Chapter 8
A Role for Fetal Testosterone in Human Sex Differences

Implications for Understanding Autism

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Abstract Autism spectrum conditions (ASCs) may be an extreme manifestation of specific male-typical characteristics. Evidence for this theory is provided by the empathizing–systemizing (E–S) theory of sex-typical behavior, which suggests ASCs as an extreme form of the male brain (EMB). In this chapter, we review the evidence supporting EMB theory and examine the effect of hormones on the development of sex differences related to ASCs. An important candidate mechanism for the development of sex-typical behavior is the effect of fetal testosterone (fT) during pregnancy. Evidence that elevated levels of fT may be a risk factor for ASC is also discussed. Many neurodevelopmental conditions occur in males more often than females, including autism, dyslexia, attention-deficit hyperactivity disorder (ADHD), and early onset persistent antisocial behavior [1]). Autism in particular has been described as an extreme manifestation of some sexually dimorphic traits or an "extreme male brain" [2]. In this chapter, we review the reasons why this condition in particular has been viewed in this light and the evidence related to it.

Keywords Fetal testosterone · sex differences · autism

The Extreme Male Brain Theory of Autism

Autism, high-functioning autism, Asperger syndrome, and pervasive developmental disorder (not otherwise specified, PDD/NOS) are thought to lie on the same continuum and can be referred to as autism spectrum

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conditions (ASCs)[1]. These conditions are characterized by impairments in reciprocal social interaction, in verbal and nonverbal communication, alongside strongly repetitive behaviors and unusually narrow interests [3]. Recent epidemiological studies have shown that as many as 1% of people could have an ASC [4]. The incidence of ASC is strongly biased toward males [5, 6, 7] with a male: female ratio of 4:1 for classic autism [8] and as high as 8:1 for Asperger syndrome [9]. The cause of the observed sex difference in ASC remains a topic of debate. It is possible that males have a lower threshold for expressing the condition [10]. ASCs have a strong neurobiological and genetic component [11]; however, the specific factors (hormonal, genetic, or environmental) that are responsible for the higher male incidence in ASC are still unclear.

The extreme male brain (EMB) theory of autism is an extension of the empathizing–systemizing (E–S) theory of typical sex differences [2, 12] which proposes that females on average have a stronger drive to empathize (to identify another person's emotions and thoughts and to respond to these with an appropriate emotion) while males tend to have a stronger drive to systemize (to analyze or construct rule-based systems, whether mechanical, abstract, or another type) [12]. The empathizing quotient (EQ) [13] and systemizing quotient (SQ) [14] were developed to measure these dimensions in an individual. Using the difference between a person's EQ and SQ, individual "brain types" can be calculated [15, 16], where individuals who are equal in their E and S are said to have a balanced (B) brain type (E = S). The type S (S > E) brain type is more common in males while the type E (E > S) is more common in females [16]. Extreme types are also found [16], and the majority (61.6%) of adults with ASC fall in the extreme S (S >> E), compared to 1% of typical females [16] (Fig. 8.1).

Experimental evidence at the psychological level relevant to the EMB theory of autism includes the following:

Individuals with ASC score higher on the SQ, an instrument on which typical males score higher than typical females in both adults [14, 16] and children [17]. Individuals with ASC are superior to controls on the embedded figures task (EFT), a task on which typical males perform better than typical females [18, 19]. The EFT requires good attention to detail, a prerequisite of systemizing. Individuals with ASC have also been found to have either intact or superior functioning on tests of intuitive physics [20, 21], a domain which shows a sex difference in favor of males in adulthood [21]. Sex differences have been found on the block design subscale of the WISC-R intelligence test,

[1] The American Psychiatric Association uses the term ASD for autism spectrum disorders. We prefer the use of the term ASC as those at the higher functioning end of the autistic spectrum who do not necessarily see themselves as having a "disorder," and the profile of strengths and difficulties in ASC can be conceptualized as atypical but not necessarily disordered. ASC remains a medical diagnosis, hence the use of the term "condition," which signals that such individuals need support. We feel that the use of the term ASCs is more respectful to differences; recognizes that the profile in question does not fit a simple "disease" model but includes areas of strength (e.g., in attention to detail) as well as areas of difficulty; and does not identify the individual purely in terms of the latter.

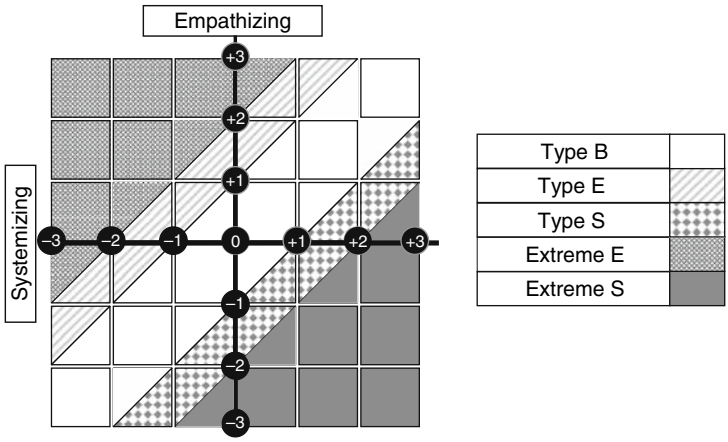

Fig. 8.1 The Empathizing–Systemizing Model of Typical Sex Differences. The main brain types are illustrated on axes of Empathizing (E) and Systemizing (S) dimensions (numbers represent standard deviations from the mean). Balanced brain (Type B); female brain (Type E), male brain (Type S); the extreme Types E and S lie at the outer borders. According to the 'extreme male brain' theory of autism, people with ASC will generally fall in the Extreme Type S region. Modified from Ref. [153]

with typical males performing better than females [22], and children with autism demonstrating superior functioning on this test [23, 24, 25].

Studies using the EQ [13] report that individuals with ASC score lower on both the adult and the child versions than the control groups [13, 17], where typical females score higher than typical males [13, 17]. Individuals with ASC are also impaired on certain measures where women tend to score higher than men. For example, individuals with ASC score lower than control males in the "Reading the Mind in the Eyes" task (considered to be an advanced test of empathizing) [26], the Social Stories Questionnaire [21], which involves recognition of complex emotions from videos of facial expressions or audios of vocalizations [27], and on the Friendship and Relationship Questionnaire (which tests the importance of emotional intimacy and sharing in relationships).

The Childhood Autism Spectrum Test (CAST) [28, 29], (formerly known as the childhood Asperger syndrome test, renamed because it can be used for all subgroups on the autistic spectrum [30]) is a parent-report measure developed to screen for Asperger syndrome and ASCs in a typical population on which [28] boys score higher than girls [31], and children with ASC score higher than typically developing children [29]. Another measure is the Autism Spectrum Quotient (AQ), which was developed to help quantify the number of autistic traits an individual displays [32], and individuals with Asperger syndrome or high-functioning autism score higher than those without a diagnosis [32]. Among controls, males again score higher than females [32], and these results are consistent in adults, adolescents, and children [32, 33, 34] as well as

cross-culturally [35, 36, 37, 38]. Furthermore, similar results have been found using the Social Responsiveness Scale (SRS), a 65-item rating scale designed to measure the severity of autistic symptoms, demonstrating that individuals with an ASC diagnosis score higher than typical males, who in turn score higher than typical females [39].

In addition to the evidence at the psychological level, it has been suggested that characteristics of neurodevelopment in autism, such as larger overall brain volumes and greater growth of the amygdala during childhood, may also represent an exaggeration of typical sex differences in brain development [40]. Studies using fMRI indicate that typical females show increased activity in the extrastriate cortex during the Embedded Figures Test and increased activity bilaterally in the inferior frontal cortex during the "Reading the Mind in the Eyes" test. Parents of children with ASC also tend to show hyper-masculinization of brain activity [41], suggesting that hyper-masculinization may be part of the broader autism phenotype.

It remains important to identify the biological mechanisms that cause such sexual dimorphism. One study has shown sexual dimorphism in looking preferences in 102 newborn infants who were approximately 37 hours old. Boys were found to exhibit a preference for mobiles while girls tended to prefer looking at faces [42]. Although these simple experiments with stimuli are not an indication of ASC, these early sex differences suggest a biological sex difference in behavior, as there had been no opportunity for postnatal influence of social or cultural factors. One possible biological mechanism is the effect of prenatal exposure to hormones, in particular the androgen testosterone [43].

Hormones and Sexual Differentiation

Hormones are essential to reproduction, growth and development, maintenance of the internal environment and the production, use and storage of energy [44]. There are marked physical and behavioral consequences of exposure to hormones throughout life. Prenatally, the presence or absence of specific hormones (or their receptors) is known to be essential to the sexual differentiation of the fetus. In addition to stimulating development of physical characteristics such as genitalia [45, 46, 47, 48, 49], there is increasing evidence that prenatal hormones have a substantial effect on gender-typical aspects of behavior [48, 50]. If this is observed to be the case, then the occurrence of these hormones prenatally may have a substantial bearing on the development of an extreme male profile responsible for autistic traits.

The links between hormone levels, physical development, and behavior are complex and not yet fully understood, particularly in terms of effects on early development. Hormone levels can be measured at particular points in time but levels could vary on a daily basis [51], and prenatal measurements are very difficult (and potentially dangerous) to obtain for research purposes

alone. Furthermore, correlations with behavioral measurements are always complicated by the need to determine the presence of a particular trait, without artificially inducing the behavior or creating bias in the result. A useful way of controlling some of these variables is to examine results from animals, where it has been possible to directly manipulate and monitor the levels of hormones throughout pregnancy and to control for environmental effects. As a result, we often look to confirm effects measured in animals with similar measurements in humans. Even then, the correlation between animals and humans is not always clear cut, with the potential for quite different mechanisms.

Though genetic sex is determined at conception, it is the gonadal hormones (i.e., androgens, estrogens, and progestins [52]) that are responsible for differentiation of the male and female phenotypes in the developing human fetus [45, 46, 47, 48, 49]. Androgens such as testosterone and dihydrotestosterone (DHT—a hormone formed from testosterone) are of particular interest to the study of male-typical behavior because when these androgens and the appropriate receptors are present, the male genital phenotype will develop. If androgens (or their receptors) are not present, then the female genital phenotype will develop (such as in female fetuses or males with Androgen insensitivity syndrome) [53, 54, 55, 56]. Another hormone that forms from prenatal testosterone is the estrogen hormone estradiol, which has been observed to promote male-typical behavior in rats and other rodents [57]. The relative contributions of DHT and estradiol to development of male-typical human behaviors are less certain.

Behavioral studies in nonhuman mammals have shown that the same prenatal hormones that are involved in sexual differentiation of the body are also involved in sexual differentiation of behavior [58, 59]. In animals, higher doses of hormones have been seen to masculinize behavior more than lower doses, though the effect of concentration is not uniform for different behaviors [59]. Effects are also likely to be nonlinear and include both lower and upper threshold values, beyond which changes in concentration have no effect [50]. Interaction between hormones may also be important, as described above [59].

Atypical Fetal Hormone Environments

In humans, the manipulation or even direct measurement of hormone levels is considered unethical because of the potential dangers involved. However, some information is available from specific abnormalities which occur naturally. Such abnormalities can lead to considerable difficulties for the individual and fortunately such instances are rare. However, some studies have obtained sufficient participation to render useful information about how abnormal environments influence behavior. A detailed review of many of the studies surrounding these conditions has been provided elsewhere [43, 44, 48, 50], so we focus our discussion here on findings relevant to characteristics of ASC.

Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia (CAH) is a genetic disorder affecting both sexes that causes excess adrenal androgen production beginning prenatally [60]. CAH affects both males and females but is most clearly observed in females because of their typically low androgen levels. Female fetuses with CAH have similar androgen levels to those found in typical males [48]. Behavioral studies of females with CAH show a more masculinized profile compared to unaffected female siblings or matched controls.

In terms of specific behaviors, girls with CAH show masculinization of ability in activities typically dominated by males. These include spatial orientation, visualization, targeting, personality, cognitive abilities, and sexuality [61, 62, 63]. Females with CAH may also be more likely to be left handed [64] and are more interested in male-typical activities and less interested in female-typical activities throughout life [65, 66, 67, 68, 69].

Studies relating CAH and autism are limited. Since the condition typically introduces masculinization, effects are more apparent in girls than boys. For these girls, behavior tends to become more aligned with expectations of behavior from typical males and few cases of ASC with CAH are reported. Results from one study of girls with CAH suggest that they exhibit more autistic traits, measured by the AQ, compared to their unaffected sisters [70]. Individuals with CAH also demonstrate higher levels of language and learning difficulties compared to unaffected family members [63], as do people with ASCs. Whilst CAH provides an interesting window on additional androgen exposure, the relatively rare occurrence of CAH in conjunction with ASCs makes it difficult to obtain large enough sample sizes for generalization to the wider population. In addition, some researchers have suggested that CAH-related disease characteristics, rather than prenatal androgen exposure, could be responsible for atypical cognitive profiles [71, 72].

Complete Androgen Insensitivity Syndrome

Complete androgen insensitivity syndrome (CAIS) occurs when there is a complete deficiency of androgen receptors and is more common in males, with incidence between 1 in 60,000 and 1 in 20,000 births. At birth, genetic male infants with CAIS are phenotypically female despite an XY (male typical) complement and are usually raised as girls with no knowledge of the underlying disorder. Although breasts develop, diagnosis usually takes place when menarche fails to occur [52, 73].

Investigation of behavior such as gender identity, sexual orientation, gender role behavior in childhood and adulthood, personality traits that show sex differences, and hand preferences have suggested that males with this condition do not significantly differ from same-sex controls [72, 74]. However, other

results suggests that individuals with CAIS tend to show feminized performance on tests of visuo-spatial ability [75]. If replicable, this finding lends support to the notion that androgens enhance male-typical behaviors. Specific evidence for ASCs is not available due to the low incidence of this condition.

Idiopathic Hypogonadotropic Hypogonadism

Idiopathic hypogonadotropic hypogonadism (IHH) occurs when an individual's gonads lack sufficient stimulation to produce normal levels of hormones, and the disorder can occur congenitally or after puberty. These individuals have normal male genitalia at birth, so it can be assumed that their prenatal testosterone levels were normal [44]. Men with IHH perform worse on the Embedded Figures Test, the space relations subtests from the differential aptitude tests and the block design subtest of the Weschler Adult Intelligence Scale, when compared with normal males and males with acquired hypogonadotropic hypogonadism after puberty [76]. However, another study found that males with IHH do not show deficits on the same scale [77]. More research needs to be conducted to resolve these findings and relate the effects to ASC.

Hormonal Effects: Indirect Studies in Typical Populations

There is a steady body of evidence that indicates that fetal hormone levels influence certain physical characteristics that can be observed after birth. These "proxy" measurements have been used to indicate the levels of prenatal androgen expose and have been examined extensively in relation to behavioral traits. Several reviews of these measurements exist [49, 50], and we focus the discussion here on studies related to behaviors associated with ASC.

Digit Ratio (2D:4D)

The ratio between the length of the 2nd and 4th digit (2D:4D) has been found to be sexually dimorphic, being lower in males than in females. 2D:4D ratio is thought to be fixed by week 14 of fetal life, and it has been hypothesized that it might reflect fetal exposure to prenatal sex hormones in early gestation [78].

Measurements indicate an association between fT levels and 2D:4D ratio for the right hand after controlling for sex [79]. For subjects with CAH, females show lower (more masculinized) 2D:4D on the right hand compared to unaffected females, and men with CAH have lower 2D:4D on the left hand compared to unaffected males [80]. Results in this sample are consistent with the notion that prenatal androgen exposure masculinizes 2D:4D ratio. This measure has been widely used as a proxy for prenatal testosterone exposure due

to the ease and simplicity of measurement. However, it is likely that 2D:4D ratio is affected by multiple factors [50].

The findings in studies with 2D:4D ratio tend to support the suggestion that higher f T levels are a risk factor for ASC. Lower (i.e., hyper-masculinized) digit ratios have been found in children with autism compared to typically developing children, and this was also found in the siblings and parents of children with autism, suggesting genetically based elevated f T levels in autism [81, 82].

Dermatoglyphics

Dermatoglyphics, or fingerprints, have also been used as a proxy measure for prenatal exposure to testosterone. The number of dermal ridges is thought to be fixed by about the 4th month of gestation [83]. Researchers have used total finger ridge count and asymmetry between left and right hands. Sex differences have been observed in ridge count with males exhibiting more ridges in total than females. Sex differences have also been observed in asymmetry although both sexes have more ridges on the right hand than on the left hand (R > L). The left greater than right (L > R) pattern is more common in females than in males [49].

Studies examining total ridge count in adults and children have shown that for both men and women who exhibit L > R, performance was better for tasks that show a female superiority such as verbal fluency and perceptual speed [84, 85, 86]. The opposite pattern was found for those exhibiting the R > L pattern, who demonstrated better performance for tasks that show a male superiority [84, 85, 86].

Data from dermatoglyphic patterns and their relation to autism are limited and conflicting. In one study 78 children with autism were compared to the same number of matched controls [87]. Analysis of ridge patterns and ridge counts resulted in significant differences between the children with and without autism. Children with autism typically exhibited lower ridge count and less distinct fingerprint features [87]. However, a smaller comparison of children with autism, learning difficulties (then called "retardation"), and typical children found no significant differences for ridge counts [88]. It was argued that dermatoglyphics may be ineffective in delineating autism from other typical populations [88].

As with the 2D:4D ratio, studies using dermatoglyphics may be useful, but more evidence is needed to establish whether there is a link between dermatoglyphics and prenatal hormone exposure. In addition, further studies are needed to understand the potential links with ASCs. The few studies of dermatoglyphics in ASCs are quite old, and in more recent decades, diagnostic clinics have become more alert to detecting autism in higher functioning individuals (such as those with Asperger syndrome), and it would be of interest to repeat these early studies with the range of subgroups on the autistic spectrum.

Lateralization

It has been proposed that some observable sex differences in human behavior and cognition may be accounted for by differences in cerebral lateralization [89]. In addition to research investigating functional asymmetries in the brain, body asymmetries (other than fingerprint asymmetries) have been associated with prenatal sex hormones [49].

Fetal testosterone (fT) has been implicated in left-handedness and asymmetrical lateralization [90, 91, 92, 93]. Left-handedness and ambidexterity are more common in typical males [94] as well as in individuals with autism [95]. In addition, the typical male brain is heavier than the female brain [96], a difference that may in part be due to early fT exposure [48].

Pubertal Onset

Pubertal onset has been used to investigate variations in hormones. Females typically enter puberty earlier than males [50]. Research examining the physical indicators of hormone exposure and autism has found that a subset of male adolescents with autism show hyper-androgeny, or elevated levels of androgens, and precocious puberty [97]. These findings suggest that individuals with autism have atypical hormonal activity around this time. Other research has also shown that androgen-related medical conditions such as polycystic ovary syndrome (PCOS), ovarian growths, and hirsutism [98] occur with elevated rates in both women with Asperger syndrome and in mothers of children with autism [98]. Delayed menarche has also been observed in females with Asperger syndrome [98, 99]. These may reflect early abnormalities in level of fT, though this would require testing in a longitudinal study.

Co-Twin Sex

Other indirect studies of the relationship between prenatal hormones and behavior come from studies examining the effects of having an opposite or same-sex twin. Nonhuman studies examining the effects of animal position in the uterus have suggested that the sex of littermates can affect the development of sex-typical behaviors [100]. For rodents, masculinization of females was seen to occur when they were between two males in the uterus. For multiple littermates, the blood supply is channeled between fetuses, and in another study it was found that females developed more male typical traits if they were "downstream" of their male littermates [48].

For human twins, it is thought that females adjacent to a male will demonstrate masculinized behavior as a result of testosterone from the male [101, 102, 103]. There is also some evidence that human males with an opposite-sex

twin exhibit feminized gender-role behavior [104]. However, most studies have not observed feminization [105, 106, 107, 108, 109]. Other investigations of gender-typical play have also failed to find opposite-sex twin effects [108, 110]. Such findings in humans are difficult to interpret because these findings may be a result of being reared with a female, rather than an effect of hormonal exposure during gestation [50].

It is widely accepted that genes play a role in the etiology of autism. In the absence of any known gene or genes, the main support for this is derived from family and twin studies. Two recent studies [111, 112] suggest that the twinning process itself may be an important risk factor in the development of autism. Both studies compared the number of affected twin pairs among affected sibling pairs to expected values in two separate samples and reported a significant excess of twin pairs. However, data from other studies do not support twinning as a substantial risk factor in the etiology of autism [113, 114, 115]. The high proportion of twins found in affected-sib-pair studies could be explained by the high ratio of concordance rates in monozygotic (MZ) twins versus siblings [114]. Researchers have suggested that environmental factors associated with various demographic characteristics such as sex, multiple births, maternal age, and education may interact with genetic vulnerability to increase the risk of autism [113] but no firm conclusions can be drawn at the present time.

Hormonal Effects: Measurements of Fetal Testosterone

Whilst many convenient methods have been recommended, the ability to infer prenatal hormone exposure through abnormal environments or proxy measures has obvious limitations. Although evidence for theories surrounding the influence of androgens can be obtained, there is (as yet) little direct support for these predictors as a way of studying prenatal hormone influence.

Ideally, we would like to make direct measurements of testosterone at regular intervals throughout gestation and into postnatal life. Whilst some indication of fetal exposure to androgens might be gained from maternal samples, there is little evidence to suggest that these correlate well with the fetal environment that is protected by the placenta [48].

The timing of hormonal effects is also crucial when studying lasting effects on development. There are thought to be two general types of hormonal effects: organizational and activational [116]. Organizational effects are most likely to occur during early development when most neural structures are becoming established and produce permanent changes in the brain [116], whereas activational effects are short term and are dependent on current hormone levels. Since ASC are typically persistent with an early onset, any hormonal influence on the development of ASC is likely to be organizational in nature.

It is widely thought that organizational effects are maximal during sensitive periods, which are hypothetical windows of time in which a tissue can be formed

[48]. Outside the sensitive period, the effect of the hormone will be limited, protecting the animal from disruptive influences. This means, for example, that circulating sex hormones necessary for adult sexual functioning does not cause unwanted alterations to tissues even though the same hormones might have been essential to the initial development of those tissues. Different behaviors may also have different sensitive periods for development [117]. The importance of sensitivity to organizational effects was seen by Goy et al., who showed that androgens masculinize different behaviors at different times during gestation for rhesus macaques [117].

For typical human males, there is believed to be a surge in fT at around 8–24 weeks of gestation [43, 48, 57], with a decline to barely detectable levels from the end of this period until birth. As a result, any effects of fT on development are most likely to be determined in this period. For typical human females, levels are typically very low throughout pregnancy and childhood [48].

In addition to the fetal surge, two other periods of elevated testosterone have been observed in typical males. The first takes place shortly after birth and lasts for approximately 3–4 months [118], after which levels return to very low levels until puberty. Results show that neonatal testosterone is important for genital development [119], but the evidence for its role in behavioral development is unclear. Early pubertal effects are the first visible effects of rising androgen levels in childhood and occur in both boys and girls. Due to the early onset of ASC, the pubertal surge in testosterone is of little interest in determining etiology of these conditions. Few studies have been conducted on the effects of neonatal testosterone; however, there is an increasing body of evidence that suggests that prenatal androgens may be involved in determining sexually dimorphic traits. In the remainder of this chapter, we discuss direct measurements of testosterone and our ability to correlate this with the development of ASC.

Maternal Sampling During Pregnancy

Various studies have measured testosterone levels in maternal blood during pregnancy [120, 121, 122]. One study found that androgen exposure in the second trimester was positively associated with male-typical behavior in adult females [121]. Similar findings in another study revealed that higher levels of testosterone in mothers were associated with masculinized gender-role behavior in 3.5-year-old girls, but not boys. These findings may be a result of a genetic predisposition for women with high testosterone levels to pass these genes on to their daughters [120]. Another possibility is that raised maternal testosterone levels promote more male-typical behavior in girls [120]. No study to date has used maternal testosterone levels to investigate the development of autistic traits, and it would be interesting to examine whether maternal testosterone during pregnancy is related to fT or future development of autistic traits.

Samples from the Umbilical Cord

A series of studies have examined relationships between umbilical cord (perinatal) hormones and later behavior such as temperament and mood. High perinatal testosterone and estradiol levels were significantly related to low timidity in boys [123, 124, 125]. In girls, no relationships were observed. Other studies of umbilical cord hormones have shown inconsistent results [126, 127, 128].

An important factor to consider when using umbilical samples is that fT levels are typically at very low levels from about week 24 of gestation, whereas the neonatal peak has not yet appeared. In addition, the cord contains blood from the mother as well as the fetus, and hormone levels may vary due to labor itself [124].

Amniotic Fluid

One of the most promising methods for obtaining information about the fetal exposure to androgens appears to be the direct sampling of fT levels in amniotic fluid, obtained from routine diagnostic amniocentesis. This is performed for clinical reasons to detect genetic abnormalities in the fetus. As a result, it is typically performed in a relatively narrow time window that is thought to coincide with the peak in fT for male fetuses. This peak is also apparent in amniotic fluid, and several studies have documented a large sex difference in amniotic androgens [129, 130, 131, 132, 133]. There are significant risks associated with the procedure itself, so that it cannot be performed solely for research. However, the process itself does not appear to have any negative effects for later development [131].

The origins of androgens in amniotic fluid are not fully understood, but the main source seems to be the fetus itself [50]. Hormones enter the amniotic fluid in two ways: via diffusion through the fetal skin in early pregnancy and via fetal urine in later pregnancy [131, 134]. Given the risk entailed in obtaining blood from the fetus, there are very limited data directly comparing testosterone in amniotic fluid to that in fetal blood. Androgens in amniotic fluid are unrelated to androgens measured in maternal blood in the same period, as shown in studies in early and mid-gestation [51, 135]. Based on these findings, testosterone obtained in amniotic fluid appears to be a good reflection of the levels in the fetus and represents an alternative to direct assay of the more risky process of collecting fetal serum [50].

Finegan et al. [136] conducted the first study that explored the relationship between prenatal hormone levels in amniotic fluid and later behavior on a broad range of cognitive functions at age 4. The findings are difficult to interpret since the authors used measures that did not show sex differences. However, the same children were followed up at 7 years of age, and associations between spatial ability and fT were examined [137]. A significant positive

association between fT levels and faster performance on a mental rotation task was observed in a small subgroup of girls, but not boys. At 10 years of age, prenatal testosterone levels were found to relate to handedness and dichotic listening tasks [138], and the results were interpreted as providing support for the hypothesis that higher levels of prenatal sex hormones are related to lateralization in boys and girls [139].

Cambridge Fetal Testosterone Project

The Cambridge Fetal Testosterone Project is an ongoing longitudinal study investigating the relationship between fT levels and the development of behaviors relating to ASC [43, 140]. Mothers of participating children had all undergone amniocentesis for clinical reasons between 1996 and 2001 and gave birth to healthy singleton infants. To date, these children have been tested postnatally at 12 months, 18 months, 24 months, 4 years, and 6–8 years of age.

Fetal Testosterone and Eye Contact at 12 Months

The first study aimed to measure fT and estradiol levels in relation to eye contact for a sample of 70 typically developing, 12-month old children [141]. Reduced eye contact is a characteristic common in children with autism [141, 142]. Frequency and duration of eye contact were measured using videotaped sessions. Sex differences were found, with girls making significantly more eye contact than boys. The amount of eye contact varied quadratically with fT levels when the sexes were combined. Within the sexes, a relationship was only found for boys [141]. No relationships were observed between the outcome and estradiol levels. Results were taken to indicate that fT may play a role in shaping the neural mechanisms underlying social development [141].

Fetal Testosterone and Vocabulary at 18 and 24 Months

Another study (of 87 children) focused on the relationship between vocabulary size in relation to fT and estradiol levels from amniocentesis. In some subgroups within ASCs, such as classic autism, vocabulary development is also delayed [143]. Vocabulary size was measured using the Communicative Development Inventory that is a self-administered checklist of words for parents to complete [144]. Girls were found to have a significantly larger vocabulary than boys at both time points [145]. Results showed that levels of fT inversely predicted the rate of vocabulary development in typically developing children between the ages of 18 and 24 months [145]. Within sex analyses showed no significant relationships in boys or girls, which the authors believe may have been due to the relatively small sample sizes. No relationships between estradiol and vocabulary size were found. Despite the lack of significant results within sex,

the significant findings in the combined sample suggest that fT may be involved in shaping the neural mechanisms underlying communicative development [145].

Fetal Testosterone and Empathy at Age 4

These children were next followed-up at 4 years of age. Thirty-eight children completed a "moving geometric shapes" task where they were asked to describe cartoons with two moving triangles whose interaction with each other suggested social relationships and psychological motivations [146]. Sex differences were observed with girls using more mental and affective state terms to describe the cartoons compared to boys; however, no relationships between fT levels and mental or affective state terms were observed. Girls were found to use more intentional propositions than males, and a negative relationship between fT levels and frequency of intentional propositions was observed when the sexes were combined and in boys. Boys used more neutral propositions than females, and fT was related with the frequency of neutral propositions when the sexes were combined. However, no significant relationships were observed when boys and girls were examined separately. In addition, no relationships with estradiol were observed. These results are consistent with the EMB theory since other studies have found that individuals with ASC perform lower than typical males on a similar moving geometric shapes task [147].

Fetal Testosterone, Restricted Interests, and Social Relationships at Age 4

A second follow-up at 4 years of age in this same cohort of children utilized a measure called the Children's Communication Checklist [148]. The quality of social relationships subscale demonstrated higher fT levels to be associated with poorer quality of social relationships for both sexes combined but not individually. A limitation to within-sex analysis was the sample size ($n = 58$).

fT levels were also associated with more narrow interests when the sexes were combined and in boys only [149]. Sex differences are reported, with males scoring higher (i.e., having more narrow interests) than females [149]. Individuals with ASC demonstrate more restricted interests as well as difficulties with social relationships [149].

Fetal Testosterone, Systemizing, Empathizing at Ages 6–8

In 2004, the Cambridge fT project sample size was increased by recruiting more mothers who had undergone amniocentesis during the same period. The parents of these children ($n = 204$) were asked to complete children's versions of the Systemizing Quotient (SQ-C) and Empathy Quotient (EQ-C).

In systemizing, boys scored higher than girls on the SQ-C, and levels of fT positively predicted SQ-C scores in boys and girls individually [150]. Sex differences were observed in EQ-C scores, with girls scoring higher than boys.

A significant negative correlation between fT levels and EQ-C was observed when the sexes were combined and within boys.

A regression analysis was used to identify the main contributions to EQ-C. Whilst the main effect of sex was found, there was no main effect of fT. However, the effect of fT cannot be disregarded, since sex and fT are strongly correlated [151]. A subset of these children ($n = 78$) were also invited to participate in further cognitive tests, and the children's version of the Reading the Mind in the Eyes task (Eyes-C) was administered. No significant differences were found between sexes though a significant relationship between fT levels and Eyes-C was observed for both boys and girls [151].

Behaviors Associated with ASC

In the study outlined above, a series of measurements go some way toward experimentally linking direct measurements of fT and behaviors associated with ASC. At ages 12 months, 18 months, 24 months, and 4 years, behavioral traits associated with empathizing appear to be linked to lower levels of fT. At 6–8 years of age, SQ and EQs both appear to show sexual dimorphism – consistent with the E–S theory. In addition, fT was positively correlated with systemizing for both boys and girls.

The lack of ability to empathize and drive to systemize appear to be characteristic of ASC [2]. Whilst these behaviors do not confirm a clear link between fT and ASC, the results are broadly consistent with a role for fT in shaping sexually dimorphic behavior.

Fetal Testosterone and Autistic Traits

In light of some of the above results, a more direct approach of evaluating the links between autistic traits and fT was implemented. In this study, effects of fT were directly evaluated against autistic traits as measured by the CAST [28, 29] and the Child Autism Spectrum Quotient (AQ-C) [152]. The CAST was used because it has shown good test-retest reliability, good positive predictive value (50%), and high specificity (97%) and sensitivity (100%) for ASCs [29]. The AQ-C has also shown good test-retest reliability, high sensitivity (95%), and high specificity (95%) [34].

fT levels were positively associated with higher scores (indicating greater number of autistic traits) on the CAST as well as on the AQ-C. For the AQ-C, this relationship was seen within sex as well as when the sexes were combined, suggesting that this is an effect of fT rather than an effect of sex. The relationship between CAST scores and fT was also seen within males, but not within females [152]. These findings, from two measures of autistic traits, are consistent with the notion that higher levels of fT may be associated with the development of autistic traits.

Limitations of Amniocentesis Methods

Research suggests that amniotic fluid provides the best, direct measurement of fetal hormones compared to maternal serum and is therefore probably the best choice for studying the behavioral effects of variations in prenatal androgen exposure [51, 136]. Using this method, research has shown that fT levels are significantly associated with behaviors associated with ASC, providing strong evidence for a role for prenatal hormones on typical development.

A drawback of amniocentesis is that it can only be conducted for purposes of diagnosing fetal anomalies. This means that the samples studied are selected in several ways that may influence the generalizability of the results. In addition, in these amniotic fluid studies, total extractable (or free) testosterone is utilized. However, free testosterone may not be directly responsible for the interactions which masculinize behavior [48]. A more detailed understanding of the chemistry of masculinization would be useful in extrapolating the effects of free testosterone in the development of conditions such as ASC. Against these limitations should be weighed the obvious strengths of amniocentesis, which mainly concern its timing and measurement of the fetal environment whilst avoiding unnecessary additional risk.

Conclusions

ASCs are characterized by social impairments, restricted and repetitive interests accompanied by language delay. ASC are believed to lie on a spectrum, reflecting the range of individual ability in each of these areas.

Many of the behaviors that are characteristic of ASC have also been linked to extremes of certain male-typical behaviors. Evidence includes superior performance on a range of tasks where male individuals typically outperform females but increased impairment compared to typical males on tasks with female superiority. Additional evidence linking ASC to an extreme form of the male brain comes from measurements of physical characteristics where males and females typically differ.

Physical sexual differentiation is largely attributed to the gonadal hormones, and in particular testosterone and its derivatives. Animal studies have suggested that the same hormones might also control the development of sex-typical behaviors. In humans, the direct manipulation of hormones in early development is not possible. Studies of atypical hormone environments yield some information about the role of testosterone in behavioral development, but sample sizes are very limited, particularly for individuals who also have an ASC.

More information about the hormones affecting ASC can be extrapolated from indicators of prenatal hormone levels such as finger length ratio and lateralization. These measurements are typically easy to obtain but their links to hormone levels are not clear.

Direct study of the effects of testosterone is difficult because levels rise and fall in the fetal environment over the course of gestation. In addition, maternal levels are not representative of fetal levels. The optimal way to directly measure fT appears to be via amniotic fluid obtained during clinical amniocentesis. This method is not ideal because it limits the sample available. However, some results have been obtained linking behavior and fT levels in a group of typically developing children in the UK.

In this sample, findings suggest that behaviors known to be affected by ASC also tend to be related to elevated fT levels. These behaviors include measures of social and communicative development, empathy and systemizing. In addition, a more recent study using the CAST and the Autism Spectrum Quotient-Children's Version (AQ-C) has demonstrated a relationship between the number of autistic traits a child exhibits and the fT levels measured via amniocentesis.

The findings presented in this chapter lend support to the "Extreme Male Brain" theory of ASC and its link to fT. However, a proper evaluation of this theory will require testing not just for associations between fT and autistic traits, but between fT and clinically diagnosed ASC. The latter will require much larger samples than have previously been available. Nevertheless, exposure to elevated levels of fT is indicated to be a possible factor in the development of ASCs, a hypothesis that needs more definitive testing.

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