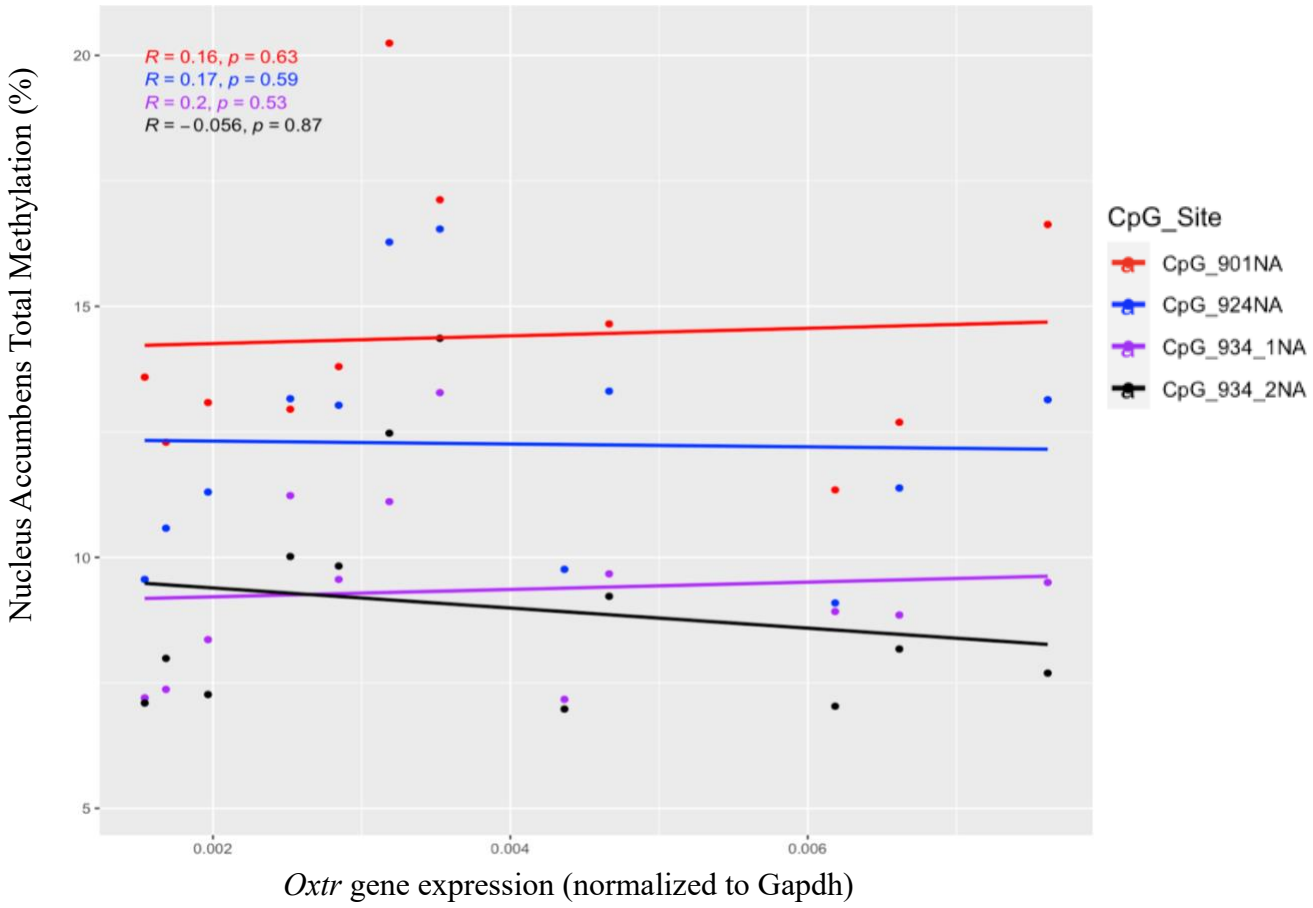

B
Saline Vehicle
R = −0.021, p = 0.96
R = 0.17, p = 0.6
R = 0.23, p = 0.47
R = 0.07, p = 0.83
Nucleus Accumbens Total Methylation (%)
20
15
10
5
0.002
0.003
0.004
0.005
0.006
Oxtr gene expression (normalized to Gapdh)
CpG_Site
CpG_901NA
CpG_924NA
CpG_934_1NA
CpG_934_2NA

Figure 2. No significant relationships are observed between *Oxtr* total DNA methylation and *Oxtr* gene expression at any of the four CpG sites in the low-dose OXT (A) and the medium-dose OXT group (B). A trending positive relationship is observed in the high-OXT group at CpG site -934_1 (C). CpG sites -934_2, -924, and -901 in the high-dose OXT group indicate a shift toward a positive relationship though they do not reach statistical significance (C). Spearman' s correlation: + $p < 0.1$.



B Medium-Dose OXT



C

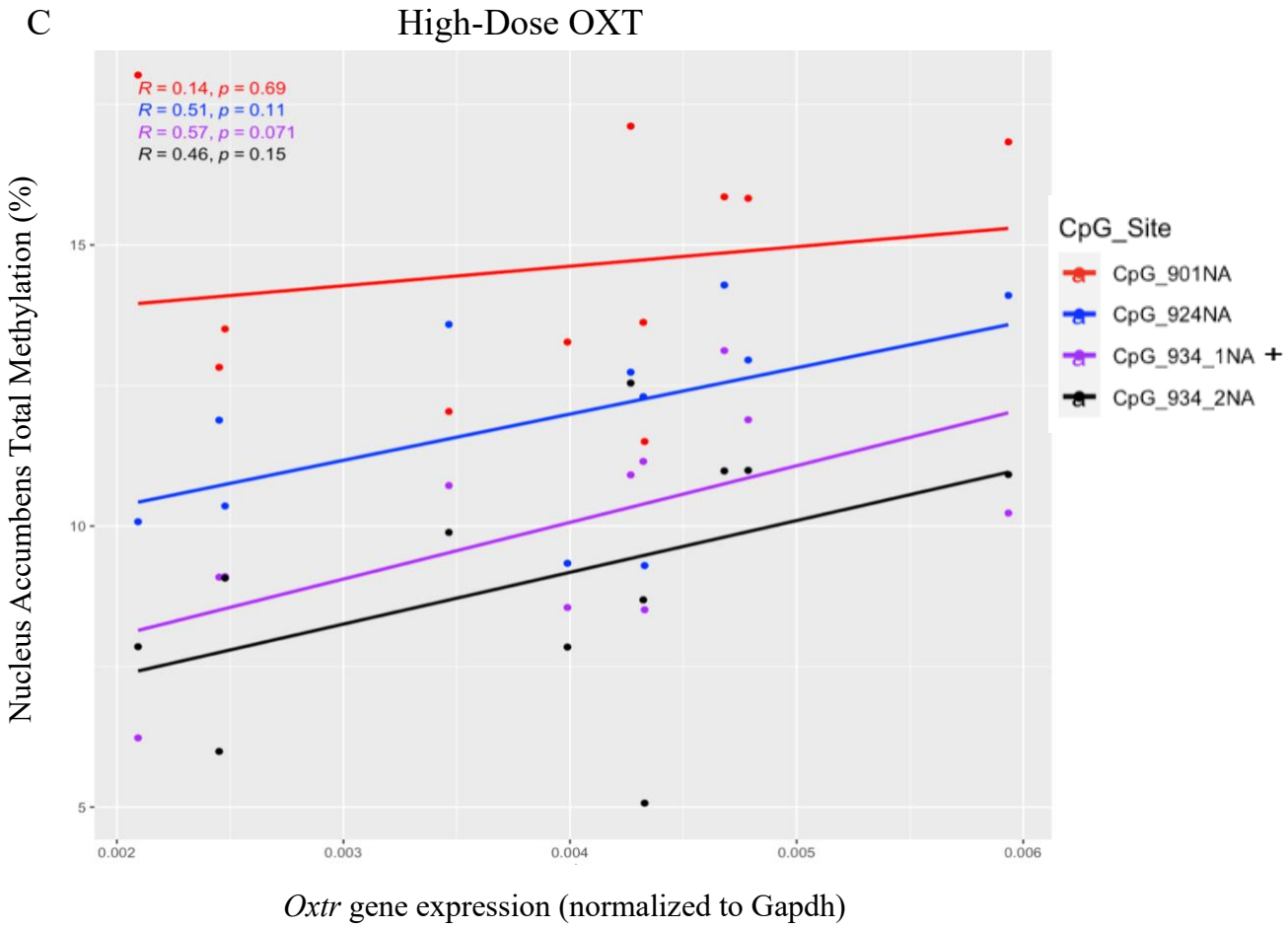

High-Dose OXT
R = 0.14, p = 0.69
R = 0.51, p = 0.11
R = 0.57, p = 0.071
R = 0.46, p = 0.15
Nucleus Accumbens Total Methylation (%)
15
10
5
0.002
0.003
0.004
0.005
0.006
Oxtr gene expression (normalized to Gapdh)
CpG_Site
CpG_901NA
CpG_924NA
CpG_934_1NA
CpG_934_2NA

Figure 3. A spearman's correlation coefficient showed no significant relationships in the high-dose OXT group between 5mC at each CpG site and *Oxtr* gene expression in the nucleus accumbens of term pregnant female prairie voles.

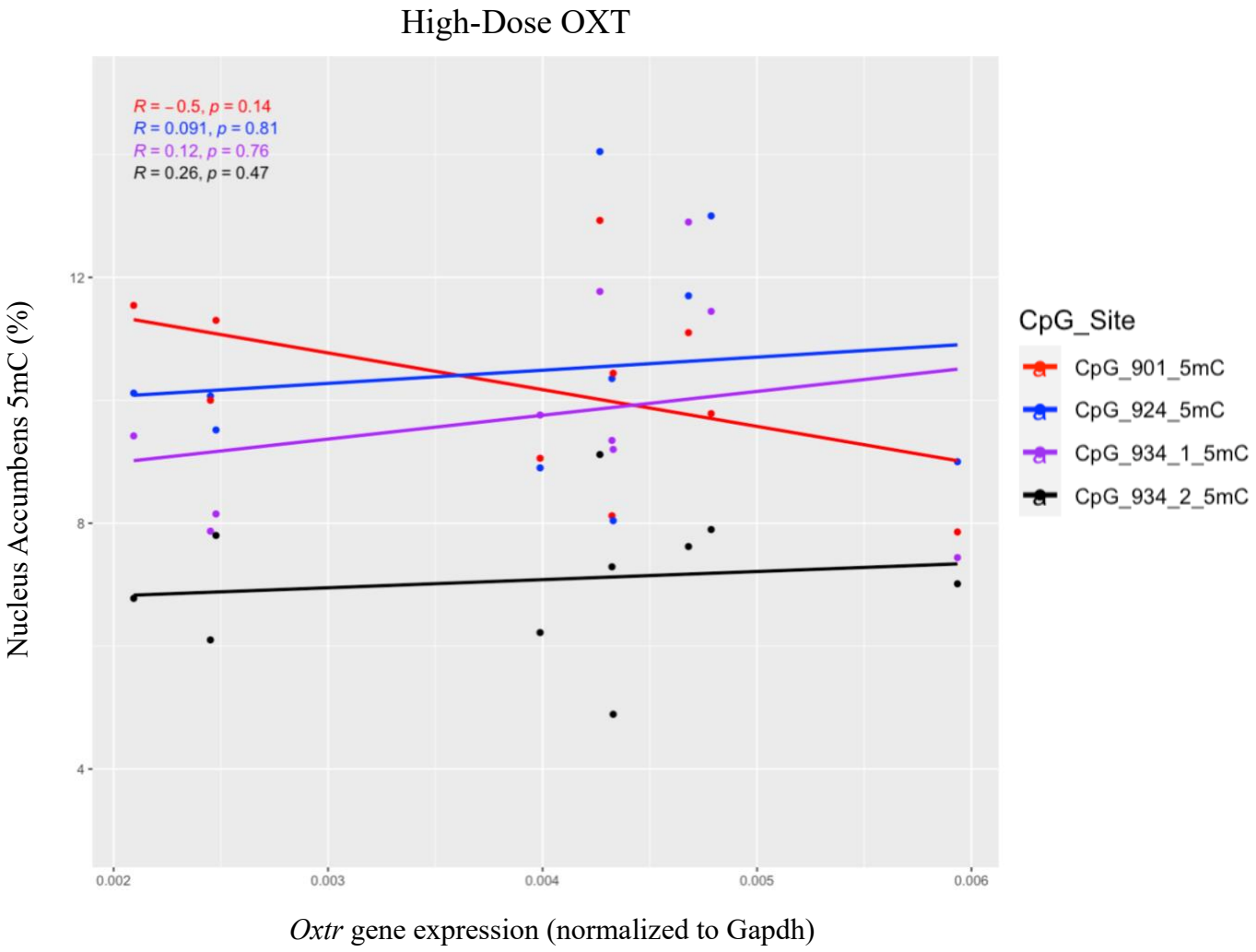


Figure 4. A significant positive relationship is found in the high-dose OXT group between 5hmC and *Oxtr* gene expression at CpG site -901. A positive relationship is also observed at CpG site -934_1, -934_2 and -924, however, the correlations are not statistically significant. Spearman's correlation: * $p < 0.05$

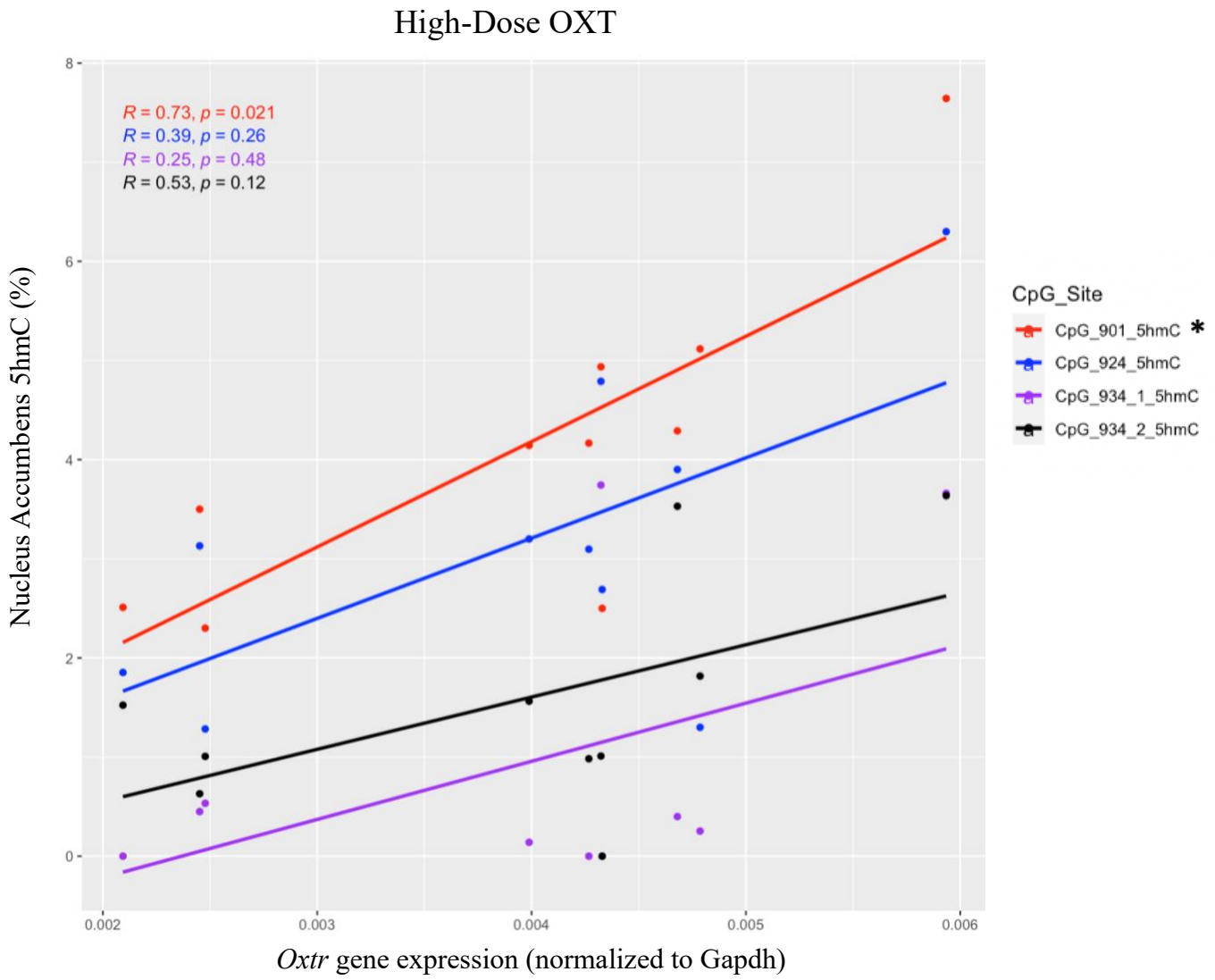
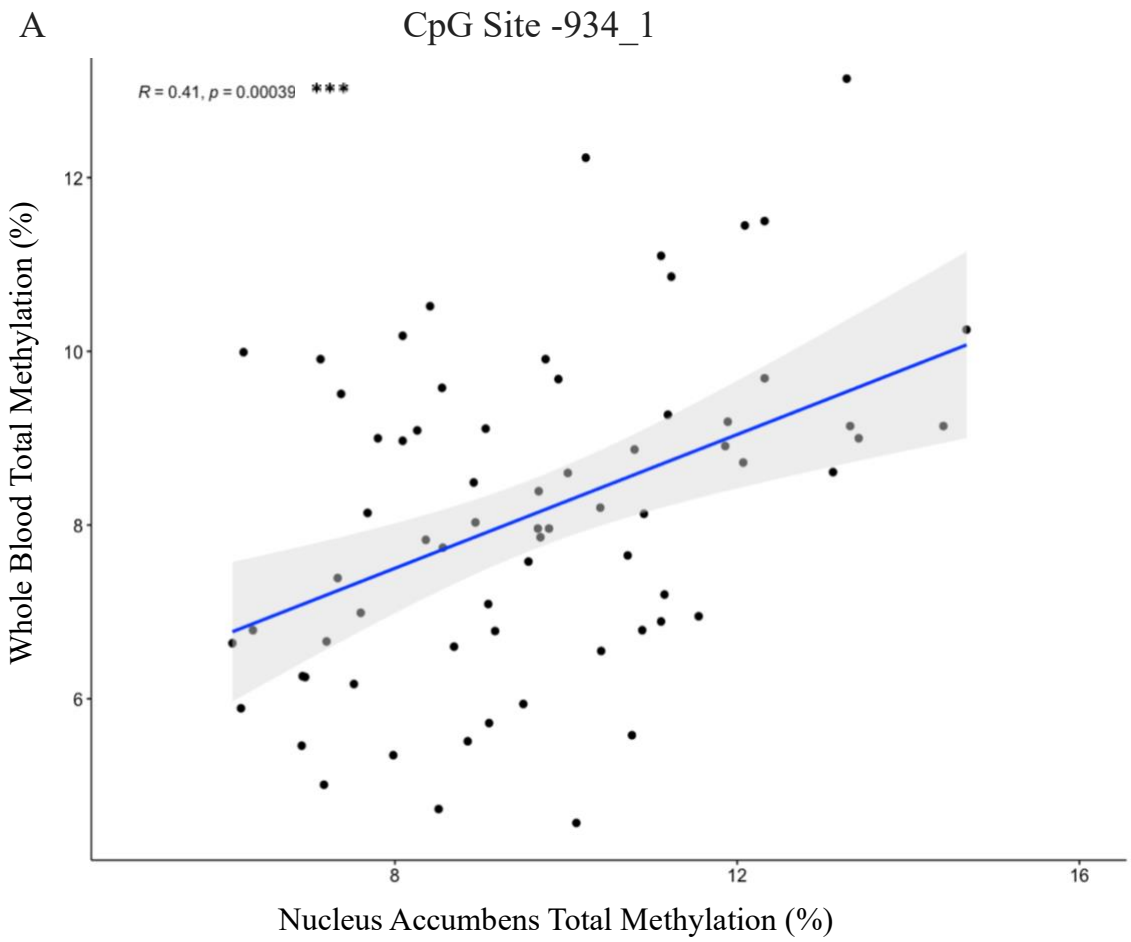
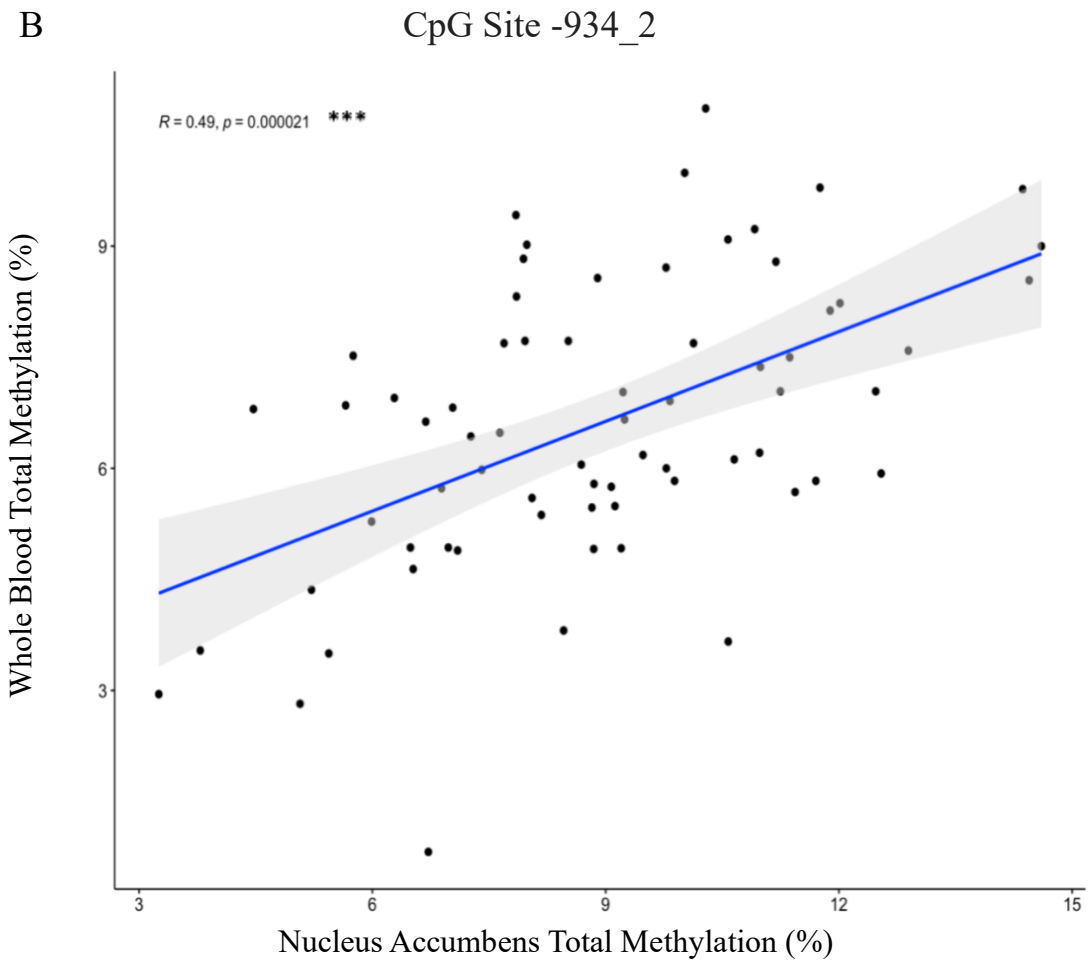
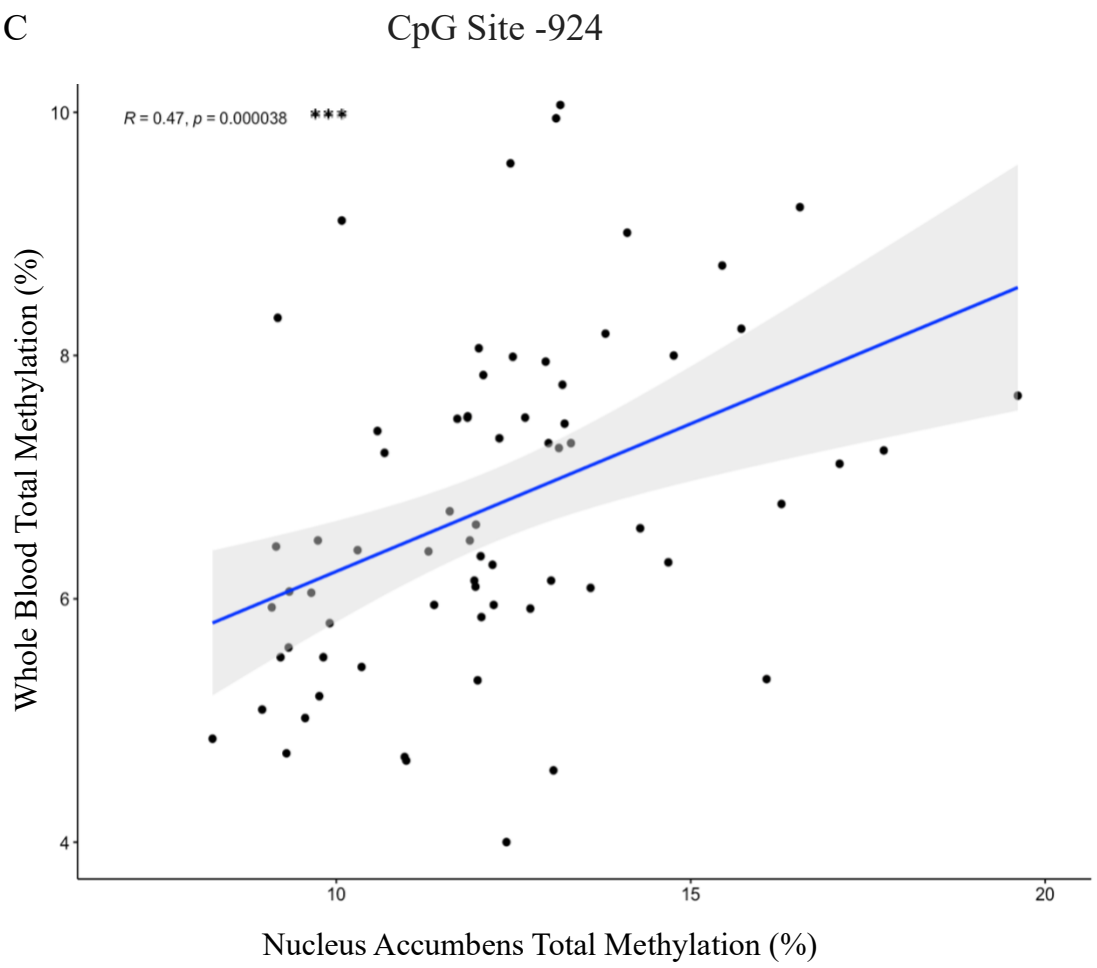


Figure 5. There is a significant positive correlation between whole blood and nucleus accumbens *Oxtr* total DNA methylation at CpG Site -934_1, -934_2, and -924 across all treatment groups (see Figure 5A, B and C); however, we observed no relationship at CpG site -901 (see Figure D). Spearman's correlation: *** $p < 0.001$. Note the differences in the scale between DNA methylation in whole blood and in the nucleus accumbens across CpG sites.




B
CpG Site -934_2
R = 0.49, p = 0.000021 ***
Whole Blood Total Methylation (%)
9
6
3
3
6
9
12
15
Nucleus Accumbens Total Methylation (%)


C
CpG Site -924
R = 0.47, p = 0.000038
***
Whole Blood Total Methylation (%)
10
8
6
4
10
15
20
Nucleus Accumbens Total Methylation (%)

D

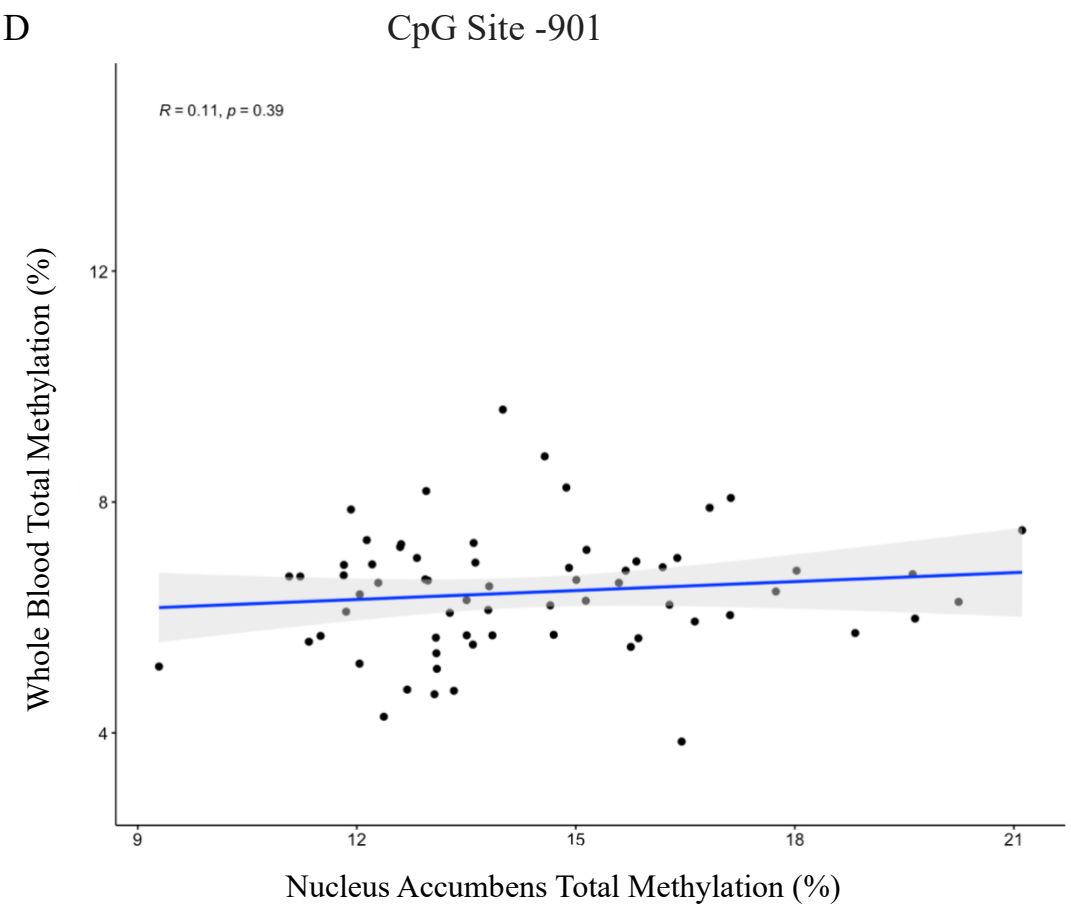
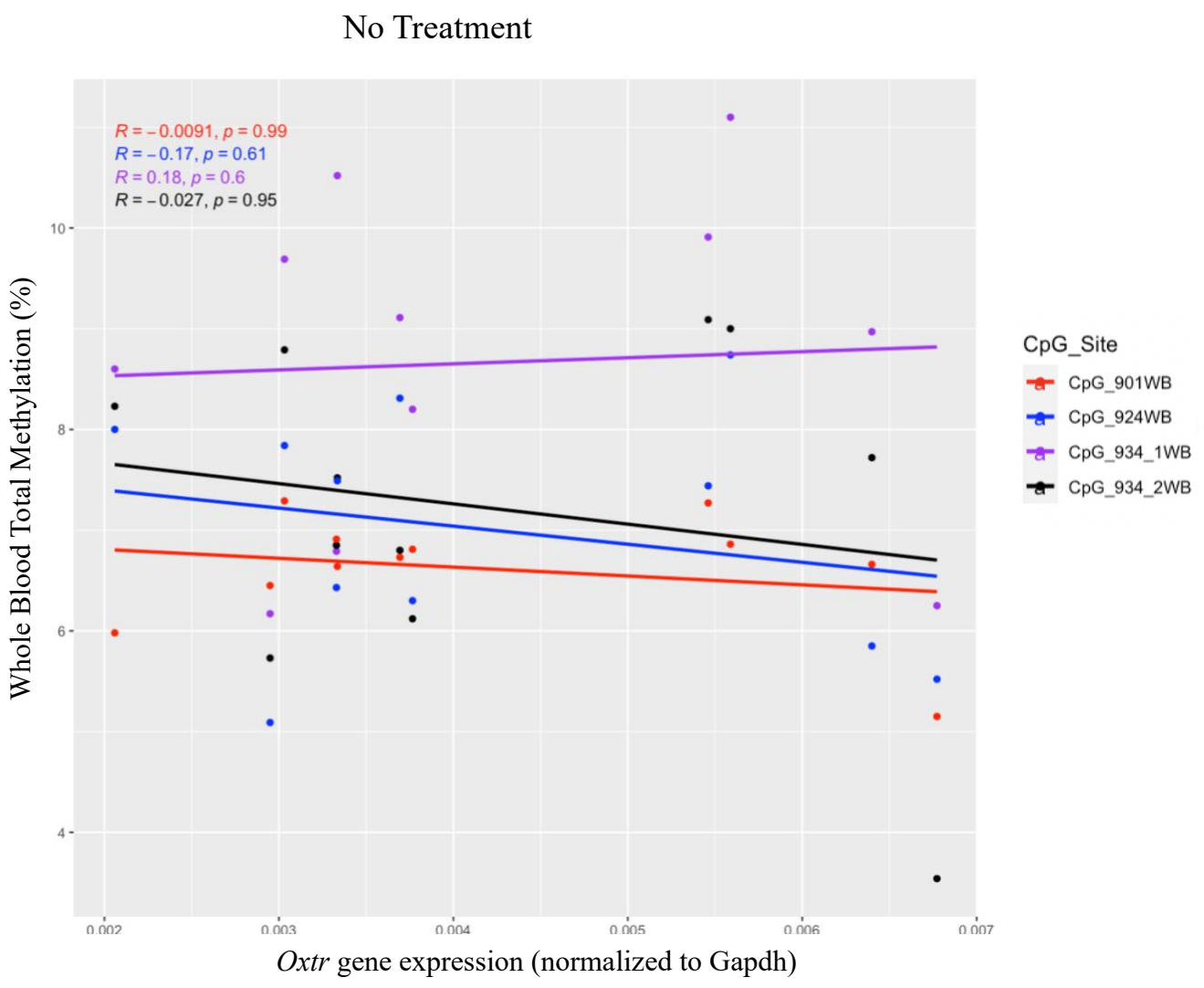
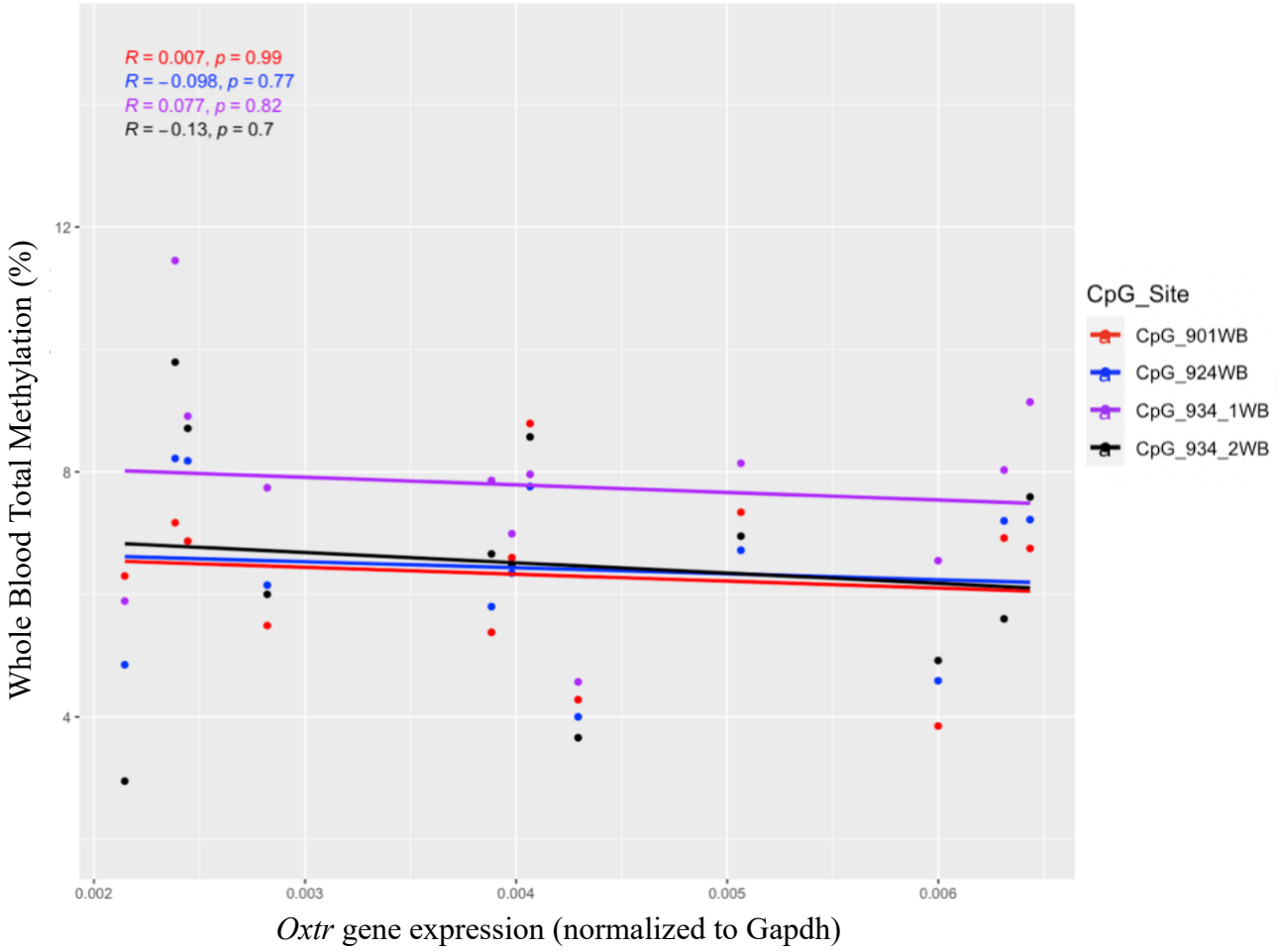

CpG Site -901
R = 0.11, p = 0.39
Whole Blood Total Methylation (%)
12
8
4
9
12
15
18
21
Nucleus Accumbens Total Methylation (%)

Figure 6. A significant negative correlation between *Oxtr* total DNA methylation in whole blood and *Oxtr* gene expression in the nucleus accumbens is found in the low-dose OXT group at CpG site -901 (see Figure 6C). Conversely, a trending positive relationship is observed in high-dose OXT group at CpG site -901 (see Figure 6E). No other significant relationships were observed across treatment groups (see Figure 5A, B, C, D and E). Spearman's correlation: * $p < 0.05$, + $p < 0.1$.

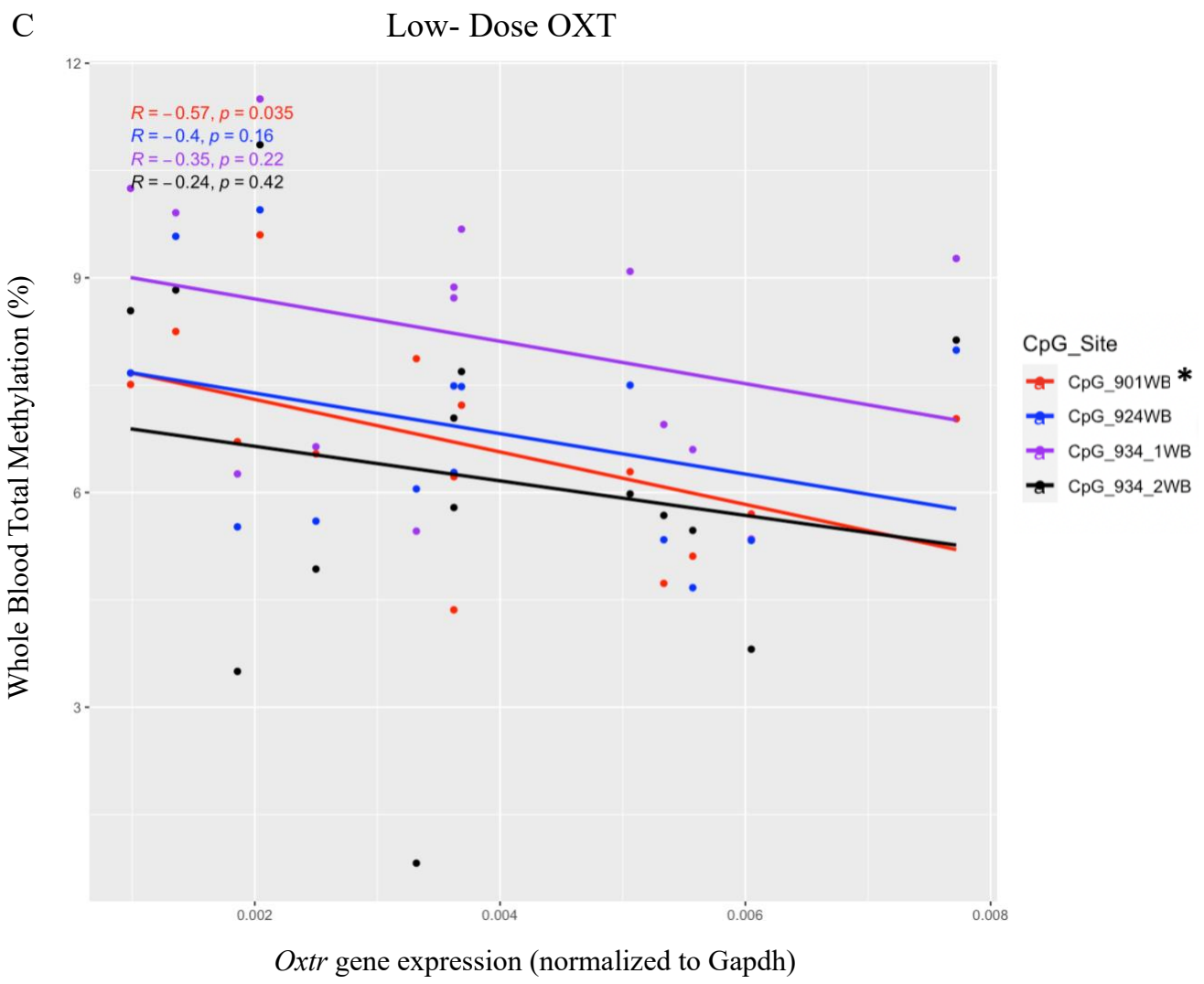


B

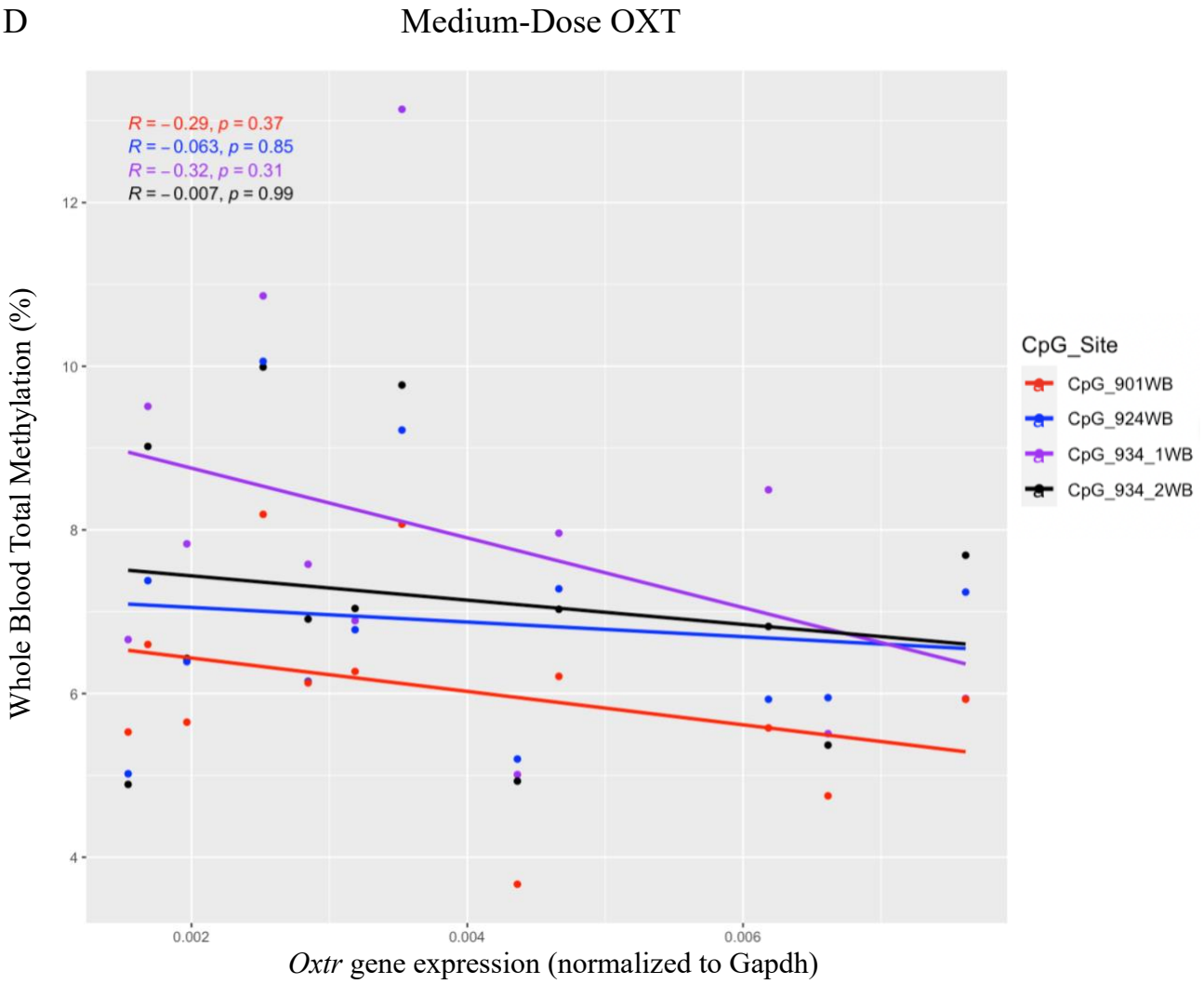
Saline Vehicle



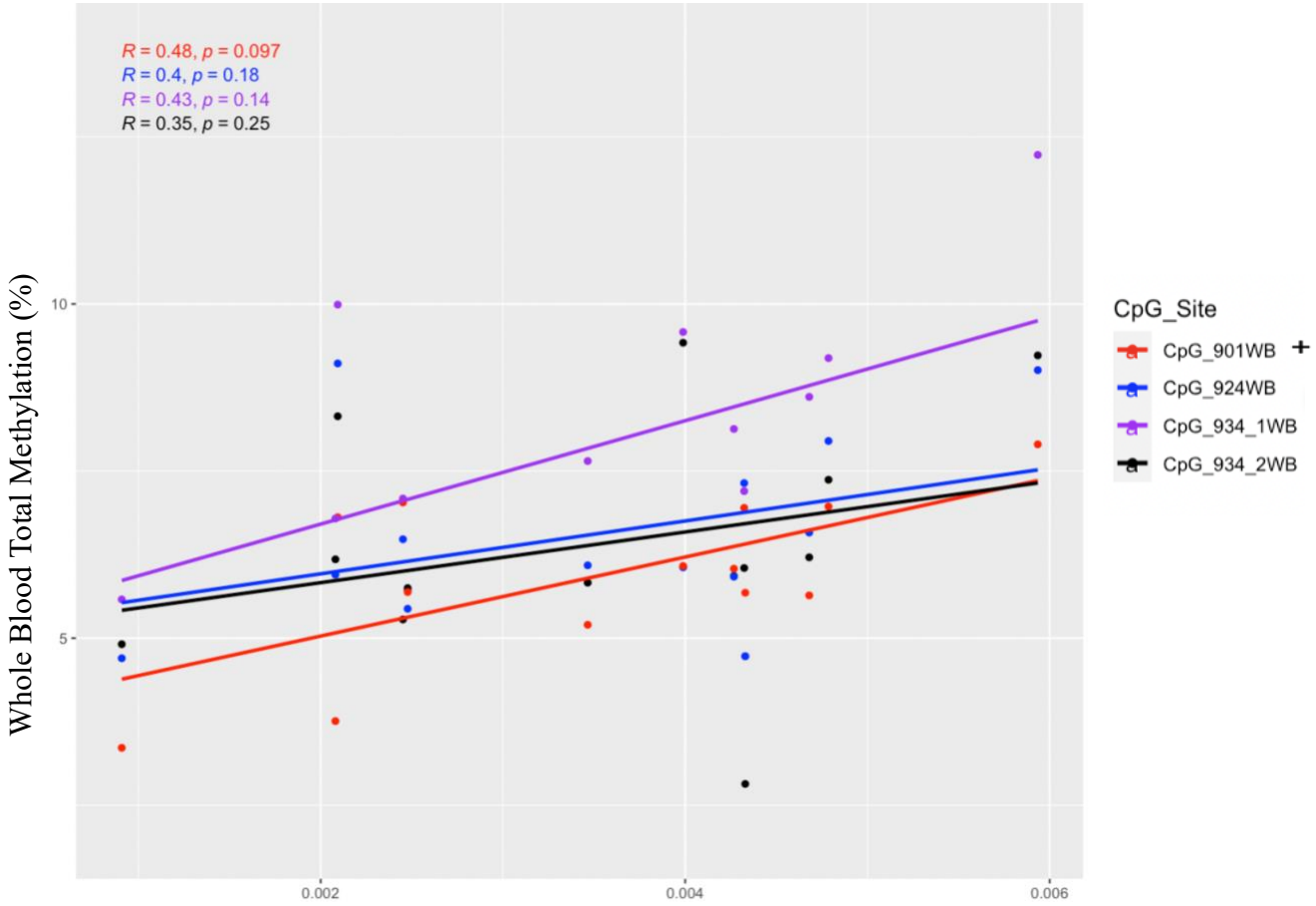
C


Low- Dose OXT
R = −0.57, p = 0.035
R = −0.4, p = 0.16
R = −0.35, p = 0.22
R = −0.24, p = 0.42
Whole Blood Total Methylation (%)
12
9
6
3
0.002
0.004
0.006
0.008
Oxtr gene expression (normalized to Gapdh)
CpG_Site
CpG_901WB *
CpG_924WB
CpG_934_1WB
CpG_934_2WB

D


Medium-Dose OXT
R = −0.29, p = 0.37
R = −0.063, p = 0.85
R = −0.32, p = 0.31
R = −0.007, p = 0.99
Whole Blood Total Methylation (%)
12
10
8
6
4
0.002
0.004
0.006
Oxtr gene expression (normalized to Gapdh)
CpG_Site
CpG_901WB
CpG_924WB
CpG_934_1WB
CpG_934_2WB

E High-Dose OXT



Oxtr gene expression (normalized to Gapdh)

Supplement

Figure S1. The oxytocin receptor gene (*OXTR*) is homologous in humans and in prairie voles with conserved CpG sites -934, -924, and -901.

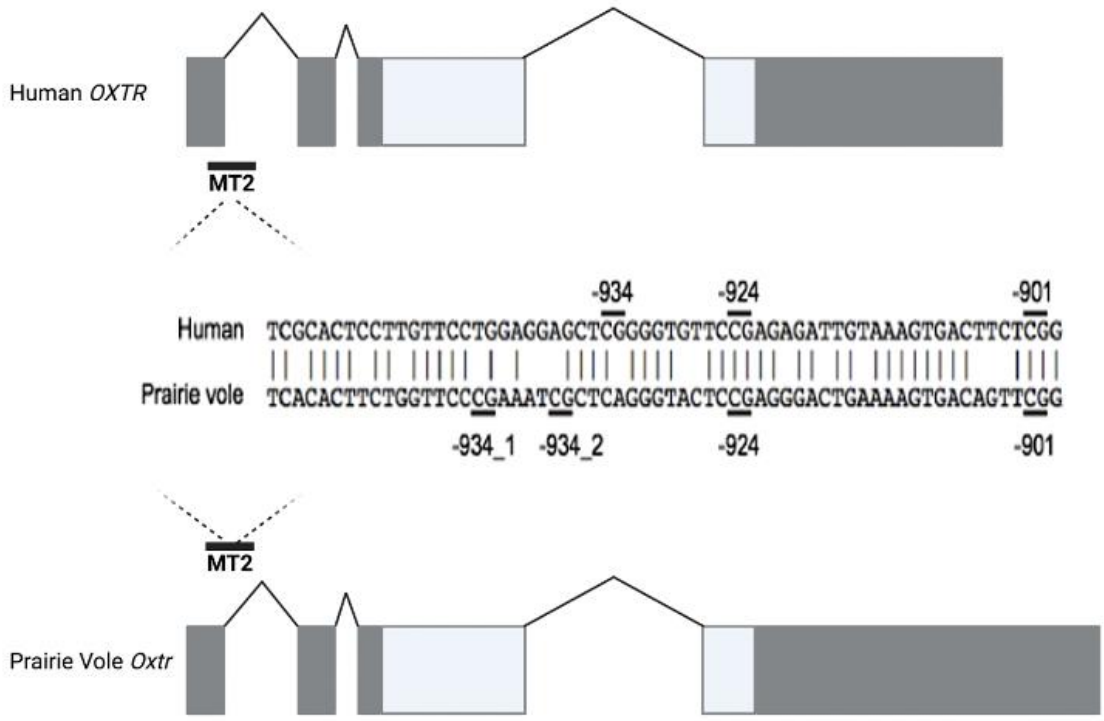


Figure S2. No significant differences are found in the methylation state of the four CpG sites (-901, -924, -934_1, -934_2) assessed in the nucleus accumbens 90 minutes after synthetic oxytocin administration, a saline vehicle, or no treatment.

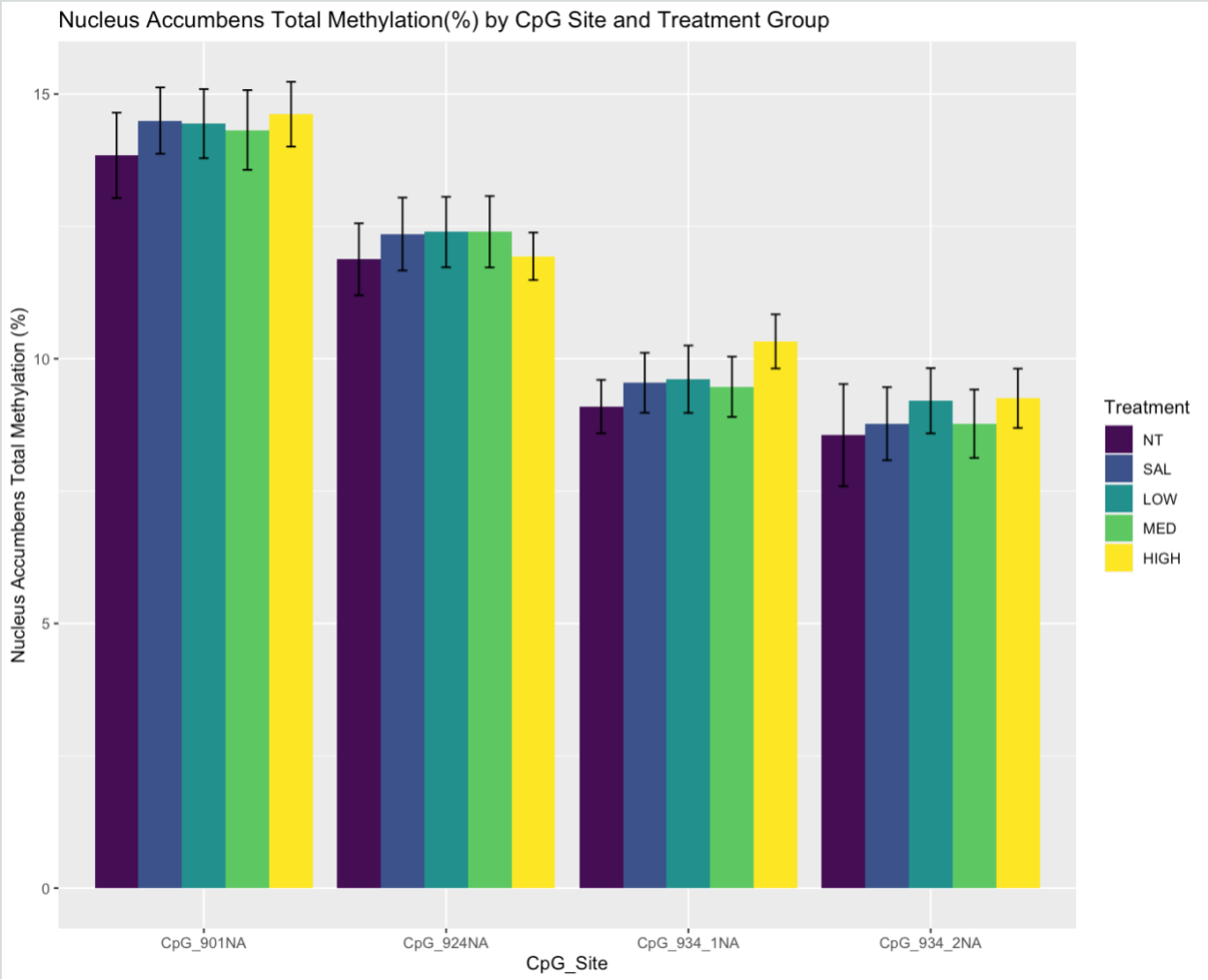


Figure S3. No significant differences are found in *Oxtr* gene expression in the nucleus accumbens 90 minutes after synthetic oxytocin administration, a saline vehicle, or no treatment.

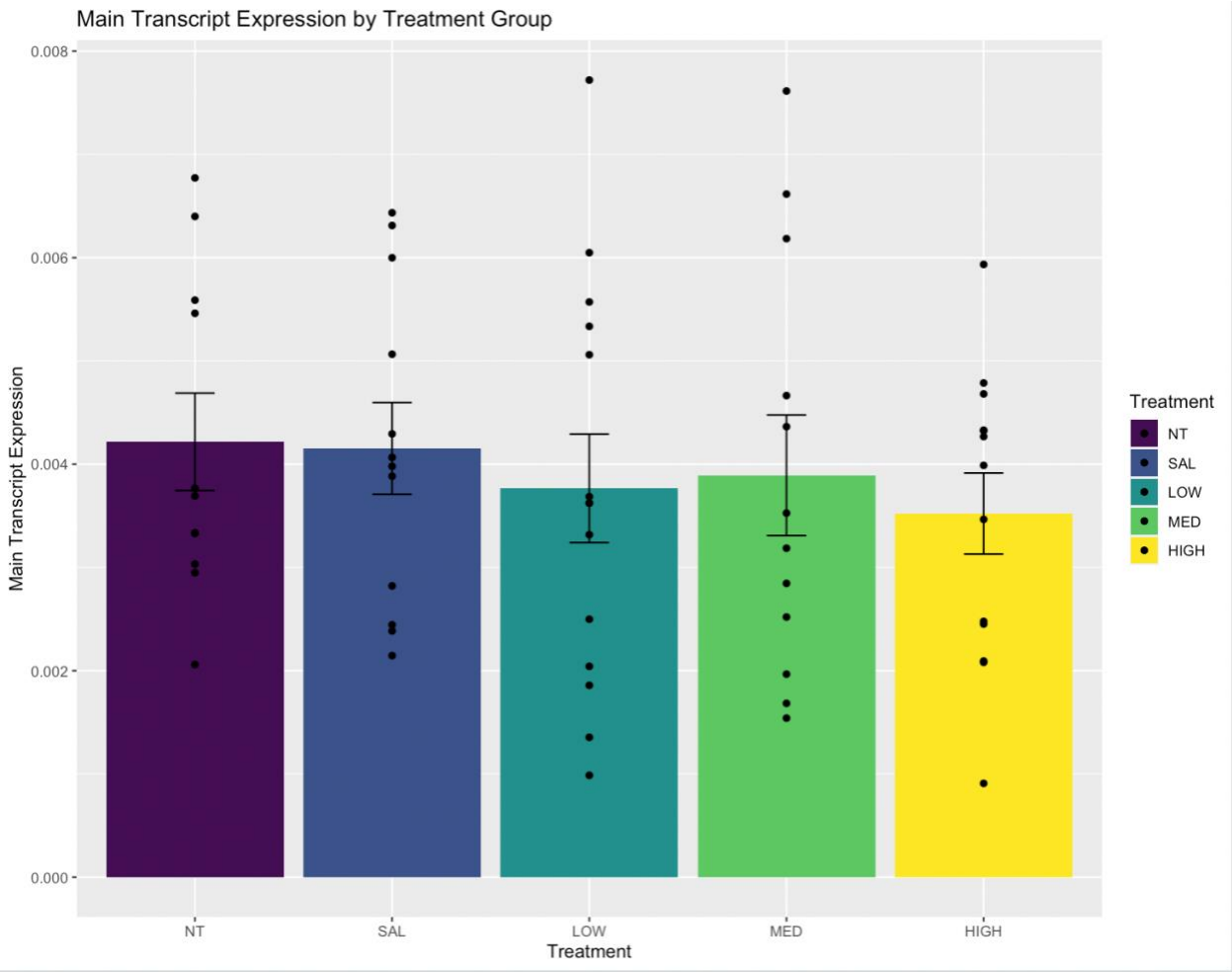
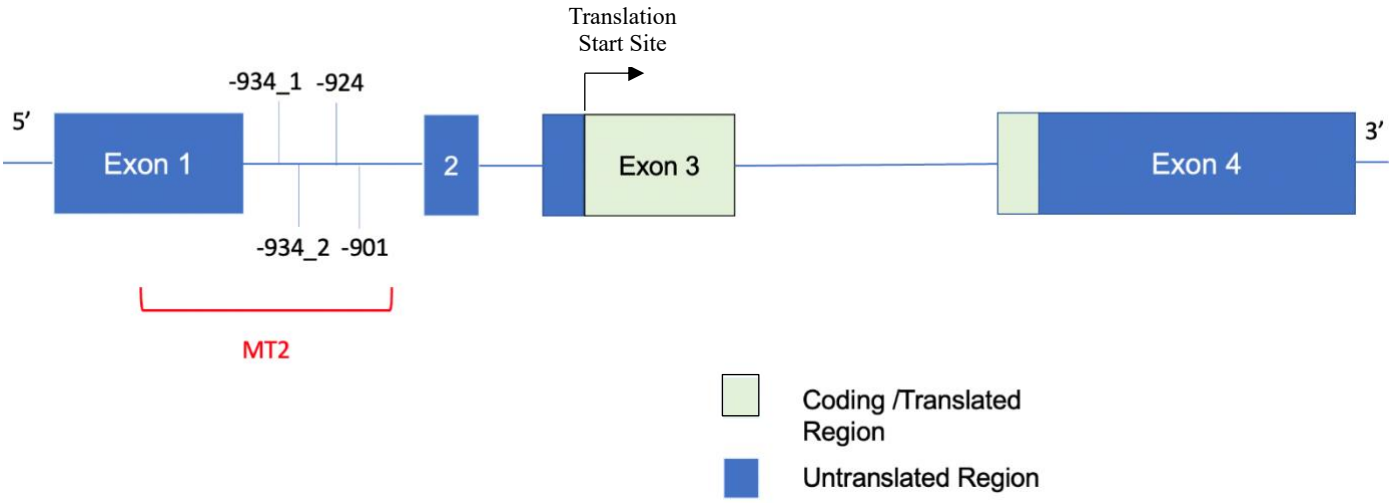


Figure S4. A schematic of the oxytocin receptor gene where the boxes denote the four exons, and the lines between exons represent the three intronic regions of the gene. Also included are homologous CpG-sites in the MT2 region of *Oxtr* and coding and noncoding regions of the gene.



References

Bell, A., Carter, S., Steer, C., Golding, J., Davis, J., Steffan, A., Rubin, L., Lillard, T., Gregory, S., Harris, J., & Connelly, J. (2015). Interactions between oxytocin receptor DNA methylation and genotype is associated with risk of postpartum depression in women without depression in pregnancy. *Frontiers in Genetics, 6*(243). https://doi.org/10.3389/fgene.2015.00243

Bleuler, M. (1963). Conception of Schizophrenia within the last fifty years and today [Abridged]. *Proceedings of the Royal Society of Medicine, 56*(10), 945–952.

Branco, M., Ficz, G., & Reik, W. (2011). Uncovering the role of 5-hydroxymethylcytosine in the epigenome. *Nature Reviews Genetics, 13,* 7-13. https://doi.org/10.1038/nrg3080

Carter, S., Witt, D., Schneider, J., Harris, Z., & Volkening, D. (1987). Male stimuli are necessary for female sexual behavior and uterine growth in prairie voles (Microtus ochrogaster). *Hormones and Behavior, 21(*1), 74-82. https://doi.org/10.1016/0018-506X(87)90032-8

Costa, B., Pini, S., Gabelloni, P., Abelli, M., Lari, L., Cardini, A., Muti, M., Gesi, C., Landi, S., Galderisi, S., Mucci, A., Lucacchini, A., Cassano, G., & Martini, C. (2009). Oxytocin receptor polymorphisms and adult attachment style in patients with depression. *Psychoneuroendocrinology, 34*(10), 1506-1514. https://doi.org/10.1016/j.psyneuen.2009.05.006

Daly D, Minnie K, Blignaut A, Blix, E., Nilsen, A., Dencker, A., Beeckman. K., Gross, M., Pehlke-Milde, J., Grylka-Baeschlin, S., Koenig-Bachmann, M., Clausen, J., Hadjigeorgiou, E., Morano, S., Iannuzzi, L., Baranowska, B., Kiersnowska, I., & Uvnas-Moberg, K. (2020). How much synthetic oxytocin is infused during labour? A review and

analysis of regimens used in 12 countries. *PLOS ONE,* 15(7). https://doi.org/10.1371/journal.pone.0227941

Danoff, J., Wroblewski, K., Graves, A., Quinn, G., Perkeybile, A., Kenkel, W., Lillard, T., Parikh, H., Golino, H., Gregory, S., Carter, S., Bales, K., & Connelly, J. (2021). Genetic, epigenetic, and environmental factors controlling oxytocin receptor gene expression. *Clinical Epigenetics, 23.* https://doi.org/10.1186/s13148-021-01017-5

Erickson, E., Myatt, L., Danoff, J., Krol, K., & Connelly, J. (2023). Oxytocin receptor DNA methylation is associated with exogenous oxytocin needs during parturition and postpartum hemorrhage. *Communications Medicine, 3*(11). https://doi.org/10.1038/s43856-023-00244-6

Food and Drug Administration. (2014). Pitocin. Accessed September 17, 2022. https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/018261s031lbl.pdf

Gregory, S., Connelly, J., Towers, A., Johnson, J., Biscocho, D., Markunas, C., Lintas, C., Abramson, R., Wright, H., Ellis, P., Langford, C., Worley, G., Delong, G., Murphy, S., Cuccaro, M., Perisco, A., & Pericak-Vance, M. (2009). Genomic and epigenetic evidence for oxytocin receptor deficiency in autism. *BMC Medicine, 7*(62). https://doi.org/10.1186/1741-7015-7-62

Gu, V., Feeley, N., Gold, I., Hayton, B., Robins, S., Mackinnon, A., Samuel, S., Carter, S., & Zelkowitz, P. (2016). Intrapartum synthetic oxytocin and its effects on maternal well-being at 2 months postpartum. *Birth, 43*(1), 28-35. https://doi.org/10.1111/birt.12198

Grotegut, C., Lewis, L., Manuck, T., Allen, T., James, A., Seco, A., Deneux-Tharaux, C. (2017). The oxytocin product correlates with total oxytocin received during labor: A research

methods study. *American Journal of Perinatology, 35*(1), 78-83. https://doi.org/10.1055/s-0037-1606119

Jack, A., Connelly, J., & Morris, J. (2012). DNA methylation of the oxytocin receptor gene predicts neural response to ambiguous social stimuli. *Frontiers in Human Neuroscience, 6*(280). https://doi.org/110.3389/fnhum.2012.00280

Keebaugh, A., & Young, L. (2011). Increasing oxytocin receptor expression in the nucleus accumbens of pre-pubertal female prairie voles enhances alloparental responsiveness and partner preference formation as adults. *Hormones and Behavior*, *60*(5), 498–504.

Kenkel, W., Ortiz, R., Yee, J., Perkeybile, A., Kulkarni, P., Carter, S., Cushing, B., & Ferris, C. (2023). Neuroanatomical and functional consequences of oxytocin treatment at birth in prairie voles. *Psychoneuroendocrinology, 150*. https://doi.org/10.1016/j.psyneuen.2023.106025

Kenkel, W., Perkeybile, A., Yee, J., Pournajafi-Nazarloo, H., Lillard, T., Ferguson, E., Wroblewski, K., Ferris, C., Carter, S., & Connelly, J. (2019). Behavioral and epigenetic consequences of oxytocin treatment at birth. *Science Advances, 5*(5). https://doi.org/10.1126/sciadv.aav2244

Kimmel, M., Clive, M., Gispen, F., Guintivano, J., Brown, T., Cox, O., Beckmann, M., Kornhuber, J., Fasching, P., Osbourne, L., Binder, E., Payne, J., & Kaminsky, Z. (2016). Oxytocin receptor methylation in postpartum depression. *Psychoneuroendocrinology, 69,* 150-160. https://doi.org/10.1016/j.psyneuen.2016.04.008

King, L., Robins, S., Chen, G., Yerko, V., Zhou, Y., Nagy, C., Feely, N., Gold, I., Hayton, B., Turecki, G., & Zelkowitz, P. (2017). Perinatal depression and DNA methylation of

oxytocin-related genes: A study of mothers and their children. *Hormones and Behavior, 96,* 84-94. https://doi.org/10.1016/j.yhbeh.2017.09.006

Kroll-Desrosiers, A., Nephew, B., Babb, J., Guilarte-Walker, Y., Simas, T., & Deligaiannidis, K. (2017). Association of peripartum synthetic oxytocin administration and depressive and anxiety disorders within the first postpartum year. *Depress Anxiety, 34*(2), 137-146. https://doi.org/10.1002%2Fda.22599

Kusui, C, Kimura, T, Ogita, K, Nakamura, H., Matsumura, Y., Koyama, M., Azuma, C., & Murata, Y. (2001). DNA methylation of the human oxytocin receptor gene promoter regulates tissue-specific gene expression. *Biochemical and Biophysical Research Communications, 289*(3), 681-686. https://doi.org/10.1006/bbrc.2001.6024

Liu, C., Jiao, C., Wang, K., & Yuan, N. (2018). DNA methylation and psychiatric disorders. *Progress in Molecular Biology and Translational Science, 157*, 175-232. https://doi.org/10.1016/bs.pmbts.2018.01.006

McCullough, M., Churchland, P., & Mendez, A. (2013). Problems with measuring peripheral oxytocin: Can the data on oxytocin and human behavior be trusted? *Neuroscience and Biobehavioral Reviews, 37,* 1485-1492. http://dx.doi.org/10.1016/j.neubiorev.2013.04.018

Olazabal, D., & Young, L. (2006). Oxytocin receptors in the nucleus accumbens facilitate "spontaneous" maternal behavior in adult female prairie voles. *Neuroscience, 141*(2), 559-568. https://doi.org/10.1016/j.neuroscience.2006.04.017

Osterman, M., Hamilton, B., Martin. J., Discroll, A., & Valenzuela, C. (2023). Births: Final data for 2021. *National Vital Statistics Reports.* Retrieved from https://www.cdc.gov/nchs/data/nvsr/nvsr72/nvsr72-01.pdf

Perkeybile, A., Carter, S., Wroblewski, K., Puglia, M., Kenkel, W., Lillard, T., Karaoli, T., Gregory, S., Mohammadi, N., Epstein, L., Bales, K., & Connelly, J. (2019). Early nurture epigenetically tunes the oxytocin receptor. *Psychoneuroendocrinology, 99,* 128-136. https://doi.org/10.1016/j.psyneuen.2018.08.037

Perkeybile, A., Kenkel, W., Yee, J., Lillard, T., Ferris, C., Carter, S., & Connelly, J. Pregnancy and birth epigenetically shape the maternal oxytocin receptor. *(In prep).*

Phaneuf, S., Rodríguez Liñares, B., TambyRaja, R., MacKenzie, I., & Bernal, A. (2000). Loss of myometrial oxytocin receptors during oxytocin-induced and oxytocin-augmented labour. *Journal of Reproduction and Fertility,* 120(1), 91-97. https://doi.org/10.1530/jrf.0.1200091

Puglia, M., Lillard, T., Morris, J., & Connelly, J. (2015). Epigenetic modification of the oxytocin gene receptor influences the perception of anger and fear in the human brain. *PNAS, 112*(11), 3308-3313.

Richa, R., & Sinha, R. (2014). Hydroxymethylation of DNA: An epigenetic marker. *EXLI Journal, 13,* 592-610. https://pubmed.ncbi.nlm.nih.gov/26417286

Simpson, K. (2022). Trends in labor induction in the United States, 1989 to 2020. *The American Journal of Maternal Child Nursing, 47*(4), 235. https://doi.org/10.1097/NMC.0000000000000824

Thul, T., Corwin, E., Carlson, N., Brennan P., & Young, L. (2020). Oxytocin and postpartum depression: A systematic review. *Psychoneuroendocrinology, 120.* https://doi.org/10.1016/j.psyneuen.2020.104793

Tichelman, E., Warmink-Perdijk, W., Henrichs, J., Peters, L., Schellevis, F., Berger, M., & Burger, H. (2021). Intrapartum synthetic oxytocin, behavioral and emotional problems in

children, and the role of postnatal depressive symptoms, postnatal anxiety and mother-to infant bonding: A Dutch prospective cohort study. *Midwifery, 100.* https://doi.org/10.1016/j.midw.2021.103045

Toepfer, P., O'Donnell, K., Entringer, S., Garg, E., Heim, C., Lin, D., MacIsaac, J., Kobor, M., Meaney, M., Provencal, N., Binder, E., Wadhwa, P., & Buss, C. (2019). Dynamic DNA methylation changes in maternal oxytocin gene locus (OXT) during pregnancy predict postpartum maternal intrusiveness. *Psychoneuroendocrinology, 103,* 156-162. https://doi.org/10.1016/j.psyneuen.2019.01.013

Trost, S., Beauregard, J., Chandra, G., Njie, F., Berry, J., Harvey, A., & Goodman, D. (2022). Pregnancy-related deaths: Data from maternal mortality review committees in 36 US States, 2017-2019. *Centers for Disease Control and Prevention, US Department of Health and Human Services.* Retrieved from https://www.cdc.gov/reproductivehealth/maternal-mortality/erase-mm/data-mmrc.html

Turnpin, M., & Salbert, G. (2022). 5-methylcytosine turnover: Mechanisms and therapeutic implications in cancer. *Frontiers in Molecular Biosciences, 9.* https://doi.org/10.3389/fmolb.2022.976862

World Health Organization. (2012). WHO recommendations for the prevention and treatment of postpartum hemorrhage. Accessed September 17, 2022. https://apps.who.int/iris/bitstream/handle/10665/75411/97?sequence=1

Zhang, J., Branch, D., Ramirez, M., Laughon, S., Reddy, U., Hoffman, M., Bailit, J., Kominiarek, M., & Chen, Z. (2011). Oxytocin regimens or labor augmentation, labor progression, perinatal outcomes. *Obstetrics and Gynecology, 118*(201), 249-256. https://doi.org/10.1097%2FAOG.0b013e3182220192

CHAPTER FIVE: DISCUSSION

Synthetic oxytocin is one of the most widely used medications during parturition; however, since its introduction in the 1950s, there has been little agreement regarding an optimal regimen for synthetic oxytocin administration. Guidelines for use of synthetic oxytocin vary with continued debate regarding the benefits and risks of high-dose and low-dose oxytocin regimens during labor induction and augmentation (Daly et al., 2020). Per Daly and colleagues (2020), recommendations are to use the lowest effective dose during parturition to achieve the desired outcome and to mitigate the risk of adverse effects.

Short-term reproductive risks are often associated with high doses of synthetic oxytocin including uterine hypertonicity, fetal distress, uterine atony, and an increased risk for invasive procedures such as cesarean deliveries (Food and Drug Administration [FDA], 2014). In addition, evidence suggests maternal exposure to synthetic oxytocin during parturition may be associated adverse maternal psychological outcomes such as postpartum depression (PPD) (Gu et al., 2016; Kroll-Desrosiers et al., 2017; Tichelman et al., 2021). Specifically, maternal exposure to synthetic oxytocin has been found to increase a woman's risk of developing PPD or being prescribed an antidepressant or anxiolytic medication within the first year after delivery (Kroll-Desrosiers et al., 2017).

PPD is one of the most common complications of pregnancy and childbearing and can lead to poor maternal health habits, decreased breastfeeding initiation, poor maternal-infant bonding, decreased parental safety practices, and increased maternal suicidal ideation (Gavin et al., 2005; Kingston et al., 2012; Szegda et al., 2014). In the era of evidenced-based and personalized medicine, it is important to assess the dose-dependent effects of synthetic oxytocin on maternal psychological outcomes.

The goal of the present study was to examine associations between maternal exposure to synthetic oxytocin during parturition and outcomes of *Oxtr* DNA methylation and *Oxtr* gene expression in the maternal brain using the prairie vole as a model organism. We further explored the relationship between *Oxtr* DNA methylation and *Oxtr* gene expression across multiple doses of synthetic oxytocin to identify maternal neurobiological effects based on the amount of oxytocin administered. The study consisted of 75 term pregnant female prairie voles divided into five treatment groups (low-dose oxytocin (OXT), medium-dose OXT, high-dose OXT, saline vehicle, and no treatment) and examined tissues including the nucleus accumbens and whole blood for all subjects. Homologous CpG sites between humans and prairie voles were assessed including CpG sites -934_1, -934_2, -924, -901, for future translation of this work to human participants. Correlations between the methylation state in the nucleus accumbens and whole blood were also examined to demonstrate the potential utility of whole blood to serve as a proxy for understanding central neurobiological changes in human mothers.

The specific aims of the study were as follows:

(1) Examine the relationship between synthetic oxytocin dose (low-dose OXT, medium-dose OXT, high-dose OXT, saline vehicle, and no treatment), DNA methylation in the 3' MT2 region of *Oxtr* (CpG sites -934_1, -934_2, -924, -901) and *Oxtr* gene expression in the nucleus accumbens of term pregnant female prairie voles

(2) Analyze correlations between *Oxtr* DNA methylation in the nucleus accumbens and in whole blood of term pregnant prairie voles.

This chapter provides a synopsis of the findings in the dissertation study. Results will be discussed by specific aim and will be followed by a discussion of the strengths and limitations of this research. Further discussion will include the clinical implications of this research, translational considerations with use of the prairie vole labor induction model, and recommendations for future study.

Summary of Specific Aim 1

The purpose of aim 1 was to examine the relationship between synthetic oxytocin dose (low-dose OXT, medium-dose OXT, high-dose OXT, saline vehicle, and no treatment), DNA methylation in the 3' MT2 region of *Oxtr* (CpG sites -934_1, -934_2, -924, -901) and *Oxtr* gene expression in the nucleus accumbens of term pregnant female prairie voles. No significant treatment group differences were found regarding average *Oxtr* DNA methylation or average *Oxtr* gene expression in the nucleus accumbens 90 minutes post-injection with synthetic oxytocin, saline, or no treatment. However, a Spearman correlation coefficient analysis revealed differences in the relationship between *Oxtr* DNA methylation and *Oxtr* gene expression in the nucleus accumbens 90 minutes after treatment with synthetic oxytocin.

As consistent with previous data in the lab revealing no relationship between methylation and gene expression in term pregnant females receiving no treatment, the present study also demonstrated no significant relationships between *Oxtr* total DNA methylation and *Oxtr* gene expression in the nucleus accumbens at CpG sites -934_1, -934_2, and -924 in the no treatment group. A trending negative relationship was observed at CpG site -901 in the no treatment group which may indicate a natural shift toward the negative relationship observed in the maternal postpartum brain (Perkeybile et al., *in prep*). In the present study, the saline vehicle group also demonstrated no significant relationships at any of the four examined CpG sites.

Across the oxytocin-treated groups, we observed a shift toward a positive relationship in the high-dose OXT group where higher levels of DNA methylation showed a trend toward higher levels of *Oxtr* gene expression in the nucleus accumbens. This was an interesting finding as previous evidence in human studies demonstrates higher levels of DNA methylation and downregulation of *OXTR* gene expression in the maternal uterus when mothers were exposed to higher doses of synthetic oxytocin during parturition (Erickson et al., 2023; Phaneuf et al., 2000). Upon further delineation of true methylation (5mC) and hydroxymethylation (5hmC) in the high-dose OXT group, we found a significant positive relationship between 5hmC and *Oxtr* gene expression such that higher 5hmC was associated with higher *Oxtr* gene expression in the nucleus accumbens of term pregnant female prairie voles 90 minutes post-injection. A significant positive relationship was specifically observed at CpG site -901 demonstrating an important site for further study.

Summary of Specific Aim 2

The purpose of aim 2 was to analyze correlations between *Oxtr* total DNA methylation at CpG sites -934_1, -934_2, -924, -901 in the nucleus accumbens and in whole blood of term pregnant female prairie voles 90 minutes post-injection with a dose of synthetic oxytocin, a saline vehicle, or no treatment. In the initial assessment, a Spearman correlation coefficient analysis revealed a significant positive correlation between *Oxtr* DNA methylation in the nucleus accumbens and whole blood at three CpG sites (-934_1, -934_2, and -924); however, we did not observe a significant relationship at CpG site -901.

Interestingly, upon further analysis, we found a significant negative relationship between *Oxtr* total DNA methylation at CpG site -901 in whole blood and *Oxtr* gene expression in the nucleus accumbens for females provided a low dose of synthetic oxytocin. Conversely, a

trending positive relationship was observed at the same CpG site in females provided a high dose of synthetic oxytocin. It is possible that the significant negative relationship observed in the low-dose OXT group indicates an early shift toward the negative relationship observed in the maternal postpartum brain (Perkeybile et al., *in prep*). In the high dose OXT group, however, a positive relationship at the 90-minute time point may mean that a shift toward the maternal postpartum state is prolonged.

Importantly, methylation and hydroxymethylation are cell-type specific and can reveal unique relationships across tissue types. Although we found a significant correlation between DNA methylation in the nucleus accumbens and whole blood at CpG sites -934_1, -934_2 and -924, total DNA methylation in whole blood may not reflect the same relationships with *Oxtr* gene expression as total DNA methylation in the nucleus accumbens. Future studies are needed to differentiate 5mC and 5hmC in whole blood to further assess dose-dependent effects of synthetic oxytocin on the relationship between DNA methylation in whole blood and *Oxtr* gene expression in central tissues such as the nucleus accumbens.

Strengths and Limitations

This study had two important limitations. Seven samples had to be excluded from the RNA expression analysis due to low RNA yield during isolation. The interpretation of results in epigenetic studies can be limited by small sample sizes and often requires larger studies to confirm associations (Brenton et al., 2017; Carnevali & Buoli, 2021). Though this study revealed interesting findings, we recommend the use of larger studies to replicate these findings and identify additional variations in dose-dependent outcomes.

For the second limitation, we found five samples with a unique variation in their genetic sequence which altered their level of *Oxtr* total DNA methylation and *Oxtr* gene expression

across CpG sites and treatment groups. In the present study, these samples were excluded; however, we acknowledge that there may be important genetic by epigenetic interactions that underpin the relationship between maternal exposure to synthetic oxytocin during parturition and maternal neurobiological outcomes. Future studies assessing the effects of synthetic oxytocin in mothers should consider the likely effect of genetic underpinnings and utilize larger datasets to examine genetic by epigenetic interactions.

A major strength of this study was the use of a prairie vole labor induction model to examine the effects of synthetic oxytocin administration during parturition in the maternal brain. Previous studies using a time mating paradigm and prairie vole labor induction model have identified physiological and neurobiological effects of synthetic oxytocin in offspring; however, to our knowledge, this is the first study to assess neurobiological effects in mothers considering mechanisms of methylation and hydroxymethylation (Kenkel et al., 2019; Kenkel et al., 2023). We further examined dose-dependent effects of synthetic oxytocin in both central and peripheral tissues which has been an important gap in the literature. This work provides a foundation for future research examining dose-dependent effects of synthetic oxytocin on outcomes including maternal neurobiology and subsequent maternal postpartum behaviors.

The Prairie Vole Model and Clinical Translation

Using the prairie vole as a model organism provides a beneficial method to examine clinically derived questions for future translational research. The prairie vole has been widely studied in various contexts to assess environmental effects on parental and social behaviors (Danoff et al., 2021, Perkeybile et al., 2019, Tabbaa et al., 2017, Young et al., 2011). In recent years, the prairie vole has been further used to examine the effects of synthetic oxytocin administration in term pregnant females on offspring physiology, neurodevelopment, and long-

term social behaviors (Kenkel et al., 2019; Kenkel et al., 2023). Findings from these studies have provided a foundation for future translational research in the clinical setting.

Regarding the maternal effects of synthetic oxytocin administration during parturition, previous studies involving human participants have identified adverse reproductive and psychological risks including higher rates of postpartum hemorrhage and maternal PPD (Erickson et al., 2023; Gu et al., 2016; Kroll-Desrosiers et al., 2017; Tichelman et al., 2021). Using the prairie vole as a model system, the present dissertation study examined maternal neurobiological outcomes using five treatment groups to mimic the doses of synthetic oxytocin often provided in hospital settings. The doses of synthetic oxytocin were determined based on previous animal studies involving the prairie vole labor induction model and clinical data reporting average amounts of synthetic oxytocin provided during labor induction (Kenkel et al., 2019; Kenkel et al., 2023; Daly et al., 2020). As an example, the medium-dose OXT group was administered 0.25mg/kg of synthetic oxytocin which is equivalent to 5 IU or an average dose for labor induction used across hospital systems (Daly et al., 2020; Grotegut et al., 2017; Uvanas-Moberg et al., 2019; Zhang et al., 2011).

There are important factors researchers are recommended to consider when using the prairie vole labor induction model in translational research. In human mothers, moderate to high doses of synthetic oxytocin would be expected to induce labor; however, all comparative doses are beneath the threshold for labor induction in the prairie vole. There are two possible reasons for this outcome. First, prairie voles have a relatively high plasma oxytocin level and likely require higher doses of synthetic oxytocin for labor onset (Kramer et al., 2009). Secondly, the use of a single intraperitoneal (IP) injection, rather than the continuous intravenous (IV) infusion commonly used in the clinical setting, may further contribute to variations in labor induction

outcomes. While medications delivered IV are not limited by mechanisms of metabolism and absorption, those delivered IP will often undergo first pass metabolism leading to lower amounts of the drug in the systemic vasculature (Lukas et al., 1971; Shoyaib et al., 2019). This can present a limitation for studying the clinical impact of synthetic oxytocin in prairie voles as they do not have a readily accessible vein for IV administration. Conversely, the IP route provides important benefits in animal studies including a less stressful environment, higher bioavailability, and rapid absorption of molecules compared to other routes of administration such as oral or subcutaneous methods (Shoyaib et al., 2019).

Subsequent translational research should explore common findings between studies using the prairie vole model and those involving human participants. Studies examining clinical outcomes in human mothers have focused on epigenetic changes in the maternal uterus and have found a downregulating impact of high doses of synthetic oxytocin on *OXTR* (Erickson et al., 2023; Phaneuf et al., 2000; Robinson et al., 2003). The prairie vole labor induction model provides an opportunity to assess outcomes across multiple tissue types, including the maternal uterus, to compare findings to studies involving human mothers. In addition, the examination of *OXTR* DNA methylation in whole blood provides another useful and accessible tissue type for translational research in prairie voles and humans.

Nursing Implications

There are important implications of this research in clinical practice with a particular impact on nursing care. Per Simpson (2022), increasing trends of labor induction in the United States require an increase in nurse staffing to provide safe and effective care for laboring women. In 2007, synthetic oxytocin was labeled a high-alert medication due to maternal and fetal health risks and a lack of standardization of oxytocin regimens (Simpson & Knox, 2009). Specifically,

for labor augmentation mechanisms, the World Health Organization (WHO) (2014) provides guiding principles to maintain ethical and safety standards of practice. Among these principles, the WHO recommends that women undergoing labor augmentation with synthetic oxytocin should not be left unattended. Further, it is recommended that labor augmentation only occur in settings with the capacity to manage adverse maternal and fetal health outcomes (World Health Organization [WHO], 2014).

Nurses have an important role in managing the delivery of synthetic oxytocin at the bedside. Errors in medication management can occur due to delays in responding to oxytocin-related complications (e.g., uterine spasms, tetanic contractions, hypertonicity); thus, nurses must maintain patient safety and observe for signs of medication overuse. In addition to maternal reproductive risks, studies highlight potential adverse maternal psychological risks associated with exposure to synthetic oxytocin during parturition (Gu et al., 2016; Kroll-Desrosiers et al., 2017; Tichelman et al., 2021). Clinicians and nurses should be aware of the potential risks and provide evidenced-based education to patients.

Regarding maternal PPD, recommendations are to screen for depressive symptoms at least once during the perinatal period using a standardized and validated screening tool (American College of Obstetricians and Gynecologists [ACOG], 2015). Many obstetrician-gynecologists and nurse midwives will conduct an initial evaluation for symptoms of maternal PPD at four-to-six-weeks post-delivery and are recommended to discuss appropriate treatment options if necessary (ACOG, 2015). Evidence suggests mothers who are exposed to synthetic oxytocin during parturition may be particularly susceptible to outcomes of PPD, so it is important for providers to assess for symptoms of depression particularly in this patient population (Gu et al., 2016). Additional research is needed to understand how doses of synthetic

oxytocin might differentially impact the mental health of mothers. The present dissertation study provides a basis to expand this knowledge in future studies.

Future Research Directions

The findings in this study reveal several avenues for continued research. In the present study, we assessed epigenetic regulatory factors in the maternal brain ninety minutes post-injection with a dose of synthetic oxytocin, a saline vehicle, or no treatment. This timepoint was previously assessed in the prairie vole labor induction model described in Kenkel et al., 2019 and was chosen for the current study. At this ninety-minute timepoint, we observed dose-dependent differences, specifically at CpG site -901, in the nucleus accumbens and in whole blood. It is possible, however, that we may have observed an impact of methylation and hydroxymethylation at other CpG sites if tissues were examined at multiple timepoints post-injection. Future studies should consider the potential time-dependent effects of synthetic oxytocin on epigenetic regulation of the *Oxtr* gene in the maternal brain. In addition, differentiating DNA methylation and hydroxymethylation in whole blood may provide additional insight regarding the utility of whole blood as a proxy for examining epigenetic changes in central tissues.

The present study did not assess outcomes of maternal behavior, and this would be an appropriate next step in understanding long-term implications of synthetic oxytocin administration. Importantly, the prairie vole model included subclinical doses of oxytocin that were not intended to induce labor. It will be important to assess variations in maternal behavior involving both subclinical and clinical doses of synthetic oxytocin. Additional studies examining maternal behaviors using the prairie vole model can be useful to provide a foundation for future translational research involving human mothers.

During the prairie vole labor induction procedures, we collected various maternal and fetal tissues that may also be useful for understanding maternal reproductive and fetal neurobiological outcomes. A complementary theoretical framework for this study is the fetal programming theory which suggests that stressful exposures incurred during pregnancy may pose an increased risk for poor fetal development and subsequent adverse health outcomes. Studies have assessed the impact of maternally administered synthetic oxytocin on offspring physiological and neurobiological outcomes and reported a positive relationship between *Oxtr* DNA methylation and *Oxtr* gene expression particularly in heavier offspring (Kenkel et al., 2019; Kenkel et al., 2023). It would be interesting to further assess mechanisms of hydroxymethylation in the offspring as methylation and demethylation processes are important for neuroplasticity across development (Richa & Sinha, 2014).

As previously noted, we found a few female subjects with a unique genetic sequence that impacted outcomes of DNA methylation and *Oxtr* gene expression. Future studies should involve larger sample sizes to account for genetic differences and to assess genetic by epigenetic interactions. In addition, future research should consider the role of stress as a confounding factor during parturition on maternal and fetal health outcomes. Previous studies in the lab demonstrate changes in the epigenetic regulation of *Oxtr* gene expression in the nucleus accumbens of voles exposed to adverse early life experiences (Danoff et al., 2020; Perkeybile et al., 2019). In the context of parturition, human mothers who develop PPD may have combined risk factors including exposure to birth interventions and various psychosocial stressors (e.g., poor social relationships, experiences of abuse or intimate partner violence, financial stress, stressful life events) (Alhusen et al., 2014; Yim et al., 2015). These experiences may be associated with symptoms of depression during pregnancy as well as PPD and should be

considered. Future studies examining the effect of stress as a potential confounding factor during parturition will be important to understand the broader experiences of human mothers.

Conclusion

Clinical administration of synthetic oxytocin has become a standard practice across many hospital systems and has demonstrated life-saving benefits. However, the overuse of synthetic oxytocin has demonstrated important adverse outcomes including reproductive and maternal psychological risks. Specifically, evidence suggests a higher incidence of maternal PPD may occur in the context of maternal exposure to synthetic oxytocin during parturition and raises concern regarding long-term consequences for both the mother and child. Continued research is needed to understand the underlying risk factors for maternal PPD including maternal exposure to synthetic oxytocin during parturition. Studies examining dose-dependent effects of synthetic oxytocin will be particularly beneficial for improved personalized use of this medication in clinical practice settings.

References

Alhusen, J., Bullock, L., Sharps, P., Schminkey, D., Comstock, E., & Campbell, J. (2014). Intimate partner violence during pregnancy and adverse neonatal outcomes in low-income women. *J Womens Health (Larchmt), 23*(11), 920-926. https://doi.org/10.1089%2Fjwh.2014.4862

The American College of Obstetricians and Gynecologists (ACOG). (2015). *Screening for Perinatal Depression.* https://www.acog.org/clinical/clinical-guidance/committee-opinion/articles/2018/11/screening-for-perinatal-depression

Brenton, C., Marsit, C., Faustman, E., Nadeau, K., Goodrich, J., Dolinoy, D., Herbstman, J., Holland, N., LaSalle, J., Schmidt, R., Yousefi, P., Perera, F., Joubert, B., Wiemels, J., Taylor, M., Yang, I., Chen, R., Hew, K., Freeland, D., Miller, R., & Murphy, S. (2017). Small-magnitude effect sizes in epigenetic end points are important in children's environmental health studies: The Children's Environmental Health and Disease Prevention Research Center's Epigenetics Working Group. *Environmental Health Perspectives, 125*(4), 511-526. https://doi.org/10.1289/ehp595

Carnevali, G., & Buoli, M. (2021). The role of epigenetics in perinatal depression: Are there any candidate biomarkers. *Journal of Affective Disorders, 280*(1), 57-67. https://doi.org/10.1016/j.jad.2020.11.056

Daly D, Minnie K, Blignaut A, Blix, E., Nilsen, A., Dencker, A., Beeckman. K., Gross, M., Pehlke-Milde, J., Grylka-Baeschlin, S., Koenig-Bachmann, M., Clausen, J., Hadjigeorgiou, E., Morano, S., Iannuzzi, L., Baranowska, B., Kiersnowska, I., & Uvnas-Moberg, K. (2020). How much synthetic oxytocin is infused during labour? A review and

analysis of regimens used in 12 countries. *PLOS ONE,* 15(7). https://doi.org/10.1371/journal.pone.0227941

Danoff, J., Wroblewski, K., Graves, A., Quinn, G., Perkeybile, A., Kenkel, W., Lillard, T., Parikh, H., Golino, H., Gregory, S., Carter, S., Bales, K., & Connelly, J. (2021). Genetic, epigenetic, and environmental factors controlling oxytocin receptor gene expression. *Clinical Epigenetics, 23.* https://doi.org/10.1186/s13148-021-01017-5

Erickson, E., Myatt, L., Danoff, J., Krol, K., & Connelly, J. (2023). Oxytocin receptor DNA methylation is associated with exogenous oxytocin needs during parturition and postpartum hemorrhage. *Communications Medicine, 3*(11). https://doi.org/10.1038/s43856-023-00244-6

Food and Drug Administration. (2014). Pitocin. Accessed September 17, 2022. https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/018261s031lbl.pdf

Gavin, N., Gaynes, B., Lohr, K., Meltzer-Brody, S., Gartlehner, G., & Swinson, T. (2005). Perinatal depression: A systematic review of prevalence and incidence. *Obstetrics and Gynecology, 106*(5), 1071-1083. https://doi.org/10.1097/01.aog.0000183597.31630.db

Grotegut, C., Lewis, L., Manuck, T., Allen, T., James, A., Seco, A., Deneux-Tharaux, C. (2017). The oxytocin product correlates with total oxytocin received during labor: A research methods study. *American Journal of Perinatology, 35*(1), 78-83. https://doi.org/10.1055/s-0037-1606119

Gu, V., Feeley, N., Gold, I., Hayton, B., Robins, S., Mackinnon, A., Samuel, S., Carter, S., & Zelkowitz, P. (2016). Intrapartum synthetic oxytocin and its effects on maternal well-being at 2 months postpartum. *Birth, 43*(1), 28-35. https://doi.org/10.1111/birt.12198

Kenkel, W., Ortiz, R., Yee, J., Perkeybile, A., Kulkarni, P., Carter, S., Cushing, B., & Ferris, C. (2023). Neuroanatomical and functional consequences of oxytocin treatment at birth in prairie voles. *Psychoneuroendocrinology, 150*. https://doi.org/10.1016/j.psyneuen.2023.106025

Kenkel, W., Perkeybile, A., Yee, J., Pournajafi-Nazarloo, H., Lillard, T., Ferguson, E., Wroblewski, K., Ferris, C., Carter, S., & Connelly, J. (2019). Behavioral and epigenetic consequences of oxytocin treatment at birth. *Science Advances, 5*(5). https://doi.org/10.1126/sciadv.aav2244

Kingston, D., Tough, S., & Whitfield, H. (2012). Prenatal and postpartum maternal psychological distress and infant development: a systematic review. *Child Psychiatry Hum Dev, 43*(5), 683–714. https://doi.org/10.1007/s10578-012-0291-4

Kramer, K., Cushing, B., Carter, C., Wu, J., & Ottinger, M. (2004). Sex and species differences in plasma oxytocin using an enzyme immunoassay. *Canadian Journal of Zoology, 82*(8), 1194-1200. http://dx.doi.org/10.1139/z04-098

Kroll-Desrosiers, A., Nephew, B., Babb, J., Guilarte-Walker, Y., Simas, T., & Deligaiannidis, K. (2017). Association of peripartum synthetic oxytocin administration and depressive and anxiety disorders within the first postpartum year. *Depress Anxiety, 34*(2), 137-146. https://doi.org/10.1002%2Fda.22599

Lukas, G., Brindle, S., & Greengard, P. (1971). The route of absorption of intraperitoneally administered compounds. *The Journal of Pharmacology and Experimental Therapeutics, 178*(3), 562-566.

Phaneuf, S., Rodríguez Liñares, B., TambyRaja, R., MacKenzie, I., & Bernal, A. (2000). Loss of myometrial oxytocin receptors during oxytocin-induced and oxytocin-augmented labour.

Journal of Reproduction and Fertility, 120(1), 91-97. https://doi.org/10.1530/jrf.0.1200091

Perkeybile, A., Carter, S., Wroblewski, K., Puglia, M., Kenkel, W., Lillard, T., Karaoli, T., Gregory, S., Mohammadi, N., Epstein, L., Bales, K., & Connelly, J. (2019). Early nurture epigenetically tunes the oxytocin receptor. *Psychoneuroendocrinology, 99,* 128-136. https://doi.org/10.1016/j.psyneuen.2018.08.037

Perkeybile, A., Kenkel, W., Yee, J., Lillard, T., Ferris, C., Carter, S., & Connelly, J. Pregnancy and birth epigenetically shape the maternal oxytocin receptor. *(In prep).*

Richa, R., & Sinha, R. (2014). Hydroxymethylation of DNA: An epigenetic marker. *EXLI Journal, 13,* 592-610. https://pubmed.ncbi.nlm.nih.gov/26417286

Robinson, C., Schumann R., Zhang, P., & Young, R. (2003). Oxytocin-induced desensitization of the oxytocin receptor. *American Journal of Obstetrics & Gynecology, 188*(2), 497-502. https://doi.org/10.1067/mob.2003.22

Shoyaib, A., Archie, S., & Karamyan, V. (2020). Intraperitoneal route of drug administration: Should it be used in experimental animal studies. *Pharmaceutical Research, 37*(12). https://doi.org/10.1007/s11095-019-2745-x

Simpson, K. (2022). Trends in labor induction in the United States, 1989 to 2020. *The American Journal of Maternal Child Nursing, 47*(4), 235. https://doi.org/10.1097/NMC.0000000000000824

Simpson, K., & Knox, G. (2009). Oxytocin as a high-alert medication: Implications for perinatal patient safety. *MCN Am J Matern Child Nurs, 34*(1), 8-15. https://doi.org/10.1097/01.nmc.0000343859.62828.ee

Szegda., K, Markenson., G, Bertone-Johnson, E., Chasan-Taber, L. (2014). Depression during pregnancy: a risk factor for adverse neonatal outcomes? *J Matern Fetal Neonatal Med*, *27*(9), 960–967. https://doi.org/10.3109/14767058.2013.845157

Tabbaa, M., Paedae, B., Liu, Y., & Wang, Z. (2017). Neuropeptide regulation of social attachment: The prairie vole model. *Compr Physiol, 7*(1), 81-104. https://doi.org/10.1002%2Fcphy.c150055

Tichelman, E., Warmink-Perdijk, W., Henrichs, J., Peters, L., Schellevis, F., Berger, M., & Burger, H. (2021). Intrapartum synthetic oxytocin, behavioral and emotional problems in children, and the role of postnatal depressive symptoms, postnatal anxiety and mother-to infant bonding: A Dutch prospective cohort study. *Midwifery, 100.* https://doi.org/10.1016/j.midw.2021.103045

Uvnas-Moberg, K., Ekstrom-Bergstrom, A., Berg, M., Buckley, S., Pajalic, Z., Hadjigeorgiou, E., Kotlowska, A., Lengler, L., Kielbratowska, B., Leon-Larios, F., Magistretti, C., Downe, S., Lindstrom, B., & Dencker, A. Maternal plasma levels of oxytocin during physiological childbirth – a systematic review with implications for uterine contractions and central actions of oxytocin. *BMC Pregnancy and Childbirth, 19*(285). https://doi.org/10.1186/s12884-019-2365-9

World Health Organization (WHO). (2014). WHO recommendations for augmentation of labor. Retrieved on May 15, 2023 from https://www.ncbi.nlm.nih.gov/books/NBK258883/

Yim, I., Stapleton, L., Guardino, C., Hahn-Holbrook, J., & Schetter, C. (2015). Biological and psychosocial predictors of postpartum depression: Systematic review and call for integration. *Annual Review of Clinical Psychology, 11,* 99-137. https://doi.org/10.1146/annurev-clinpsy-101414-020426

Young, K., Gobrogge, K., Liu, Y., & Wang, Z. (2011). The neurobiology of pair bonding: Insights from a socially monogamous rodent. *Frontiers in Neuroendocrinology, 32*(1), 53-69. https://doi.org/10.1016/j.yfrne.2010.07.006

Zhang, J., Branch, D., Ramirez, M., Laughon, S., Reddy, U., Hoffman, M., Bailit, J., Kominiarek, M., & Chen, Z. (2011). Oxytocin regimens or labor augmentation, labor progression, perinatal outcomes. *Obstetrics and Gynecology, 118*(201), 249-256. https://doi.org/10.1097%2FAOG.0b013e3182220192