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Maternal estrogen exposure may be linked to an increased risk of autism spectrum disorder

Sarah Bunker

Trinity College, Hartford Connecticut, sarah.bunker@trincoll.edu

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**MATERNAL ESTROGEN EXPOSURE MAY BE LINKED TO AN INCREASED
RISK OF AUTISM SPECTRUM DISORDER**

BY

Sarah N. Bunker

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**MATERNAL ESTROGEN EXPOSURE MAY BE LINKED TO AN INCREASED RISK
OF AUTISM SPECTRUM DISORDER**

BY

Sarah N. Bunker

Honors Thesis Committee

Thesis Advisor: Molly Helt, Ph.D.

Thesis Committee: Luis Martinez, Ph.D.

Director, Neuroscience Program: Sarah Raskin, Ph.D.

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ABSTRACT:

The current study explored the possibility that maternal estrogen dominance serves as a risk factor for having a child with autism. An online survey was sent to both biological mothers of autistic children (n=253) and biological mothers of non-autistic children (n=221). The survey presented a series of questions pertaining to both endogenous and exogenous factors and exposures that could increase estrogen levels. The data on exogenous exposures is treated in another paper (Helt, Bocobo, Bunker, & Lasky, in progress). The current paper presents the findings on exogenous maternal estrogen exposure and autism risk (e.g., previous history of trauma, stress, diet, & toxin exposure). Mothers of children with ASD reported higher consumption of non-hormone free animal products, reported putting more endocrine mimicking chemicals on their bodies daily than control mothers, and reported having “very high” levels of stress during their pregnancies compared to control mothers. Furthermore, mothers of children with ASD reported a higher incidence of traumatic events during their pregnancies, specifically during the pregnancy with their child that received an ASD diagnosis, and a higher lifetime incidence of PTSD. Overall, there are significant differences related to maternal estrogen exposure between the pregnancies of mothers with and without a child with ASD. These results suggest that future research should investigate the possibility of a direct link between maternal estrogen exposure and autism risk.

INTRODUCTION:

Autism Spectrum Disorder (ASD) is one of the most severe and fastest-growing developmental disabilities in the United States (CDC, 2016). Autism is characterized by a triad of core symptoms: social impairment, verbal and non-verbal language impairment, and repetitive/stereotyped activities (Figure 1) (CDC, 2016). As of 2010, there was a worldwide prevalence of ASD of 0.1%, with an apparent ten-fold increase in prevalence over the past four decades (CDC, 2016). This increase raises the question of whether there is a change in environmental exposures that may relate to the reported increase in children with ASD. Furthermore, there is an estimated 1 in 68 children on the autism spectrum with approximately 1 in 42 boys diagnosed and 1 in 189 girls diagnosed in the United States (CDC, 2016). This accounts for a roughly five times higher incidence in males than females (CDC, 2016), raising the question of whether sex hormones may be involved in the development of ASD.

Although molecular studies have been performed to account for the genetic basis of ASD, research into the biological causes have yet to yield conclusive results. This is likely because children with ASD are heterogeneous and symptoms are not well mapped regarding neurotransmitter activity or brain scans. However, recent work has implicated hormones in the etiology of ASD. During pregnancy, the mother's placenta acts as a protection system for the fetus, only allowing diffusion of certain gases and nutrients (Bowen & Hunt, 2000). The placenta also acts as an endocrine organ, producing both steroid and peptide hormones (Bowen & Hunt, 2000). Steroid hormones, such as androgens, estrogens, and progestins, are then able to readily pass the blood-brain barrier and impact fetal brain development (Figure 2) (Bowen & Hunt, 2000).

Hormones & Autism Risk

In relation to the possibility of hormones being implicated in the manifestation of ASD, an androgen theory of autism was proposed by Baron-Cohen and colleagues. This theory suggests that autism is a manifestation of an “extreme male brain,” accounting for the 5:1 ratio seen between males and females on the autism spectrum (Baron-Cohen et al., 2011). The proposed “extreme male brain” is an extension of typical sex differences found in the Empathizing-Systemizing theory (Baron-Cohen et al., 2011). Typically developing females tend to have higher empathy scores, whereas typically developing males tend to have higher systemization scores (Auyeung et al., 2009).

The Extreme Male Brain (EMB) hypothesis proposes that early exposure to androgens, such as testosterone, affects brain development and produces sex differences in behavior, cognition, and function (Baron-Cohen et al., 2011). Between weeks 8 and 24 of gestation, male fetuses are exposed to a significant surge in fetal testosterone levels to the point that pubertal levels are almost reached (Baron-Cohen et al., 2011). Typically developing fetuses are exposed to normal levels of testosterone *in utero* and, therefore, have standard levels of systemization and empathy. However, when the fetus is exposed to abnormally high levels of testosterone, the risk of developing autism increases by producing hyper-masculinized brains and resulting in increased systemization and decreased empathy (Baron-Cohen, 2002). It is suggested that this surge of testosterone affects brain development and eventually leads to a decrease in social interaction and cognitive development (Baron-Cohen et al., 2011). The EMB theory is supported by previous findings that children with autism scored significantly higher on tests for systemization and well below the average level of typically-developing children in terms of empathy, supporting the proposed “hyper-masculinized profile” of autism (Auyeung et al., 2009).

Baron-Cohen's research was the first of its time in terms of investigating hormonal exposure in relation to autism occurrence. It is possible that for this reason other hormones, such as estrogen, have been overlooked and focus has predominately been on testosterone. Nevertheless, the effects of estrogen are important to examine considering estrogen levels are directly related to testosterone levels. Estradiol, the most potent member of the estrogen family, is derived via aromatization of testosterone by estradiol synthase (McCarthy, 2008). In rat studies, it has been found that sexual behavior is masculinized following administration of estradiol (McCarthy, 2008), suggesting that high levels of estrogen may also result in hyper-masculinity. In fact, it was determined that estradiol administration was more effective than testosterone at masculinizing the brains of newborn female rats (McCarthy, 2008). Considering the evidence, further research into the role of estrogen is warranted.

Estrogen & Autism Risk

Nevertheless, there may be a correlation between estrogen exposure and autism occurrence. Estrogen is a steroid hormone and a neuro-hormone that affects the development and daily functioning of the brain (Barth, Villringer, & Sacher, 2015). Estrogen, being a steroid hormone, has both the ability to pass through the placenta and modulate gene transcription in target cells. Therefore, estrogen may affect the developing fetus' DNA, providing a potential mechanism whereby estrogen could influence genes associated with ASD risk. Presently, one correlation studied between estrogen and autistic epigenetic alterations regards the β -catenin gene, which is believed to be of relevance in the development of ASD (Mbadiwe & Millis, 2013). β -catenin forms a complex with the glycogen synthase kinase 3 beta (GSK3B) gene, which is a target of the estrogen receptor alpha ($ER\alpha$) (Mbadiwe & Millis, 2013). When $ER\alpha$ is

activated by estradiol, a form of estrogen, β -catenin is released from the complex and results in an increased cytosolic concentration of β -catenin (Mbadiwe & Millis, 2013). This increase in concentration alters gene expression via the Wnt pathway during critical periods of prenatal development, and may be related to the development of ASD and other neurodevelopmental disorders (De Ferrari & Moon, 2006). Considering epigenetic associations do exist between estrogen and the gene expression of genes related to ASD, it is important to further examine the relationship.

Being a steroid hormone also allows estrogen the ability to cross the blood-brain barrier and, therefore, allows maternal estrogen levels to directly influence fetal estrogen levels (Troisi et al., 2003). Estrogen levels can be influenced by both endogenous factors, including one's body mass index (BMI) and estrogen sensitive cancers, and exogenous factors, including stress level, diet, and exposure to endocrine mimickers, such as Bisphenol-A (BPA), detergents, and creams (de Cock, Maas, & van de Bor, 2012). Regarding estrogen's influence on fetal brain development in humans, a number of papers have been cited that report findings consistent with the hypothesis that elevated maternal estrogen levels may constitute as a risk factor for the development of ASD.

Ovulation Inducing Drug Use and Autism Risk:

In 2010, a study by Lyall and colleagues reported findings of a potential link between prenatal exposure to ovulation inducing drugs (OID) and autism. OIDs work to increase the release of gonadotropin-releasing hormone (GnRH), which in turn increases the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Kumar & Sait, 2011). It is this increase in LH and FSH that induces ovulation and, as a result, alters estrogen levels in the

body (Kumar & Sait, 2011). This study compiled data from 3,985 female participants who had delivered their first child between 1993 and 2003 (Lyll, Pauls, Santangelo, Spiegelman, & Ascherio, 2010). Every two years, starting in 1993, the women self-reported their use of OIDs to counteract their history of infertility. In 2005, two years after the cut off of child births, the women were asked whether any of their children had been diagnosed with any disorder on the autism spectrum. After data analysis was completed, it was determined that 111 women reported having a child with ASD (Lyll et al., 2010). It was demonstrated that a history of infertility combined with the use of OID resulted in an almost 2-fold increased risk of having a child with ASD, with an overall trend toward a greater risk for ASD for women who reported OID use versus women who did not report any OID use (Lyll et al., 2010).

Lyll and colleagues' study was supported by a study done by Zachor, Lahat, and Itzchak in 2010. Zachor and colleagues found that in a study of 438 children with ASD, 10.2% of the mothers reported use of in vitro fertilization (IVF) (Zachor, Lahat, & Itzchat, 2010). IVF can involve the use of hormonal drugs, including some that trigger the release of estrogen. Finally, in 2011 a study was completed that connected the findings of both the Lyll and Zachor studies. This research examined the use of both OIDs and IVF and the possible link to ASD in a population of 555,828 Danish children born between the years of 1995 and 2003 (Hvidtjørn et al., 2010). In total, 33,139 children were born as a result of assisted conception and 0.68% (n = 225) were born with an ASD diagnosis (Hvidtjørn et al., 2010). This represents an increased risk of an ASD diagnosis in the subgroup of the study that was born using assisted conception (Hvidtjørn et al., 2010). More significantly, there was a higher risk for children born after OID than those born after IVF (Hvidtjørn et al., 2010), presumably because the drugs used between the two methods differ in terms of their impact on maternal estrogen release. In conclusion, these

three studies suggest that a potential link may exist between ovulation inducing drugs and having a child diagnosed with ASD.

Endocrine Disruptor Chemical Exposure and Autism Risk:

Another explored estrogen-related factor is the exposure to endocrine disruptors, such as BPA in plastics and phthalates in cosmetics and personal care products. These endocrine disrupting chemicals are known to not only mimic estrogen, but are also associated with developmental and reproductive health problems (de Cock et al., 2012). Furthermore, within the last decade, it has been discovered that the average woman in the United States puts on six different cosmetic creams and products in a single day (de Cock et al., 2012). Products that contain substances such as phthalates mimic estrogen and disrupt the normal balance of hormones within the pregnant mother's body. In a study by Weiss and colleagues published in 2009, it was reported that Swedish infants who lived in homes with vinyl floors, which can emit phthalates, are twice as likely to develop autism than children raised in homes with wood or linoleum flooring (Larsson, Weiss, Jensen, Sundell, & Bornehag, 2009). This study included 4,779 children between the ages of 6 and 8. In total, 72 of the children were diagnosed autism, 60 of which were males (Larsson et al., 2009). In support of these findings, another study was completed in 2011 by Miodovnik and colleagues that tested prenatal exposure to both BPA and phthalates in relation to the potential impact on early brain development (Miodovnik et al., 2011). In a population of 404 families, 137 of which included a child with autism, it was discovered that there was a positive association between phthalate exposure and social deficits, such as poor social cognition, communication, and awareness (Miodovnik et al., 2011). Although no significant findings of BPA exposure were identified in this study, it has been found that BPA

exposure in rodent studies is associated with aggressive behavior and increased levels of anxiety and hyperactivity (Kawai et al., 2003; Tian, Baek, Lee, & Jang, 2010).

Maternal Diet and Autism Risk:

A pregnant mother's diet may have significant implications in the development of ASD. Previous research has connected maternal diet with several mechanisms of action, including hormonal estrogen activity, the regulation of gene expression, and effects on the cell signaling pathways (Román, 2007). These effects are influenced by the consumption of polyphenols, which include flavonoids, phenolic acids, and lignans. Soy beans and leaves contain significant amounts of both lignans and flavonoids, indicating that diets high in soy derivatives are likely to increase estrogen activity and alterations in gene expression (Román, 2007). Román postulated that the most significant effect of soy ingestion is their ability to inhibit thyroperoxidase, the enzyme that catalyzes thyroid hormone biosynthesis and ultimately results in permanent alternations in the cerebral cortical structure of the fetal brain similar to those that have been seen in children with autism (Román, 2007). Similar to Román's review of diet and estrogen, it has been found that iodine deficiency and hypothyroidism promote estrogen-sensitive cell growth (King, 2011). Between the mid-first trimester and the early-second trimester, there is a critical period of development that is especially sensitive to estradiol (King, 2011). Estradiol stimulation in higher cognition brain areas can be expected to affect later-developing brain regions, thus impacting cognition in the newborn (King, 2011). Children with autism have altered sensitivity to hormones such as estrogen and testosterone (Sharpe, Gist, & Baskin, 2013), which could likely stem from the mother's consumption of foods that impact estrogen levels, such as the aforementioned lignans and flavonoids in soy.

Red meat also has an effect on hormone levels. A longitudinal study found that consumption of red meat was linked to development of estrogen-sensitive cancers, such as breast cancer (Forman & Silverstein, 2012). It has been suggested that mothers with a history of estrogen-sensitive cancers have an increased risk of having a child with autism, suggesting that red meat consumption could be related to estrogen-sensitive cancers through increased estrogen levels, and thus developmental abnormalities leading to autism (Foreman & Silverstein, 2012). Furthermore, maternal intake of fats prior to and during pregnancy may also be correlated with increased risk of autism in the subsequent generation (Lyll, Munger, O'Reilly, Santangelo, & Ascherio, 2013). In an analysis of the Nurses' Health Study II, 317 mothers reported having a child with ASD. Of the 317 mothers, it was found in Lyll and colleagues' study that mothers with a lower intake of ω -3 fatty acids had a 53% increase in risk of having a child with autism compared to mothers who were in the middle 90% distribution of ω -3 fatty acid intake (Lyll et al., 2013). ω -3 fatty acids are a type of polyunsaturated fatty acid, commonly found in fish, that alter the metabolism of sex hormones such as estrogen (Young, Raatz, Thomas, Redmon, & Kurzer, 2013). Using this information, it can be said that mothers who have a healthy intake of fatty acids have a decreased risk of having a child with autism. Each of these relevant studies suggest a significant implication of maternal diet on estrogen levels, and in relation, on autism development.

Maternal Stress and Autism Risk:

Finally, chronic maternal stress has been found to influence cell growth within the uterus and ovaries (Gunin, 1996). These changes are mediated by estrogen levels, in which there is an increase in estrogen when an individual is chronically stressed (Gunin, 1996). These findings were observed in rats, however in humans, chronic or long term stress can be rooted in sources such as experiencing trauma, high level occupational stressors, financial struggles, etc. When one is chronically stressed, their levels of estradiol are increased (Gunin, 1996). Estradiol the predominant form of estrogen found in mammals, which, as discussed earlier, may lead to development of autism in the fetus as maternal estrogen levels directly impact the estrogen levels that the fetus is exposed to. In conjunction with this finding, it has been found that women who were exposed to abuse during their childhood were at an increased risk of having a child with autism (Roberts, Lyall, Rich-Edwards, Ascherio, & Weisskopf, 2013). The higher the level of abuse, the greater the prevalence of autism was found in the subsequent generation (Roberts et al., 2013). Furthermore, maternal stress prior to pregnancy is also correlated with an increased risk of having a child with autism (Rai et al., 2013). Each of these correlations provides a larger target for future research on causality of autism occurrence.

The aforementioned research provides a wealth of background regarding exogenous estrogen exposure and autism occurrence. However, in previous studies estrogen has not been proposed as the mechanism linking these findings. Previous research did not interpret their findings as related to estrogen, it is instead our interpretation that estrogen may be the common thread that links together these findings. The research that we are expecting to conduct is novel, as we are specifically looking for a variety of indicators of the presence of estrogen dominance within the same cohort, not in individualized cases. We hypothesize that mothers of children with autism will have higher rates of estrogen-related health problems, such as increased levels

of stress, depression, anxiety, and abuse, and increased consumption and/or exposure to endocrine disruptors such as soy, BPA, and phthalates.

MATERIALS & METHODS:

To collect data for our study, the biological mothers of both autistic children and non-autistic children were recruited to complete an online survey, which posed simple multiple choice questions and short answer questions. The study was approved by the Institutional Review Board at Trinity College and conducted through the Neuroscience Program in Professor Helt's Social Learning and Developmental Laboratory. Prior to starting the online survey, a statement was provided about the purpose and length of the study, and participants could choose to either consent to continue the study, or were able to exit the study without completion. Additionally, all participants were able to end the study at any point.

Women were recruited via Connecticut listervs, specifically aimed at parents with children either with Autism Spectrum Disorder (ASD) or any other special need between the ages of 5 and 18 years old. Each mother that agreed to take the survey was then asked to recruit a friend or neighbor who had a typically developing child to complete the survey as a control. In total, 253 mothers of autistic children and 221 mothers of non-autistic children submitted the survey. To verify that there was no significant variance in demographics, the Hollingshead Index was used to measure socio-economic status (SES). It was determined that there were no significant differences in SES in mothers with and without children with ASD ($t(461) = 1.027$, $p = 0.98$). There were also no significant differences in the average age of the mothers ($M = 41.2$, $M = 42.5$) ($t(461) = 1.33$, $p = 1.03$) or age of the children ($M = 9.98$, $M = 10.5$) ($t(461) = 0.975$, $p = 0.245$).

The entirety of the online survey focused on solely the biological mother and child. Much of the beginning questions regarded the demographics of the family, including gender of the child, age of both mother and child, number of children, developmental outcome of all children, education background, and employment status. However, the depth of the survey focused on the mother's exogenous and endogenous exposures to estrogen before and during each pregnancy. In terms of endogenous factors, the survey questions the mother's menstrual cycles, use of contraceptives, history of hormone related medications and disease, and body mass index (BMI). As for exogenous exposure during pregnancy, drug use, exposure to endocrine mimicking chemicals and toxins, diet during pregnancy, and stress levels were all questioned.

Responses were excluded if the survey was not finished to completion or if the children did not fall within the appropriate age category. In addition, responses were excluded if they were completed by a father or a non-biological mother. In total, 474 responses were submitted successfully. Of these surveys, 183 of the mothers had both a child with autism and a typically-developing child, 70 mothers' only child had autism, and 221 mothers had only typically developing children.

Once the survey was completed and enough responses were collected, the categorical data was coded and analyzed using Statistical Package for the Social Sciences (SPSS) software. Chi-Square analysis was used to measure whether specific occurrences were more likely in one within each group versus another. T-tests were used to examine the variance and trends of the non-categorical data and the different averages between the two groups of data.

RESULTS:

Diet:

Women were asked to describe their level of soy consumption, as well as their consumption of animal products that included hormones (i.e. the default assumption unless the participant indicated via checking a box that they bought hormone free products). No statistical differences were found for high levels of soy consumption (> 25 grams per day) between groups ($\chi(1) = 1.35, p = 1.54$). However, more children with ASD had been exclusively fed soy formula as infants (Figure 3, $n = 5$ vs. $n = 1$), ($\chi(1) = 5.23, p = 0.04$). In addition, mothers of children with ASD reported higher average daily consumption of non-hormone free animal products (76g/day vs. 59g/day), compared with mothers of non ASD children (Figure 4, $t(461) = -2.29, p = 0.05$).

Drug Use:

Within the group of mothers of children with ASD, mothers were no more likely to have used OIDs to conceive their child with ASD as they were to have conceived their typically developing children ($\chi(1) = 1.89, p = 0.22$). Between groups, mothers of children with ASD were no more likely to have used OIDs than mothers of typically developing children and children with other disabilities ($\chi(1) = 1.039, p = 0.44$). There was also no difference between groups on ingestion of marijuana prior to or during pregnancy ($\chi(1) = 0.967, p = 0.10$).

Toxin Exposure:

Women were asked to list the creams, products, shampoos, lotions, make-ups, etc. that they use on a daily basis. Unless they checked a box indicating that they make an attempt to buy phthalate free product, it was assumed that the products contained phthalates. The average number of potentially endocrine mimicking chemicals the mothers of children with ASD put on

their bodies on a daily basis (6.7) was greater than the average number of potentially endocrine mimicking chemicals mothers of non ASD children put on their bodies each day (5.2) (Figure 5, $t(468) = -3.2, p = 0.039$).

Stress Levels, Traumatic Events, and Diagnoses:

Women were asked to report on their stress levels during their pregnancies, whether any traumatic events happened during their pregnancies, whether they received a clinical diagnosis (such as depression or anxiety) before or during their pregnancies, whether they had ever previously been diagnosed with PTSD, and whether they had been victims of long term or short term abuse as children.

More mothers of children with ASD reported “very high” levels of stress during their pregnancies ($\chi(1) = 3.65, p = 0.01$), with an average stress level of 3.91 on a 1-5 Likert scale, compared with mothers of non-ASD children, at 3.01 (Figure 6, $t(468) = 5.1, p < 0.01$).

In a potentially related finding, mothers of ASD children reported a higher incidence of traumatic events during their pregnancies than their counterparts ($\chi(1) = 3.89, p = 0.01$). In total, 7 mothers of children with ASD reported at least one traumatic event, whereas only one mother in the control group reported experiencing a traumatic event. Within the mothers of children with ASD group who had both typically developing and ASD children ($n = 183$), mothers trended toward being more likely to have a traumatic event occur during the pregnancy with their child later diagnosed with ASD ($n = 6, 3.8\%$) than with other pregnancies ($n=3, 1.6\%$) ($\chi(1) = 2.43, p = 0.054$). Whereas mothers of children of ASD ($n = 12$) were no more likely than their counterparts ($n = 14$) to have experienced short term abuse as a child (e.g., single incident), ($\chi(1)$

= 0.967, $p = 1.02$), they were more likely to have experienced long term abuse (e.g., repeated incidents) ($n = 21$ in ASD group versus 12 in non-ASD group) (Figure 7, $\chi(1) = 2.49$, $p = 0.026$).

Mothers of children with ASD were more likely to have received a diagnosis of depression or anxiety prior to or during their pregnancies ($n = 49$, 19.4%), compared to controls ($n = 20$, 9%) (Figure 8, $\chi(1) = 4.3$, $p < 0.01$), as well as more likely to have received a diagnosis of PTSD at any point in their lives ($n = 20$ in ASD group versus $n = 9$ in control group) (Figure 9, $\chi(1) = 3.1$, $p = 0.01$). In terms of antidepressant use, 2.7% of the mothers of ASD children reported use during pregnancy (14% of depressed mothers in this group); in contrast, 0.45% of these mothers in the non-ASD group reported antidepressant use during pregnancy (5% of depressed mothers in this group) (Figure 8).

DISCUSSION:

The findings gathered from this study support the estrogen theory of autism that was proposed in the beginning of this paper. The findings suggest that mothers of children with autism have a higher occurrence of health issues related to an increase in endogenous and exogenous estrogen exposure, which is consistent with our initial hypothesis. The main health concerns that were highlighted were increased consumption of non-hormone free animal products, increased toxin exposure, increased reported stress levels, PTSD, trauma in their lives, and an increased diagnosis of anxiety and/or depression.

Implications of Diet:

Soy consumption has been considered controversial for many years. Soy contains lignans and flavonoids, both of which increase estrogen activity and alter hormone synthesis, which may result in permanent alterations in cortical development (Romàn, 2007). Therefore, many mothers have been warned to avoid diets high in soy derivatives, as there may be implications down the line of altered estrogen activity (Cox & Phelan, 2008). In the current study, women were asked to describe their consumption of both soy productions and other animal products that may include hormones. It was found that there was no significant difference between the experimental and the control groups in terms of maternal soy consumption. However, it was reported that children who later received a diagnosis of ASD were more likely to be fed exclusively soy formula as infants (Figure 3). Furthermore, mothers of children with ASD reported higher average daily consumption of non-hormone free animal products compared with mothers of non-ASD children (Figure 4). Non-hormone free animal products are produced from animals that have been raised with additional hormones added to their diet, ultimately causing an increased concentration of hormones (such as estrogen) that may throw off the normal balance within the body (Greger, 2016). In previous studies, it has been reported that both maternal obesity and maternal starvation are linked to increased rates of disability, such as ADHD, in their children (Rodriguez et al., 2008). Therefore, the current study is not the first of its kind that link maternal diet to implications in the neurodevelopment of offspring. Overall findings indicate that diet may play a significant role in not only maternal health, but the long-term development of their child as well.

Toxin Exposure:

Of additional concern is the toxins that are being applied daily on mothers' bodies. As stated previously, it has been found that in the last decade, the average woman puts on six different cosmetic creams and products daily (Lortez et al., 2005). These cosmetic creams are known to contain chemicals, such as phthalates, that are endocrine disrupting chemicals and mimic estrogen, ultimately causing an imbalance of hormones (Kim et al., 2010). In our survey, mothers were asked to list the products that were used on a daily basis. This includes any sort of lotion, cream, shampoo, or make-up included in their daily routine. In this study, it was reported that mothers of children with ASD put an average of 6.7 creams on their body per day, which is significantly higher than the 5.2 creams that mothers of typically developing children apply to their bodies per day (Figure 5). Each of these products contain endocrine mimicking chemicals, which are likely to alter the hormone balance within the mother's bodies and therefore alter the hormones to which the fetus is exposed. Specifically, endocrine mimicking chemicals disrupt estrogen balance, therefore potentially increasing levels of estrogen and further altering the fetus' estrogen balance and brain development (de Cock et al., 2012). Although there has been no research regarding the rise in autism diagnosis in relation to the rise in cosmetic product use, it would be interesting in future research to see if there is a connection between self-image, beauty care, and autism occurrence considering it has been found that mothers of children with autism are less aware of the dangers of chemicals than control mothers (Kim et al., 2010).

Implications of Stress:

Chronic stress results in a significant increase in estrogen levels (MacNiven, DeCatanzaro, & Younglai, 1992). When someone has experienced repeated episodes of abuse or trauma, they have a prolonged elevation of estrogen due to the rise in cortisol levels and the

interactions between the hypothalamic-pituitary-adrenal (HPA) axis feedback loop and sex hormones, such as estrogen (Pasquali, 2012). Therefore, if a mother experienced a traumatic event even years before their pregnancy or they have been diagnosed with PTSD, their estrogen levels are likely to be elevated, resulting in their fetus being exposed to higher than normal levels of estrogen due to the stress and/or trauma experienced. It is important to note that in this study, stress levels during pregnancy were inquired about, as opposed to the stress of having a newborn child or a child with autism. Mothers of children with ASD reported a significantly higher level of stress compared to the control group. However, what is important to mention here is the possibility of recollection bias, in which a mother may be more likely to report their stress levels as high now that they have a child with autism compared to if they had a typically developing child. A more accurate response may have been collected had the survey been delivered to the mother during the pregnancy, not years later after their child had already developed and aged.

Traumatic Events and Abuse:

Mothers of children with ASD also reported a higher incidence of traumatic events compared to control mothers. Furthermore, when analyzing mothers of both children with ASD and typically developing children, it was found that 3.8% of the mothers experienced a traumatic event during their pregnancy with their child that later received an ASD diagnosis (n=6), compared to only 1.6% of mothers experiencing said traumatic event during their pregnancy with their typically developing child (n=3). Again, when interpreting these results, the possibility of recollection bias must be considered. Because the survey was given after giving birth to and raising a child diagnosed with autism, the mothers may be more likely to consider, in retrospect, an event related to their pregnancy traumatic. For example, a divorce or separation may not have

been considered traumatic prior to the development of their child with autism, but was considered traumatic while taking the survey years later because they considered the autism diagnosis to be causative of such a “traumatic” event.

Also relating to long-term abuse and estrogen dominance, mothers of children with ASD reported having higher incidences of long-term abuse compared to their counterparts but with no apparent difference in short-term abuse (Figure 7). In conjunction with this finding, it is reported that significantly more mothers who have children with ASD have a PTSD diagnosis compared to control mothers (Figure 9). These findings are supported by the findings of Roberts and colleagues’ study from 2013 that examined the association of maternal exposure to childhood abuse to having a child with autism. In this study, the data on a population of 116,430 women were examined in the Nurses’ Health Study II to investigate the correlation between abuse and a child’s autism status (Roberts et al., 2013). They concluded that there is in fact a correlation between the two occurrences (Roberts et al., 2013). Mothers who suffered the greatest amount of childhood abuse had the greatest risk of having a child with autism, whereas mothers who never experienced any form of abuse were less likely to have a child whom later developed autism (Roberts et al., 2013). The findings of Roberts and colleagues and the findings from the present study agree, representing that early abuse is significant not only to the mother, but to later generations as well.

Depression & Anxiety Diagnosis:

Not only are mothers of children with ASD more likely to have a lifetime diagnosis of PTSD, but they are also more likely have received a diagnosis of depression or anxiety prior to or during their pregnancy. Just as more mothers of children with ASD were diagnosed with

depression and/or anxiety, more of these mothers also reported taking antidepressants during their pregnancy compared to the control mothers (Figure 8). However, in total, there was a very small sample of mothers who reported antidepressant use overall. This small percentage of mothers who took antidepressants could be because mothers did not want any form of drug interactions during pregnancy or they merely did not want to be medicated during pregnancy. Nevertheless, for such a high population of depressed mothers it is surprising to see such a low percentage of antidepressant use. In an aim to further understand the connection between depression and estrogen levels, previous research examined antidepressants' role on estrogen regulation. Using AroER tri-screen technology, Chen and colleagues observed that paroxetine (Paxil), a selective serotonin reuptake inhibitor (SSRI) commonly prescribed as an antidepressant, modulates the expression of genes regulated by estrogen (Chen et al., 2014). This finding suggests that Paxil may act as an estrogen receptor agonist (Chen et al., 2014), therefore potentially pushing the body into estrogen dominance by promoting estrogen activity. Furthermore, there was a significant difference in PTSD diagnosis, in which mothers of children with ASD were more likely to have been diagnosed with PTSD than controls mothers (Figure 9).

The findings regarding stress and depression seem to be most intriguing because of the connection between the health issues. Mothers who have received a diagnosis of PTSD, or who have experienced trauma/long-term abuse, may understandably have a higher level of stress throughout their lives. This increase in stress may result in further depression and therefore elevate estrogen levels even more. Ultimately, the occurrence of stress, trauma, and depression may all build upon one another in a mother and increase the chances of having a child with autism drastically because of the role that each condition has on estrogen within the body. What is most interesting is the finding that mothers of children with autism reported more occurrences

of long term abuse, but there was no significant difference between mothers in terms of short term abuse. This is significant because it supports the theory that chronic stress, which is provoked from long-term abuse but not a single abusive episode, may shift the body into a state of estrogen dominance. Likewise, the same interpretation can be made from the finding that more mothers of children with autism reported having a PTSD diagnosis compared to mothers of typically developing children. This is certainly a field that requires more research to see how stress, depression, autism, and possibly estrogen, are all related.

Although in the context of the current study, these results are consistent with overall estrogen profiles differentiating the two groups, it is important to consider additional possible explanations for this correlation. First, perinatal complications are a possibility that could relate to having a child that later develops autism. Future research may consider whether mothers who experienced child abuse or who received a diagnosis of depression and/or anxiety reported higher rates of perinatal complications. In fact, there has been research done that shows mothers with untreated depression were more likely to face perinatal risks, potentially due to unhealthy behavior as a consequence of the depression (Bonari et al., 2004). Nevertheless, more research needs to be completed before a confident link can be confirmed, especially in regard to autism. Second, there is evidence that extreme maternal stress may result in epigenetic changes during pregnancy. A study in 1995 reported on the increased occurrence of schizophrenia in personal accounts of individuals who were conceived during the Dutch Hunger Winter between 1944 and 1945 (Brown, Susser, Lin, Neugebauer, & Gorman, 1995). It was reported that the risk of having a child with schizophrenia, or any affective psychosis for that matter, increased in women who were in their second trimester of pregnancy during the Dutch Hunger Winter due to the alterations that extreme famine had on the epigenetics of the fetus (Brown et al., 1995;

Heijmans et al., 2008). The fetuses that survived and went on to grow into adults were studied six decades later, and it was found that they had less DNA methylation of certain genes that may relate to their schizophrenia diagnosis (Heijmans et al., 2008). Therefore, the prenatal famine experienced may have altered the fetus' genetic makeup and influenced their development, as well as the development of generations to come (Carey, 2012). Not only did the Dutch Hunger Winter push the population into extreme famine, but also caused a great deal of stress as mothers and families searched for food and survival. Therefore, it could be interpreted that not only the famine, but the stress resulting from the famine, may influence the epigenetics of the child in utero that ultimately results in the eventual development of schizophrenia. Likewise, this can be related to maternal stress resulting in epigenetic effects that eventually lead to an autism diagnosis as investigated in the present study.

Of additional importance is the role that SES may have on the outcome of this study. Although there was no significant difference in SES between groups, one's socioeconomic background certainly does play a role in certain factors that were tested for. For instance, beauty products used, diets followed, and environmental toxin exposure may all be impacted by one's location and background. Furthermore, stress levels may be influenced by education, employment, and income. Future analysis of this data will include SES as a co-variate to control for the effect it may have on the results. Including it as a covariate in an ANOVA test will improve the validity of the findings.

CONCLUSION:

Although previous research has suggested that such factors that we have identified are seen in mothers with autism, there has never been a connection made between the given exposures, estrogen dominance, and the occurrence of having a child with autism. This is the first study of its kind that suggest there may be a positive link between maternal estrogen exposure and having a child later receive an autism diagnosis. Future research in the field must be directed toward examining this theory at a deeper level.

FIGURES:

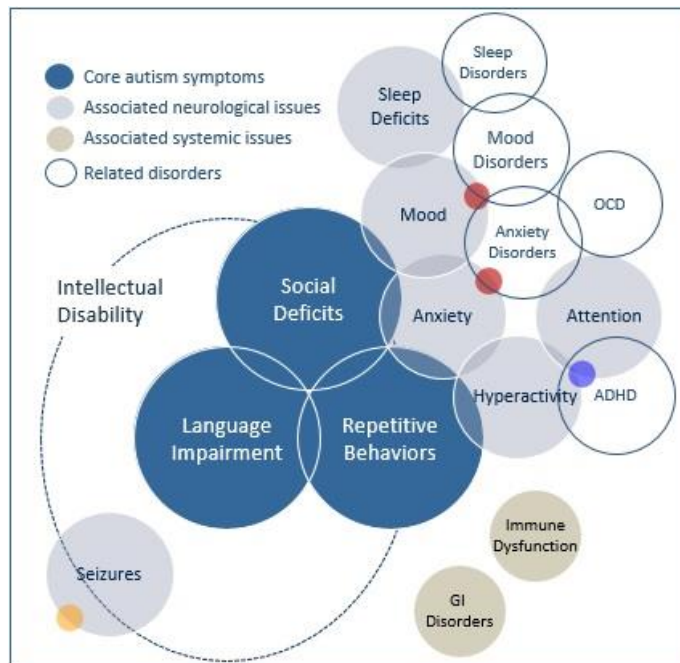


Figure 1: The core symptoms of autism spectrum disorder (ASD) and common comorbidities associated with the disorder.

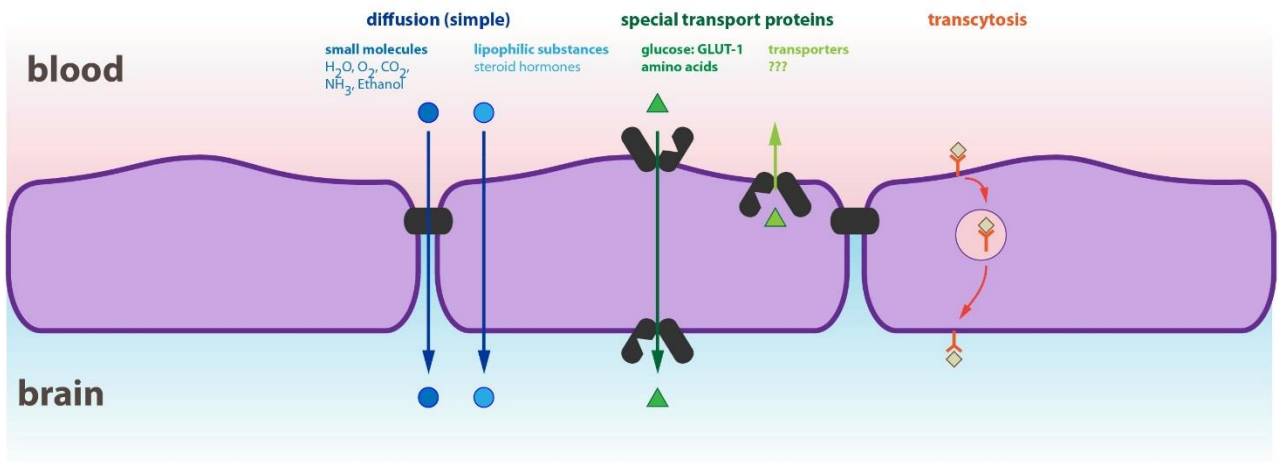


Figure 2: Representation of blood brain barrier permeability.

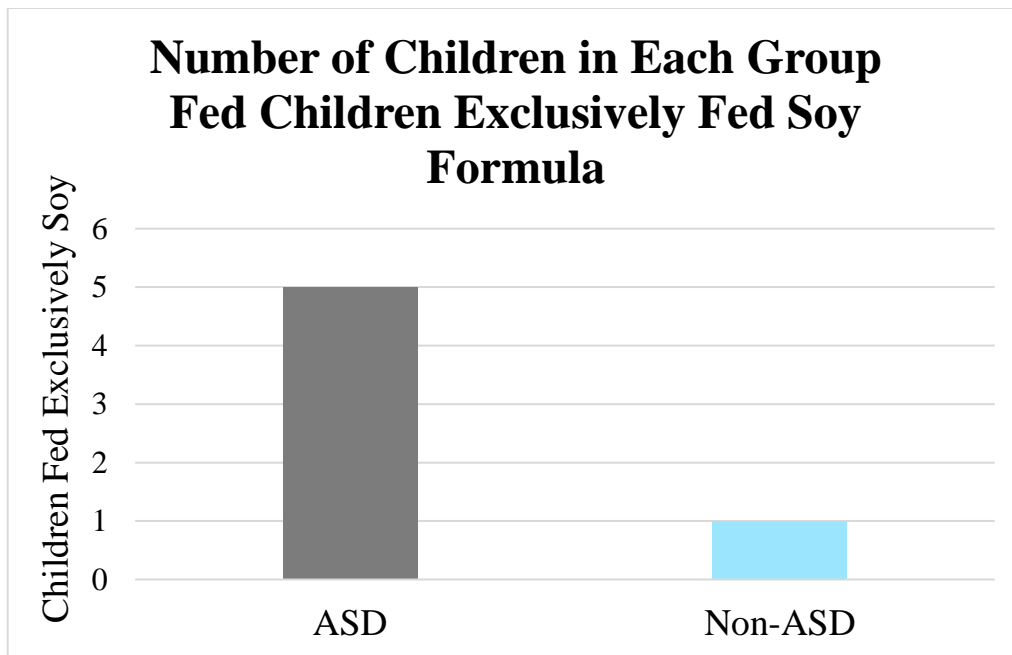


Figure 3: Significant difference between the early diets of children with and without a diagnosis of ASD in terms of soy consumption ($\chi(1) = 5.23, p=0.04$).

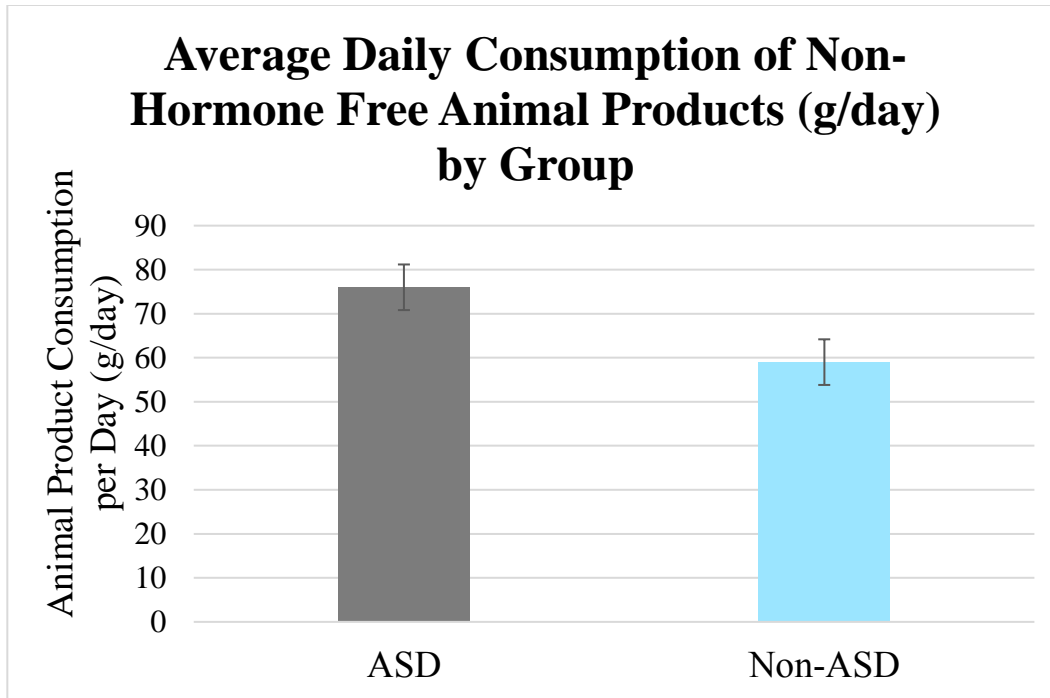


Figure 4: Significant differences in dietary habits between mothers of children with autism versus mothers of typically developing children ($t(461) = -2.29, p = 0.05$).

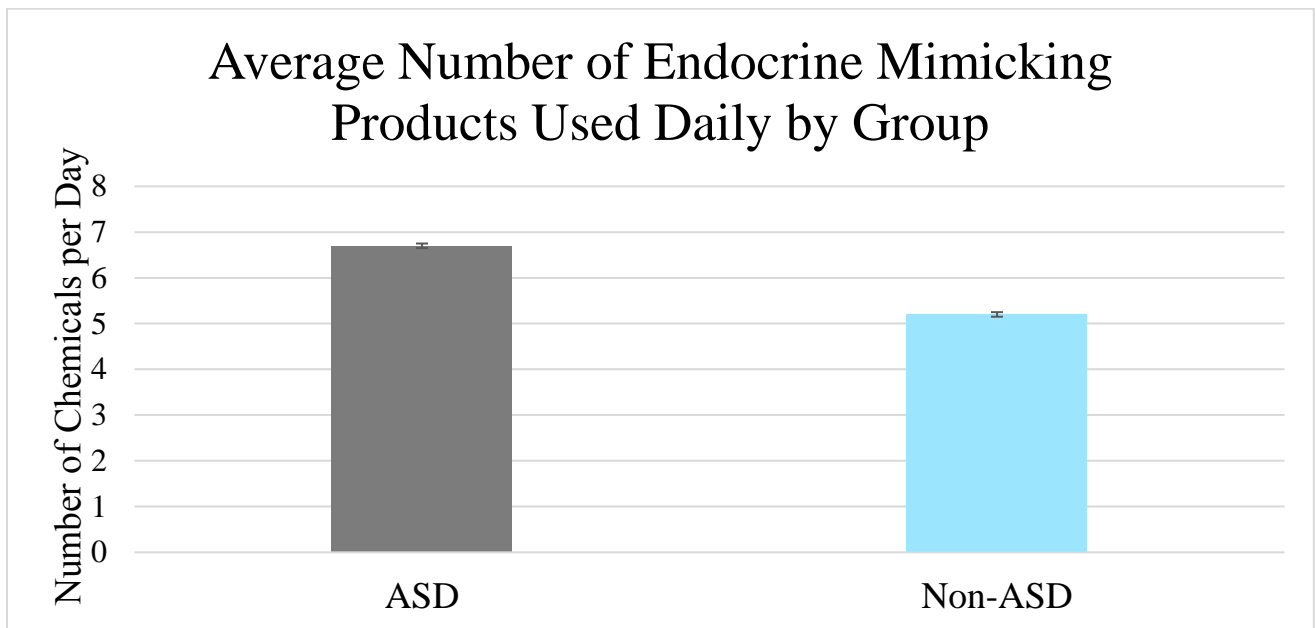


Figure 5: Significant difference in toxin exposure between groups ($t(468) = -3.2, p = 0.039$).

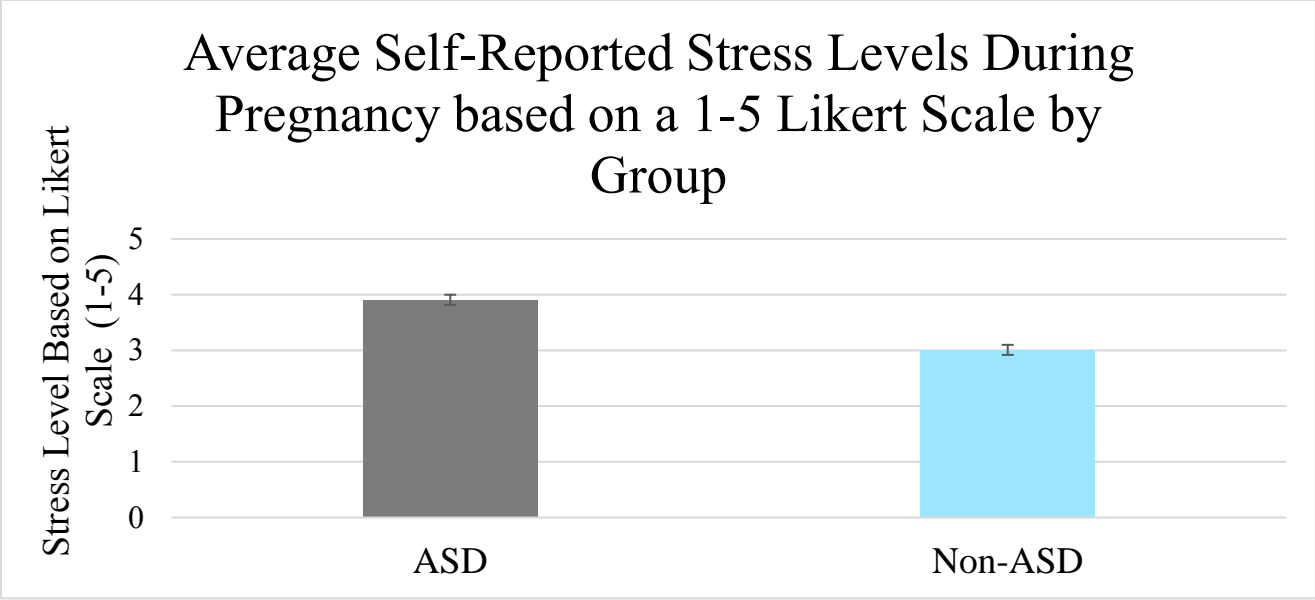


Figure 6: More mothers of children with autism reported having higher levels of stress based on the 1-5 Likert Scale compared to mothers of non-ASD children ($t(468) = 5.1, p < 0.01$).

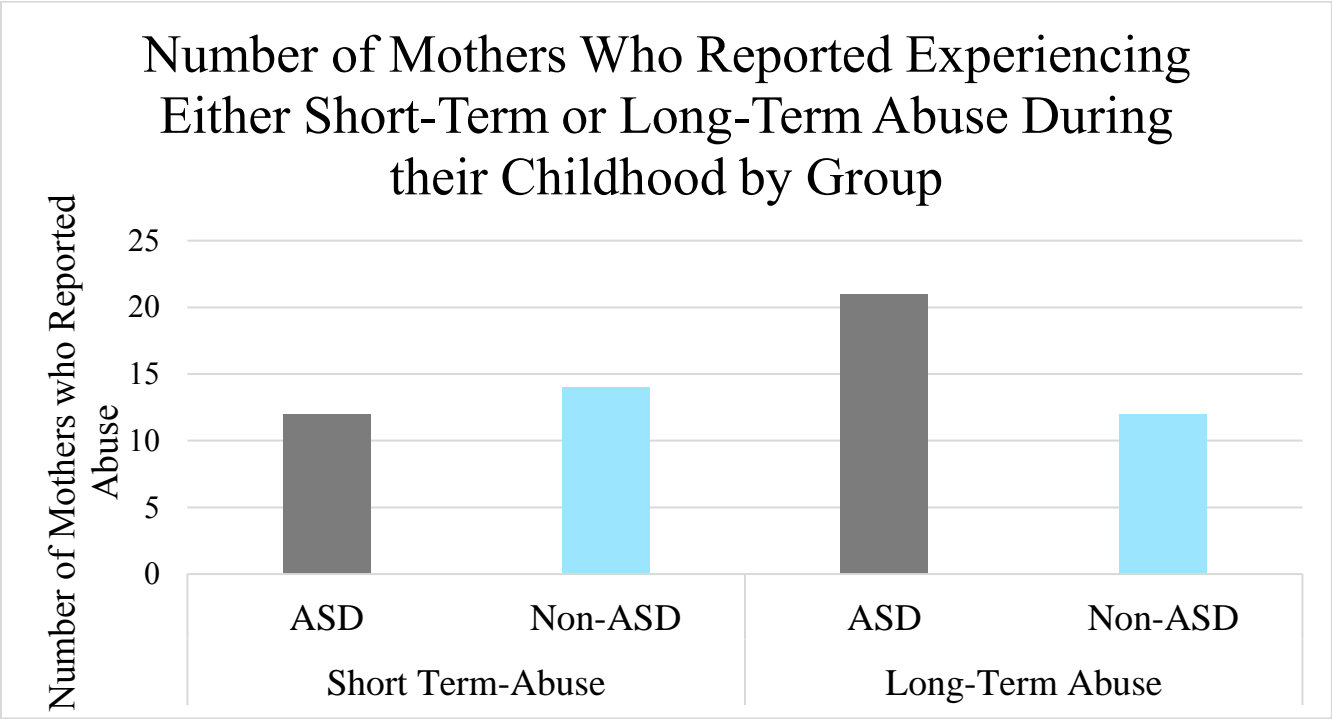


Figure 7: Mothers of children with autism reported a higher incidence of experiencing a long-term abuse ($\chi(1) = 2.49, p = 0.026$), but no more likely to have experienced short-term abuse ($\chi(1) = 0.967, p = 1.02$).

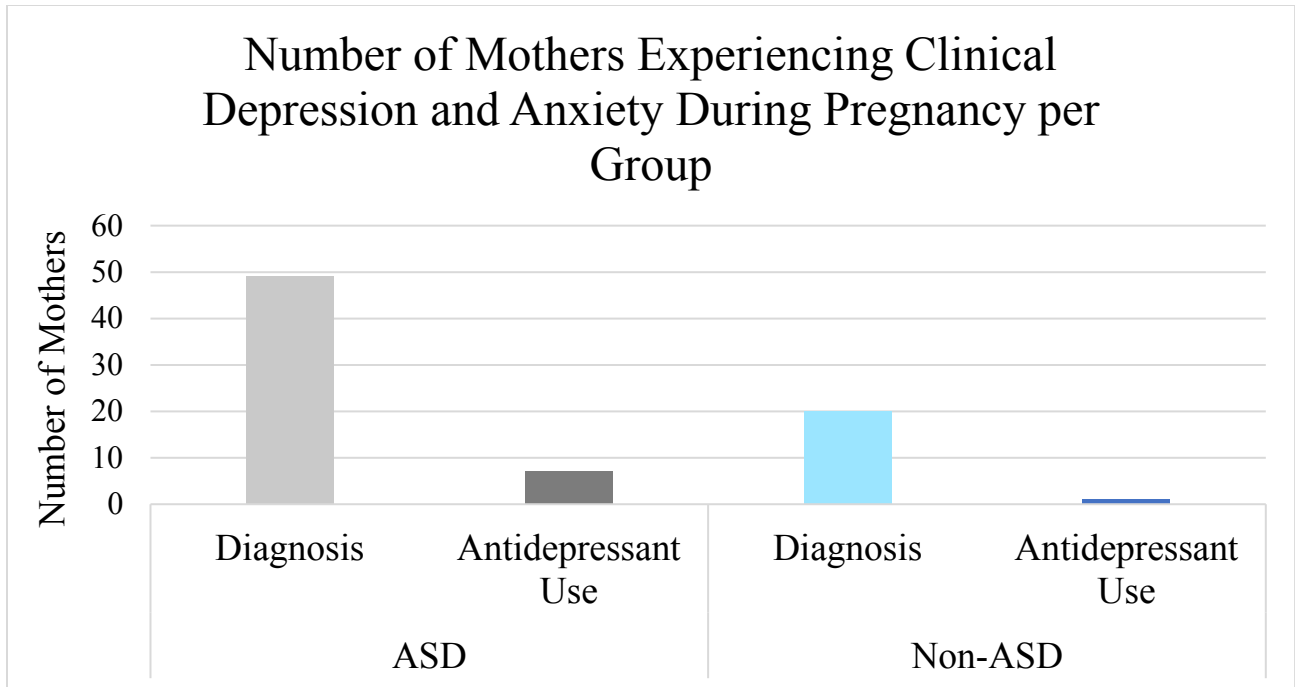


Figure 8: Mothers of children with autism reported higher diagnosis of depression and/or anxiety ($\chi(1) = 4.3, p < 0.01$). Interestingly, there was no significant difference in antidepressant use. Low use of antidepressants was consistent between both groups of mothers.

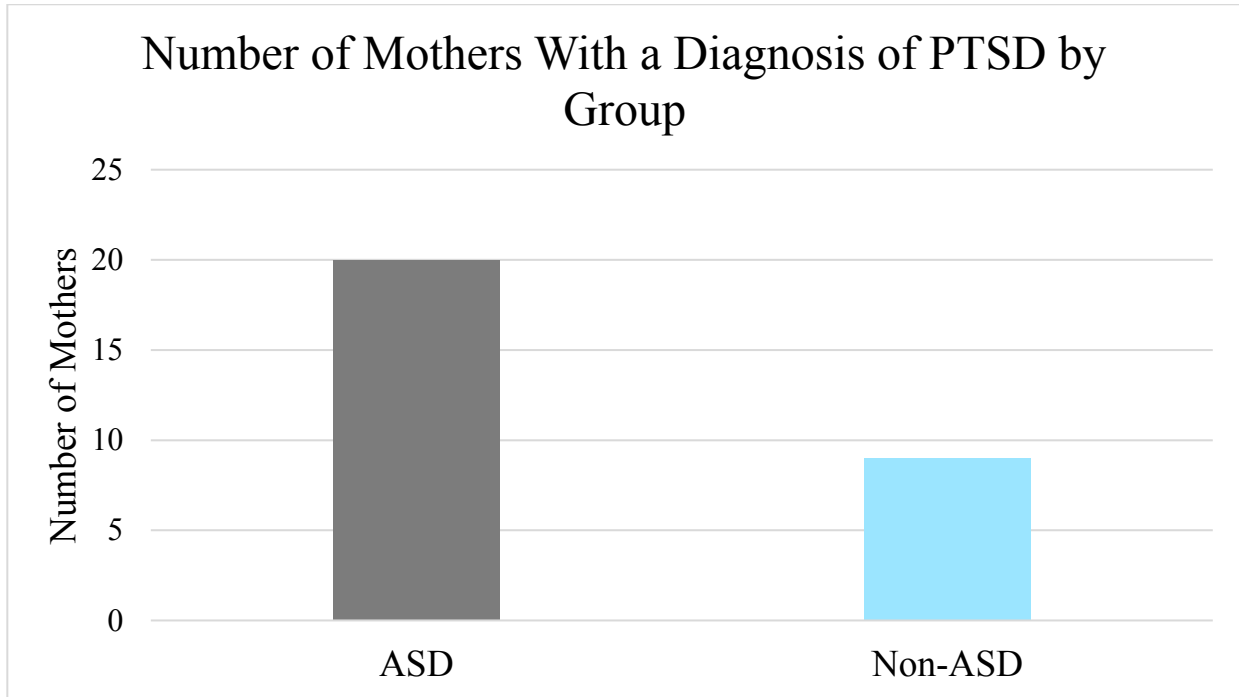


Figure 9: Mothers of children with autism reported higher diagnosis of PTSD ($\chi(1) = 3.1, p = 0.01$).

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