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## Modeling the Psychiatric Aspects of Polycystic Ovary Syndrome and Induced Stress

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MODELING THE PSYCHIATRIC ASPECTS OF POLYCYSTIC OVARY  
SYNDROME AND INDUCED STRESS

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A Thesis

Presented to

The Graduate Faculty

Central Washington University

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In Partial Fulfillment

of the Requirements for the Degree

Master of Science

Experimental Psychology

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by

Danielle Leigh Peecher

May 2018

CENTRAL WASHINGTON UNIVERSITY

Graduate Studies

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## ABSTRACT

### MODELING THE PSYCHIATRIC ASPECTS OF POLYCYSTIC OVARY SYNDROME AND INDUCED STRESS

by

Danielle Leigh Peecher

May 2018

Polycystic ovary syndrome (PCOS) is an endocrine disorder in women that is characterized by excess testosterone and is associated with increased risk of infertility. Women with PCOS also show higher rates of depression and anxiety. Modeling PCOS in mice via administration of dihydrotestosterone (DHT) results in physiological alterations that are consistent with the physiological symptoms of PCOS in women. While some studies have investigated behavioral changes in PCOS mouse models, findings have varied. Thus, the current research examined whether a PCOS model with an additional stress factor resulted in behavioral changes. To create a stress condition, subjects were implanted with corticosterone (CORT) pellets to model the complex interaction of stress and PCOS on depression- and anxiety-like behavior. Data analysis for depression-like behavior on the forced swim test showed a significant main effect of time, with subjects spending more time immobile across the 5-minute test,  $F(4, 108) = 104.43, p < .001$ . Anxiety-like behavior on the elevated zero maze showed a significant time x prenatal treatment interaction,  $F(4, 108) = 2.71, p = .034$ . The hypothesis that the prenatal PCOS model in conjunction with the stress condition would better model the human condition

and result in behavioral alterations was only partially supported. These findings are an important step toward understanding the intersection of stress, mental health, and PCOS for the approximately five million women in the United States with this disorder.

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## CHAPTER I

### INTRODUCTION

Of endocrine disorders in reproductive-aged women, polycystic ovary syndrome (PCOS) is the most often diagnosed (Yu, Hao, Wang, Shen, & Kang, 2016). PCOS is a disorder associated with metabolic, reproductive, and hormonal changes. Many negative health outcomes have been associated with PCOS, including infertility, obesity, insulin resistance, and an increased risk of diabetes (Ressler, Grayson, Ulrick-Lai, & Seeley, 2015). Other common symptoms of PCOS include irregular menstrual cycles, increased levels of the hormone testosterone, and ovarian cysts (as cited in Yu et al., 2016). As will be discussed in depth in the follow sections, PCOS in women is often associated with higher than normal rates of psychiatric illness. As a result, understanding the connection between PCOS as a biological disorder and its psychological components is crucial. The etiology for women with PCOS developing psychological disorders is currently unclear. Research on how anxiety, depression, and other mental disruptions are related to PCOS is important for both medical and psychological fields (Rassi et al., 2010).

The purpose of the current research was to induce a prenatal model of PCOS in rodents to understand the physiology and psychiatric components of the disorder. In addition, a factor of stress was added to attempt to better model the human condition and understand the complexities of PCOS. The hypothesis that androgen elevation would produce significant anxiety-like and depression-like behaviors, as measured with mouse models for anxiety and depression, was examined. Utilizing behavioral models for anxiety and depression with the prenatal mouse model—we hoped to identify possible effects of PCOS and androgen elevation in offspring. Overall, answering questions

related to PCOS and the development of psychiatric illness is necessary for treatment and better understanding of the disorder.

## CHAPTER II

### LITERATURE REVIEW

#### **Physiology of PCOS**

Hyperandrogenism is a medical condition that creates excess testosterone hormone in females and is a factor in PCOS. Increased testosterone is also related to ovarian and reproductive disruptions (Rasgon et al., 2005). Testosterone is predominately associated with masculinity and high testosterone in women tends to increase facial hair, aggression, muscle mass, and general masculine features. Hahn and colleagues (2005) studied a sample of 120 women with PCOS and a demographic-matched control group and found those with PCOS had significantly higher levels of serum testosterone. Another study, using a hormone assay, again found increased total testosterone levels in PCOS women (Barth, Field, Yasmin, & Balen, 2010). Amiri and colleagues (2014) examined metformin and flutamide in treating PCOS and measured testosterone levels along with hirsutism scores (i.e., abnormal hair growth and facial hair) that appeared to correlate with increased testosterone. Therefore, hyperandrogenism functions in PCOS by increasing testosterone levels, which may associate with decreased fertility and manifestations of masculine characteristics or behaviors.

An example of reproductive disruptions includes the increased risk of infertility in women with PCOS. According to a PCOS review article, infertility often results from the lack of ovulation (i.e., anovulation) in respective women (Goodarzi, Dumensic, Chazenbalk, & Azziz, 2011). An important longitudinal cohort study examined self-reports on infertility, infertility hormone treatments, and regularity of ovulation in women with and without PCOS (Joham, Teede, Ranasinha, Zoungas, & Boyle, 2015). According

to participants' reports, 72% and 16% of women with PCOS and without, respectively, experienced infertility. Infertility rates were significantly higher in women with PCOS; however, Joham and colleagues (2015) discussed the limitation that these infertility rates only come from women who have attempted pregnancy, which may underestimate actual infertility rates. As proposed by Tian, Shen, Lu, Norman, and Wang (2007), infertility in women with PCOS also interacts with insulin resistance as supported by their findings of a 30.8% risk for spontaneous abortion in women with PCOS and a 47.8% for those only with insulin resistance. Described by Tian et al., insulin resistance is predicted to mediate the relationship of obesity, PCOS, and spontaneous abortion. More specifically, both obese women and women with PCOS are at risk for insulin resistance. Women with PCOS have higher levels of obesity and, thus, these factors contribute to difficulty in achieving or maintaining a pregnancy.

Beyond infertility, insulin resistance also increases the risk of diabetes in women with PCOS. Although the exact prevalence of Type 2 diabetes in women with and without PCOS varies across studies (Moran, Misso, Wild, & Norman, 2010), Talbott et al. (2007) documented that 12.8% of women with PCOS had Type 2 diabetes, significantly more than the 3.6% of women without PCOS. Importantly, in a review by Ressler et al. (2015), obesity was reported to be prevalent in PCOS populations with rates ranging between 61 to 76% of those in diagnosed samples (Ching, Burke, & Stuckey, 2007; Glueck et al., 2005). Animal research indicates that obesity can intensify PCOS symptomology with rats fed a high fat diet having higher insulin resistance compared to rats on a low fat diet (Ressler et al., 2015). Unfortunately, higher insulin resistance may be an inescapable symptom of PCOS because rats modeling PCOS that were fed a low fat

diet still exhibited insulin resistance, just to a lesser extent than did rats modeling PCOS that were fed a high fat diet. As these researchers concluded, dietary changes may not be effective in improving insulin resistance in women with PCOS.

PCOS is a heterogeneous condition and, therefore, it has been difficult for clinicians and researchers to understand all of its associated metabolic, reproductive, and hormonal changes, making consistent diagnostic criteria difficult to establish. For example, March et al. (2010) utilized three possible PCOS criteria, from the National Institutes of Health (NIH), the Rotterdam, and the Androgen Excess Society (AES), to determine which produced a higher estimate of the prevalence of the disease. The results yielded prevalence rates of PCOS ranging from 8.7% to 11.9% using NIH and AES criteria, respectively. The Rotterdam criteria provided a prevalence rate of 10.2% (March et al., 2010). More research on PCOS is clearly needed to improve diagnostic criteria as well as to enhance research on treatment options.

### **PCOS and Psychiatric Disorders**

Often mental illness is dually diagnosed with other health concerns. For example, depression is often associated with other mental health disorders or medical conditions, and PCOS's wide variety of symptoms includes psychiatric disturbances (e.g., Yu et al., 2016). Determining how anxiety, depression, and other potential psychological, behavioral, or cognitive changes are related to PCOS is important for both medical and psychological fields (Rassi et al., 2010). Rassi and colleagues (2010) found that 55.9% of sampled women with PCOS were also diagnosed with a psychiatric disorder. The most commonly diagnosed disorders in that sample were depression (26.4%) and anxiety (9.7%). Jedel and colleagues (2010) used a small sample of 60 women, 30 of whom were

diagnosed with PCOS and found self-reported anxiety on the Brief Scale for Anxiety to be significantly higher in the PCOS group. Another study found that depression, as measured by a psychiatrist, had a 2.93% higher prevalence in women with PCOS compared to controls (Hung et al., 2014). Thus, the research indicates that women with PCOS are more likely to exhibit depression or other mood disorders, but the etiology of psychiatric disorders in PCOS remains unclear.

Investigating the connection between PCOS and depression, Yu et al. (2016) measured depressive-like behaviors and changes in monoamines (i.e., serotonin, dopamine, norepinephrine) in a PCOS mouse model that used androgen treatment to induce PCOS-like symptoms and found significantly higher levels of serotonin in the hippocampus, striatum, hypothalamus, and dorsal raphe nucleus brain regions in treated mice compared to controls. Accordingly, it is possible that depression and other psychological disorders develop in PCOS due to androgen excess and subsequent biochemical changes in the brain.

Thus, while the hyperandrogenism associated with PCOS may underlie both the physical and psychological symptoms of the disorder, another potential pathway in the etiology of psychological disorders in women with PCOS may be that coping with the physical symptoms of PCOS, such as infertility and obesity, leads to psychological disorders such as anxiety and depression. Supporting such a possibility is research by Annagur et al. (2013) who reported that testosterone levels were not significantly different in those with or without depression and PCOS. Cwikel, Gidron, and Sheiner (2004) suggested that stressors associated with infertility manifest as depression or anxiety in women with PCOS, and Hahn et al. (2005) found that women with PCOS



reported decreased quality of life in physical, emotional, social, and psychological areas. In contrast, a meta-analysis of research on depression and PCOS found that, although there was an increased risk of depression in women with PCOS, that risk was independent of obesity (Dorkas, Clifton, Futterweit, & Wild, 2011). Regardless of the mechanism of action, PCOS is clearly interconnected with mood disturbances and, ultimately, affects the quality of life for those living with the disease. However, understanding the mechanism of action and how the physical symptoms of PCOS are related to psychological disorders is crucial in developing and investigating potential treatments for anxiety and depression in women with PCOS.

### **Stress, Psychological Disorders, and PCOS**

In attempting to explain the relationship between PCOS and depression, Benson et al. (2009) suggested that stress response differences in women with PCOS might contribute to the observed increased rates of depression among patients. In that study, women with PCOS had significantly increased heart rate and cortisol levels when stress was induced via a public speaking task. It remains important to understand this interconnection between stress, physiological changes, and mental health.

Many risks factors and processes of chronic stress are related to and intertwined with the etiology of mental illness. It is accepted among mental health and biological fields that depression is caused by a combination of biopsychosocial factors (e.g., Dinan, 1994; Duman, Aghajanian, Sanacora, & Krystal, 2016; Menke & Binder, 2014; Nester, 2014). For example, studies in epigenetics identify genetic risk factors for depression being ‘turned-on’ in reaction to stress (Nester, 2014) potentially through the actions of the glucocorticoid stress hormones, cortisol in humans and corticosterone in rodents.

Cortisol is involved in acute and chronic stress responses and is the end product of the hypothalamic-pituitary-adrenal (HPA) axis, which responds to physiological and psychological stressors with increased plasma cortisol levels (Varghese & Brown, 2001). Physiological effects of cortisol include altering "...basal processes such as fat and glucose, metabolism, blood pressure, inflammatory, and immune responses..." (as cited in Staufenbiel et al., 2013, p. 1221). While sympathetic arousal reallocates immediate energy resources to systems designed to enhance survival during stress (i.e., fight-or-flight), cortisol continues the reallocation of energy away from non-crucial body functions during longer acute stressors or under chronic stress (Boersma & Tamashiro, 2015). Importantly, elevated cortisol levels are part of an inhibitory feedback loop in which cortisol crosses the blood-brain barrier, binds to glucocorticoid receptors in the hypothalamus, and inhibits further HPA activity, bringing the stress response to an end and restoring homeostasis.

Notably, cortisol may play a role in some psychological disorders. For example, neuroendocrine studies have identified a relationship between decreased serotonin and increased cortisol in some cases of depression (Dinan, 1994). Graeff, Guimaraes, De Andrade, and Deakin (1996) report that by targeting serotonin receptors (i.e., pharmaceutically) they were able to increase public speaking anxiety in humans as well as anxiety-like behaviors in mice. Thus, Graeff et al. reduced serotonin using antagonist drugs and, subsequently, may have increased cortisol. Dinan (1994) reviewed the complexities of increased levels of cortisol binding to glucocorticoid receptors in limbic, hippocampal, and hypothalamic brain regions; and concluded, "If this hypothesis is correct ... these abnormalities in central monoamines are primary and induce secondary

endocrine disturbance” (Dinan, 1994, p. 369). As described, glucocorticoid receptors in these regions (i.e., limbic, hippocampal, and hypothalamic) link cortisol to monoamine changes and, possibly, psychological disorders. Connections between cortisol, HPA dysfunction, and depression have been demonstrated by the dexamethasone suppression test (Carroll, 1982). Dexamethasone is a synthetic cortisol that crosses the blood-brain barrier and should reduce endogenous cortisol levels via inhibition of further HPA activity. However, in a proportion of individuals with depression, cortisol levels remain elevated following dexamethasone administration, suggesting neuroendocrine dysfunction in depression.

### **HPA and Hypothalamic-Pituitary-Gonadal (HPG) Interactions**

Cortisol release from the adrenal gland in response to stress reallocates energy to prepare for physical exertion, and part of that process alters HPG axis activity. Reviewing the connection between the HPA and HPG axis, Whirledge and Cidlowski (2010) describe three contributions of the HPA axis onto the HPG system. First off, the hypothalamus, when stimulated by excess cortisol, decreases production of gonadotropin-releasing hormone (GnRH), which is an initial step in HPG activation. Secondly, Whirledge and Cidlowski reported that the pituitary gland can inhibit secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which together are responsible for ovulation and reproductive processes. The third interaction is the gonadal aspect and decreased formation of proper hormones and gamete production (i.e., estrogen and oocytes). Divyashree and Yajurvedi (2016) reviewed the impact of stress-induced HPA activity on HPG function, finding significant increases in adrenal gland weight and serum cortisol concentrations associated with decreased estrous cycles and decreased

ovary weight following exposure to eight weeks of stress in rats. Thus, reductions in gonadal functions have been documented in response to chronic stress, and such pathways may contribute to exacerbation of HPG disruptions in PCOS, given the psychological and physiological stress associated with infertility and anovulation.

**PCOS and Stress.** Considering the marked effects on androgen hormone levels, reproductive functions, and metabolic systems in PCOS, it may be possible to draw connections between HPG alterations and stress systems. Supporting the connection between stress and PCOS, Ressler et al. (2015) measured HPA activation in a mouse model of PCOS by a novel environment stressor. Surprisingly, Ressler et al. found that, in novel environment tests, PCOS mice had lower corticosterone responses than did controls. However, these mice also demonstrated anxiety-like behavior in an elevated plus maze; a behavioral finding that would typically correlate with increased corticosterone levels. Measuring corticosterone levels in animal models becomes important when attempting to parse out these discrepancies. These results point to possible differences between PCOS and controls in processing or responding to stressful events.

Unfortunately, as previously noted, variations of physical symptoms in women with PCOS have made it difficult for researchers to identify a common etiology (Abbott, Tarantal, & Dumesic, 2009). Additionally, psychological symptoms such as anxiety and depression may stem from underlying physiological differences in HPA and/or HPG systems in women suffering from the disorder or from stressors caused by PCOS symptomology (i.e., infertility). Fortunately, animal models of PCOS may allow

researchers to disentangle the potential causes of psychological disorders associated with PCOS.

## **Animal Models**

**Biological Models.** Animal models, especially in rodents, are widely used in PCOS research and, while these models all involve androgen administration, they vary in the timing of that androgen exposure. PCOS models either involve prenatal or peri/postnatal androgen administration. Thus, prenatal and postnatal PCOS models target differences in organizational and activational processes in the HPG system, respectively (Foecking, McDevitt, Acosta-Martinez, & Horton, 2008). For example, androgens can play an organizational or activational role in “masculinization and defeminization of the neuroanatomy, behavior, and endocrinology of male rats” (as cited in Foecking et al., 2008, p. 3). Prenatal models focus on the hypothesis that PCOS, although diagnosed in women post-puberty, is actually due to differences in hormonal systems that were organized and established during prenatal development. Roland, Nunemaker, Keller, and Moenter (2010) state that dysfunction of reproduction in prenatally androgenized (PNA) mice relates to “...androgen programming of the reproductive axis...” (p. 221). Therefore, prenatal models alter pathology earlier in development than do postnatal models, changing the organization structure of pathways that will then be activated in puberty.

Only one study to date directly compares the effects of prenatal and postnatal mouse PCOS models involving androgen administration and focuses on physiological phenotypes, including ovarian weights and cycles, insulin resistance, and adipose tissue

changes (Caldwell et al., 2014). Caldwell and colleagues (2014) found that mice in a postnatal PCOS model underwent fewer estrous cycles in two weeks than did those in a prenatal PCOS model. In contrast, mice in a prenatal PCOS model exhibited a higher percentage of unhealthy ovarian follicle cells than did those in a postnatal PCOS model. Thus, either postnatal or prenatal models can be utilized when modeling PCOS phenotypes and, as previously mentioned, organizational and activational androgen administration is the most critical distinction between pre- and postnatal PCOS models.

Other physiological changes that are important to metabolic and endocrine changes associated with PCOS include vaginal opening and anogenital distance measures. Marking the beginning of the estrous cycle, vaginal opening typically occurs at the fifth postnatal week (Rodriguez, Araki, Khatib, Martinou, & Vassalli, 1997); however, this opening has been found to occur earlier in some PNA models of PCOS (Roland et al., 2010). Rodriguez et al. (1997) found that mouse models would undergo vaginal opening earlier if they were exposed to an estrogen precursor. It seems, then, that hormone regulation is important for vaginal opening. Prenatal models of PCOS have not reported vaginal opening as a standard measure. Schober, Dulabon, Martin-Alguacil, Kow, and Pfaff (2006), in a study unrelated to PCOS, reported vaginal fusion in young girls and describe it as fusion of the labia together and red tissue surrounding the vagina. Schober et al. also discussed the function of lower levels of estrogen at birth playing a role in the vaginal fusion event. Connecting this concept to the study done by Rodriguez and colleagues, if PNA mice have elevated testosterone and lower levels of estrogen, then, the lack of vaginal opening is a reasonable outcome given that reproductive hormone dysfunction could result in vaginal fusion. Cases of vaginal fusion may be underreported

in PCOS animal models. In addition, anogenital distance, which is normally longer in males, is a measure that increases in women exposed to androgens during *in utero* development (Wu, Zhong, Chen, Zheng, Liao, & Xie, 2017). One prenatal PCOS rat model found that PNA-treated subjects had longer anogenital distance (Wu et al., 2010). Because PCOS is characterized by increased testosterone in females, measuring anogenital distance has become a validation measurement for the model. Both vaginal opening and anogenital distance are sensitive physiological metrics for reproductive hormone changes.

PCOS models also vary in their utilization of testosterone and related hormones to simulate different processes of the disease. Caldwell et al. (2014) identified “4 distinct hyperandrogenized models,” that include “androgens, estrogens, aromatase inhibitors, antiprogestines, changes in light exposure, and genetic manipulations” (p. 3147). Testosterone, in healthy women, is processed by the enzyme aromatase into estrogen; thus, estrogen and aromatase levels are typically higher in females (Goodarzi et al., 2011). Interrupting the process of converting testosterone to estrogen is accomplished by using dihydrotestosterone (DHT) or dehydroepiandrosterone (DHEA) derivatives that remain as testosterone. As a reminder, serum testosterone levels are higher in women with PCOS. The purpose of these models is to alter biological processes and produce excess androgens to simulate PCOS.

Walters, Allan, and Handelsman (2012) summarized the effects of DHEA on metabolic and reproductive symptoms. DHEA is similar to DHT in that both are derivatives of testosterone and will not convert to estrogen in women. The authors suggested that recent research has utilized DHEA models for studying “acyclicity,

abnormal maturation of ovarian follicles, and anovulation” (p. 1). DHEA is primarily used in postnatal models. For example, Walters et al. (2012) reviewed the literature on several postnatal DHEA rodent models, and found these studies consistently produced irregular or acyclic estrus cycles and ovarian cysts. Another review of PCOS animal models emphasized that DHEA, as a long-term postnatal treatment, is “the best approach to simulate the breadth of reproductive, endocrine, and metabolic features of human PCOS” (Caldwell et al., 2014, p. 3155). In regards to DHEA, Caldwell et al. found effects on androgen levels to approach higher levels in the DHEA postnatal model compared to DHEA in the prenatal model. As mentioned previously, Caldwell and colleagues (2014) studied DHEA and DHT in both pre- and postnatal models.

The other commonly studied model for simulating excess androgen uses DHT, a derivative of testosterone. Unlike DHEA, which has mostly been examined in postnatal models, DHT has been examined in both pre- and postnatal models. Caldwell et al. (2014) demonstrated that DHT animal models resulted in fewer completed estrous cycles, reduced ovary weight, and a significantly higher percentage of unhealthy follicle cells. DHT has also been found to produce features of ovarian morphology similar to PCOS with unhealthy follicles and atretic cyst-like formations (Caldwell et al., 2014). Additionally, DHT in prenatal models mimics “androgen excess... of human PCOS” closely (Caldwell et al., 2014, p. 3156). Thus, based on the current literature, DHT is supported as a prenatal model of PCOS.

In conclusion, both pre- and postnatal models have been used to simulate PCOS physiological phenotypes in animals. Recently, Yu and colleagues (2016) studied brain structure and monoamines in relation to the effects of androgens (i.e., mimicking PCOS)



on depression and mood disorders. Nonetheless, research on the relationship between psychological disorders and PCOS is limited to only a few studies. In prenatal models, researchers have historically examined biological PCOS effects, but studies on the psychological symptoms of PCOS have been published recently. Determining the interplay between these areas of symptomology is important to increase PCOS knowledge and to further understand PCOS models.

**Behavioral Models.** In order to understand the impacts of PCOS in humans, researchers have used behavioral models to observe emotional and psychological distress in animal subjects. Animal models such as the elevated zero maze (EZM) and forced swim test have predictive validity for research in biomedical fields (Yan, Cao, Das, Zhu, & Gao, 2010; Bell, Duke, Gilmore, Page, & Begue, 2013). Therefore, pharmaceutical companies have validated these models of anxiety- and depression-like behavior to investigate potential clinical anxiolytic and antidepressant drug efficiency. Such behavioral models are short tests (e.g., 5- or 15-minute intervals) and consist of coding inactivity or activity changes across the time-period (Castagne, Moser, Roux, & Porsolt, 2011). For example, forced swim tests in which time spent struggling in a small, room-temperature pool of water is measured, have been validated for their ability to predict clinical efficacy of antidepressant medications, namely fluoxetine, in humans. Antidepressant efficacy in clinical trials is correlated with decreased time spent immobile in the forced swim apparatus in mice that received a single acute pre-administration of the drug (Page, Detke, Dalvi, Kirby, & Lucki, 1999).

It is important to note that these behavioral models for anxiety- and depression-like behaviors also show face validity (i.e., giving up and not swimming or struggling on

the forced swim test resembles hopelessness) as well as pharmaceutical predictive validity. For example, the EZM is a circular maze consisting of two open and two closed sections. Rodents appear to prefer the closed sections and, under standard conditions, spend more time in those sections. Time spent in the open sections is interpreted to reflect decreased anxiety-like or fear-like behavior; an interpretation further supported by findings that anxiolytic medications increase the percentage of time spent in open-arms of the maze (Shepherd et al., 1994). Other behavioral changes on the EZM which include exploratory behavior such as head-dips, rearing, and stretched attend posture are also sensitive to anxiolytic manipulation (Molewijk, van der Poel, & Oilvier, 1995). Given that behavior on the EZM and forced swim test changes in predictable ways in response to anxiolytic and antidepressants agents, respectively, these models are commonly used as measures of anxiety and depression.

In investigating behavioral changes in a prenatal PCOS model (i.e., PNA mice), Yu et al. (2016) utilized the forced swim and tail suspension test to observe “depression-like behavior” (p. 1) and the open field test to investigate activity and anxiety-like behavior. Yu et al. found that PCOS animals exhibited significantly increased immobility on both the forced swim and tail suspension tests, which is interpreted as depression-like behavior. On the open field test, PNA mice had significantly lower activity levels. Therefore, these findings indicate that animal models of PCOS may show behavioral changes, including lower activity levels and higher depression-like behavior.

Another related study administered DHT in a rat prenatal model examined anxiety-like behavior in the offspring (Hu et al., 2015). These researchers found a significant decrease in activity in the open arms of the EZM in PCOS animals compared

to controls. Likewise, Ressler and colleagues (2015) discovered mice treated with DHT postnatally exhibited anxiety-like behavior represented by decreased time spent in the open arms of an EZM. Additionally, this same study found increased immobility for PNA mice on a forced swim test. Taken together, these findings suggest that animals exposed to treatments designed to model PCOS show alterations in anxiety- and depression-like behaviors: however, such investigations have not been widespread nor have they considered the additional effects of stress on behavior.

**Order effects.** Behavioral test history and carryover effects in test batteries have been previously reported in animal research. The prevailing rule of thumb has been that less invasive tests should be conducted first, followed by more invasive testing (i.e., Blokland et al., 2012; McIlwain, Merriweather, Yuva-Paylor, & Paylor, 2001; Voikar, Vasar, & Rauvala, 2004). It is important to understand the differences between test naive and experienced mice when running behavioral tests. Voikar, Vasar, & Rauvala (2004) found that naive C57BL/6J mice took a significant shorter time to enter open areas of an elevated plus maze and spent a longer amount of time in open arms. The elevated plus maze is similar to the EZM and this finding is important for interpreting the results of the current research. Whereas these carryover effects may occur in the elevated plus maze, McIlwain et al. (2001) found that carryover effects were test dependent, limiting generalizations across tests. In considering the possibility of carryover effects and test history, the current research followed the concept of using the least invasive test first, followed by the more invasive test.

## **Animal Models and Stress**

As previously mentioned, acute stress is temporary, whereas chronic stress is more prolonged and, often, more pronounced than normal day-to-day stress functions. In humans, the nature, number, and persistence of stressors are associated with the onset and relapse of psychological disorders as well as the intensity of symptomology (Schneiderman, Ironson, & Siegel, 2005). However, stress responses are also adaptive and serve as a biological mechanism for regulating basic bodily processes and fight-or-flight fear responses (Mastorakos, Pavlatou, & Mizamtsidi, 2006). One interesting study, found that chronic stress exposure had physiological changes that were similar to a PCOS phenotype (Divyashree & Yajurvedi, 2016). In this model, after 12 weeks of stress exposure, the stress condition mice showed lower body weights, increased adrenal gland weights, and elevated serum corticosterone. There are multiple physiological measures that can be taken to validate a stress model, and these models can also be created in various ways.

Examining stress in animal models can take various forms. One such example uses physical restraint to induce stress. Divyashree and Yajurvedi (2016) recently used an “open-ended cylindrical glass restrainer” and forced swim test to induce chronic stress in rat subjects (p. 766). In that study, chronic stress resulted in increased adrenal gland activity, cortisol concentration, and decreased body weight after eight weeks of stress. Similar studies have used other forms of stress models, including but not limited to cold-restraint exposure, physical restraints, and social defeat stress (Divyashree & Yajurvedi, 2016; Golden, Covington, Berton, & Russo, 2011).

Such physical stressors may represent environmental stress, however, such studies have yielded conflicting results. For example, Golden and colleagues (2011) used a social defeat paradigm that produced stress-related behavior as measured by social interaction and avoidance; however, the authors suggested they might have, instead, modeled depression-like behavior. Even in human research, the concept of stress as a scientific idea continues to evolve as researchers acknowledge that stress does not exist as a uniform response to challenges to homeostasis but that, in fact, different stressors induce difference patterns of sympathetic and HPA activity (Goldstein & Kopin, 2009). Thus, when investigating the impact of cortisol and/or HPA activity, one possible remedy to inconsistent results and variations in models of stress would be to use cortisol or corticosterone pellets to induce a steady-state elevation in glucocorticoids.

Hermann and colleagues (2009) compared the effectiveness of administering corticosterone with subcutaneous injection, implantation of hormone-releasing pumps, and implantation of slow-release pellets. Administering corticosterone via pellets appeared to maintain consistent plasma levels with rapid increases in corticosterone within hours of administration. However, these authors discussed the need to replace corticosterone pellets after 7 and 14 days to maintain heightened and consistent corticosterone levels. Importantly, Hermann and colleagues measured corticosterone levels on a daily basis, and demonstrated a narrow range of levels between subjects (i.e., 1200 and 1400  $\mu\text{g/L}$ ) with low variation between subjects. Thus, in regards to evaluating the physiological and behavioral effects of HPA activation, using corticosterone pellets provides the most control and consistency over corticosterone levels which may be more

desirable for reasons of internal validity. As was done by Herrmann et al., it is necessary to measure serum levels of corticosterone to verify the validity of the treatment.

### **Hypotheses**

The purpose of the current study was to investigate the potential of a mouse model of PCOS, in particular a model using prenatal administration of DHT, to induce excessive androgen levels and to examine the physiological and psychological symptoms associated with PCOS. The impact of elevated corticosterone (CORT) in both PCOS and control mice was also evaluated. It was hypothesized that PNA mice would show more anxiety- and depression-like behaviors than prenatal saline mice due to organizational changes produced by androgen elevation in the prenatal PCOS mouse model. It was further anticipated that inducing stress-like conditions in mice would produce heightened anxiety- and depression-like behaviors, but that those effects would be more pronounced in animals that had been prenatally exposed to androgen (i.e., PNA or PCOS-model mice).

## CHAPTER III

### METHODS

#### **Subjects**

The current study's prenatal model of PCOS used C57BL/6J mice because of their applicability in comparative studies and because C57BL/6J dams are successful breeders as measured by litter size and survival rates (Weber, Hultgren, Algers, & Olsson, 2016). Additionally, C57BL/6J mice demonstrate moderate baseline activity levels appropriate for behavioral measures (de Visser, van den Bos, Kuurman, Kas, & Spruijt, 2006). Twenty-four female C57BL/6J mice were obtained from Jackson Laboratory along with males for breeding purposes. Dams were randomly assigned to the DHT or saline conditions, with successful pregnancies in 10 and 6 dams, respectively. Breeding proceeded in two separate rounds. The first round of breeding produced 10 DHT and 12 prenatal saline female offspring. For the second round of breeding, we obtained 11 DHT and 4 prenatal saline female offspring. The resulting number of subjects for each experimental condition were:  $n = 12$  (PNA-CORT),  $n = 9$  (PNA-Control),  $n = 5$  (Prenatal Saline-CORT), and  $n = 11$  (Prenatal Saline-Control). Central Washington University's Institutional Animal Care and Use Committee approved all experimental procedures (Protocol #).

**Care and Housing.** Animals were housed in climate-controlled (i.e., 75-77° F), 12-hour light/dark cycle rooms. Initial housing and breeding occurred in the Science Building vivarium at Central Washington University. Animals were pair-housed with same-sex animals in the same condition in clear polypropylene cages (22.2 x 30.80 x 16.24 cm) and provided with CareFresh bedding (Kaytee Aspen Bedding), Mazuri rodent

diet chow (6F), and water *ad libitum*. After weaning and pellet implantation, mice were transferred to the Department of Psychology vivarium, with conditions matched to the Science vivarium. The principle investigator was responsible for animal care, including daily cage checks and weekly cage changing.

**Dams and Breeding.** Female C57BL/6J mice were used in a time-mating protocol, which ensured the offspring for testing were born within a two-day period. Male C57BL/6J mice were paired with a female mouse for two consecutive evenings. Breeding occurred in the familiar cage of the female. In order to determine gestation date, dams were checked daily for the presence of copulation plugs; the presence of such a plug designated day 0 of gestation. Dams delivered pups at day 19.5 to 20.5. Once litters were produced, the pups remained with their dam until weaning on postnatal day 21 (PN21).

**Physiological Measures.** Seven days after birth, group weights of female pups were taken for each treatment group. This method of body weight was continued for PN14 and PN21. After weaning on PN21, individual weights were taken once a week until undergoing pellet surgery on PN36. During surgical recovery, female offspring were weighed daily until behavioral testing. Final weights were taken immediate to euthanasia. Vaginal openings were checked daily after weaning and until all subjects showed openings or until surgery, whichever came first. For some subjects, qualitative reports of vaginal redness were collected. Anogenital distance was measured for all subjects after euthanasia.



## **Materials**

**PNA Model.** At day 0 of gestation, dams were randomly assigned to either the PNA or saline group. Following a protocol from Caldwell et al. (2014), dams in the PNA condition received DHT (i.e., 250  $\mu$ g) prepared in sesame oil, which was administered subcutaneously in a volume not exceeding 100  $\mu$ L per injection. Saline was also prepared in sesame oil and administered in 100  $\mu$ L injections. The DHT and saline injections were administered on days 16-18 of pregnancy. The DHT treatment is designed to produce organizational changes in the dam's female offspring that result in a mouse model of PCOS in those offspring.

**Stress Model.** Female offspring of each litter were randomly assigned to CORT pellet or a control pellet treatment. CORT pellet preparation followed a published protocol using low-cost pellets (Sahores et al., 2013). Referring to Sturm, Becker, Schroeder, Bilkei-Gorzo, and Zimmer (2015), 20 mg of corticosterone was used in the pellets. Following Sahores et al.'s (2013) protocol, 600 mg corticosterone was mixed with 3.6 mg of silicone adhesive. Control pellets were made using only silicone adhesive. CORT pellets were pressed and kept overnight between glass slides. The pellets were, then, cut into equal 4 mm diameter pieces. Surgery and subcutaneous implantation of CORT pellets were followed by use of vet bond and a staple to close the wound. Subjects were allowed seven days of recovery before conducting behavioral tests. In future studies, serum CORT levels will be measured in subjects to verify this model.

## **Behavioral Models**

Some models for psychological disturbances in mice include the EZM and forced swim test, which provide operationalized variables of anxiety, depression, and baseline

activity. Behavioral testing occurred between PN43 to 47 for female offspring. Each behavioral model measured subject activity in 5 min increments. Subject activity was recorded using a video camera placed above or across from the apparatus. Trained observers coded activity of mice after all behavioral testing was complete. To ensure reliability, scores for each video came from the average of two coders. Scores needed to be in 70% agreement or higher to be included in the average. If the agreement was below 70%, a third coder's data were, instead, used in the average calculation. If 70% agreement was not achieved after the third coder, the average per condition and time interval was included. To clarify, if a PNA-CORT subjects' score for minute 1 did not reach 70% agreement then the average for all other subjects in both PNA and CORT treatment conditions for that minute was averaged and included in that cell.

**EZM.** The EZM measures anxiety-like behavior based on the time spent in open compared to closed areas as well as providing measures of total activity in the form of total arms entered. Subjects in all four experimental conditions (i.e., PNA-CORT, PNA-Control, Prenatal Saline-CORT, Prenatal Saline-Control) underwent the EZM first. The EZM apparatus was elevated 50 cm off the floor and is an annular maze (e.g., circular). The maze was 60 cm in diameter and consists of four distinct quadrants. Open quadrants and closed quadrants, with ~28 cm clear acrylic walls, are opposite each other in the EZM maze. See Appendix A for an image of the EZM. Mice were placed in the maze for 5 min. Lumens in the open areas were approximately 22 to 40 lux and in the closed areas were 6 to 10 lux. Movement between closed and open areas and time spent in the open and closed arms were coded. An entry into an open arm occurred if the mouse crossing all 4 paws into the open arm. Counting the number of entries into closed and open arms

followed the same requirement; that all 4 paws needed to cross into the new arm. If mice spend time mostly in closed areas, this is interpreted as indicative of anxiety-like behavior. Again, this behavioral model has predictive validity for pharmaceutical drugs, such as anxiolytics. Each subject was tested on the EZM before testing on the forced swim apparatus.

**Forced swim.** As a test of depression-like behavior, subjects in all four experimental conditions (i.e., PNA-CORT, PNA-Control, Prenatal Saline-CORT, Prenatal Saline-Control) underwent a forced swim test 24 hr after the EZM. Mice were placed in a 5000 mL container of room-temperature water. The container was filled with 3500 mL of water so the mouse could not touch the bottom or climb out. See Appendix A for an image of the forced swim apparatus. Mice naturally can swim; however, they prefer to escape water. Mice can either choose to swim (e.g., attempt to get out) or float. Floating behavior is coded as immobility and is taken at face value to resemble hopelessness behavior (i.e., depression-like behavior). Floating behavior was coded, resulting in immobility data for each of the 5 min of the test. Floating was determined if the animal was not propelling through the water and only had minimal paw movements (i.e., only paw movement to stay above water not to propel it through the water). As discussed previously, the forced swim test has been validated as a depression-like behavioral model for testing antidepressant drugs.

## **Design**

PCOS was operationalized in the current experiment by the concept of prenatal exposure to androgen excess. More specifically, female mice in the experimental group should have mimicked PCOS symptomology following the prenatal administration of

DHT (i.e., a testosterone derivative). Body weight measures were collected weekly to determine percent body weight changes, as other studies have found differences in body weight in response to DHT or CORT treatment, separately. As mentioned, vaginal opening determines sexual maturity in female subjects. After euthanasia, anogenital distance was measured to determine if prenatal exposure to androgen resulted in larger anogenital distances compared to control subjects.

Measures of depression- and anxiety-like behavior were collected for all subjects to compare psychological effects of prenatal treatment (i.e., PNA, Prenatal Saline) and postnatal stress condition (i.e., CORT, Control). Overall, it was expected that mice in the DHT group would spend less time in the open arms of the EZM (i.e., increased anxiety-like behavior) and have higher levels of immobility in the forced swim test (i.e., increased depression-like behavior). Each subject completed the EZM and, then, after 24 hr, the forced swim test. As noted, for each test, data were collected for each minute of the 5 min test to determine if behavioral patterns rather than overall behavior differed across conditions. Therefore, 2 (PNA, Prenatal Saline) x 2 (CORT, Control) x 5 (1-5 min) mixed ANOVAs were used to analyze behavior on the EZM and immobility on the FST. In other words, each behavioral measure followed a repeated measures design of activity and immobility (i.e., repeated measures were scored at each minute in the interval).

### **Statistical Analysis**

As previously noted, behavioral data consisted of averages from two coders per video recording. On measures in which coders did not show over 70% agreement, a third coder's data were included in the average. Lastly, when 70% was not reached, the average score for the condition and time interval was included.

Mixed factor ANOVAs examined the effects of PNA and postnatal CORT administration on behavioral measures in all subjects. When appropriate, an additional factor of minute in the test (i.e., 1-5 min) was utilized, for resulting separate 2 (PNA, Prenatal Saline) x 2 (CORT, Control) x 5 (1-5 min) ANOVAs to analyze time spent in the open arms of the EZM, total arm entries on the EZM, and immobility on the forced swim test. As noted, increased time in the open arms is interpreted as decreased anxiety-like behavior while increased immobility on the forced swim test is interpreted as depression-like behavior. Bonferroni correction was used as a post-hoc analysis for all statistics.

Physiological measures were analyzed using separate independent samples *t*-tests or ANOVAs. The physiological measures and analysis included the number of pups born to each treatment condition dam (independent samples *t*-test), which was collapsed within each cage to account of pair housing of dams. Weights before pellet implantation [2 (PNA, Prenatal Saline) x 3 (PN7, PN14, PN21) ANOVA], final weight at the time of euthanasia [2 (PNA, Prenatal Saline) x 2 (CORT, Control) ANOVA], vaginal opening [(PNA, Prenatal Saline) ANOVA], and anogenital distance [2 (PNA, Prenatal Saline) x 2 (CORT, Control) ANOVA], were also analyzed. Two analyses were included for vaginal opening. The first measured the percentage of true vaginal opening in subjects for each group. The second counted vaginal opening as the first day of the qualitative observation of red tissue around the vagina (i.e., vaginal redness) and, similarly, used percentage of opening for each treatment condition. Bonferroni correction was used as a post-hoc analysis.

## CHAPTER IV

### RESULTS

The total number of animals in each condition ranged from 5 to 12 (PNA-CORT:  $n = 12$ ; PNA-Control:  $n = 9$ ; Prenatal Saline-CORT:  $n = 5$ , and Prenatal Saline-Control:  $n = 11$ ). There was a non-significant trend for fewer female pups in DHT-treated ( $M = 3.5$ ,  $SD = 1.38$ ) compared to prenatal saline-treated ( $M = 5.33$ ,  $SD = 1.53$ ) litters,  $t(7) = 1.83$ ,  $p = .056$ . As a reminder, litters were collapsed within each pair housed cage of dams. Data for body weights, vaginal openings/vaginal redness, anogenital distances, forced swim immobility, and time spent in the open arms and total arm entries in the EZM are discussed below.

#### **Body Weight**

PNA and prenatal saline pups were not significantly different in body weight before weaning and analyzed by a 2 (PNA, Prenatal Saline) x 3 (PN7, PN14, PN21) ANOVA; however, there was a significant main effect of PN day,  $F(2, 14) = 44.71$ ,  $p < .001$ , with a post-hoc comparison indicating that body weights increased significantly across days,  $ps < .05$ , as shown in Table 1. Body weights were also collected daily after pellet implantation and the average body weight across those days was analyzed via a 2 (PNA, Prenatal Saline) x 2 (CORT, Control) ANOVA, which found no significant effects or interaction, as shown in Figure 1. For body weight at euthanasia, the prenatal condition did not significantly affect body weight, but postnatal pellet implantation did significantly influence body weight,  $F(1, 33) = 5.22$ ,  $p < .05$ , with the CORT condition having lower body weights as shown in Table 2.

Table 1

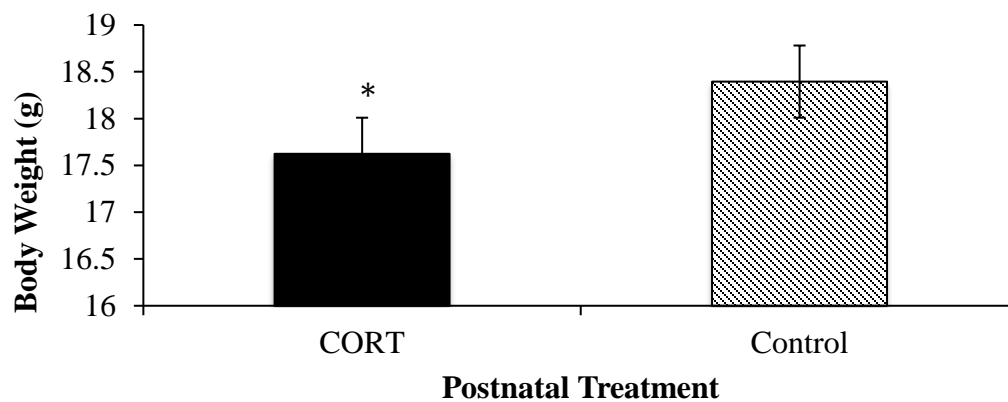
*Average Female Body Weights (g) at PN7, PN14, and PN21*

Group	<i>n</i>	PN7 <i>M (SD)</i>	PN14 <i>M (SD)</i>	PN21 <i>M (SD)</i>
PNA	21	4.94 (2.45)	7.22 (1.21)	8.33 (1.64)
Prenatal- Saline	16	4.60 (1.72)	6.84 (0.73)	8.15 (0.92)

Table 2

*Average Body Weights (g) at Time of Euthanasia*

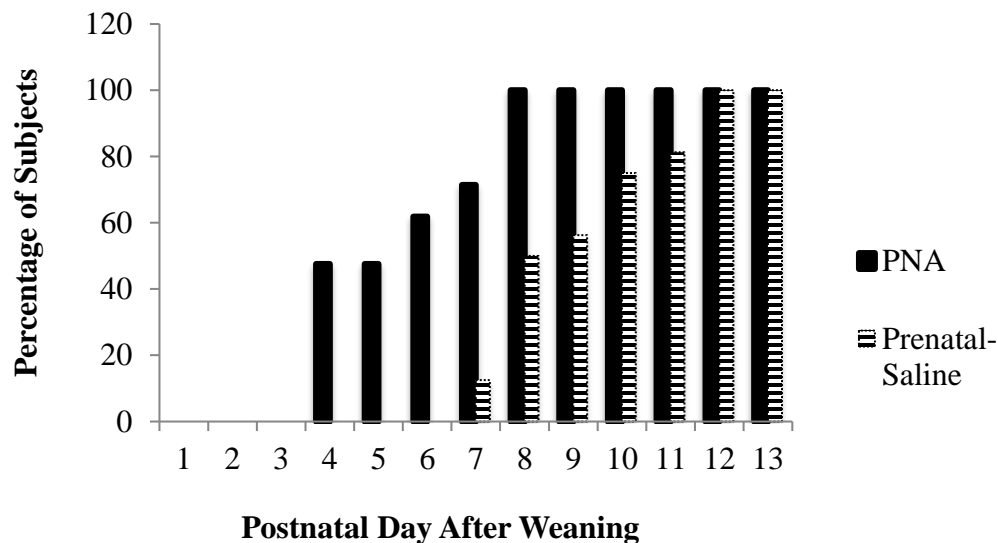
Group	<i>n</i>	<i>M (SD)</i>
PNA-CORT	12	17.67 (0.68)
PNA-Control	9	18.33 (0.72)
Prenatal Saline-CORT	5	17.52 (1.23)
Prenatal Saline-Control	11	18.45 (0.93)



*Figure 1.* Average body weights (g ± SEM) at time of euthanasia collapsed across prenatal conditions. \*Difference between CORT and control,  $p < .05$ .

## Vaginal Openings

An ANOVA for the factor of prenatal treatment on vaginal opening did not reveal any differences. However, PNA mice showed vaginal redness in some cases, as described previously, instead of vaginal opening. If the redness were coded as the day of vaginal opening, then prenatal treatment did significantly affect vaginal opening (i.e., vaginal redness),  $F(1, 35) = 6.04$ ,  $p < .05$ , as shown in Figure 2. Of the PNA mice, 50% showed vaginal opening or vaginal redness at day 4, whereas 50% of prenatal saline mice showed vaginal opening later at day 8. At day 8, 100% of PNA mice showed vaginal opening or vaginal redness, whereas prenatal saline mice reached 100% vaginal opening at day 13.



*Figure 2.* Percentage of subjects on each post-weaning day showing vaginal opening or vaginal redness (for PNA subjects); differences between PNA and prenatal saline as noted in text.

## Anogenital Distance

A 2 (PNA, Prenatal Saline) x 2 (CORT, Control) ANOVA for anogenital distance immediately after euthanasia revealed a significant effect of prenatal treatment,  $F(1, 29)$



= 6.79,  $p < .05$ , but no effect of postnatal manipulation nor an interaction. PNA mice had significantly longer anogenital distances than prenatal saline mice, as detailed in Table 3.

Data is collapsed within prenatal treatment groups (i.e., PNA and Prenatal Saline).

Table 3

*Average Anogenital Distance (mm) at Euthanasia*

Group	<i>n</i>	<i>M (SD)</i>
PNA	20	5.9 (1.0)
Prenatal-Saline	13	5.0 (0.4)

### **EZM and Anxiety-like Behavior**

**Time Spent in the Open Arms.** Outliers, represented by scores greater or less than two standard deviations from the mean of each condition, were removed prior to analysis, resulting in  $n = 10$ ,  $n = 7$ ,  $n = 5$ , and  $n = 9$  for the PNA-CORT, PNA-Control, Prenatal Saline-CORT, and Prenatal Saline-Control conditions, respectively.

A 2 (PNA, Prenatal Saline) x 2 (CORT, Control) x 5 (1-5 min) ANOVA revealed a significant main effect of test minute on time spent in the open arms,  $F(4, 108) = 3.42$ ,  $p < .05$ , in addition to a test minute x prenatal treatment interaction,  $F(4, 108) = 2.71$ ,  $p < .05$ . A post-hoc comparison of the interaction revealed no differences among prenatal conditions at any specific test minute but did reveal that time spent in the open arms increased significantly from test minute 2 to test minute 3 and from test minute 3 to test minute 4,  $ps < 0.05$ , as detailed in Table 4. Follow up ANOVAs were conducted for the factors of prenatal and postnatal treatments at each of the 5 test minutes, with only test minute 4 yielded a significant difference between prenatal treatments,  $F(1, 29) = 314.08$ ,

$p < .05$ . PNA subjects showing increased time spent in open arms of the maze. Mean and standard deviations collapsed across postnatal treatment conditions into PNA and prenatal saline groups,  $M = 11.53$ ,  $SD = 7.5$ , and  $M = 5.56$ ,  $SD = 6.3$ , respectively.

Table 4

*Time Spent in Open Arms (sec) in the EZM by Test Minute and Group*

Groups	<i>n</i>	Minute 1	Minute 2	Minute 3	Minute 4	Minute 5
		<i>M (SD)</i>	<i>M (SD)</i>	<i>M (SD)</i>	<i>M (SD)</i>	<i>M (SD)</i>
PNA-CORT	10	3.6 (6.3)	2.9 (4.1)	6.2 (6.7)	9.0 (9.2)	6.9 (6.2)
PNA-Control	7	5.5 (5.0)	3.6 (4.0)	4.2 (7.2)	14.4 (5.8)	5.0 (2.5)
Prenatal Saline-CORT	5	3.4 (5.5)	4.7 (7.2)	4.8 (6.2)	8.4 (7.5)	13.3 (9.0)
Prenatal Saline-Control	9	4.6 (6.1)	4.1 (5.2)	8.8 (7.9)	2.8 (3.6)	7.5 (7.4)

**Total Entries into Arms.** Analysis of total entries into arms on the EZM did not reveal any significant effects of prenatal or postnatal treatments nor on test minute on the apparatus as partially depicted in Table 5.

Table 5

*Total Entries into Open and Closed Arms on the EZM*

Groups	<i>n</i>	Open Arms Entries	Closed Arms Entries
		<i>M (SD)</i>	<i>M (SD)</i>
PNA-CORT	10	7.1 (4.2)	6.8 (4.6)
PNA-Control	7	7.0 (2.6)	6.2 (2.8)
Prenatal Saline-CORT	5	5.6 (2.2)	4.9 (2.4)
Prenatal Saline-Control	9	4.8 (2.6)	4.9 (2.8)

**Forced Swim Test: Depression-like behavior**

Outliers, represented by scores greater or less than two standard deviations from the mean of each condition, were removed prior to analysis, resulting in  $n = 9$ ,  $n = 7$ ,  $n = 4$ , and  $n = 11$  for the PNA-CORT, PNA-Control, Prenatal Saline-CORT, and Prenatal Saline-Control conditions, respectively.

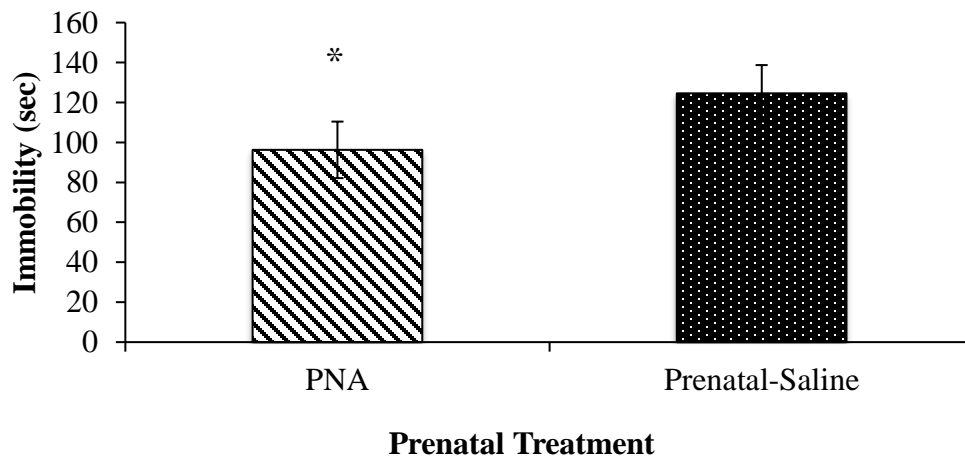
A mixed 2 (PNA, Prenatal Saline) x 2 (CORT, Control) x 5 (1-5 min) ANOVA for immobility on the forced swim test revealed a significant main effect of test minute,  $F(4, 108) = 104.43$ ,  $p < .001$ ; post-hoc comparisons revealed increases in immobility across the test such that test minute 1 had less immobility than test minutes 2-5 and test minute 2 had less immobility than test minutes 3-5,  $ps < .001$ , as detailed in Table 6. There was also a significant effect of prenatal manipulation,  $F(1, 27) = 9.30$ ,  $p < .01$ , with PNA animals having lower immobility than prenatal saline animals, as depicted in Figure 3. Prenatal manipulation for the total time spent immobile is collapsed across postnatal treatments, for example, PNA-CORT and control treatments combined and prenatal

saline-CORT and control treatment ( $M = 19.25$ ,  $SD = 12.26$  and  $M = 27.79$ ,  $SD = 14.54$ , respectively).

Table 6

*Immobility Time (sec) by Test Minute and Group on the Forced Swim Test*

Groups	<i>n</i>	Minute 1	Minute 2	Minute 3	Minute 4	Minute 5
		<i>M (SD)</i>	<i>M (SD)</i>	<i>M (SD)</i>	<i>M (SD)</i>	<i>M (SD)</i>
PNA-CORT	9	1.4 (1.5)	14.2 (4.7)	22.1 (5.0)	25.1 (6.4)	26.7 (9.0)
PNA-Control	7	1.9 (1.5)	14.7 (7.2)	23.8 (11.8)	33.7 (7.3)	30.7 (6.7)
Prenatal Saline-CORT	4	4.1 (1.7)	22.3 (9.2)	35.0 (5.6)	36.1 (9.9)	31.4 (5.5)
Prenatal Saline-Control	11	2.3 (1.4)	18.6 (9.6)	31.5 (9.6)	33.1 (10.4)	36.2 (8.2)



*Figure 3.* Time (sec  $\pm$  SEM) spent immobile in the forced swim test by prenatal condition.

Prenatal condition (PNA or Prenatal-Saline) is collapsed across postnatal stress (CORT or control) conditions. \*Difference between PNA and prenatal saline animals,  $p < .01$ .

## CHAPTER IV

### DISCUSSION

In the current study, the PNA model resulted in decreased anxiety-like (i.e., increased time in the open arms on the EZM at test minute 4) and depression-like (i.e., decreased time spent immobile in the forced swim test) behavior. Neither decreased anxiety-like behavior on the elevated zero maze nor, decreased depression-like behavior on the forced swim test was expected in PNA mice. Effects of the prenatal model were observed on the physiological measures of vaginal redness and anogenital distance with earlier vaginal redness and longer anogenital distances in PNA mice compared to saline controls. The postnatal treatment also altered body weight at the time of euthanasia, with animals who were implanted with a CORT pellet having lower body weight than animals implanted with a control pellet. Applications of the current findings to future research will be important for understanding the interconnections between PCOS and mental illness. In addition, limitations of the current study may be overcome in future research.

#### **PNA Model and Anxiety-like behavior**

As noted, PNA-treated mice showed decreased anxiety-like behavior during the 4<sup>th</sup> minute of the EZM test and did not alter activity levels as measured by total arm entries. These findings do not support the hypothesis that our PCOS model or our postnatal stress condition would result in increased anxiety-like behavior. Given that prior research has demonstrated the EZM to be a sensitive measure for anxiety-like behavior in rodents (Bell et al., 2014), it might be argued that the current prenatal PCOS model did not alter anxiety-like behavior. Our prenatal PCOS and stress manipulations did not impact anxiety-like behavior as expected. The lack of expected findings may be

due to differences in lab protocols for measuring anxiety-like behavior (i.e., different anxiety-like testing procedures).

As previously noted, Ressler et al. (2015) found total percent time spent in the open arms to be significantly different between control and DHT-treated animals on the EZM, supporting a conclusion of increased anxiety-like behavior in a PCOS animal model. The contradictory findings in the current study may be due to differences in the timing of DHT treatment (i.e., postnatal vs. prenatal, respectively) between Ressler et al.'s study and the current research. The etiology of anxiety and depression in PCOS might not be due to developmental factors but, instead, to environmental or epigenetic changes that occurs after birth, allowing Ressler et al. (2015) to find changes in his postnatal model because elevating testosterone levels postnatally have a stronger impact on the stress system and, subsequently, on anxiety-like behavior.

The EZM analysis only coded the total amount of time spent in the open arms and the number of total arm entries. However, exploratory behavior on the EZM may also provide useful measures of anxiety-like behavior (Rodgers, Cao, Dalvi, & Holmes, 1997). Commonly reported exploratory behaviors include head dips (i.e., looking over the side of the maze) and stretch-attend posture (i.e., extended posture and minimal movement; Molewijk, van der Poel, & Olivier, 1995). For example, protected-head dipping and sniffing in the closed area, in a factor analysis, loaded highly with anxiety (Rodgers et al., 1997). Another study found that a dose of the anxiolytic drug, diazepam, increased the number of open arm entries as well as head dips in the open areas but decreased the frequency of stretch-attend postures (Matto, Harro, & Allikmets, 1997). Thus, head dips in the open arms might be interpreted as decreased anxiety-like behavior, but increased

stretch-attend postures and closed-arm head dipping might indicate increased anxiety-like behavior (Rodgers et al., 1997). Studying exploratory behaviors may provide more nuanced measures of anxiety-like behavior in rodent models and, therefore, future studies should consider coding these measures.

### **PNA Model and Depression-like Behavior**

Behavior on the forced swim test in the current study aligned with prior reports of increased immobility across time on this behavioral test (Petit-Demouliere, Chenu, & Bourin, 2005). With approximately 4,300 published papers using the forced swim test, it appears to be a popular measure for depression-like behavior (Molendijk & de Kloet, 2015). Despite the ubiquitous use of the forced swim test, neither prenatal nor postnatal manipulations associated with DHT and CORT, respectively, changed immobility as expected in the current study. In fact, contrary to our expectation, PNA mice showed lower immobility than prenatal saline controls (i.e., less depression) while our postnatal manipulation did not have any effects on immobility.

Previous studies have found increased immobility in postnatal models of PCOS (Yu et al., 2016), suggesting that, similarly to our findings regarding anxiety-like behavior, postnatal but not prenatal DHT treatment may yield PCOS models that more strongly impact behavior. Yu et al. (2016) also correlated changes on their behavioral tests to decreased levels of dopamine, serotonin, and norepinephrine in their DHT-treated animals, indicating potential neurochemical mechanisms that could underlie the observed behavioral changes. Using measures of neurotransmitter function may be particularly relevant as a biological endpoint and collecting brain tissue samples in a prenatal model of PCOS may be an important step in the development of PCOS animal models. The

majority of PCOS research focuses on metabolic features of the disorder and examining psychological components of the disorder in animals remains a novel and relatively recent pursuit, resulting in limited information on brain-related changes. Investigating changes in neurochemistry would be an important step to understanding the etiology of depression or other mental illness in PCOS.

A recent publication further complicates our ability to interpret the current findings in that that article calls into doubt the use of the forced swim test as a true measure of depression-like behavior (Molendijk & de Kloet, 2015). According to those authors, the forced swim test more accurately represents a response to an acute stressor (i.e., rodent being placed in water they cannot escape) than a test of depression-like behavior. Thus, our mice may actually be demonstrating dampened stress reactivity in the PNA conditions similar to those described by Ressler et al. (2015) in women with PCOS. If the DHT prenatal treatment does decrease stress reactivity, then we would expect to see an increase in mobility (i.e., less depression-like behavior) compared to prenatal saline controls which was observed in the current study.

Applying this argument provides an explanation for the contradictory findings between the current study and previous work, and further suggests that future assessments of psychological and/or behavioral changes in animal models of PCOS might consider utilizing different tests to measure depression-like behavior. One example of an alternative test for depression-like behavior is the sucrose preference test. Snyder, Soumier, Brewer, Pickel, & Cameron (2012) used a stress paradigm, not related to a PCOS model, and examined depression-like behavior using the forced swim and sucrose preference test; finding changes in depression-like behavior on both tasks. The sucrose



preference test is based on anhedonia, or lack of enjoyment from activities, which is a common symptom of depression. In a similar vein, Kim, Yang, Kwon, Woo Lee, and Kim (2017) used an unpredictable shock stressor and found that the sucrose preference test showed changes in anhedonia-like behavior that were not visible in the forced swim test. Thus, sucrose preference tests may allow researchers to study other depression-like symptoms (i.e., anhedonia or lack of pleasure) in animal models and using a test with construct validity for depression-like behavior may provide a more accurate assessment of the effects of PCOS models.

### **PNA model**

In the current study, our PNA model resulted in a narrow change in anxiety-like behavior that was not in the anticipated direction and a change in depression-like behavior that was contrary to prior findings. The PNA model also resulted in increases in anogenital distance and qualitative differences in sexual maturity as measured by vaginal redness, indicating that physiological changes were associated with the prenatal treatment. Future studies might consider more thoroughly evaluating the redness and vaginal fusion present in PNA mice. Alternatively, another measure related to sexual maturity is the estrous cycle, which can provide biological information about the hormones present at different stages of the cycle (Caligioni, 2009). For example, Caldwell et al. (2014) reported that PNA mice completed fewer estrous cycles. Anogenital distance is another revealing measure used in PCOS models because female rodents treated with testosterone should show longer anogenital phenotypes (Wu et al., 2017) as observed in the current study.

As a model of PCOS, prenatal treatments have been well studied in regard to physiological phenotypes in animal models, while investigations of anxiety- and depression-like behavior are largely understudied. Thus, it may also be important to include estrous cycle information in order to ensure hormonal changes are not impacting behavior on tests like the EZM or forced swim test. Meziane, Ouagazzal, Aubert, Wietrzyk, and Krezel (2007) used a battery of behavioral tests to compare strain and estrous cycle differences on behavior and found their largest differences on a despair-like behavioral test similar to the forced swim test. In particular, C57BL/6J mice showed increased immobility on the despair-like behavioral test while in the stage of metestrous. Thus, potential effects of estrous cycle stage on behavioral measures may be especially important to evaluate in a PCOS model, which is known to impact hormone regulation and estrous cycle. Measuring estrus cycle could also help to identify the acyclicity often found in models of PCOS. Ovarian measures (i.e., ovarian weight and presence of cysts) are also commonly reported as physiological properties of PCOS models (i.e., Caldwell et al., 2014; Walters et al., 2012) and future research could include ovarian measures to evaluate prenatal PNA models for both behavior and physiological changes.

### **PNA and Stress**

As mentioned previously, prenatal exposure to DHT as a model for PCOS resulted in narrow and contradicting differences in behavior on the EZM; as such, it is interesting to consider the interplay of the PNA and CORT treatments. Ressler et al. (2015) discussed how DHT treatment resulted in a dampened CORT response during novel environment exposure. That finding was not replicated in the current study, because if PNA were to dampen reactivity to CORT, then it would be expected that

CORT pellet implantation in DHT-treated animals would result in increased time spent in the open arms due to less fear or anxiety. These scenarios present the plausibility that the complex interaction between CORT and PNA treatment is more complex than a test such as the EZM can unmask. Moreover, an important aspect in understanding the interplay of PNA and CORT is to analyze CORT serum levels. Performing this serum analysis might help in understanding the difference in CORT reactivity in models of PCOS.

Behavioral findings from our prenatal PCOS model support further study into behavioral differences using other anxiety- and depression-like tests. It is likely that the depression experienced in women with PCOS arises from hormonal dysfunction and alterations to the HPA axis which have been found in some people with depression (Varghese & Brown, 2001). In particular, the dexamethasone suppression test (i.e., Carroll et al., 1982) show that a subset of individuals with depression respond abnormally to administration of the synthetic cortisol and do not decrease their own endogenous cortisol production. Importantly, depression resulting from dysfunctional neuroendocrine systems has not been validated in classical behavioral tests like the forced swim test which should be a consideration when selecting behavioral tests in PCOS rodent models.

Genetic factors have been found that influence the connection between stress and mental illness. A longitudinal study by Caspi et al. (2003) found that some participants with depression also had a serotonin transporter allele variant that altered the functioning of serotonin, a neurotransmitter related to depression. In their study, life stress events were measured and correlated with the allele variant, and the authors concluded that the interaction of stress with this genetic polymorphism enhanced the development of mental illness (Caspi et al., 2003). In a meta-analysis evaluating depression and stress, Karg,

Burmeister, Shedden, and Sen (2011) found strong connections between the same allele described by Capsi et al. (2003) and stress and depression. Thus, genetic variability among women with PCOS or between animal strains used in PCOS models may influence the appearance of anxiety- or depression-like behaviors.

If the depression observed in women with PCOS results from an underlying physiological basis rather than from sociocultural stressors, then hormone alterations may underpin depression in these women and classic behavioral tests for depression-like behavior in mice may not be sensitive models for this type of depression. To note, the tests used in the current research do have predictive validity in pharmaceutical research but, as a result, may not have generalizability when applied to circumstances other than that associated with monoamine-altering antidepressants (Petit-Demouliere, Chenu, & Bourin, 2005). As mentioned previously, other tests such as the sucrose preference test could add complexity and accuracy to the depression-like behaviors measured in rodent models.

### **Validating the Physiological Model**

This study is in agreement with previous reports that body weight does not increase in PNA mice either prior to weaning or during young adulthood (i.e., Caldwell et al., 2014; Roland et al., 2010; Walters et al., 2012), although these findings do contrast with a report from Caldwell et al. (2014) of increased weight gain in a postnatal model of PCOS. For example, in examining animals at 3, 4, and 5 months of age (i.e., later ages than measured in the current study), Roland and colleagues (2010) did not report weight differences in a prenatal PCOS model. One explanation for the lack of weight differences in prenatal PCOS models may be based on reabsorption that occurs in rodents to optimize

survival of offspring. Jafari et al. (2017) examined reabsorption rates in C57Bl/6J mice exposed to an auditory stress and found dams to have higher levels of reabsorption and fewer birthed pups. If the prenatal DHT treatment were a perceived stressor, either physiologically or environmentally, those mice of a lower fetal weight may have undergone reabsorption, with the remaining offspring displaying higher resiliency to environmental or physiological stressors. As we see a trend towards smaller DHT litters this could be an important consideration.

CORT treatment decreased body weight in the current study. Because stress responses involve the body diverting energy to the sympathetic system and away from functions like building body weight, it would be expected that subjects in the stress condition weighed less than controls (Divyashree & Yajurvedi, 2016) although a lack of body weight differences following CORT pellet implantation has been reported (Sturm et al., 2015). CORT serum level analysis will be undertaken on samples collected during the current study and are expected to confirm higher CORT levels in those mice implanted with CORT pellets as observed by Sturm and colleagues' (2015). Future research should verify serum CORT levels from pellets. Understanding and measuring these specific changes are necessary for further validating the CORT treatment.

### **Limitations**

Several limitations to this study were identified upon completion. There were a restricted number of prenatal saline subjects due to small litter sizes in saline-treated dams. Due to multiple within subject ANOVA analyses, inherent type I error is probable (i.e., false-positive) and explains the marginal change in decreased anxiety-like behavior in PNA mice at minute 4 on the EZM. This difference in anxiety-like behavior could be

produced by this false-positive result. Nonetheless, the ANOVA tests were used appropriately and the type I error or alpha inflation is a result of the test itself. Large differences in scores were also found in minute 4 of the elevated zero maze. Another limitation occurred due to difficulties in coding the videos from the forced swim test due to low room lighting, which were reduced because Huang, Zhou, and Zhang (2012) identified changes in anxiety-like behavior and performance on the Morris Water Maze in reaction to bright lighting conditions. Based upon these prior findings and in order to prevent overhead lights from altering behavior, the light in our testing room was decreased which, unfortunately, resulted in poor video quality for the forced swim test.

### **Future research**

Several suggestions regarding future research have already been made. To reiterate, for the EZM, future research should consider including coding exploratory behaviors for a potentially more robust measure of anxiety-like behavior. As for the forced swim test and depression-like behavior, it may be important to include other behavioral assays; for example, the sucrose preferences test. As well, the importance of measuring neuronal tissue in relation to behavioral changes would be an important physiological end point. The confirmation of vaginal redness as a measure of vaginal opening in PNA mice would also be important, as would measures of estrous cycle and ovarian morphology for validating the PNA model. Furthermore, estrous cycle measures may be important in enhancing our understanding of behavior variations on the EZM and/or forced swim test and could potentially allow us to unmask differences due to prenatal or postnatal treatments.

Another important area of PCOS research, which remains unexplored, involves examining the impact of prenatal DHT treatment on male offspring behavior. Only female offspring were used in the current study; however, there may be changes to PNA males as well. Male offspring from the treated dams were also run on behavioral testing and analysis will be done in the future. Elevated *in utero* testosterone was shown, in a study comparing women with and without PCOS, to increase androstenedione in male and female offspring (Daan, Koster, Steegers-Theunissen, Eijkemans, & Fauser, 2017). Moreover, offspring from women with PCOS showed that elevated levels of testosterone impacted androgen hormone levels in their offspring. A related study found that male rats exposed to prenatal testosterone showed increased hypertension features later in life (Chinnathambi, Balakrishnan, Yallampalli, & Sathishkumar, 2012). Male offspring that have experienced an elevated testosterone environment *in utero* also demonstrate higher levels of testosterone in adulthood when compared to controls (as cited in Chinnathambi et al., 2012). Lastly, in a study using male sheep as a model of elevated *in utero* testosterone, altered responsiveness of hormones for gonadal development and regulation was found (i.e., gonadotropin-releasing hormone; Recabarren et al., 2012). These findings indicate that male reproductive systems show abnormalities following elevated prenatal testosterone, and, thus, it may be useful to explore behavioral changes in males exposed to our prenatal PCOS model.

Studies on carryover effects in behavioral testing have yielded mixed results across various tests and rodent models. Blokland et al. (2012) found that the forced swim test was more sensitive than the EZM to testing history in rats, which would indicate that the opposite order should have been employed in the current study. However, the forced

swim test was the only assay for which Blokland et al. controlled the ordering of testing, limiting the applicability of their findings. Future research may find differences if they staggered the order of EZM testing. Alternatively, testing order may not have impacted behavior in the current study; McIlwain et al., (2001) found that mice showed similar behavior in the open-field test regardless of testing history. Given that either our prenatal or postnatal manipulations may have altered animal sensitivity to the behavioral tests, it may be useful to investigate order of testing effects in future PCOS research.

## **Conclusion**

The prenatal model of PCOS used in the current research attempted to examine changes in anxiety- and depression-like behaviors. In addition to the standard prenatal model of PCOS, a stress condition was induced to more accurately model the human experience of PCOS. Behavioral tests were used to analyze differences due to the prenatal model of PCOS and the postnatal stress manipulation. Behavioral differences were found in both the EZM and forced swim test, with decreased anxiety-like behavior found in one of the five minutes of testing on the EZM and decreased depression-like behavior found in PNA as compared to prenatal saline mice. Differences were also found in physiological measures, including anogenital distance, body weight, and vaginal redness that are consistent with what was expected for our prenatal and postnatal treatments. Modeling PCOS in animal models is crucial to creating a better understanding of the etiology, biological mechanisms, and potential treatments for this syndrome. Women with PCOS suffer from various metabolic and reproductive difficulties, including increased risk of diabetes, obesity, infertility, and ovarian cysts. Not only do women with PCOS suffer from physical symptoms associated with the disorder but they also have an



increased risk for developing mental illness. Thus, it remains essential to research the connections between PCOS and mental health in order to create better treatments.

#### References

- Abbott, D. H., Tarantal, A. F., & Dumesic, D. A. (2009). Fetal, infant, adolescent and adult phenotypes of polycystic ovary syndrome in prenatally androgenized female rhesus monkeys. *American Journal of Primatology*, 71, 776-784. doi: 10.1002/ajp.20679
- Annagur, B. B., Tazegul, A., Uguz, F., Kerimoglu, O. S., Tekinarslan, E., & Celik, C. (2013). Biological correlates of major depression and generalized anxiety disorder in women with polycystic ovary syndrome. *Journal of Psychosomatic Research*, 74, 244-247. doi: 10.1016/j.jpsychores.2013.01.002
- Amiri, M., Golsorkhtabaramiri, M., Esmailzadeh, S., Ghofrani, F., Bijani, A., Ghorbani, L., & Delavar, M. A. (2014). Effect of metformin and flutamide on anthropometric indices and laboratory tests in obese/overweight PCOS women under hypocaloric diet. *Journal of Reproductive Infertility*, 15(4), 205-213.
- Barth, J. H., Field, H. P., Yasmin, E., & Balen, A. H. (2010). Defining hyperandrogenism in polycystic ovary syndrome: Measurement of testosterone and androstenedione by liquid chromatography-tandem mass spectrometry and analysis by receiver operator characteristic plots. *European Journal of Endocrinology*, 162(3), 611-615. doi: 10.1530/EJE-09-0741
- Bell, R., Duke, A. A., Gilmore, P. E., Page, D., & Begue, L. (2014). Anxiolytic-like effects observed in rats exposed to the elevated zero-maze following treatment

- with 5-HT<sub>2</sub>/5-HT<sub>3</sub>/5-HT<sub>4</sub> ligand. *Scientific Reports*, 4(3881), 1-7. doi: 10.1038/srrep03881
- Benson, S., Arck, P. C., Tan, S., Hahn, S., Mann, K., Rifaie, N., ... Elsenbruch, S. (2009). Disturbed stress responses in women with polycystic ovary syndrome. *Psychoneuroendocrinology*, 34, 727-735. doi: 10.1016/j.psyneuen.2008.12.001
- Blokland, A., ten Oever, S., van Gorp, D., van Draanen, M., Schmidt, T., Nguyen, E., ... Klinkenberg, I. (2012). The use of a test battery assessing affective behavior in rats: Order effects. *Behavioural Brain Research*, 228, 16-21. doi: 10.1016/j.bbr.2011.11.042
- Boersma G. J., & Tamashiro, K. L. (2015). Individual differences in the effects of prenatal stress exposure in rodents. *Neurobiology of Stress*, 1, 100-108. doi: 10.1016/j.ynstr.2014.10.006
- Caldwell, A. S. L., Middleton, L. J., Jimenez, M., Desai, R., McMahon, A. C., Allan, C. ... Walters, K. A. (2014). Characterization of reproductive, metabolic, and endocrine features of polycystic ovary syndrome in female hyperandrogenic mouse models. *Endocrinology*, 155(8), 3146-3159. doi:10.1210/en.2014-1196
- Caligioni, C. (2009). Assessing reproductive status/stages in mice. *Current Protocols in Neuroscience*, Appendix 4, Appendix 4I. doi: 10.1002/0471142301.nsa04is48
- Capsi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., ... Poulton, R. (2003). Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science*, 18, 386-389. doi: 10.1126/science.1083968
- Carroll, B. J. (1982). The dexamethasone suppression test for melancholia. *British Journal of Psychiatry*, 140, 292-304.

- Castagne, V., Moser, P., Roux, S., & Porsolt, R. D. (2011). Rodent models of depression: Forced swim and tail suspension behavioral despair tests in rats and mice. *Current Protocols in Neuroscience*, 55(1), 1-8. doi: 10.1002/0471142301.ns0810as55
- Ching, H. L., Burke, V., & Stuckey, B. G. A. (2007). Quality of life and psychological morbidity in women with polycystic ovary syndrome: Body mass index, age and the provision of patient information are significant modifiers. *Clinical Endocrinology*, 66(3), 373-379. doi: 10.1111/j.1365-2265.2007.02742.x
- Chinnathambi, V., Balakrishnan, M., Yallampalli, C., & Sathishkumar, K. (2012). Prenatal testosterone exposure leads to hypertension that is gonadal hormone dependent in adult rat male and female offspring. *Biology of Reproduction*, 86(5), 1-7. doi: 10.1095/biolreprod.111.097550
- Cwikel, J., Gidron, Y., & Sheiner, E. (2004). Psychological interactions with infertility among women. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, 117, 126-131. doi: 10.1016/j.ejogrb.2004.05.004
- Daan, N. M. P., Koster, M. P. H., Steegers-Theunissen, R. P., Eijkemans, M. J., & Fauser, B. C. J. M. (2017). Endocrine and cardiometabolic cord blood characteristics of offspring born to mother with and without polycystic ovary syndrome. *Fertility and Sterility*, 107(1), 261-268.
- de Visser, L., van den Bos, R., Kuurman, W. W., Kas, M. J. H., & Spruijt, B. M. (2006). Novel approach to the behavioural characterization of inbred mice: Automated home cage observations. *Genes, Brain and Behavior*, 5, 458-466. doi: 10.1111/j.1601-183X.2005.00181.x

- Dinan, T. G. (1994). Glucocorticoids and the genesis of depressive illness: A psychobiological model. *British Journal of Psychiatry*, 164, 365-371.
- Divyashree, S., & Yajurvedi, H. N. (2016). Long-term chronic stress exposure induces PCO phenotype in rat. *Reproduction*, 152, 765-774. doi: 10.1530/REP-16-0404
- Dokras, A., Clifton, S., Futterweit, W., & Wild, R. (2011). Increased risk for abnormal depression scores in women with polycystic ovary syndrome. *Obstetrics & Gynecology*, 117(1), 145-152. doi: 10.1097/AOG.0b013e318202b0a4
- Duman, R. S., Aghajanian, G. K., Sanacora, G., & Krystal, J. H. (2015). Synaptic plasticity and depression: New insights from stress and rapid-acting antidepressants. *Nature Medicine*, 22(3), 238-249. doi: 10.1038/nm.4050
- Foecking, E. M., McDevitt, M. A., Acosta-Martinez, M., & Horton, T. H. (2008). Neuroendocrine consequences of androgen excess in female rodents. *Hormones and Behavior*, 53(5), 673-692.
- Glueck, C. J., Dharashivkar, S., Wang, P., Zhu, B., Gartside, P. S., Tracy, T., & Sieve, L. (2005). Obesity and extreme obesity, manifest by ages 20-24 years, continuing through 32-41 years in women, should alert physicians to the diagnostic likelihood of polycystic ovary syndrome as a reversible underlying endocrinopathy. *European Journal of Obstetrics, Gynecology, and Reproductive Biology*, 122(2), 206-212. doi: 10.1016/j.ejogrb.2005.03.010
- Golden, S. A., Covington, H. E., Berton, O., & Russo, S. J. (2011). A standardized protocol for repeated social defeat stress in mice. *National Protocol*, 6(8), 1183-1191. doi:10.1038/nprot.2011.361

- Goldstein, D. S. & Kopin, I. J. (2009). Evolution of concepts of stress. *The International Journal of the Biology of Stress*, 10(2), 109-120. doi: 10.1080/10253890701288935
- Goodarzi, M. O., Dumesic, D. A., Chazenbalk, G., & Azziz, R. (2011). Polycystic ovary syndrome: Etiology, pathogenesis and diagnosis. *Nature Reviews Endocrinology*, 7(4), 219-231. doi: 10.1038/nrendo.2010.217
- Graeff, F. G., Guimaraes, F. S., De Andrade, T. G. C. S., & Deakin, J. F. W. (1996). Role of 5-HT in stress, anxiety, and depression. *Pharmacology Biochemistry and Behavior*, 54(1), 129-141.
- Hahn, S., Janssen, O. E., Tan, S., Pleger, K., Mann, K., Schedlowski, M., ... Elsenbruch, S. (2005). Clinical and psychological correlates of quality-of-life in polycystic ovary syndrome. *European Journal of Endocrinology*, 153, 853-860. doi: 10.1530/eje.1.02024
- Herrmann, M., Henneicke, H., Street, J., Modzelewski, J., Kalak, R., Buttgereit, F., ... Seibel, M. J. (2009). The challenge of continuous exogenous glucocorticoid administration in mice. *Steroid*, 74(2), 245-249. doi: 10.1016/j.steroids.2008.11.009
- Huang, Y., Zhou, W., & Zhang, Y. (2012). Bright lighting conditions during testing increases thigmotaxis and impair water maze performance in BALB/6 mice. *Behavioural Brain Research*, 226(1), 26-31. doi: 10.1016/j.bbr.2011.08.043
- Hung, J., Hu, L., Tsai, S., Yang, A. C., Huang, M., Chen, P., ... Shen, C. (2014). Risk of psychiatric disorders following polycystic ovary syndrome: A nationwide

- population-based cohort study. *PLoS ONE*, 9(5), 1-6. doi: 10.1371/journal.pone.0097041
- Hu, M., Richard, J. E., Maliqueo, M., Kokosar, M., Fornes, R., Benrick, A., ... Stener-Victorin, E. (2015). Maternal testosterone exposure increases anxiety-like behavior and impacts the limbic system in the offspring. *PNAS*, 112(46), 14348-14353. doi: 10.1073/pnas.1507514112
- Jafari, Z., Faraji, J., Agha, B. M., Metz, G. A. S., Kolb, B. E. & Mohajerani, M. H. (2017). The adverse effects of auditory stress on mouse uterus receptivity and behaviour. *Scientific Reports*, 7, 1-11. doi: 10.1038/s41598-017-04943-8
- Jedel, E., Waern, M., Gustafson, D., Landen, M., Eriksson, E., Holm, G., ... Stener-Victorin, E. (2010). Anxiety and depression symptoms in women with polycystic ovary syndrome compared with controls matched for body mass index. *Human Reproduction*, 25(2), 450-456. doi: 10.1093/humrep/dep384
- Joham, A. E., Teede, H. J., Ranasinha, S., Zoungas, S., & Boyle, J. (2015). Prevalence of infertility and use of fertility treatment in women with polycystic ovary syndrome: Data from a large community-based cohort study. *Journal of Women's Health*, 24(4), 299-307. doi: 10.1089/jwh.2014.5000
- Karg, K., Burmeister, M., Shedden, K., & Sen, S. (2011). The serotonin transporter promoter variant (5-HTTLPR), stress, and depression meta-analysis revisited: Evidence of genetic moderation. *Archives of General Psychiatry*, 68(5), 444-454. doi: 10.1001/archgenpsychiatry.2010.189
- Kim, J. Y., Yang, S. H., Kwon, J., Lee, H. W., & Kim, H. (2017). Mice subjected to uncontrollable electric shocks show depression-like behaviors irrespective of their

- state of helplessness. *Behavioural Brain Research*, 322, 138-144. doi: 10.1016/j.bbr.2017.01.008
- March, W. A., Moore, V. M., Willson, K. J., Phillips, D. I. W., Norman, R. J., & Davies, M. J. (2010). The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. *Human Reproduction*, 25(2), 544-551. doi: 10.1093/humrep/dep399
- Mastorakos, G., Pavlatou, M. G., & Mizamtsidi, M. (2006). The hypothalamic-pituitary-adrenal and the hypothalamic-pituitary-gonadal axes interplay. *Pediatric Endocrinology Reviews*, 3(1), 172-181.
- Matto, V., Harro, J., & Allikmets, L. (1997). The effects of cholecystokinin A and B receptor antagonists on exploratory behavior in the elevated zero-maze in rat. *Neuropharmacology*, 36(3), 389-396.
- McIlwain, K. L., Merriweather, M. Y., Yuva-Paylor, L. A., & Paylor, R. (2001). The use of behavioral test batteries: Effects of training history. *Physiology & Behavior*, 73, 705-717. doi: 10.1016/S0031-9384(01)00528-5
- Menke, A., & Binder, E. B. (2014). Epigenetic alterations in depression and antidepressant treatment. *Dialogues in Clinical Neuroscience*, 16(3), 395-404.
- Meziane, H., Ouagazzal, A. M., Aubert, L., Wietrzyk, M., & Krezel, W., (2007). Estrous cycle effects on behavior of C57BL/6J and BALB/cByJ female mice: Implications for phenotyping strategies. *Genes, Brain, and Behavior*, 6(2), 192-200. doi: 10.1111/j.1601-183X.2006.00249.x

- Molendijk, M. L., & de Kloet, E. R. (2015). Immobility in the forced swim test is adaptive and does not reflect depression. *Psychoneuroendocrinology*, 62, 389-391. doi: 10.1016/j.psyneuen.2015.08.028
- Molewijk, H. E., van der Poel, A. M., & Olivier, B. (1995). The ambivalent behavior 'stretched approach posture' in the rat as a paradigm to characterize anxiolytic drugs. *Psychopharmacology*, 121(1), 81-90. doi: 10.1007/BF02245594
- Moran, L. J., Misso, M. L., Wild, R. A., & Norman, R. J. (2010). Impaired glucose tolerance, type 2 diabetes and metabolic syndrome in polycystic ovary syndrome: A systematic review and meta-analysis. *Human Reproduction Update*, 16(4), 347-363. doi: 10.1093/humupd/dmq001
- Nester, E. J. (2014). Epigenetic mechanism of depression. *JAMA Psychiatry*, 71(4), 454-456. doi: 10.1001/jamapsyhiatry.2013.4291
- Page, M. E., Detke, M. J., Dalvi, A., Kirby, L. G., & Lucki, I. (1999). Serotonergic mediation of the effects of fluoxetine, but not desipramine, in the rat forced swimming test. *Psychopharmacology*, 147, 162-167.
- Petit-Demouliere, B., Chenu, F., & Bourin, M. (2005). Forced swimming test in mice: A review of antidepressant activity. *Psychopharmacology*, 177, 245-255. doi: 10.1007/s00213-044-2048-7
- Rasgon, N. L., Alshuler, L. L., Fairbanks, L., Elman, S., Bitran, J., Labarca, R., ... Mintz, J. (2005). Reproductive function and risk for PCOS women treated for bipolar disorder. *Bipolar Disorders*, 7, 246-259. doi: 10.1111/j.1399-5618.2005.00201.x
- Rassi, A., Veras, A. B., dos Reis, M. D., Pastore, D. L., Bruno, L. M., Bruno, R. V., ... Nardi, A. E. (2010). Prevalence of psychiatric disorders in patients with



- polycystic ovary syndrome. *Comprehensive Psychiatry*, 51, 599-602. doi: 10.1016/j.comppsy.2010.02.009
- Recarbarren, S. E., Lobos, A., Figueroa, Y., Padmanabhan, V., Foster, D. L., SirPetermann, T. (2007). Prenatal testosterone treatment alters LH and testosterone responsiveness to GnRH agonist in male sheep. *Biological Research*, 40, 329-338.
- Ressler, I. B., Grayson, B. E., Ulrich-Lai, Y. M., & Seeley, R. J. (2015). Diet-induced obesity exacerbates metabolic and behavioral effects of polycystic ovary syndrome in a rodent model. *American Journal of Endocrinology Metabolism*, 308, 1076-1084. doi: 10.1152/ajpendo.00182.2014
- Rodgers, R. J., Cao, B.-J., Dalvi, A., & Holmes, A. (1997). Animal models of anxiety: An ethological perspective. *Brazilian Journal of Medical and Biological Research*, 30(3), 289-304.
- Rodriguez, I., Araki, K., Khatib, K., Martinou, J.-C., & Vassalli, P. (1997). Mouse vaginal opening is an apoptosis-dependent process which can be prevented by the overexpression of Bcl2. *Developmental Biology*, 184, 115-121.
- Roland, A. V., Nunemaker, C. S., Keller, S. R., & Moenter, S. M. (2010). Prenatal androgen exposure programs metabolic dysfunction in female mice. *Journal of Endocrinology*, 207, 213-223. doi: 10.1677/JOE-10-0217
- Sahores, A., Luque, G. M., Wargon, V., May, M., Molinolo, A., Becu-Villalobos, D., ... Lamb, C. A. (2013). Novel, low cost, highly effective, handmade steroid pellets for experimental studies. *PLoS One*, 8(5). doi: 10.1371/journal.pone.0064049

- Schneiderman, N., Ironson, G., Siegel, S. D. (2005). Stress and health: Psychological, behavioral, and biological determinants. *Annual Review of Clinical Psychology*, 1, 607-628. doi: 10.1146/annurev.clinpsy.1.102803.144141
- Schober, J., Dulabon, L., Martin-Alguacil, N., Kow, L.-M., & Pfaff, D. (2006). Significance of topical estrogens to labial fusion and vaginal introital integrity. *Journal of Pediatric Adolescent Gynecology*, 19, 337-339. doi: 10.1016/j.jpog.2006.06.004
- Shepherd, J. K., Maidenhead, E., Grewal, S. S., Fletcher, A., Bill, D. J., & Dourish, C. T. (1994). Behavioural and pharmacological characterization of the elevated 'zero-maze' as an animal model of anxiety. *Psychopharmacology*, 116(1), 56- 64. doi: 10.1007/BF02244871
- Snyder, J. S., Soumier, A., Brewer, M., Pickel, J., & Cameron, H. A. (2012). Adult hippocampal neurogenesis buffers stress responses and depressive behavior. *Nature*, 476(7361), 458-461. doi: 10.1038/nature10287
- Staufenbiel, S. M., Penninx, B. W. J. H., Spijker, A. T., Elzinga, B. M., & van Rossum, E. F. C. (2013). Hair cortisol, stress exposure, and mental health in humans: A systematic review. *Psychoneuroendocrinology*, 38(8), 1220-1235. doi: 10.1016/j.psyneuen.2012.11.015
- Sturm M., Becker, A., Schroeder, A., Bilkei-Gorzo, A., & Zimmer, A. (2015). Effect of chronic corticosterone application on depression-like behavior in C57BL/6N and C57BL/6J mice. *Genes, Brain and Behavior*, 14, 292-300. doi: 10.1111/gbb.12208

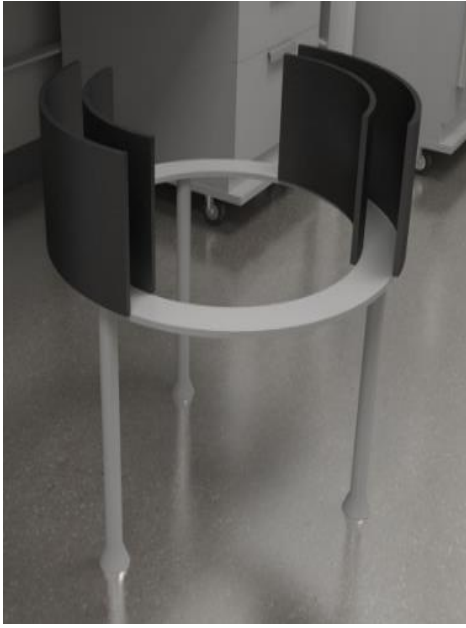
- Talbott, E. O., Zborowski, J. V., Rager, J. R., Kip, K. E., Xiaohui, X., & Orchard, T. J. (2007). Polycystic ovarian syndrome (PCOS): A significant contributor to the overall burden of type 2 diabetes in women. *Journal of Women's Health, 16*(2), 191-197. doi: 10.1089/jwh.2006.0098
- Tian, L., Shen, H., Lu., Q., Norman, R. J., & Wang, J. (2007). Insulin resistance increase the risk of spontaneous abortion after assisted reproduction technology treatment. *The Journal of Clinical Endocrinology and Metabolism, 92*(4), 1430-1433. doi: 10.1210/je.2006-1123
- Varghese, F. P., & Brown, E. S. (2001). The hypothalamic-pituitary-adrenal axis in major depression disorder: A brief primer for primary care physicians. *Primary Care Companion Journal of Clinical Psychiatry, 3*(4), 151-155.
- Voikar, V., Vasar, E., & Rauvala, H. (2004). Behavioral alterations induced by repeated testing in C57BL/6J and 129S2/Sv mice: Implications for phenotyping screens. *Genes, Brain, and Behavior, 3*(1), 27-38. doi: 10.1046/j.1601-183X.2003.0044x
- Walters, K. A., Allan, C. M., & Handelsman, D. J. (2012). Rodent models for human polycystic ovary syndrome. *Biology of Reproduction, 86*(5), 1-12. doi: 10.1095/biolreprod.111.097808
- Weber, E. M., Hultgren, J., Algers, B., & Olsson, A. S. (2016). Do laboratory mouse females that lose their litters behave differently around parturition? (2016). *PLOS One, 11*. doi: 10.1371/journal.pone.0161238
- Whirledge, S. & Cidlowski, J. A. (2010). Glucocorticoids, stress, and fertility. *Minerva Endocrinologica, 35*(2), 109-125.

- Wu, X. Y., Li, Z. L., Wu, C. Y., Liu, Y. M., Lin, H., Wang, S. H., & Xiao, W. F. (2010). Endocrine traits of polycystic ovary syndrome in prenatally androgenized female Sprague-dawley rats. *Endocrine Journal*, 57(3), 201-209. doi: 10.1507/endocrj.K09E-205
- Wu, Y., Zhong, G., Chen, S., Zheng, C., Liao, D., & Xie, M. (2017). Polycystic ovary syndrome is associated with anogenital distance, a marker of prenatal androgen exposure. *Human Reproduction*, 32(4), 937-943. doi: 10.1093/humrep/dex042
- Yan, H.-C., Cao, X., Das, M., Zhu, X.-H., & Gao, T.-M. (2010). Behavioral animal models of depression. *Neuroscience Bulletin*, 26(4), 327-337. doi: 10.1007/s12264-010-0323-7
- Yu, Q., Hao, S., Wang, H., Shen, Q., & Kang, J. (2016). Depression-like behavior in a dehydroepianodrosterone-induced mouse model of polycystic ovary syndrome. *Biology of Reproduction*, 95(4), 1-10. doi: 10.1095/biolreprod.116.142117

## Appendix A

### Behavioral Assays Used for Depression- and Anxiety-like Behavior

Elevated zero maze



Forced swim test

