Developmental Risks of Paternal Age During Adrenarche

Jimin Lew

Integrated Program in Neuroscience

McGill University

Montreal, Quebec, Canada

April 2021

A thesis submitted to McGill University in partial fulfillment

of the requirement of the degree of Master of Science

TABLE OF CONTENTS

ABSTRACT

There is a large body of evidence supporting the notion that maternal factors, such as age and mental health, affect her child's development. In contrast, much less is known about the paternal contribution to child outcomes, though preliminary investigations have identified specific developmental risks linked to extremes of paternal age, such as lower IQ and/or behavioral problems. Hence, fathers, like mothers, influence their child's development, whether through prenatal biological alterations in the father's sperm cells, postnatal variations in the household and learning environment, and/or quality of the father-child or couple relationships. Any developmental risks linked to paternal age may be amplified during middle childhood (6-8 years old) as children graduate from kindergarten and adjust to the more demanding environment of elementary school. This developmental stage also coincides with a unique endocrine event shown to influence brain development, adrenarche.

As such, this project aims to investigate whether the child's androgen and cortisol production during adrenarche moderates the relationships between father's age (alone or in relation to mother's age) and the child's cognition and behavior during the school transition. Data from a sub-cohort of the 3D study (n=61) was collected on parents and children from the 1st trimester of pregnancy to 6-8 years postpartum. We found extremes of paternal age and parental age gaps to interact with the child's androgen to cortisol ratio in moderating behavioral risk, especially measurable differences in externalizing symptoms such as conduct or inattention/hyperactivity symptoms. Based on these findings, we propose a new conceptual model for father-child risk transmission based on the level of "fitness" between a father's age and his child's hormonal profile and discuss future implications of this model in research and clinical practice.

RÉSUMÉ

Un nombre important de recherches démontrent des effets reliés aux facteurs maternels tels que l'âge et la santé mentale sur le développement de son enfant. Pourtant, cette même relation est très peu étudiée chez le père, bien que des enquêtes préliminaires ont indiqué des risques développementaux spécifiques liés à des extrêmes d'âge paternel, tels qu'un QI plus bas et/ou des problèmes comportementaux chez l'enfant. Par conséquent, les pères, semblables aux mères, influencent le développement de leur enfant, que ce soit par les altérations biologiques périnatales des spermatozoïdes du père, les variations postnatales du foyer et de l'environnement d'apprentissage et/ou la qualité de la relation père-enfant ou du couple. Tout risque développemental lié à l'âge paternel peut être amplifié au cours de la période intermédiaire de l'enfance (6-8 ans) puisqu'elle marque le passage de la maternelle à l'école primaire durant laquelle les enfants s'adaptent à un environnement scolaire plus exigeant. Cette période coïncide également avec un événement endocrinien unique, nommé l'adrénarche, qui exerce une influence au niveau du développement cérébral.

Ainsi, cette étude vise à déterminer si la production d'androgènes et de cortisol par l'enfant au cours de l'adrénarche modère les relations entre l'âge du père (seul ou en relation avec l'âge de la mère) et la cognition et le comportement de l'enfant pendant la transition scolaire. Dans le contexte de l'étude paternelle (une sous-cohorte de l'étude 3D), les données (n=61) ont été collectées sur les parents et leurs enfants couvrant le 1er trimestre de la grossesse jusqu'à 6-8 ans après l'accouchement. Nous avons trouvé que des extrêmes d'âge paternel ainsi que des écarts d'âge des parents interagissent avec les rapports d'androgène-cortisol de l'enfant dans la modération du risque comportemental chez l'enfant, entraînant des différences mesurables dans les symptômes d'extériorisation tels que les problèmes de conduite ou les déficits de

l'attention/hyperactivité. Pris ensemble, nous proposons un nouveau modèle conceptuel de transmission du risque père-enfant basé sur le niveau de « concordance » entre l'âge du père et le profil hormonal de son enfant et discutons des implications futures de ce modèle dans le domaine de recherche et de pratique clinique courante.

ACKNOWLEDGEMENTS

I would like to express my sincerest gratitude and warm appreciation to all the wonderful people and organizations I've worked with throughout my Master's degree, this thesis would not have been possible without their support.

First and foremost, I would like to thank my supervisor, Dr. Tuong-Vi Nguyen for her continuous support and guidance, invaluable insight and feedback throughout the course of my graduate studies. Under her supervision, I acquired indispensable skills and expertise. Furthermore, her kindness and compassion have created a wholesome environment for learning, allowing me to thrive as a research trainee and achieve personal and professional growth. My experience of working with her will be remembered for many years to come.

I would also like to thank my committee members, Drs. Sherif Karama and Jacquetta Trasler. Their insightful questions, advice and assessment expanded my learning and have greatly improved the quality of this thesis.

In addition, I am grateful for the longstanding support I've received from Dr. Sherri Lee Jones, especially for her help and guidance in data cleaning and analyses, interpretation, thesis writing and revisions. Her kindness, patience and mentorship are cherished and greatly appreciated. I would like to acknowledge Guillaume Elgbeili's help as well for his aid in data cleaning and analyses, interpretation, and training in statistics.

I would like to also express my appreciation to our lab members who provided indispensable help with participant recruitment and data collection as well as running the research visits with me: my fellow MSc student, Christina Caccese, as well as our research assistants, Tu Anh Nguyen, Denis Rompotinos, Mia Wu, Ally Yu and Michelle Klausner. I would like to also acknowledge the help we received from our psychologists Drs. Catherine Serra Poirier, Judith Le

Gallais and Stéphanie Sylvain-Roy, our imagining team Antonys Melek, Francine Duval and Jacinthe Baril as well as our research coordinator Laya Fayyazi. Their assistance was paramount to the successful completion of the Paternal Study.

The Paternal Study would not have been possible without the contribution of our collaborators and investigators from the 3D (Design, Develop, Discover) Study: Drs. Marie Brossard-Racine, Patricia Monnier, Jacquetta Trasler, Sherif Karama, William Fraser, Sophie Parent, Catherine Herba and Jean R. Séguin. Many thanks to Catherine Allard, Isabelle Krauss, Maria Rosa, Julie Miserere and Karine Després for your help with participant recruitment and data accessment.

I am also grateful to all parents and children who have generously offered their time for the 3D and Paternal Studies. They have all shown high interest and dedication to our projects and I greatly enjoyed working with them.

Last but not least, this research was made possible due to the generous funding received from the Research Institute of the McGill University Health Centre (RI-MUHC), Merck, Sharp & Dohme Corp., Canadian Institutes of Health Research, Montreal General Hospital Foundation, McGill University Health Center Foundation and Fonds de Recherche du Québec (FRQ). Additionally, I would like to personally thank the following institutions, the RI-MUHC and the FRQ-Santé, from which I was awarded scholarships in support of my MSc training.

CONTRIBUTION OF AUTHORS

Dr. Tuong-Vi Nguyen conceived and designed the study. The data used in this thesis was in part collected prenatally by the 3D team at the Ste-Justine Hospital and postnatally at the Research Institute of the McGill University Health Centre. I assisted with participant recruitment and data collection by running the research visits with a team of research assistants. Guillaume Elgbeili provided statistical support and reviewed my results section as well as the tables and figures. I analyzed the data and wrote the present thesis by carefully taking into account the feedback and suggestions provided by Drs. Tuong-Vi Nguyen, Sherri Lee Jones, Sherif Karama and Jacquetta Trasler.

1. INTRODUCTION

Intergenerational transmission of risk from mother-to-child linked to maternal age, diet, or mental health has been shown to outlast the gestational period, impacting postnatal development in a long-lasting manner. In contrast, few studies have considered whether a similar risk transmission occurs from father-to-child, despite significant shifts in socio-demographic characteristics in paternal age in the last decade. Indeed, Canadian fathers now delay procreation for an unprecedented length of time, with potential repercussions on sperm quality (see **2.1**). In parallel, expectations for fathers to be fully engaged in the cognitive and emotional development of their offspring are higher than ever before, particularly around 5-6 years of age when the child gains more autonomy from the mother and transitions from kindergarten to elementary school.

This academic transition coincides with an endocrine event unique to middle childhood, named adrenarche (see **2.2**). Adrenarche heralds a period of major neuroendocrine plasticity and susceptibility to paternal influences, whether previously inherited or linked to the quality of the father-child relationship. Therefore, any developmental risks of paternal age expressed by the child during adrenarche may have significant public health ramifications.

As such, this project aimed to investigate: 1) whether intergenerational transmission of neurodevelopmental risk from father-to-offspring linked to paternal age is shaped by the surge in androgen levels typical of adrenarche; and 2) whether those developmental effects of paternal age (if confirmed) remain apparent in the context of parental age gap calculations and interact with the child's level of neuroendocrine maturity, by using parental and child data from the 3D (Design, Develop, Discover) cohort, prospectively collected from the 1st trimester of pregnancy onward (see **4.1**).

2. BACKGROUND

2.1 Paternal Age at Conception and Child Development

A gradual increase in parental age at first birth has been observed since the late 1960s in Canada, reaching 28.7 years in 2012 and 29.2 years in 2016 for mothers and 31.6 years in 2012 and 32.2 years in 2016 for fathers (Provencher et al., 2018). These changes have been ascribed to longer life expectancy and various societal and socioeconomic factors, such as increased use of contraceptive methods, changing gender roles, and pursuit of education- and career-related goals as opposed to reproductive planning (Provencher et al., 2018). Maternal age at conception has been an early focus of research on the intergenerational transmission of risk from parent-to-child and there is now a large body of research suggesting that both early (e.g., <20 years old, so called teenage mothers) and advanced (e.g., >35 years old) maternal age may confer increased neurodevelopmental risks to their offspring (Chang et al., 2014; McGrath et al., 2014). While some of these effects have been traditionally considered to come from biological changes in the mother's reproductive cells (e.g., genetic/epigenetic alterations with advanced maternal age), variation in maternal age likely impacts the child through a mix of biological, psychological and social pathways. For example, an older mother may carry more biological risk (e.g., linked to autism and schizophrenia) but also offer a rich learning environment for her children, perhaps accounting for the mixed effects of advanced maternal ages on child's development reported in the current literature (Barclay & Myrskylä, 2016; Duncan et al., 2018; Han et al., 2018; Kim et al., 2018; McGrath et al., 2014; Pariente et al., 2019). In contrast, very young mothers tend to give birth to children with a higher risk of developing externalizing disorders such as Attention Deficit Hyperactivity Disorder (ADHD) and also tend to offer a poor childhood environment that is

common in young mothers (e.g., financial difficulties and poor education) while they themselves undergo various life changes (Chang et al., 2014; Fergusson & Lynskey, 1993).

Paternal age at conception has received less attention in comparison, despite the accumulating evidence from the field of evolutionary psychology that support unique, flexible, and increasingly central contributions from fathers to their children's development over the course of human history (Machin, 2019; Maher, 2015). Similar to the mother's, paternal age at conception may also influence child's development through both prenatal biological alterations in the father's sperm cells as well as postnatal variations in the level of emotional engagement and the quality of the learning environment they offer to their children (McGrath et al., 2014). In particular, advanced paternal age has been associated with an accumulation of genetic and epigenetic alterations during the process of spermatogenesis. This process occurs in a continuous manner over the lifespan, increasing the susceptibility of sperm cells to genetic and epigenetic changes over time (Hehar & Mychasiuk, 2015). Notably, age-related *de novo* genetic mutations of single nucleotide variants (dnSNVs) are 3-4 times more prevalent in paternal, compared to maternal, germ cells (Taylor et al., 2019). In addition, as fathers get older, higher levels of dnSNVs have been detected in their offspring, supporting the notion that age-related genetic alterations in male germ cells are transmissible from father to child (Herati et al., 2017; Milekic et al., 2015). These genetic alterations due to advanced paternal age may lead to neurodevelopmental risk in their offspring to some extent, for example through a higher prevalence of autism, schizophrenia, and bipolar disorder (McGrath et al., 2014). Still, this genetic mutation process in the male germline occurs slowly over several decades and is thought to be only responsible for a small portion (i.e., about 10% increased risk of autism and schizophrenia and about 20% increased risk of intellectual disability) of neurodevelopmental disorders in the child (Taylor et al., 2019). In the context of

young fathers, similar to the mother's, a mix of reproductive, socioeconomic and familial factors pertaining to the early parenthood may explain the association between young paternal age and increased risk of ADHD (Janecka et al., 2019).

In addition to the increased risk of neurodevelopmental disorders observed in the offspring, paternal age may also influence their offspring's cognitive development. Notably, Saha et al. (2009) examined 33 437 children during infancy (at 8 months old) and in early childhood (4 years and 7 years of age) to test the relationship between paternal age and the child's developmental milestones (using the Bayley scales for Infant Development), general intelligence (using the Standford Binet Intelligence Scale and the Wechsler Intelligence Scale for Children), conceptual and perceptual motor ability (using the Graham-Ernhart Block Sort Test) as well as academic performance (using the Wide Range Achievement Test) (Saha, Barnett, Foldi, et al., 2009). In this study, advanced paternal age was found to be associated with lower cognitive scores in children over the entire age range examined and for all the selected developmental measures, except the Bayley Motor and Mental scales (the adjusted R-squared ranging from 2.9% for Bayley Mental to 29.5% for WISC full scale IQ; Saha, Barnett, Foldi, et al., 2009). Paternal age may also influence their offspring's behavioral development during middle childhood. Upon examining 21 753 7-year-old children from the US Collaborative Perinatal Project, the authors found a significant association between advanced paternal age and increased risk of adverse externalizing behavior in the offspring (Saha, Barnett, Buka, et al., 2009b). In another study, Janecka et al. (2017) reported a U-shaped relationship between father's age at delivery and their offspring's social development (as measured by the Strength and Difficulty Questionnaire -SDQ-), with children of fathers at both age extremes (<25 and >51 years old) showing higher initial sociability scores but less developmental change over time (from 4 to 16 years of age) compared to the offspring of fathers who were 25-50 years

old at delivery (Janecka et al., 2017). Interestingly, Weiser et al. (2008) observed a similar U-shaped pattern in a separate study of male adolescents 16-17 years of age, with lower social functioning in the offspring of younger (<20 years) (odds ratio (OR)=1.27, 95% confidence interval (CI) 1.08-1.49) as well as older fathers (>45 years) (OR=1.52, 95% CI 1.43-1.61) (Weiser et al., 2008).

As such, there has been increasing interest for the role that other age-related paternal factors (e.g., epigenetic alterations in the germline and differences in the emotional and cognitive environment provided to the child) and individual factors in the child (prenatal hormonal programming and postnatal neuroendocrine function during subsequent developmental stages (Auyeung et al., 2010; Parner et al., 2012)) may play in the development of complex neurodevelopmental disorders like autism and schizophrenia (de Kluiver et al., 2017). Paternal age likely interacts with a number of bio-psycho-social factors (e.g., maternal age, socio-economic status, age-related decline in sex hormones and reproductive function, etc.) in altering both sperm parameters (Eisenberg & Meldrum, 2017; Herati et al., 2017) and offspring's cognitive and behavioral development.

2.1.1 Knowledge Gaps and Summary: Paternal Age at Conception

As we have seen, currently available evidence does support the existence of specific cognitive and behavioral risks for the offspring of fathers at age extremes, i.e., early or advanced ages. However, there is insufficient information to understand whether/in which way paternal age may interact with individual factors in the child such as prenatal hormonal programming and postnatal neuroendocrine function. Similarly, it is unclear whether paternal age interacts with maternal age in determining neurodevelopmental risk in the child, or whether either parent's chronological clock independently affects their offspring. Interestingly enough, it has been

recently suggested that there is an association between larger parental age gap (e.g., a combination of an older father and a younger mother) and increased risk of ADHD and autism in the child (Janecka et al., 2019; Lopez-Castroman, 2014; Sandin et al., 2016). It remains to be elucidated whether these developmental effects linked to paternal age become apparent in the context of parental age gap calculations and as to how they interact with individual factors in the child.

2.2 Adrenarche: A Critical Developmental Period for the Child

Intergenerational transmission of risk from parent-to-offspring is particularly likely to affect cognitive and behavioral function in the child during critical stages of neuroendocrine development that occur at specific times from fetal life to late adulthood. The neuroendocrine period relevant to us is adrenarche, which coincides with the large shift in cognitive and emotional demands during the transition from kindergarten to elementary school in middle childhood (6-8 years old).

Neuroendocrine development is predominantly engineered by two brain-endocrine axes: the hypothalamo-pituitary-adrenal (HPA) and the hypothalamo-pituitary-gonadal (HPG) axes (Campbell, 2011). Adrenarche marks the onset of significant, pulsatile activity in the HPA axis, with marked adrenal secretion of dehydroepiandrosterone (DHEA), corticosteroids such as cortisol (C) and to a lesser extent, androstenedione (A) and testosterone (T) (Bremer & Miller, 2014; Kamin & Kertes, 2017). Adrenarche is distinct, though it partially overlaps, with the onset of pulsatile HPG axis activity, or gonadarche (of which the onset is around 10-15 years of age) (Campbell, 2011). These neuroendocrine stages significantly alter the brain's sensitivity to steroid hormones, through both long-standing alterations in the brain's structural organization (i.e., organizational effects) or transient changes in brain function (i.e., activational effects) (Phoenix et al., 1959; Schulz & Sisk, 2016). As a result, neuroendocrine development in a particular child will

likely interact with any cognitive or mental health vulnerabilities previously inherited from the father.

Adrenarche also heralds the onset of physical transformations typical of early sexual maturation, such as axillary or pubic hair development, increases in sebum production, and changes in body odor (Campbell, 2011). Most interesting to us, though, is its potential protective and trophic properties with regards to brain growth. Indeed, adrenarche is an event unique to humans and the great apes thought by many to represent an adaptation for the prolonged period of brain development characteristic of our species (Campbell, 2011). There is recent evidence that indeed, the hormones secreted during adrenarche may promote learning, cognition and emotional regulation during middle childhood (Campbell, 2011).

In particular, the large increase in DHEA-driven anabolic activity, unmatched by a corresponding increase in glucocorticoid-driven catabolic activity, is likely to have evolved in humans to protect the rapidly developing brain against the adverse effects of cortisol during a period of rapid physical stresses and transformations (Maninger et al., 2009). As a result, any disruption in the timing of or rate at which adrenarche progresses in a particular child has adverse cognitive and behavioral consequences for the child (Feldman Witchel & Plant, 2009). Notably, premature adrenarche (i.e., secondary sexual hair emerging in girls younger than 8 and in boys younger than 9 years old) may be associated with an adverse hormonal "priming" effect that leads to cognitive (verbal, spatial, executive function) and behavioral impairments (anxiety, depression, aggression), even in the context of normal ovarian or testicular maturation (Dorn et al., 1999; Nass et al., 1990; Sontag-Padilla et al., 2012; Tissot et al., 2012; Williams et al., 2012).

One of the most striking features of neuroendocrine development is the fact that its main products -steroid hormones- are readily converted (and re-converted) from one to the other (for

the human steroidogenic pathway, see Bremer & Miller, 2014), ensuring rapid, adaptive and flexible adjustments with the external environment with varying degrees of hormonal potency and receptor binding affinity. In this case, androgen potency can be defined as the efficacy and the degree to which an androgen hormone binds to its receptor (androgen receptor affinity) to elicit responses (Allolio et al., 2012). During adrenarche, DHEA can be converted to androstenedione; and similarly, androstenedione can be converted to testosterone; going from a hormone of lower to a hormone of greater androgen potency (DHEA < androstenedione < testosterone) (Allolio et al., 2012; S. G. Beck & Handa, 2004; Bremer & Miller, 2014; Fink, 2007; Maninger et al., 2009). Because of the conversion properties of the steroid system, any steroid hormone can shift from having neuroprotective to neurotoxic properties by being rapidly converted to another hormone along several possible pathways, underlining the importance of evaluating hormones together rather than in isolation (Foradori et al., 2008). One way to characterize androgen potency in a specific child (and its effects on the central nervous system; CNS) is to calculate the relative levels of one androgen hormone vs. another, in order to yield an androgen ratio, e.g., DHEA/testosterone ratio.

There is evidence that steroid hormones play an important role in fine-tuning the balance between neuroprotection and neurotoxicity within the CNS. For example, DHEA can tip the balance toward neuroprotection, buffering against sudden, large or chronic, stress-related increases in cortisol (Kamin & Kertes, 2017). In contrast, other androgens such as androstenedione and testosterone may only be partially protective or even have detrimental effects on brain development and cortical functioning (Allolio et al., 2012).

Adding to this complexity is the fact that the distinct role of each androgen hormone varies across different brain regions, cognitive functions and behavioral domains. For instance, our group

has previously shown that within a sample of children and adolescents of 6-22 years old, higher DHEA levels and higher DHEA/cortisol ratios favor a greater divergence in cortical vs. amygdala/hippocampal growth patterns, resulting in optimization of cortex-based cognitive functions (e.g., executive functions) and impairment in perceptual tasks (e.g., detection of emotional or spatial stimuli) driven by the amygdala or hippocampus (Farooqi et al., 2018a, 2018b, 2019; Nguyen, Wu, et al., 2017). Furthermore, we showed that higher testosterone levels and higher testosterone/cortisol ratios favor greater coordination in the growth of the cortex and amygdala/hippocampus, resulting in optimization of amygdala-/hippocampal-driven short-term spatial or verbal memory (Nguyen, Lew, et al., 2017). However, these benefits come with significant downsides, such as a higher risk of amygdala-driven aggressive behaviors and a greater impairment in cortex-based executive functions (Nguyen et al., 2018; Nguyen, Lew, et al., 2017).

2.2.1 Knowledge Gaps and Summary: Adrenarche

The dynamic interactions within the steroid system during adrenarche (androgen-to-androgen as well as androgen-to-cortisol) create a fertile ground for brain growth, defining a period of rapid, adaptive, and flexible neuroplasticity that promotes the development of a host of cognitive and behavioral abilities. In addition, adrenarche coincides with significant psychosocial changes that are uniquely characterized by the period of middle childhood (transition from kindergarten to elementary school, greater autonomy gained by the child from the parents, increasing motor, cognitive and perceptual demands as well as possible onset of psychopathology). Thus, the partial or full expression of paternally inherited factors in the context of middle childhood will likely interact with the significant neuroendocrine alterations that occur at this stage of a child's development. Few studies, however, have addressed both parental and child factors in order for us

to better understand the relationship between intergenerational transmission of risk and its neurobehavioral expression during adrenarche.

3. RATIONALE, AIMS, HYPOTHESES

In sum, the available literature supports the notion that extremes of paternal age (early or advanced) may be transmitted to the offspring and expressed as cognitive or behavioral alterations. In turn, the expression of paternally inherited factors during middle childhood is likely to be influenced by the hormonal shifts of adrenarche, which themselves determine the degree of neuroplasticity, vulnerability and resilience of a particular child to neurodevelopmental disorders. As such, the overarching goal of this project is to test for the interactions between paternal age at conception and the child's hormonal, cognitive and behavioral profile during adrenarche, using prospectively collected parental and child data from a small sub-sample of an existing cohort (3D study) (see **4.1** and **4.2**). Because of the exploratory nature of our study, our results should be considered as hypothesis-generating as opposed to hypothesis-confirming as the latter can only be accomplished by examining data from larger cohort studies (Gould, 2010). Specific aims are:

AIM #1: To determine whether paternal age at conception interacts with the child's adrenarcheal hormones to influence cognition and behavior between 6-8 years of age, in a manner distinct from that of maternal age.

Hypothesis: Hormones of adrenarche will moderate the effects of paternal age on child's cognition and behavior such that the presence of two risk factors (i.e., extremes of paternal age and lower levels of androgens relative to cortisol levels or higher levels of high-potency androgens relative to low-potency androgens) will be associated with worse developmental outcomes than either in isolation.

AIM #2: To determine whether the age gap between fathers and mothers interacts with the hormones of adrenarche in the child in influencing their cognition and behavior between 6-8 years of age.

Hypothesis: Hormones of adrenarche will moderate the effects of parental age gap on child's cognition and behavior, such that the presence of three risk factors (greater age gap (older fathers and younger mothers) and lower levels of androgens relative to cortisol levels or higher levels of high-potency androgens relative to low-potency androgens) will be associated with worse developmental outcomes than either in isolation.

4. METHODS

4.1 Participants

Participants to this study were initially recruited as part of the 3D (Découvrir, Développer, Devenir) cohort created by the IRNPQEO (Integrated Research Network in Perinatology of Quebec and Eastern Ontario). The 3D cohort is an ongoing prospective study of 2366 families that includes sociodemographic and clinical information (e.g., parental age, substance use, and levels of depression and anxiety), as well as biospecimens (e.g., placenta samples, umbilical cord blood, and blood samples from both parents and their child at two years old) (Fraser et al., 2016). The 3D study was set up to investigate an array of intrauterine determinants of adverse birth outcomes (i.e., prematurity, intrauterine growth retardation, and birth defects) including environmental, psychosocial, nutritional and genetic factors (Fraser et al., 2016). Initial recruitment took place between May 25, 2010 and August 30, 2012 from nine different centres, of which seven were located in Montreal, one in Quebec City, and the last one in Sherbrooke, Quebec (Fraser et al., 2016). Families were recruited during the 1st trimester of pregnancy and followed prospectively

throughout pregnancy, delivery, when the child turned 2 years old, and when the child transitioned from kindergarten to elementary school (5-6 years old, Transition follow-up study).

The sample used in this project ("paternal study") is a sub-cohort of the Transition study, itself a follow-up study of the initial 3D cohort. Inclusion criteria for the paternal study were: 1) complete data from 3D child-father-mother trios; 2) active participation in 3D data collection and the Transition study; 3) being available for data collection between January 2018 and January 2020, 6-8 years after the index pregnancy and delivery of the child. In addition to the 3D study's initial exclusion criteria (significant medical illness in the mother, such as multiple gestation pregnancy, human immunodeficiency virus (HIV), etc. (Fraser et al., 2016)), the paternal study also excluded pregnancies arising from assisted reproductive techniques and families in which either parent had a history of significant substance use disorder (e.g., heroin, cocaine, etc.) or in which the child displayed a history of neurological disorders affecting brain function. The Transition team identified 221 eligible participants, of which 209 were successfully reached at least once by phone or email by our research team and 76 families expressed interest in taking part to the study. At last, 61 families consented to participate and completed all the data collection pertinent to the paternal study (see **Table 1** and **Figure 1**). The study obtained approval from the Research Ethics Board (REB) of the Research Institute-McGill University Health Centre (RI-MUHC) and conformed to the principles of the Declaration of Helsinki. Verbal assent was obtained from children and participating parents provided written consent for their own as well as their child's participation to the study. Further details and results from the 3D study as well as the exhaustive list of publications can be found on the IRNPQEO website (https://www.irnpqeo.ca/en/).

4.2 Measures

As part of the paternal study, additional data were collected in participating families through a one-time visit at the RI-MUHC site. In the child, data collection included: 1) endocrine data (repeated salivary samples; they were used to measure the levels of steroid hormones (i.e., dehydroepiandrosterone, androstenedione, testosterone and cortisol)), 2) cognitive and behavioral data (evaluation of verbal and non-verbal cognitive abilities through Wechsler Intelligence Scale for Children-V; age-appropriate evaluation of behavior through Strengths and Difficulty Questionnaire) and 3) clinical data (health history, physical exam, vital signs, height and weight, and anthropometrics). In the parent, we collected additional self-report of paternal and maternal depressive and anxious symptoms at the time of the research visit or shortly (2-4 weeks) prior to or after the visit. Some of the potential confounding variables such as maternal age, parental education, and gestational age were derived in part from the data already collected in the original 3D study (for the full list, please refer to 4.3.1).

4.2.1 Paternal Age Calculations

By using information on (1) paternal age at test date (acquired in years, converted to days), (2) child's age (in days) at test day derived from their date of birth and date of testing, and (3) gestational days, paternal age at conception was computed as: paternal age at conception = (1) – (2) – (3). Everything was then converted back into years. This variable named "paternal age at conception" was subsequently used in our first set of statistical analyses as the main predictor.

4.2.2 Paternal to Maternal Age Gap Calculations

Age gap variable was computed by subtracting maternal age at conception from paternal age at conception. Thus, a positive value would signify that fathers are older than their partners while a negative value would signify that mothers are older than their partners. A value of zero

would signify that both parents are of same age. This variable named "paternal to maternal age gap" was subsequently used in our second set of statistical analyses as the main predictor.

4.2.3 Hormone Sampling, Assays and Imputation

Throughout the visit, two saliva samples were collected each time (within a minute apart) across three timepoints (at baseline (pre-MRI; magnetic resonance imaging), post-MRI, and 1h post-MRI), totaling six samples at the end. Of note, for the purpose of this paper, only the two baseline hormones were examined. All samples were collected for a duration of 60-90 seconds each with respect to the standard salivary collection time in children, in order to maximize saliva volume collection for hormonal detection (Tryphonopoulos et al., 2014). Immediately after collection, test tubes were spun (n=366 samples) to optimize sample volume and were frozen at a -20°C freezer for a duration that does not exceed four months. Special care was taken to the saliva collection times as adrenal hormones follow specific diurnal patterns in response to adrenocorticotropic and gonadotropin-releasing hormones (Matchock et al., 2007). Strict attention was also paid to limiting the age range and pubertal status of children included in our sample (using pubertal development scale, see next section **4.2.4**) to ensure that all were within the same developmental window. Additionally, a saliva journal was completed prior to saliva collection to inform ourselves ahead of all potential confounding factors (e.g., hours of sleep, food/drink consumption, medications, and potential elements to saliva contamination such as blood) in order to increase the accuracy of our hormonal measurements while minimizing all known confounding factors. Finally, all sampling took place on the same day at a time of limited hormonal variability, i.e., over 3.5 hours in early (1-3PM) afternoon (as opposed to morning collection) (Schultheiss & Stanton, 2009).

Children's saliva (~1mL) was collected using Cortisol-Salivette (SARSTEDT, #51.1534.500) and DHEA, androstenedione, testosterone and cortisol levels were measured using enzyme-linked immunosorbent assay (ELISA) kits from Salimetrics (DHEA: catalog. #1-2212-5; testosterone: cat. #1-2402-5; cortisol: cat. #1-3002-5;) and Abcam (androstenedione: cat. #ab178609). The % cross-reactivity between hormones using ELISA was found to be low (refer to Salimetrics protocols for the exact %s; Salimetrics, 2020). The kit's intra-assay and inter-assay coefficients of variations (COVs) were 5.3-5.8% and 7.9-8.5% for DHEA, 8.5% and 11% for androstenedione, 2.5-6.7% and 5.6-14.1% for testosterone, and 4-7% and 3-11% for cortisol, respectively. Following the assays, saliva samples that had sufficient quantity to run the assay but with a concentration level that was not detectable by the kit (noted in the dataset as NF=not found or BLQ=below the limit of quantification) were quantified as one unit below the kit's lower detection limit or one unit below the detectable minimum of the hormone of interest if that minimum was found to be smaller than the kit's lower detection limit (see **Appendix Table A1**). Furthermore, when assayed samples demonstrated values beyond the upper limit, the samples were re-assayed, and the concentration closest to the kit's detection limits was included in the analyses.

In order to minimize our chance of losing data (due to possible insufficient volume) and improve the validity and accuracy of our hormonal measures, few additional steps were taken. First, for each hormone (DHEA, A, T and C) and timepoint separately, its mean was calculated by taking the levels of both samples collected. In other words, if both samples had sufficient volume, the average was computed or else, the single available value was used. In the case where both values were missing (i.e., samples with insufficient volume to test for a particular analyte at both collections ("insufficient" samples)), the average for that timepoint was set as a missing value. Next, using the Expectation-Maximization method (Dong & Peng, 2013), the means from all

hormones at all timepoints were imputed at the same time, adjusting for the variables that may be highly correlated with our hormones or are, in theory, associated with them: child's sex, season, paternal and maternal ethnicity (white vs. non-white), gestational age at birth, arm and back skinfold measures, lean body mass, BMI percentile, child's age at testing and time of first saliva collection. This method was applied to minimize possible problems that may arise due to missing values in our dataset (e.g., loss of valuable information, reduced statistical power and biased estimates of parameters) (Dong & Peng, 2013). In the exceptional case where all six samples were insufficient in volume (this only occurred for two participants in the context of androstenedione; see **Table 1**), they were left with no values. Additionally, if imputed values were considered to be impossible (i.e., negative hormonal values), they were replaced by the smallest detectable value selected for that particular hormone (as we did with NF or BLQ samples). Following imputation, hormonal ratios (DHEA/C, A/C, T/C, DHEA/A, DHEA/T and A/T) at baseline were computed as our main interest was to examine hormone levels in relation to another. Thus, each hormone was examined separately and together by looking at the ratios. At last, all distributions were checked for normality, and square root transformations were applied to correct for skewness of our baseline hormones while log transformations were applied to correct for skewness of our hormonal ratios (with the exception of DHEA/A which did not need to be transformed) (Sollberger & Ehlert, 2016).

4.2.4 Pubertal Development Scale

The physical changes of puberty were measured using the Pubertal Development Scale (PDS), a parental self-report questionnaire on the child's physical traits. The PDS has been shown to have good reliability (coefficient α: 0.77) and validity (r^2 = 0.61-0.67) in comparison to physical examination (Petersen et al., 1988). Pubertal maturation includes both changes attributable to adrenarche (axillary and pubic hair, skin changes) and to gonadarche (e.g., facial and voice changes

in boys, and breast growth and menarche in girls). In the context of our study, PDS was used to ensure that all participating children are within the same pubertal developmental stage (i.e., adrenarche in the context of children between 6-8 years of age).

4.2.5 Cognitive and Behavioral Measures in the Child

We have selected two age-appropriate standardized measurements of verbal and non-verbal cognitive abilities (Wechsler Intelligence Scale for Children -V; WISC) and adaptive and social functioning (Strength and Difficulty Questionnaire; SDQ for children aged 4-10). These measurements have all been validated with healthy and clinical populations and show good psychometric properties.

In this study, a trained psychologist administered seven subtests of WISC-V to participating children (i.e., Block Design, Similarities, Matrix Reasoning, Digit Span, Coding, Vocabulary and Figure Weights). Its full scale (total IQ score) was computed from these subtests and verbal comprehension and fluid reasoning indices were measured by summing scores on similarities and vocabulary, and matrix reasoning and figure weights, respectively. Our analyses first prioritized the high order scales (i.e., full scale, verbal comprehension and fluid reasoning indices > seven subtests) and subsequently moved down to the subtests only when a significance was found at the higher level, this was done to delineate the subtest next in order that could be driving the effects in the relevant model. WISC has been shown to be reliable (reliability of all subtests of the full scale WISC ranging from 0.80 to 0.94) and valid (factor analysis showing WISC-V primary subtests to be associated with different aspects of cognitive ability) with its clinical relevance proven through its association with Child and Adolescent Academic Questionnaire (containing items related to risk factors for school failure; r = -0.50) and Child and

Adolescent Behavior Questionnaire (containing items related to risk factors for delinquency and criminal behavior; r = -0.12) (Pearson, 2018).

While the child underwent the cognitive testing, we asked the parents to complete the Strength and Difficulties Questionnaire, basing his or her answers on the child's behavior over the last six months. The child's overall behavioral problems (total difficulties score on SDQ) were computed by summing its five subscales (i.e., prosocial behavior, peer problems, emotional problems, conduct problems and hyperactivity/inattention problems). Of note, SDQ prosocial behavior was reverse coded as opposed to the rest of the subscales. In addition to the overall behavioral problems, internalizing (sum of peer and emotional problems) and externalizing (sum of conduct and hyperactivity/inattention problems) problems were measured and analyzed. Similar to WISC, our analyses favored the high order scales (i.e., overall behavioral problems, prosocial behavior, and internalizing and externalizing problems > subtests (peer, emotional, conduct and hyperactivity/inattention problems)) and subsequently moved to analyzing the subtests if a significance was found at the upper level. SDQ showed high internal consistency (Cronbach's alphas of ≥ 0.7 between total difficulties score and hyperactivity scale of parent completed SDQ and between total difficulties score and three out of five subscales of teacher completed SDQ) and high concurrent and divergent validity compared to Child Behavior Checklist and Teacher's Report Form subscales when used in young children (age 5-6) in a Dutch sample (Mieloo et al., 2012). When compared with corresponding sections on the Development and Well-being Assessment (instrument designed to assess diagnoses on mental disorders for children between 5-17), high diagnostic potential was observed for depressive disorders, generalized anxiety disorders, conduct disorders, hyperactive disorders, and antisocial personality disorders (Silva et al., 2015).

4.3 Statistical Analyses

4.3.1 Covariates

Statistical analyses were run using IBM SPSS version 24.0 (SPSS Inc., Chicago, Illinois). Significance level for all analyses was set at $p \leq 0.05$. Based on existing literature, to single out the unique associations between paternal age and child's development, we followed a two-step process in selecting our covariates:

1) Identification of notable factors that may affect child's development, based on existing literature and availability in our dataset, with a particular focus on those factors that may be transmitted through sperm/placental genetic or epigenetic alterations:
    a. child's sex, season of hormonal collection, race/ethnicity, and gestational age at birth;
    b. prenatal and postnatal parental depression and anxiety (Hehar & Mychasiuk, 2015; Rodgers et al., 2013), as measured prenatally in the 3D cohort by the Perceived Stress Scale (PSS), Center for Epidemiologic Studies Depression Scale (CESD) and an in-house questionnaire listing mood and anxiety symptoms based on DSM-IV criteria (STR) (Shapiro et al., 2017), and postnatally in the paternal study by Beck Depression Inventory (BDI; A. T. Beck et al., 1961) and Beck Anxiety Inventory (BAI; A. T. Beck et al., 1988), two well-validated parental self-report questionnaires measuring depression and anxiety symptoms;
    c. paternal body mass index (BMI: weight/height^2 in kg/m^2) (Yeung et al., 2017), as computed using the 3D study's paternal anthropometric measurements;
    d. paternal alcohol consumption in the year preconception (Hehar & Mychasiuk, 2015), as measured by the following question: "During the year before your partner got pregnant, how often did you drink alcoholic beverages per week?";

e. paternal smoking (Day et al., 2016), as measured by the number of days/week smoked and the number of cigarettes smoked/day in the year preconception;
f. level of parental education and household income, previously found to be highly correlated with parental IQ (Capron & Duyme, 1989; Ceci & Williams, 1997; Matarazzo & Herman, 1984; Winship & Korenman, 1997);
g. father-child relationship, as measured by father-baby relationship at 3-, 12- and 24-month postpartum (measured through PACOTIS; Parental Cognitions and Conduct Toward the Infant Scale; Boivin et al., 2005).

2) Selection of covariates that were significantly associated with the child's cognition and behavior and conversely, removal/non-inclusion of factors with no demonstrable effects on child's outcomes.

A complete list of selected covariates can be found in the appendix (see **Appendix Table A2**). Notably, sex was not associated with any of the child's outcomes of interest, so this variable was excluded from the final selection of covariates. However, because sex effects may be lost in samples mixing boys and girls, we ran additional exploratory models testing for moderated moderation effects of sex (see **Appendix Figure A1** for the conceptual model of moderated moderation) and further confirmed no moderating effect of sex on the relationships between paternal age/parental age gap and the child's neuroendocrine development (see **Appendix Tables A3-22** for our results from sex-bases analyses). Additionally, paternal education was only associated with some of the child's outcomes of interest, while others were associated with both maternal education and paternal education. We have combined maternal and paternal education into one "parental education" variable wherever possible to limit the associated loss of power with

the addition of two (vs. one) covariate. Of note, none of the results varied significantly regardless of the type of education variable included.

4.3.2 Models for Aim #1: Paternal Age and Child's Neuroendocrine Development

Moderation analyses were conducted using PROCESS software, version 2 (Hayes, 2013) to test whether child's neuroendocrine levels (hormones of adrenarche and their ratios, see **4.2.3)** moderate the association between paternal age at conception (see **4.2.1**) and developmental outcomes (child's IQ and behavior, see **4.2.5**). Of note, our models tested the interaction at large so all variables (apart from sex) were treated as continuous, not categorical variables. For visualization purposes only, we've displayed the relationship between our predictors and outcomes at the 10^{th} and 90^{th} percentiles for the reader to understand the direction of the interaction at a glance (see **Figures 2-5**) and we did not run post-hoc tests comparing the 10^{th} and 90^{th} percentiles. Following Hayes' guidelines, only the region(s) of significance that overlapped with our slopes were presented as part of the figures (Hayes, 2013).

4.3.3 Models for Aim #2: Parental Age Gap and Child Neuroendocrine Development

Moderation analyses were conducted using PROCESS software, version 2 (Hayes, 2013) to investigate whether offspring's neuroendocrine levels (hormones of adrenarche and their ratios, see **4.2.3**) moderate the association between age differences in fathers and mothers (see **4.2.2**) and child's developmental outcomes (child's IQ and behavior, see **4.2.5**). Similar to our predictor "paternal age at conception", parental age gap variable was treated as a continuous variable. Again, we've displayed the relationship between our predictors and outcomes at the 10^{th} and 90^{th} percentiles sorely for visualization purposes and no post-hoc tests have been conducted. Following Hayes' guidelines, only the region(s) of significance that overlapped with our slopes were presented as part of the figures (Hayes, 2013).

4.3.4. Correction for Multiple Comparisons

As previously mentioned, in the context of our study with an underlying exploratory nature and a limited sample size, current analyses were conducted in a hypothesis-generating setting. In consideration of this information, it was deemed more appropriate to not control for multiple comparisons. As such, our results and discussion sections focused on the primary findings obtained from moderation analyses (see **5.2** and **5.3**). Still, in order to acknowledge the importance of correcting for multiple comparisons especially within a research design like ours involving multiple hormones and (sub)indices of cognitive and behavioral measures, the Benjamini-Hochberg (B-H) False Discovery Rate (FDR) was applied to our models (where "q" was set at p=0.05, and "Q, the threshold of significance" was equal to (i/m)*q (i=rank in terms of significance, m=total number of tests being examined)) and the adjusted p-values have been included as an additional information for the readers (see **Tables 2-5** and **Appendix Tables A23-38** for adjusted p-values of our significant and non-significant findings, respectively) (Benjamini & Hochberg, 1995; Verhoeven et al., 2005).

5. RESULTS

5.1 Sample Characteristics

Sample characteristics are listed in **Table 1**. Our sample included 61 children (36 boys and 25 girls). The ranges for child age, maternal age at conception, paternal age at conception, and age gap were 5-8, 19-40, 22-53, and -4-18 years respectively. On average in our samples, mothers conceived at the age of 31 while fathers were 34 years old at time of conception; furthermore, fathers were older than mothers by a difference of 2 years. No one was excluded on the basis of Pubertal Developmental Scale and the mean baseline hormonal levels were 53.77 pg/mL for DHEA, 49.04 pg/mL for androstenedione, 29.15 pg/mL for testosterone and 723.37 pg/mL for

cortisol, before performing square root transformations (see **Appendix Figures A2-12** for scatter plots of our main variables (paternal/maternal age, age gap, baseline hormones and its ratios)). The average IQ score in participating children was 112 when measured by Wechsler Intelligence Scale for Children -V and the mean overall behavioral problems was 8 (out of 40) in participating children when measured by Strength and Difficulties Questionnaire. In terms of the highest level of education, the majority of the participating mothers had a Bachelor's (or university equivalent) degree (n=26; 42.6%) and the majority of the participating fathers had either a CEGEP/college (n=16; 26.2%) or a university degree (n=17; 27.9%). Most parents were of Caucasian ethnicity (77% of the mothers (n=47) and 78.3% of the fathers (n=47)).

Bivariate correlations between predictors, covariates and outcomes are listed in **Table 6** and **Appendix Tables A39-42**. Notably, there were no correlations between 1) paternal age and child's IQ and behavior; 2) parental age gap and child's IQ and behavior; and 3) child's neuroendocrine status and his/her IQ and overall behavior problems.

5.2 Results -Aim #1: Hormones of Adrenarche Moderate the Relationship between Paternal Age and Child Behavior

As shown in **Table 2**, moderation models controlling for maternal age at conception, maternal depression and paternal education revealed that the ratio between androstenedione and cortisol (A/C) in the child moderated the relationship between paternal age and level of child's externalizing problems (B= 0.4425, SE= 0.1932, p= 0.0262) at age 6-8 years old. Advanced paternal age was associated with higher externalizing problems only in the context of higher A/C ratios in the child (see **Appendix Figures A13** for a visual depiction of the relationship at the 10th and 90th percentiles as well as **A14,** a copy of the same figure with the addition of individual data points, clustered into 6 groups).

A closer examination revealed that this interaction was driven by the component within the externalizing subscale measuring the severity of conduct problems in the child (B= 0.2055, SE= 0.0831, p= 0.0167). Probing of this interaction showed that A/C ratio was inversely related to the severity of conduct problems (higher A/C; fewer conduct problems) in children of fathers younger than 32.3029 years old (depicted by the region of significance in yellow; see **Figure 2** and **Appendix Figure A15** to visualize individual data points).

No other associations have been found (see **Appendix Tables A23-31** for non-significant results).

5.3 Results -Aim #2: Hormones of Adrenarche Moderate the Relationship between Parental Age Gap and Child Behavior

Figure 3 shows that testosterone (T) levels moderate the relationship between parental age gap and the child's level of overall behavioral problems at 6-8 years of age (B= 0.1500, SE= 0.0679, p= 0.0313; see **Table 3**) when controlling for maternal depression. The older the father was compared to the mother, the greater the overall level of behavioral difficulties displayed by their offspring in the context of higher T levels (significant slope depicted by the black line; conditional effect of age gap on overall problems at highest 10% T: B= 0.5510, SE= 0.2552, $p< 0.05$). In addition, probing of this interaction revealed that offspring of parents with little age difference (≤2.1464 years) displayed an inverse association between T levels and overall behavioral difficulties (higher T; fewer behavioral problems; region of significance depicted in yellow).

Figure 4 shows that the moderating impact of DHEA/cortisol (DHEA/C) ratio on the relationship between parental age gap and the child's externalizing problems at 6-8 years of age (B= 0.1850, SE= 0.0874, p= 0.0388; see **Table 4**) when controlling for paternal education and

maternal depression. The older the father was compared to the mother, the greater the level of externalizing problems displayed by their offspring in the context of higher DHEA/C ratios (significant slope depicted by the black line; conditional effect of age gap on externalizing problems at highest 10% D/C ratio: $B = 0.3039$, $SE = 0.1511$, $p < 0.05$). In addition, probing of this interaction revealed that offspring of fathers older than mothers by a gap of ≥7.9013 years displayed a significant association between DHEA/C ratio and externalizing problems (higher DHEA/C; higher externalizing problems; region of significance depicted in yellow).

Figures 5A/5B/5C show the moderating impact of androstenedione and cortisol (A/C) on the relationships between parental age gap and the child's externalizing ($B = 0.7246$, $SE = 0.2881$, $p = 0.0150$), conduct ($B = 0.2572$, $SE = 0.1262$, $p = 0.0466$) and hyperactivity problems ($B = 0.4673$, $SE = 0.2008$, $p = 0.0239$) when controlling for paternal education and maternal depression (see **Table 5**). The older the father was compared to the mother, the greater the level of externalizing and hyperactivity/inattention behaviors displayed by the child at higher A/C ratios (significant slope depicted by the black lines; conditional effect of age gap on externalizing scale at highest 10% A/C ratio: $B = 0.3367$, $SE = 0.1512$, $p < 0.05$; on hyperactivity/inattention subscale: $B = 0.2339$, $SE = 0.1054$, $p < 0.05$). Probing of these interactions revealed that offspring of parents with little age difference (≤0.0921 years for externalizing scale; ≤2.55 years for conduct subscale) displayed a significant inverse association between A/C and externalizing or conduct problems (higher A/C; lower externalizing or conduct scores; region of significance depicted in yellow). Conversely, offspring of fathers older than mothers by a gap of ≥7.7196 years displayed a significant association between A/C ratio and hyperactivity/inattention behaviors (higher A/C; higher hyperactivity/inattention problems; region of significance depicted in yellow).

No other associations have been found (see **Appendix Tables A32-38** for non-significant results and **Figures A16-20** to visualize Figures 3-5 with the addition of individual data points).

6. DISCUSSION

This study's primary objective was to determine whether paternal age interacted with the hormonal shifts of adrenarche in regulating the child's cognition and behavior. A secondary objective was to probe for interactions between paternal-maternal age gap and the child's neuroendocrine and neurodevelopmental function during adrenarche. Because there is still little understanding of the process through which early pubertal maturation processes can shape brain development, our study introduces new evidence to support the role of adrenarche in shaping the intergenerational transmission of reproductive risk factors and ultimately, the child's developmental trajectory during the transition from kindergarten to elementary school.

Regardless of the specific hormonal index tested (whether baseline androgen levels or androgen-to-cortisol ratios), our models showed that advanced paternal age and greater parental age gaps are associated with greater behavioral difficulties, particularly conduct and hyperactivity/inattention problems for offspring with higher levels of androgenization. In other words, being partially consistent with prior research linking increasing paternal age to higher externalizing behaviors in 7-year-olds (Saha, Barnett, Buka, et al., 2009a), our findings show that the older the father or alternatively, the older the father compared to the mother, the more likely their offspring will display behavioral problems but only in the context of higher androgen levels and higher androgen-to-cortisol ratios. For instance, offspring of fathers older than mothers by a large age gap of about 7 years or more were found to have *more* problems related to hyperactivity-inattention in the context of higher levels of androgenization in the child. Conversely, offspring of parents with little age difference (less than 2 years) were *less* at risk of developing behavioral

problems in the context of higher levels of androgenization. Finally, *fewer* conduct problems were present in highly androgenized children of young fathers (i.e., less than 32 years old).

Overall, these findings outline the interactions between paternal age at conception and child's neuroendocrine status suggestive of a cumulative risk, or multiple-hit, model. For example, neither factor was significantly associated with child's outcomes on its own; rather, any behavioral expression of paternal age depended on the child's hormonal profile, and *vice versa*. Additionally, in our sample, the range of father's age in years (22.74-53.79) is wider than the range of mother's age in years (19.74-40.34) (this can be explained by the more restricted reproductive window in women vs. men). This means that parental age gap (father's age minus mother's age) varies mostly as a function of paternal, rather than maternal age. In other words, the age gap results provide further support for the developmental risks of paternal age, in the context of a multiple-hit model where, for example, the risk for a child with higher levels of androgenization is even greater if the age gap between the parents is large. Multiple "hits" may be therefore necessary for the full behavioral risks of paternal age to be expressed during adrenarche. In other words, the father's age and the child's hormonal status during adrenarche may only lead to measurable differences in cognition and behavior when specific paternal, maternal and child conditions are met, supporting the importance of considering the familial system as a whole.

Our initial hypotheses were only partially confirmed, notably regarding the relevance of paternal age-child hormone interactions in shaping behavior during middle childhood. While we expected higher levels of androgenization in the child (greater androgen levels or higher androgen-to-cortisol ratios) to be associated with fewer behavioral problems, we found that this only occurred in offspring of younger fathers (i.e., less than 32 years old) and not for those of older fathers. Thus, contrary to expectation, we found higher levels of androgenization to be associated

with adverse behavioral effects in the offspring of older fathers. Additionally, no effects of paternal age-child hormone interactions were seen on child's cognition. This is likely due to the closer and direct association between our steroid system and human behavior, with hormonal levels adjusting more rapidly and adaptively to the changes in the external environment.

In light of these contrasting findings, current notions of risk transmission from father-to-child may need to be reconceptualized. Indeed, our results suggest that "father-child hormonal fit" may be a more important factor than the absolute level of androgenization in a particular individual in the expression of behavioral risk during adrenarche.

Large epidemiological studies show a gradual decrease in androgens in men with age (commonly known as "adrenopause") in the age range of the fathers included our study (Allolio et al., 2012; Ellison et al., 2002; Miller & Flück, 2014). In contrast, cortisol levels remain relatively stable in adult men (Allolio et al., 2012; Miller & Flück, 2014). Therefore, we would expect *paternal* androgen-to-cortisol ratios to decrease over time as fathers reach more advanced ages at conception. As such, a child with *lower* levels of androgenization would be more "fit" for an *older* father, while a child with *greater* levels of androgenization would be more "fit" for a *younger* father.

The steroid system has evolved to provide human organisms with a way to rapidly and flexibly regulate nervous system responses to the external environment (Kamin & Kertes, 2017; McEwen, 1988). In light of this, one could speculate that a greater father-child hormonal fit would lead to more adaptive behavioral responses in the offspring. This model would be consistent with the lower risk of externalizing and conduct problems in the context of a better father-child hormonal fit observed in our study, be it for the combination of a younger father with a highly androgenized child or that of an older father with a child at lower levels of androgenization.

Parental age gap-hormone interactions add to those findings by highlighting the developmental risks incurred when an older father chooses to procreate with a younger mother, which might be even greater than those related to advanced paternal age alone. For example, age-gap-hormone interactions are associated with differences in overall behavioral difficulties -vs. externalizing symptoms alone for paternal age-, and differences in both conduct and hyperactivity-inattention symptoms -vs. conduct behaviors alone for paternal age alone-. Conversely, parents with an age gap of 2 years or less, had offspring with fewer behavioral problems in the context of higher androgenization levels in the child. In our sample, the absolute difference in maternal age between those with a higher vs. lower age gap with the fathers was negligible, perhaps due to the more restricted reproductive window in women vs. men. In contrast, fathers with a greater age gap with the mothers were significantly older than those with a smaller age gap, supporting the cumulative risks incurred by older fathers with a less adaptive fit with their child (i.e., higher levels of androgenization). On the other hand, younger fathers with highly androgenized children are likely to reap the most benefits in terms of their offspring's behavioral development.

6.1 Strengths and Limitations

6.1.1 Strengths

This is the first study to evaluate the developmental impact of paternal age on their offspring during the critical neuroendocrine transition of adrenarche. We had access to a unique dataset that included retrospectively collected parental data up to 1-year preconception as well as prospectively collected paternal, maternal and child data over almost a decade (from the 1st trimester up to 6-8 years postnatally). This allowed us to account for multiple potential confounders (e.g., gestational, parental and parent-child factors) of the interactions among paternal age and the child's neuroendocrine/neurodevelopmental status. We included a broad paternal age

span (from 22-53 years old); therefore, results are expected to be relevant for most Canadian fathers. Finally, we have captured the main components of the steroid metabolome during adrenarche, measuring the levels of several androgen hormones as well as cortisol levels.

6.1.2 Limitations

A small sample size (n=61) limits the generalizability of our results. While fathers in our study self-identified as the biological father of their child, insufficient funding did not allow us to proceed with a formal confirmation of this genetic link using blood from the father and the child. Still, the father was probed with the following question "are you this child's biological father?" four times over the entire duration of the 3D study: during pregnancy and, postnatally, when the child was 3 months, 1 year and 2 years, respectively.

Similar to other Canadian cohorts, such as the Maternal Adversity, Vulnerability and Neurodevelopment (MAVAN) (O'Donnell et al., 2014) and the Maternal-Infant Research on Environmental Chemicals (MIREC) (Arbuckle et al., 2013) cohorts, the 3D cohort is comprised of mostly Caucasian families with higher parental ages, lower parity as well as higher levels of education (i.e., CEGEP/University degrees and higher) and household income (an annual income of > $7-80 000) (Fraser et al., 2016). In light of this, results may not be applicable to a more ethnically diverse populations or those with lower socioeconomic status.

Finally, while mother-child hormonal fit may partly account for our findings, testing this model is beyond the scope of this study as adrenarche mainly involves an increase in androgens rather than estrogens or progestogens.

7. CONCLUSION

This study outlines the behavioral impact on the child of specific interactions between: 1) the parents' "biological clock" (advanced paternal age; greater age differences between an older

father and a younger mother) and 2) their offspring's neuroendocrine status (as measured by androgen levels and androgen-to-cortisol ratios) during adrenarche. These findings contribute to the literature in several ways: 1) they provide evidence that paternal age at conception is a reproductive risk factor with a distinct neurobehavioral impact compared to other paternal or maternal factors; 2) they support for the concept that extremes of paternal ages may interact with extremes of maternal age in shaping the relationship between hormonal and behavioral function in the child; 3) they further our understanding of the relationships between the steroid system and developmental changes during adrenarche; and 4) they highlight novel aspects of risk transmission from father-to-child, with a potential role for father-child "hormonal fit" in determining behavioral outcomes of the offspring during middle childhood.

7.1 Future Directions

This project has focused on levels of steroid hormones at baseline. Children's neuroendocrine reactivity was also measured in our study, with repeated sampling before and after MRI (Eatough et al., 2009). We plan to test interactions between paternal age, neuroendocrine reactivity and developmental outcomes in the child by calculating hormonal trajectories over time (area-under-the-curve (AUC)). In an effort to clarify the biological pathways through which paternal age may affect the link between the child's endocrine and brain development, we also plan to examine whether: 1) paternal age is associated with differences in placental epigenetics and brain structure in the child; 2) whether these placental and CNS differences are linked to alterations in the child's IQ and behavior during adrenarche.

8. REFERENCES

Allolio, B., Arlt, W., & Hahner, S. (2012). Dehydroepiandrosterone and androstenedione. In E. Nieschlag & H. M. Behre (Eds.), *Testosterone: Action, Deficiency, Substitution* (4th ed., pp. 437–458). Cambridge University Press. https://doi.org/DOI: 10.1017/CBO9781139003353.021

Arbuckle, T. E., Fraser, W. D., Fisher, M., Davis, K., Liang, C. L., Lupien, N., Bastien, S., Velez, M. P., von Dadelszen, P., Hemmings, D. G., Wang, J., Helewa, M., Taback, S., Sermer, M., Foster, W., Ross, G., Fredette, P., Smith, G., Walker, M., … Ouellet, E. (2013). Cohort Profile: The Maternal-Infant Research on Environmental Chemicals Research Platform. *Paediatric and Perinatal Epidemiology*, *27*(4), 415–425. https://doi.org/10.1111/ppe.12061

Auyeung, B., Taylor, K., Hackett, G., & Baron-Cohen, S. (2010). Foetal testosterone and autistic traits in 18 to 24-month-old children. *Molecular Autism*, *1*(1), 11. https://doi.org/10.1186/2040-2392-1-11

Barclay, K., & Myrskylä, M. (2016). Advanced Maternal Age and Offspring Outcomes: Reproductive Aging and Counterbalancing Period Trends. *Population and Development Review*, *42*(1), 69–94. https://doi.org/10.1111/j.1728-4457.2016.00105.x

Beck, A. T., Epstein, N., Brown, G., & Steer, R. A. (1988). An inventory for measuring clinical anxiety: psychometric properties. *Journal of Consulting and Clinical Psychology*, *56*(6), 893–897. https://doi.org/10.1037//0022-006x.56.6.893

Beck, A. T., Ward, C. H., Mendelson, M., Mock, J., & Erbaugh, J. (1961). An inventory for measuring depression. *Archives of General Psychiatry*, *4*, 561–571. https://doi.org/10.1001/archpsyc.1961.01710120031004

Beck, S. G., & Handa, R. J. (2004). Dehydroepiandrosterone (DHEA): a misunderstood adrenal hormone and spine-tingling neurosteroid? *Endocrinology*, *145*(3), 1039–1041. https://doi.org/10.1210/en.2003-1703

Benjamini, Y., & Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society. Series B (Methodological)*, *57*(1), 289–300. http://www.jstor.org/stable/2346101

Boivin, M., Pérusse, D., Dionne, G., Saysset, V., Zoccolillo, M., Tarabulsy, G. M., Tremblay, N., & Tremblay, R. E. (2005). The genetic-environmental etiology of parents' perceptions and self-assessed behaviours toward their 5-month-old infants in a large twin and singleton sample. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *46*(6), 612–630. https://doi.org/10.1111/j.1469-7610.2004.00375.x

Bremer, A. A., & Miller, W. L. (2014). Regulation of Steroidogenesis. *Cellular Endocrinology in Health and Disease*, 207–227. https://doi.org/10.1016/B978-0-12-408134-5.00013-5

Campbell, B. C. (2011). Adrenarche and Middle Childhood. *Human Nature*, *22*(3), 327–349. https://doi.org/10.1007/s12110-011-9120-x

Capron, C., & Duyme, M. (1989). Assessment of effects of socio-economic status on IQ in a full cross-fostering study. *Nature*, *340*(6234), 552–554. https://doi.org/10.1038/340552a0

Ceci, S. J., & Williams, W. M. (1997). Schooling, intelligence, and income. *American Psychologist*, *52*(10), 1051–1058. https://doi.org/10.1037/0003-066X.52.10.1051

Chang, Z., Lichtenstein, P., D'Onofrio, B. M., Almqvist, C., Kuja-Halkola, R., Sjölander, A., & Larsson, H. (2014). Maternal age at childbirth and risk for ADHD in offspring: a population-based cohort study. *International Journal of Epidemiology*, *43*(6), 1815–1824. https://doi.org/10.1093/ije/dyu204

Day, J., Savani, S., Krempley, B. D., Nguyen, M., & Kitlinska, J. B. (2016). Influence of paternal preconception exposures on their offspring: through epigenetics to phenotype. *American Journal of Stem Cells*, *5*(1), 11–18. https://pubmed.ncbi.nlm.nih.gov/27335698

de Kluiver, H., Buizer-Voskamp, J. E., Dolan, C. V., & Boomsma, D. I. (2017). Paternal age and psychiatric disorders: A review. *American Journal of Medical Genetics, Part B: Neuropsychiatric Genetics*, *174*(3), 202–213. https://doi.org/10.1002/ajmg.b.32508

Dong, Y., & Peng, C.-Y. J. (2013). Principled missing data methods for researchers. *SpringerPlus*, *2*(1), 222. https://doi.org/10.1186/2193-1801-2-222

Dorn, L. D., Hitt, S. F., & Rotenstein, D. (1999). Biopsychological and cognitive differences in children with premature vs on-time adrenarche. In *Archives of Pediatrics and Adolescent Medicine* (Vol. 153, Issue 2, pp. 137–146). https://doi.org/10.1001/archpedi.153.2.137

Duncan, G. J., Lee, K. T. H., Rosales-Rueda, M., & Kalil, A. (2018). Maternal Age and Child Development. *Demography*, *55*(6), 2229–2255. https://doi.org/10.1007/s13524-018-0730-3

Eatough, E. M., Shirtcliff, E. A., Hanson, J. L., & Pollak, S. D. (2009). Hormonal reactivity to MRI scanning in adolescents. *Psychoneuroendocrinology*, *34*(8), 1242–1246. https://doi.org/https://doi.org/10.1016/j.psyneuen.2009.03.006

Eisenberg, M. L., & Meldrum, D. (2017). Effects of age on fertility and sexual function. *Fertility and Sterility*, *107*(2), 301–304. https://doi.org/10.1016/j.fertnstert.2016.12.018

Ellison, P. T., Bribiescas, R. G., Bentley, G. R., Campbell, B. C., Lipson, S. F., Panter-Brick, C., & Hill, K. (2002). Population variation in age-related decline in male salivary testosterone. *Human Reproduction*, *17*(12), 3251–3253. https://doi.org/10.1093/humrep/17.12.3251

Farooqi, N. A. I., Scotti, M., Lew, J. M., Botteron, K. N., Karama, S., McCracken, J. T., & Nguyen, T. V. (2018a). Role of DHEA and cortisol in prefrontal-amygdalar development

and working memory. *Psychoneuroendocrinology*, *98*(February), 86–94. https://doi.org/10.1016/j.psyneuen.2018.08.010

Farooqi, N. A. I., Scotti, M., Lew, J. M., Botteron, K. N., Karama, S., McCracken, J. T., & Nguyen, T. V. (2018b). Role of DHEA and cortisol in prefrontal-amygdalar development and working memory. *Psychoneuroendocrinology*, *98*(August), 86–94. https://doi.org/10.1016/j.psyneuen.2018.08.010

Farooqi, N. A. I., Scotti, M., Yu, A., Lew, J., Monnier, P., Botteron, K. N., Campbell, B. C., Booij, L., Herba, C. M., Séguin, J. R., Castellanos-Ryan, N., McCracken, J. T., & Nguyen, T. V. (2019). Sex-specific contribution of DHEA-cortisol ratio to prefrontal-hippocampal structural development, cognitive abilities and personality traits. *Journal of Neuroendocrinology*, *31*(2), 1–18. https://doi.org/10.1111/jne.12682

Feldman Witchel, S., & Plant, T. M. (2009). *CHAPTER 17 - Puberty: Gonadarche and Adrenarche* (J. F. Strauss & R. L. B. T.-Y. & J. R. E. (Sixth E. Barbieri (eds.); pp. 395–431). W.B. Saunders. https://doi.org/https://doi.org/10.1016/B978-1-4160-4907-4.00017-6

Fergusson, D. M., & Lynskey, M. T. (1993). Maternal age and cognitive and behavioural outcomes in middle childhood. *Paediatric and Perinatal Epidemiology*, *7*(1), 77–91. https://doi.org/10.1111/j.1365-3016.1993.tb00604.x

Fink, G. (2007). *Encyclopedia of Stress*. Elsevier Inc.

Foradori, C. D., Weiser, M. J., & Handa, R. J. (2008). Non-genomic actions of androgens. *Frontiers in Neuroendocrinology*, *29*(2), 169–181. https://doi.org/10.1016/j.yfrne.2007.10.005

Fraser, W. D., Shapiro, G. D., Audibert, F., Dubois, L., Pasquier, J. C., Julien, P., Bérard, A., Muckle, G., Trasler, J., Tremblay, R. E., Abenhaim, H., Welt, M., Bédard, M. J.,

Bissonnette, F., Bujold, E., Gagnon, R., Michaud, J. L., Girard, I., Moutquin, J. M., … Luo, Z. C. (2016). 3D Cohort Study: The Integrated Research Network in Perinatology of Quebec and Eastern Ontario. *Paediatric and Perinatal Epidemiology*, *30*(6), 623–632. https://doi.org/10.1111/ppe.12320

Gould, A. L. (2010). Generating and confirming hypotheses. *Pharmacoepidemiology and Drug Safety*, *19*(5), 533–536. https://doi.org/10.1002/pds.1928

Han, W., Dong, X., Song, X., Cheng, L., Xie, L., Chen, H., & Jiang, L. (2018). Effects of advanced maternal age on cognitive and emotional development in offspring rats. *Behavioural Brain Research*, *353*, 218–226. https://doi.org/https://doi.org/10.1016/j.bbr.2018.04.003

Hayes, A. F. (2013). Introduction to mediation, moderation, and conditional process analysis: A regression-based approach. In *Introduction to mediation, moderation, and conditional process analysis: A regression-based approach.* Guilford Press.

Hehar, H., & Mychasiuk, R. (2015). Do Fathers Matter: Influencing Neural Phenotypes Through Non-Genetic Transmission of Paternal Experiences? *Non-Genetic Inheritance*, *2*(1), 23–31. https://doi.org/10.1515/ngi-2015-0004

Herati, A. S., Zhelyazkova, B. H., Butler, P. R., & Lamb, D. J. (2017). Age-related alterations in the genetics and genomics of the male germ line. *Fertility and Sterility*, *107*(2), 319–323. https://doi.org/10.1016/j.fertnstert.2016.12.021

Janecka, M., Hansen, S. N., Modabbernia, A., Browne, H. A., Buxbaum, J. D., Schendel, D. E., Reichenberg, A., Parner, E. T., & Grice, D. E. (2019). Parental Age and Differential Estimates of Risk for Neuropsychiatric Disorders: Findings From the Danish Birth Cohort. *Journal of the American Academy of Child and Adolescent Psychiatry*, *58*(6), 618–627.

https://doi.org/10.1016/j.jaac.2018.09.447

Janecka, M., Haworth, C. M. A., Ronald, A., Krapohl, E., Happe, F., Mill, J., Schalkwyk, L. C., Fernandes, C., Reichenberg, A., & Rijsdijk, F. (2017). Paternal Age Alters Social Development in Offspring. *Journal of the American Academy of Child and Adolescent Psychiatry*, *56*(5), 383–390. https://doi.org/10.1016/j.jaac.2017.02.006

Kamin, H. S., & Kertes, D. A. (2017). Cortisol and DHEA in development and psychopathology. *Hormones and Behavior*, *89*, 69–85. https://doi.org/10.1016/j.yhbeh.2016.11.018

Kim, T. H. M., Connolly, J. A., Rotondi, M., & Tamim, H. (2018). Investigating Pathways to Behavioural Problems in Children of Teenage, Middle Age, and Advanced Age Mothers in Canada. *Child Indicators Research*, *11*(5), 1631–1647. https://doi.org/10.1007/s12187-017-9500-9

Lopez-Castroman, J. (2014). Young maternal age and old paternal age induce similar risk of mental disorders in offspring. *Evidence Based Mental Health*, *17*(4), 100 LP – 100. https://doi.org/10.1136/eb-2014-101861

Machin, A. (2019). *Devenir papa: c'est quoi, être père aujourd'hui?* Paris: Larousse.

Maher, B. (2015). A battle of the sexes is waged in the genes. *Nature*.

Maninger, N., Wolkowitz, O. M., Reus, V. I., Epel, E. S., & Mellon, S. H. (2009). Neurobiological and neuropsychiatric effects of dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS). *Frontiers in Neuroendocrinology*, *30*(1), 65–91. https://doi.org/10.1016/J.YFRNE.2008.11.002

Matarazzo, J. D., & Herman, D. O. (1984). Relationship of education and IQ in the WAIS—R standardization sample. *Journal of Consulting and Clinical Psychology*, *52*(4), 631–634. https://doi.org/10.1037/0022-006X.52.4.631

Matchock, R. L., Dorn, L. D., & Susman, E. J. (2007). Diurnal and seasonal cortisol, testosterone, and DHEA rhythms in boys and girls during puberty. *Chronobiology International*, *24*(5), 969–990. https://doi.org/10.1080/07420520701649471

McEwen, B. S. (1988). Steroid hormones and the brain: Linking "nature" and "nurture." *Neurochemical Research*, *13*(7), 663–669. https://doi.org/10.1007/BF00973285

McGrath, J. J., Petersen, L., Agerbo, E., Mors, O., Mortensen, P. B., & Pedersen, C. B. (2014). A comprehensive assessment of parental age and psychiatric disorders. *JAMA Psychiatry*, *71*(3), 301–309. https://doi.org/10.1001/jamapsychiatry.2013.4081

Mieloo, C., Raat, H., van Oort, F., Bevaart, F., Vogel, I., Donker, M., & Jansen, W. (2012). Validity and reliability of the strengths and difficulties questionnaire in 5-6 year olds: differences by gender or by parental education? *PloS One*, *7*(5), e36805. https://doi.org/10.1371/journal.pone.0036805

Milekic, M. H., Xin, Y., O'Donnell, A., Kumar, K. K., Bradley-Moore, M., Malaspina, D., Moore, H., Brunner, D., Ge, Y., Edwards, J., Paul, S., Haghighi, F. G., & Gingrich, J. A. (2015). Age-related sperm DNA methylation changes are transmitted to offspring and associated with abnormal behavior and dysregulated gene expression. *Molecular Psychiatry*, *20*(8), 995–1001. https://doi.org/10.1038/mp.2014.84

Miller, W. L., & Flück, C. E. (2014). *CHAPTER 13 - Adrenal cortex and its disorders* (M. A. B. T.-P. E. (Fourth E. Sperling (ed.); pp. 471-532.e1). Content Repository Only! https://doi.org/https://doi.org/10.1016/B978-1-4557-4858-7.00022-6

Nass, R., Baker, S., Sadler, A. E., & Sidtis, J. J. (1990). The effects of precocious adrenarche on cognition and hemispheric specialization. *Brain and Cognition*, *14*(1), 59–69. https://doi.org/10.1016/0278-2626(90)90060-2

Nguyen, T. V., Jones, S. L., Elgbeili, G., Monnier, P., Yu, C., Laplante, D. P., & King, S. (2018). Testosterone-cortisol dissociation in children exposed to prenatal maternal stress, and relationship with aggression: Project Ice Storm. *Development and Psychopathology*, *30*(3), 981–994. https://doi.org/10.1017/S0954579418000652

Nguyen, T. V., Lew, J., Albaugh, M. D., Botteron, K. N., Hudziak, J. J., Fonov, V. S., Collins, D. L., Ducharme, S., & McCracken, J. T. (2017). Sex-specific associations of testosterone with prefrontal-hippocampal development and executive function. *Psychoneuroendocrinology*, *76*, 206–217. https://doi.org/10.1016/j.psyneuen.2016.12.005

Nguyen, T. V., Wu, M., Lew, J., Albaugh, M. D., Botteron, K. N., Hudziak, J. J., Fonov, V. S., Collins, D. L., Campbell, B. C., Booij, L., Herba, C., Monnier, P., Ducharme, S., & McCracken, J. T. (2017). Dehydroepiandrosterone impacts working memory by shaping cortico-hippocampal structural covariance during development. *Psychoneuroendocrinology*, *86*(September), 110–121. https://doi.org/10.1016/j.psyneuen.2017.09.013

O'Donnell, K. A., Gaudreau, H., Colalillo, S., Steiner, M., Atkinson, L., Moss, E., Goldberg, S., Karama, S., Matthews, S. G., Lydon, J. E., Silveira, P. P., Wazana, A. D., Levitan, R. D., Sokolowski, M. B., Kennedy, J. L., Fleming, A., Meaney, M. J., & Team, M. R. (2014). The maternal adversity, vulnerability and neurodevelopment project: theory and methodology. *Canadian Journal of Psychiatry. Revue Canadienne de Psychiatrie*, *59*(9), 497–508. https://doi.org/10.1177/070674371405900906

Pariente, G., Wainstock, T., Walfisch, A., Sheiner, E., & Harlev, A. (2019). Advanced Maternal Age and the Future Health of the Offspring. *Fetal Diagnosis and Therapy*, *46*(2), 139–146. https://doi.org/10.1159/000493191

Parner, E. T., Baron-Cohen, S., Lauritsen, M. B., Jørgensen, M., Schieve, L. A., Yeargin-

Allsopp, M., & Obel, C. (2012). Parental age and autism spectrum disorders. *Annals of Epidemiology*, *22*(3), 143–150. https://doi.org/10.1016/j.annepidem.2011.12.006

Pearson. (2018). WISC V- Efficacy Research Report. *Pearson*, *April*, 1–42. http://search.ebscohost.com/login.aspx?direct=true&AuthType=ip,shib&db=eric&AN=ED592520&site=ehost-live&scope=site%0Ahttps://www.pearson.com/content/dam/one-dot-com/one-dot-com/global/Files/efficacy-and-research/reports/audited/NAME-research-report.pdf

Petersen, A. C., Crockett, L., Richards, M., & Boxer, A. (1988). A self-report measure of pubertal status: Reliability, validity, and initial norms. *Journal of Youth and Adolescence*, *17*(2), 117–133. https://doi.org/10.1007/BF01537962

Phoenix, C. H., Goy, R. W., Gerall, A. A., & Young, W. C. (1959). ORGANIZING ACTION OF PRENATALLY ADMINISTERED TESTOSTERONE PROPIONATE ON THE TISSUES MEDIATING MATING BEHAVIOR IN THE FEMALE GUINEA PIG1. *Endocrinology*, *65*(3), 369–382. https://doi.org/10.1210/endo-65-3-369

Provencher, C., Milan, A., Hallman, S., & Carol, D. (2018). Fertility: Overview, 2012 to 2016. In *Statistic Canada*.

Rodgers, A. B., Morgan, C. P., Bronson, S. L., Revello, S., & Bale, T. L. (2013). Paternal stress exposure alters sperm MicroRNA content and reprograms offspring HPA stress axis regulation. *Journal of Neuroscience*, *33*(21), 9003–9012. https://doi.org/10.1523/JNEUROSCI.0914-13.2013

Saha, S., Barnett, A. G., Buka, S. L., & McGrath, J. J. (2009a). Maternal age and paternal age are associated with distinct childhood behavioural outcomes in a general population birth cohort. *Schizophrenia Research*, *115*(2–3), 130–135.

https://doi.org/10.1016/j.schres.2009.09.012

Saha, S., Barnett, A. G., Buka, S. L., & McGrath, J. J. (2009b). Maternal age and paternal age are associated with distinct childhood behavioural outcomes in a general population birth cohort. *Schizophrenia Research*, *115*(2–3), 130–135. https://doi.org/10.1016/j.schres.2009.09.012

Saha, S., Barnett, A. G., Foldi, C., Burne, T. H., Eyles, D. W., Buka, S. L., & McGrath, J. J. (2009). Advanced paternal age is associated with impaired neurocognitive outcomes during infancy and childhood. *PLoS Medicine*, *6*(3), e40. https://doi.org/10.1371/journal.pmed.1000040

Salimetrics. (2020). *Research Salivary Assay Kits*. https://salimetrics.com/assay-kits/#tab1

Sandin, S., Schendel, D., Magnusson, P., Hultman, C., Surén, P., Susser, E., Grønborg, T., Gissler, M., Gunnes, N., Gross, R., Henning, M., Bresnahan, M., Sourander, A., Hornig, M., Carter, K., Francis, R., Parner, E., Leonard, H., Rosanoff, M., … Reichenberg, A. (2016). Autism risk associated with parental age and with increasing difference in age between the parents. *Molecular Psychiatry*, *21*(5), 693–700. https://doi.org/10.1038/mp.2015.70

Schultheiss, O. C., & Stanton, S. J. (2009). Assessment of salivary hormones. In *Methods in social neuroscience.* (pp. 17–44). Guilford Press.

Schulz, K. M., & Sisk, C. L. (2016). The organizing actions of adolescent gonadal steroid hormones on brain and behavioral development. *Neuroscience and Biobehavioral Reviews*, *70*, 148–158. https://doi.org/10.1016/j.neubiorev.2016.07.036

Shapiro, G. D., Séguin, J. R., Muckle, G., Monnier, P., & Fraser, W. D. (2017). Previous pregnancy outcomes and subsequent pregnancy anxiety in a Quebec prospective cohort. *Journal of Psychosomatic Obstetrics & Gynecology*, *38*(2), 121–132.

https://doi.org/10.1080/0167482X.2016.1271979

Sollberger, S., & Ehlert, U. (2016). How to use and interpret hormone ratios. *Psychoneuroendocrinology*, *63*, 385–397. https://doi.org/10.1016/j.psyneuen.2015.09.031

Sontag-Padilla, L. M., Dorn, L. D., Tissot, A., Susman, E. J., Beers, S. R., & Rose, S. R. (2012). Executive functioning, cortisol reactivity, and symptoms of psychopathology in girls with premature adrenarche. *Development and Psychopathology*, *24*(1), 211–223. https://doi.org/10.1017/S0954579411000782

Taylor, J. L., Debost, J. C. P. G., Morton, S. U., Wigdor, E. M., Heyne, H. O., Lal, D., Howrigan, D. P., Bloemendal, A., Larsen, J. T., Kosmicki, J. A., Weiner, D. J., Homsy, J., Seidman, J. G., Seidman, C. E., Agerbo, E., McGrath, J. J., Mortensen, P. B., Petersen, L., Daly, M. J., & Robinson, E. B. (2019). Paternal-age-related de novo mutations and risk for five disorders. *Nature Communications*, *10*(1), 1–10. https://doi.org/10.1038/s41467-019-11039-6

Tissot, A., Dorn, L. D., Rotenstein, D., Rose, S. R., Sontag-Padilla, L. M., Jillard, C. L., Witchel, S. F., Berga, S. L., Loucks, T. L., & Beers, S. R. (2012). Neuropsychological functioning in girls with premature adrenarche. *Journal of the International Neuropsychological Society*, *18*(1), 151–156. https://doi.org/10.1017/S135561771100138X

Tryphonopoulos, P. D., Letourneau, N., & Azar, R. (2014). Approaches to salivary cortisol collection and analysis in infants. *Biological Research for Nursing*, *16*(4), 398–408. https://doi.org/10.1177/1099800413507128

Verhoeven, K. J. F., Simonsen, K. L., & McIntyre, L. M. (2005). Implementing false discovery rate control: increasing your power. *Oikos*, *108*(3), 643–647. https://doi.org/https://doi.org/10.1111/j.0030-1299.2005.13727.x

Weiser, M., Reichenberg, A., Werbeloff, N., Kleinhaus, K., Lubin, G., Shmushkevitch, M., Caspi, A., Malaspina, D., & Davidson, M. (2008). Advanced parental age at birth is associated with poorer social functioning in adolescent males: shedding light on a core symptom of schizophrenia and autism. *Schizophrenia Bulletin*, *34*(6), 1042–1046. https://doi.org/10.1093/schbul/sbn109

Williams, R. M., Ward, C. E., & Hughes, I. A. (2012). Premature adrenarche. *Archives of Disease in Childhood*, *97*(3), 250 LP – 254. https://doi.org/10.1136/archdischild-2011-300011

Winship, C., & Korenman, S. (1997). *Does Staying in School Make You Smarter? The Effect of Education on IQ in The Bell Curve* (pp. 215–234). https://doi.org/10.1007/978-1-4612-0669-9_10

Yeung, E. H., Sundaram, R., Ghassabian, A., Xie, Y., & Buck Louis, G. (2017). Parental Obesity and Early Childhood Development. *Pediatrics*, *139*(2), e20161459. https://doi.org/10.1542/peds.2016-1459

9. TABLES AND FIGURES

Table 1. *Sample Characteristics*

	N	M [freq; %]	SD	Range
Child Age (years)	61	6.66	0.54	5.57-8.40
Age at Conception (years)	60			
Mother		31.50	4.43	19.74-40.34
Father		34.29	5.84	22.74-53.79
Age Gap	60	2.78	4.39	-4-18
Gestational Weeks	60	38.98	1.76	31.86-41.86
Maternal Highest Level of Education	61			
Elementary		[2; 3.3%]	-	-
Secondary		[2; 3.3%]	-	-
CEGEP/etc		[15; 24.6%]	-	-
University		[26; 42.6%]	-	-
MA		[11; 18%]	-	-
PhD		[5; 8.2%]	-	-
Paternal Highest Level of Education	61			
Elementary		[2; 3.3%]	-	-
Secondary		[6; 9.8%]	-	-
Post-secondary		[2; 3.3%]	-	-
CEGEP/college		[16; 26.2%]	-	-
University		[17; 27.9%]	-	-
MA		[13; 21.3%]	-	-
PhD		[5; 8.2%]	-	-
Maternal Ethnicity	61			
White		[47; 77%]	-	-
Other (African, Asian, etc)		[14; 23%]	-	-
Paternal Ethnicity	60			
White		[47; 78.3%]	-	-
Other (African, Asian, European, etc)		[13; 21.7%]	-	-
Season of Collection	61			
Spring		[21; 34.4%]	-	-
Summer		[20; 32.8%]	-	-
Fall		[12; 19.7%]	-	-
Winter		[8; 13.1%]	-	-
Beck Depression Inventory				
Mother	61	7.20	7.84	0-36

Father	55	3.91	4.47	0-17.29
Beck Anxiety Inventory				
Mother	61	6	6.67	0-34
Father	55	3.85	4.27	0-14
Baseline Hormonal Levels (Imputed, Untransformed)				
	61	53.77	61.74	0.01-232.53
DHEA (pg/mL)				
Androstenedione (pg/mL)	59	49.04	41.35	1.00-160.26
Testosterone (pg/mL)	61	29.15	26.93	0.10-106.62
Cortisol (pg/mL)	61	723.37	604.36	6.50-3755
WISC	61			
Full Scale		112	12.87	77-140
Verbal Comprehension		113.15	13.35	84-140
Fluid Reasoning		109.53	12.59	82-147
SDQ	61			
Total Difficulties		8.10	5.64	0-28
Prosocial		8.62	1.64	3-10
Internalizing		2.44	2.41	0-12
Emotional Symptoms		1.57	1.90	0-8
Peer		0.87	1.13	0-5
Externalizing		5.66	4.37	0-19
Conduct		1.80	1.89	0-9
Hyperactivity/ Inattention		3.85	2.97	0-10

Note. Out of 61 children, 36 were males and 25 were females. DHEA: Dehydroepiandrosterone; WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaires.

Table 2. *Moderation Model With Paternal Age at Conception as the Predictor and Baseline Androstenedione/Cortisol as the Moderator*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a,b]	0.2878	0.0001	0.9362	-
WISC Verbal [a,b]	0.2422	0.0005	0.8576	0.8576
WISC Fluid [a,c]	0.1914	0.0204	0.2575	0.515
SDQ Total Difficulties [a,d]	0.2526	0.0265	0.1802	-
SDQ Prosocial [a,d]	0.1431	0.0090	0.4634	0.6951
SDQ Internalizing [a,d,e]	0.3296	0.0033	0.6165	0.6165
SDQ Externalizing [a,c,d]	0.3346	0.0684	**0.0262***	0.0786
SDQ Conduct [a,c,d]	0.3464	0.0784	**0.0167***	**0.0334***
SDQ Hyper/Inattention [a,c,d]	0.2712	0.0429	0.0893	0.0893

***p <0.05.**

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: maternal age [a], parental education [b], paternal education [c], Beck Depression Inventory (mom) [d], Beck Anxiety Inventory (mom) [e].

Table 3. *Moderation Model With Age Gap as the Predictor and Baseline Testosterone as the Moderator*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a]	0.2553	0.0026	0.6640	-
WISC Verbal [a]	0.1616	0.0031	0.6522	0.6522
WISC Fluid [b]	0.1324	0.0064	0.5282	1
SDQ Total Difficulties [c]	0.2974	0.0624	**0.0313***	-
SDQ Prosocial [c]	0.0814	0.0010	0.8042	0.8042
SDQ Internalizing [c,d]	0.3119	0.0175	0.2459	0.3689

SDQ Externalizing [b,c]	0.2602	0.0352	0.1147	0.3441

***p <0.05.**

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: parental education [a], paternal education [b], Beck Depression Inventory (mom) [c], Beck Anxiety Inventory (mom) [d].

Table 4. *Moderation Model With Age Gap as the Predictor and Baseline DHEA/Cortisol as the Moderator*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a]	0.2244	0.0124	0.3523	-
WISC Verbal [a]	0.1791	0.0099	0.4181	0.4181
WISC Fluid [b]	0.1596	0.0239	0.2163	0.4326
SDQ Total Difficulties [c]	0.2404	0.0236	0.1967	-
SDQ Prosocial [c]	0.0828	0.0062	0.5432	0.8148
SDQ Internalizing [c,d]	0.3009	0.0010	0.7817	0.7817
SDQ Externalizing [b,c]	0.2878	0.0591	**0.0388***	0.1164
SDQ Conduct [b,c]	0.2534	0.0425	0.0852	0.0852
SDQ Hyper/Inattention [b,c]	0.2553	0.0516	0.0584	0.1168

***p <0.05.**

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: parental education [a], paternal education [b], Beck Depression Inventory (mom) [c], Beck Anxiety Inventory (mom) [d].

Table 5. *Moderation Model With Age Gap as the Predictor and Baseline Androstenedione/ Cortisol as the Moderator*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a]	0.2128	0.0006	0.8427	-
WISC Verbal [a]	0.1647	0.0105	0.4184	0.8368
WISC Fluid [b]	0.1585	0.0021	0.7174	0.7174

SDQ Total Difficulties [c]	0.2764	0.0503	0.0602	-
SDQ Prosocial [c]	0.1009	0.0080	0.4958	0.7437
SDQ Internalizing [c,d]	0.3155	0.0002	0.9107	0.9107
SDQ Externalizing [b,c]	0.3108	0.0839	**0.0150***	**0.045***
SDQ Conduct [b,c]	0.2972	0.0562	**0.0466***	**0.0466***
SDQ Hyper/Inattention [b,c]	0.2693	0.0761	**0.0239***	**0.0478***

***p <0.05.**

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: parental education [a], paternal education [b], Beck Depression Inventory (mom) [c], Beck Anxiety Inventory (mom) [d].

Table 6. *Pearson Correlation Matrix Between Potential Covariates and Scores on WISC/SDQ*

	WISC Full Scale	WISC Verbal	WISC Fluid	SDQ Total Difficulties	SDQ Prosocial	SDQ Internal.	SDQ Emotion	SDQ Peer	SDQ External.	SDQ Conduct	SDQ Hyper/I
Paternal Age	-0.011	0.027	0.044	0.099	-0.223	-0.041	-0.107	0.092	0.144	0.071	0.168
Maternal Age	0.011	-0.046	0.063	0.049	-0.250	-0.049	-0.159	0.162	0.085	0.044	0.097
Age Gap	-0.026	0.082	-0.005	0.082	-0.044	-0.006	0.018	-0.041	0.107	0.050	0.125
Gestational Weeks	0.104	0.219	-0.074	0.066	-0.001	0.075	0.085	0.009	0.048	0.044	0.043
Maternal Education	**0.263***	**0.324***	0.149	0.014	-0.115	-0.047	-0.043	-0.026	0.044	0.005	0.061
Paternal Education	**0.480****	**0.313***	**0.347****	-0.232	0.016	-0.091	-0.214	0.165	-0.250	**-0.363***	-0.137
Income	**0.263***	**0.293***	0.184	-0.058	-0.134	0.020	-0.038	0.107	-0.085	-0.050	-0.094
Maternal Ethnicity 0: Other, 1: White	-0.006	0.093	-0.113	0.002	0.150	0.130	0.073	0.153	-0.069	0.010	-0.108
Paternal Ethnicity 0: Other, 1: White	0.017	0.174	-0.081	0.053	-0.120	0.040	-0.061	0.176	0.048	0.000	0.070
BDI Mom	0.049	0.006	0.144	**0.477****	**-0.297***	**0.445****	**0.452****	0.189	**0.369****	**0.262***	**0.377****
BAI Mom	0.009	-0.082	0.086	**0.360****	-0.097	**0.400****	**0.455****	0.088	0.244	0.203	0.230
BDI Dad	0.244	0.033	**0.305***	-0.027	0.144	0.164	**0.277***	-0.118	-0.124	-0.083	-0.130
BAI Dad	0.203	0.050	**0.299***	0.114	-0.151	0.182	**0.270***	-0.068	0.049	0.112	0.002

Season 1:Spring, 2:Summer, 3:Fall, 4:Winter	-0.053	-0.007	-0.206	-0.105	0.026	-0.074	-0.009	-0.143	-0.094	-0.074	-0.092
Sex 0: Males, 1: Females	-0.052	0.039	-0.054	-0.104	0.009	-0.140	-0.112	-0.110	-0.057	-0.127	-0.004
Prenatal Paternal Stress/ Depression/ Anxiety											
PSS	-0.118	-0.120	-0.013	-0.044	0.111	0.038	0.118	-0.113	-0.081	-0.065	-0.077
CESD	-0.072	-0.073	0.002	-0.222	0.205	-0.114	-0.039	-0.176	-0.231	-0.194	-0.215
STR	0.013	0.036	0.155	-0.023	0.079	0.024	0.117	-0.140	-0.045	-0.025	-0.049
Paternal BMI	-0.079	-0.104	-0.106	-0.033	-0.072	-0.041	0.003	-0.092	-0.020	0.045	-0.058
Preconception Paternal Alcohol Consumption	0.202	0.163	0.183	0.060	-0.203	0.181	0.053	**0.296***	-0.023	-0.007	-0.029
Preconception Paternal Smoking											
Days/Week Smoked	-0.041	-0.053	0.089	0.031	0.123	-0.021	0.065	-0.153	0.051	0.076	0.027
# Cigarettes/ Day Smoked	0.034	-0.005	0.112	-0.038	0.094	0.053	0.108	-0.069	-0.079	-0.040	-0.091
PACOTIS (3-month postpartum)	-0.012	-0.101	-0.004	0.165	-0.059	0.172	0.259	-0.071	0.118	0.114	0.101

***p <0.05, **p <0.01.**

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire; SDQ Inter.: SDQ Internalizing subscale; SDQ Exter.: SDQ Externalizing subscale; SDQ Hyper/I: SDQ Hyperactivity/Inattention subscale; BDI: Beck Depression Inventory; BAI: Beck Anxiety Inventory; PSS: Perceived Stress Scale; CESD: Centre for Epidemiological Studies Depression Scale; STR: Anxiety Inventory; BMI: Body Mass Index; PACOTIS: Parental Cognitions and Conduct Toward the Infant Scale.

Figure 1. *Flow Chart Depicting The Number of Participants Recruited for Each Study*

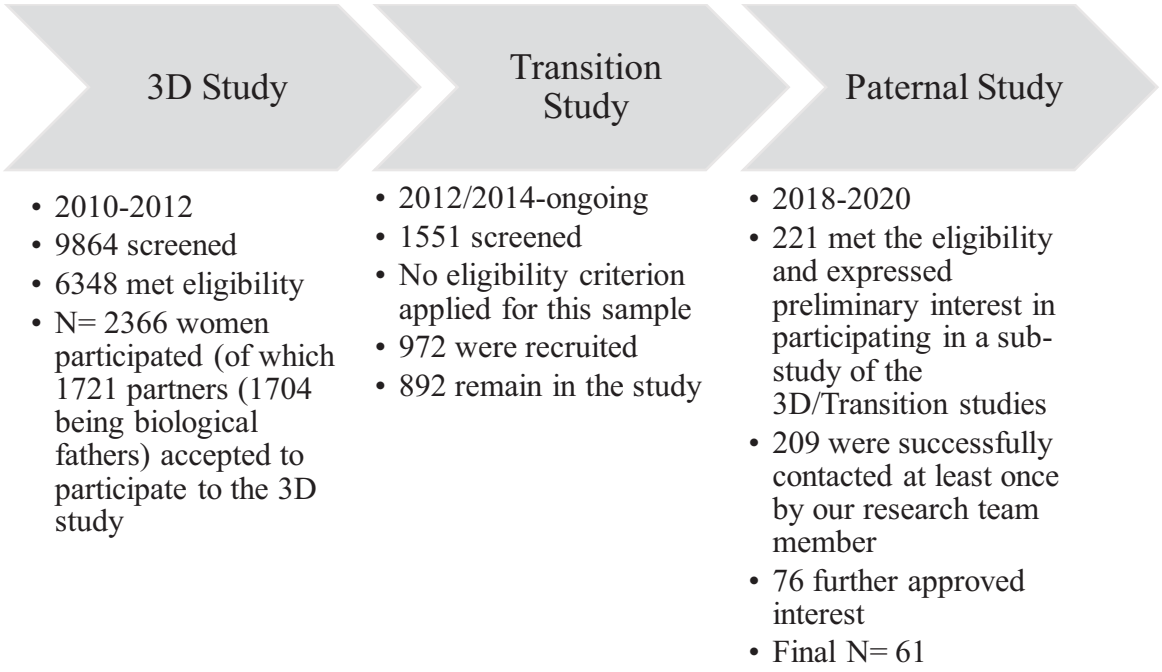
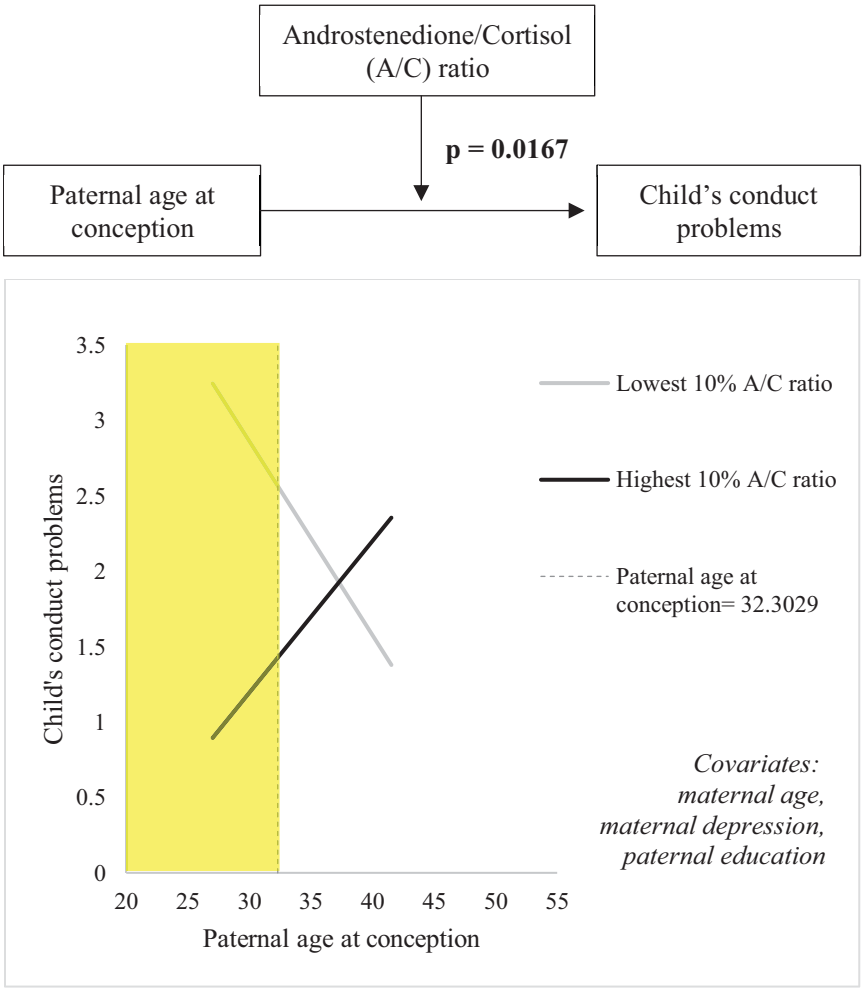


Figure 2. *Moderation Model With Paternal Age as the Predictor*



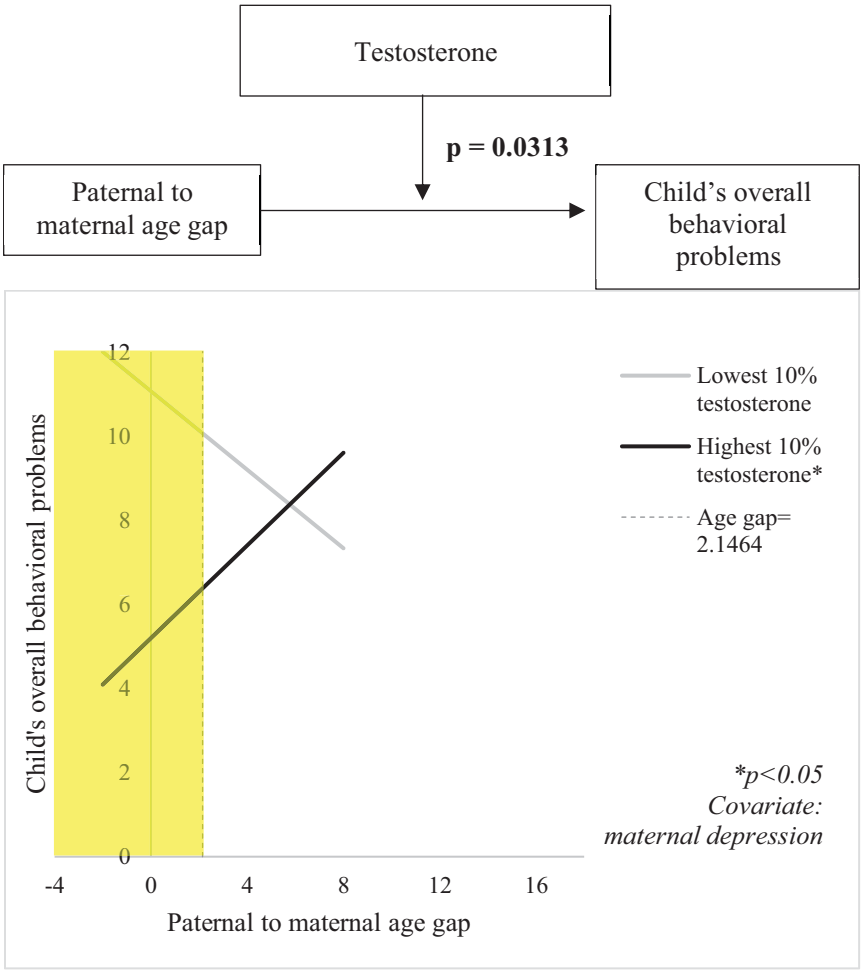
Baseline androstenedione/cortisol moderated the relationship between paternal age at conception and child's conduct problems as measured by Strength and Difficulties Questionnaire at 6-8 years of age (B= 0.2055, SE= 0.0831, p= 0.167).

The association between paternal age and child's conduct problems at different levels of baseline androstenedione/cortisol ratio is shown in the figure. The regression lines represent the top and bottom 10th percentiles of A/C as an example of the conditional effect of paternal age on conduct problems.

At highest 10% or lowest 10% of A/C, there was no association between paternal age and the offspring's conduct problems. Of note, for fathers below the age of 32.3029, there was a significant association between A/C and conduct problems, such that high A/C is associated with lower scores on conduct problems (as depicted by the region of significance in yellow).

In comparison to figure 1 from the appendix, this figure denoted that the effects of externalizing problems were primarily driven by its subscale, conduct problems, as presented here. As described in section 4.2.3, A/C ratio has been log transformed.

Figure 3. *Moderation Model With Age Gap as the Predictor*



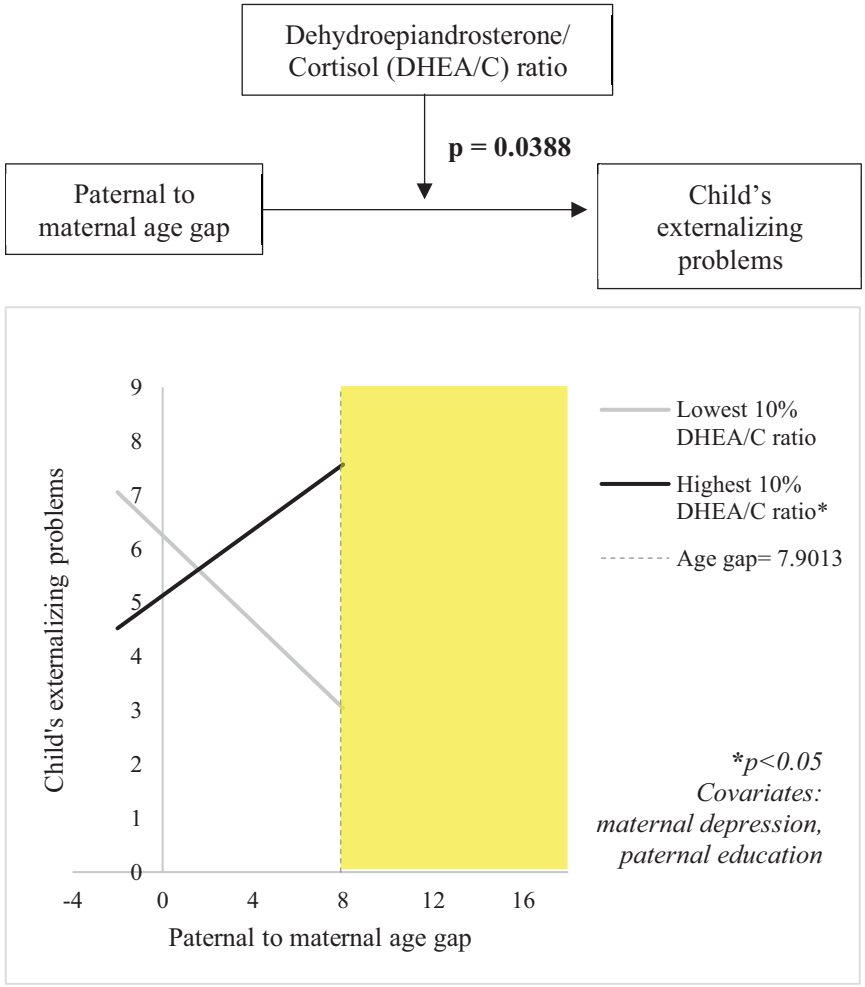
Baseline testosterone (T) moderated the relationship between paternal to maternal age gap and child's overall behavioral problems as measured by Strength and Difficulties Questionnaire (SDQ) at 6-8 years of age (B= 0.1500, SE= 0.0679, p= 0.0313).

The association between age gap and child's overall behavioral problems at different levels of baseline testosterone is shown in the figure. The regression lines represent the top and bottom 10th percentiles of testosterone as an example of the conditional effect of age gap on overall behavioral problems.

At higher T (highest 10%; black line), age gap is positively associated with the offspring's overall behavioral problems such that the older the father is compared to the mother, the higher problems are (conditional effect of age gap on overall problems at highest 10% T: B= 0.5510, SE= 0.2552, p< 0.05). Of note, for fathers who are younger than or older than their partners by <2.1464 years, there was a significant association between T and child's overall difficulties such that high T is associated with lower scores on SDQ (as depicted by the region of significance in yellow).

As described in section 4.2.3, testosterone has been square root transformed.

Figure 4. *Moderation Model With Age Gap as the Predictor*



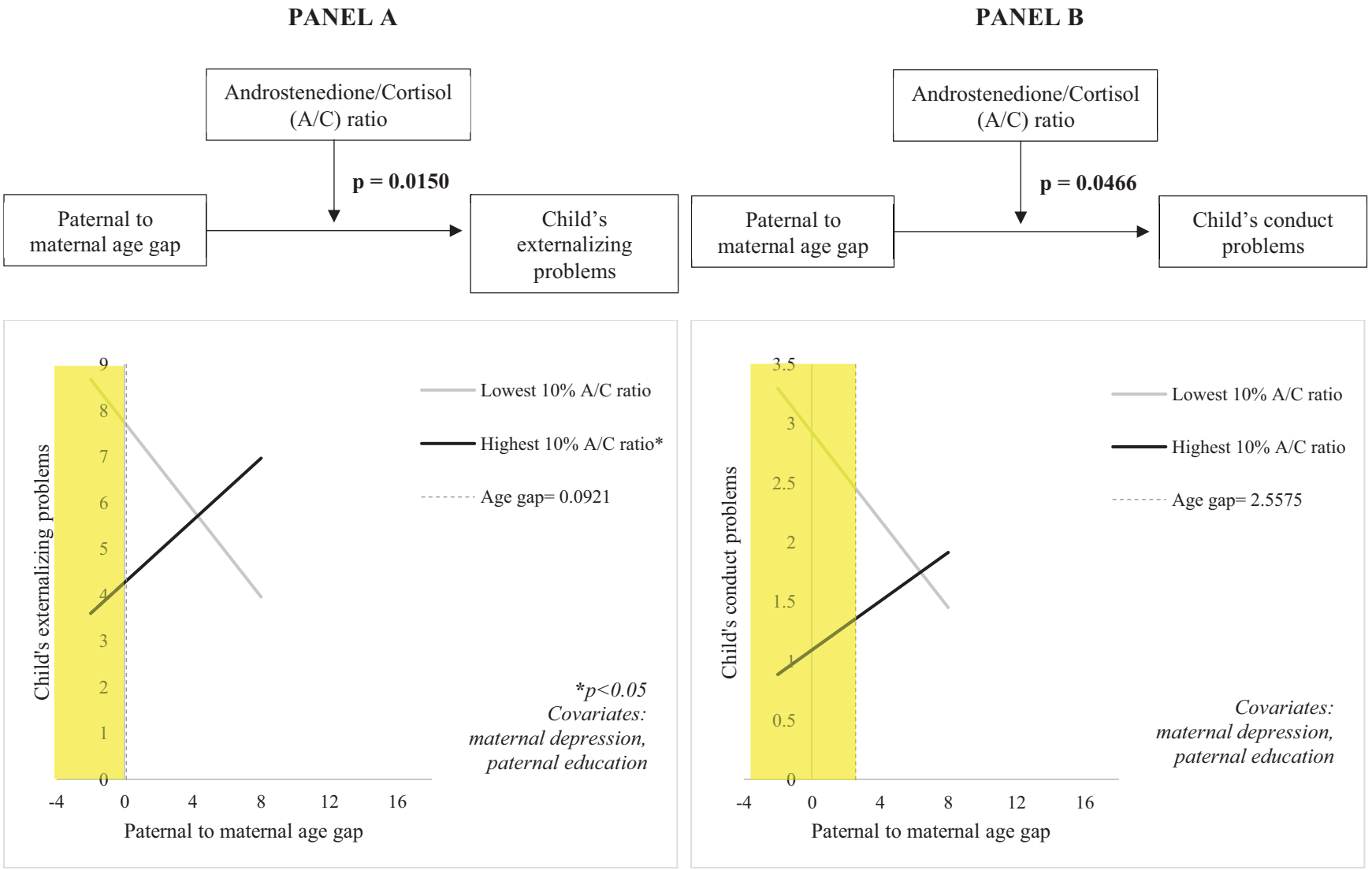
Baseline dehydroepiandrosterone/cortisol (DHEA/C) moderated the relationship between age gap and child's externalizing problems as measured by Strength and Difficulties Questionnaire at 6-8 years of age (B= 0.1850, SE= 0.0874, p= 0.0388).

The association between age gap and child's externalizing problems at different levels of baseline DHEA/C is shown in the figure. The regression lines represent the top and bottom 10th percentiles of DHEA/C as an example of the conditional effect of age gap on externalizing problems.

At higher DHEA/C ratio (highest 10%; black line), age gap is positively associated with the offspring's externalizing problems such that the older the father is compared to the mother, the higher externalizing problems are (conditional effect of age gap on externalizing problems at highest 10% DHEA/C ratio: B= 0.3039, SE= 0.1511, p< 0.05). Of note, for fathers older than their partners by 7.9013 years or higher, there was a significant association between DHEA/C and externalizing problems such that high DHEA/C is associated with higher scores on SDQ Externalizing (as depicted by the region of significance in yellow).

As described in section 4.2.3, DHEA/C has been log transformed.

Figure 5. *Moderation Model With Age Gap as the Predictor*



(**Panel A**) Baseline androstenedione/cortisol (A/C) moderated the relationship between age gap and child's externalizing problems as measured by Strength and Difficulties Questionnaire at 6-8 years of age (B= 0.7246, SE= 0.2881, p= 0.0150).

The association between age gap and child's externalizing problems at different levels of baseline androstenedione/cortisol is shown in the figure. The regression lines represent the top and bottom 10th percentiles of A/C as an example of the conditional effect of age gap on child's externalizing problems.

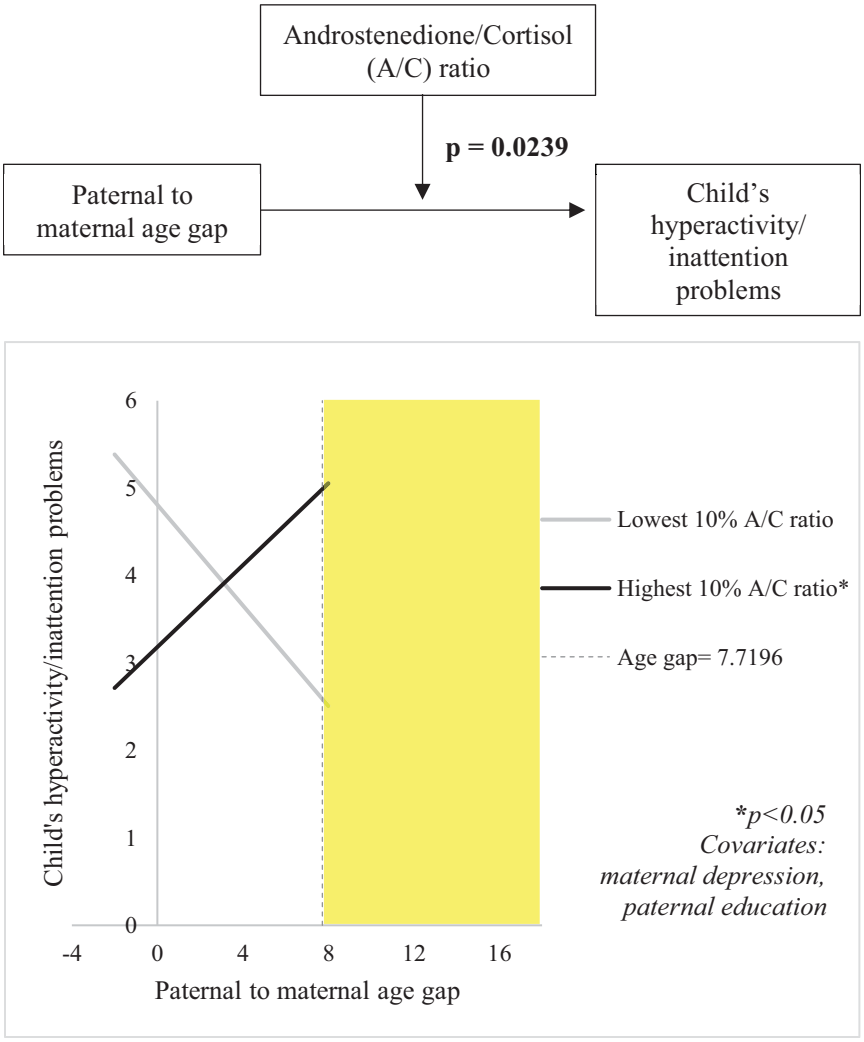
At higher A/C ratio (highest 10%; black line), age gap is positively associated with the offspring's externalizing problems such that the older the father is compared to the mother, the higher externalizing problems are (conditional effect of age gap on externalizing problems at highest 10% A/C ratio: B= 0.3367, SE= 0.1512, p< 0.05). Of note, for fathers who are younger than or of same age as their partners (age gap of 0.0921 or below), there was a significant association between A/C and externalizing problems such that high A/C is associated with lower scores on SDQ Externalizing (as depicted by the region of significance in yellow).

(**Panel B**) Baseline androstenedione/cortisol moderated the relationship between age gap and child's conduct problems as measured by Strength and Difficulties Questionnaire at 6-8 years of age (B= 0.2572, SE= 0.1262, p= 0.0466).

The association between age gap and conduct problems at different levels of baseline A/C is shown in the figure. The regression lines represent the top and bottom 10th percentiles of A/C as an example of the conditional effect of age gap on child's conduct problems.

At highest 10% or lowest 10% of A/C, there was no association between age gap and the child's performance on SDQ Conduct. Of note, for fathers younger than their partners or older by <2.55 years, there was a significant association between A/C and conduct problems such that high A/C is associated with lower scores on SDQ Conduct (as depicted by the region of significance in yellow).

PANEL C



(**Panel C**) Baseline androstenedione/cortisol moderated the relationship between age gap and child's hyperactivity/inattention problems at 6-8 years of age (B= 0.4673, SE= 0.2008, p= 0.0239).

The association between age gap and child's hyperactivity/inattention problems at different levels of baseline A/C is shown in the figure. The regression lines represent the top and bottom 10th percentiles of A/C as an example of the conditional effect of age gap on SDQ hyperactivity/inattention.

At higher A/C ratio (highest 10%; black line), age gap is positively associated with the offspring's levels of hyperactivity/inattention as measured by SDQ during middle childhood, such that the older the father is compared to the mother, the higher hyperactivity/inattention problems are (conditional effect of age gap on hyperactivity/inattention problems at highest 10% A/C: B= 0.2339, SE= 0.1054, p< 0.05). Of note, for fathers older than their partners by 7.7196 years or higher, there was a significant association between A/C and hyperactivity/inattention problems

such that high A/C is associated with higher scores on SDQ Hyperactivity/Inattention (as depicted by the region of significance in yellow).

As shown in this figure, comparison of the three figures denoted that the effects of externalizing problems were primarily driven by its subscale, conduct problems. As described in section 4.2.3, A/C ratio has been log transformed.

10. APPENDIX

Table A1. *Number of "Not Found" and "Insufficient" Hormonal Samples*

	N	Number of 'Not Found' samples	Number of 'Insufficient' samples	Replacement value for 'Not Found' samples
DHEA (pg/mL)				
At Time 1	46	9	3	0.01
At Time 2	39	11	8	
Androstenedione (pg/mL)				
At Time 1	46	1	11	1.00
At Time 2	44	4	10	
Testosterone (pg/mL)				
At Time 1	51	4	3	0.10
At Time 2	45	4	9	
Cortisol (pg/mL)				
At Time 1	54	1	3	6.50
At Time 2	49	0	9	

Note. "Not found" samples are the samples that had sufficient quantity to run the assay, but the concentration was not detectable. "Insufficient" samples are the samples that did not have sufficient quantity to run the assay. The sample size presented here is the number of hormonal samples obtained before imputation. DHEA: Dehydroepiandrostereone.

Table A2. *Full list of covariates*

	Paternal age at conception	Parental age gap
WISC		
Full Scale	Maternal age, parental education	Parental education
Verbal	Maternal age, parental education	Parental education
Fluid	Maternal age, paternal education	Paternal education
SDQ		
Total Difficulties	Maternal age, maternal depression (BDI)	Maternal depression (BDI)
Prosocial	Maternal age, maternal depression (BDI)	Maternal depression (BDI)
Internalizing	Maternal age, maternal depression (BDI), maternal anxiety (BAI)	Maternal depression (BDI), maternal anxiety (BAI)
Externalizing	Maternal age, paternal education, maternal depression (BDI)	Paternal education, maternal depression (BDI)

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire; BDI: Beck Depression Inventory; BAI: Beck Anxiety Inventory.

Table A3. *Sex-Based Analysis: Moderated Moderation Model With Paternal Age at Conception as the Predictor and Baseline DHEA and Sex as the Moderators*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a,b]	0.3295	0.0008	0.8025	-
WISC Verbal [a,b]	0.2412	0.0029	0.6638	1
WISC Fluid [a,c]	0.1709	0.0007	0.8421	0.8421
SDQ Total Difficulties [a,d]	0.4715	0.0047	0.5066	-
SDQ Prosocial [a,d]	0.1663	0.0017	0.7476	1
SDQ Internalizing [a,d,e]	0.3900	0.0001	0.9378	0.9378
SDQ Externalizing [a,c,d]	0.4836	0.0050	0.4959	1

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: maternal age [a], parental education [b], paternal education [c], Beck Depression Inventory (mom) [d], Beck Anxiety Inventory (mom) [e].

Table A4. *Sex-Based Analysis: Moderated Moderation Model With Paternal Age at Conception as the Predictor and Baseline Androstenedione and Sex as the Moderators*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a,b]	0.3778	0.0109	0.3637	-
WISC Verbal [a,b]	0.2553	0.0147	0.3355	0.3355
WISC Fluid [a,c]	0.1955	0.0204	0.2750	0.5500
SDQ Total Difficulties [a,d]	0.4438	0.0010	0.7657	-
SDQ Prosocial [a,d]	0.1529	0.0112	0.4286	1
SDQ Internalizing [a,d,e]	0.4120	0.0002	0.9012	1
SDQ Externalizing [a,c,d]	0.4496	0.0000	0.9487	0.9487

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: maternal age [a], parental education [b], paternal education [c], Beck Depression Inventory (mom) [d], Beck Anxiety Inventory (mom) [e].

Table A5. *Sex-Based Analysis: Moderated Moderation Model With Paternal Age at Conception as the Predictor and Baseline Testosterone and Sex as the Moderators*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a,b]	0.4148	0.0001	0.9468	-
WISC Verbal [a,b]	0.2676	0.0007	0.8263	0.8263
WISC Fluid [a,c]	0.1921	0.0033	0.6520	1
SDQ Total Difficulties [a,d]	0.4801	0.0031	0.5871	-
SDQ Prosocial [a,d]	0.2113	0.0260	0.2050	0.6150
SDQ Internalizing [a,d,e]	0.3745	0.0017	0.7135	0.7135
SDQ Externalizing [a,c,d]	0.4745	0.0022	0.6496	0.9744

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: maternal age [a], parental education [b], paternal education [c], Beck Depression Inventory (mom) [d], Beck Anxiety Inventory (mom) [e].

Table A6. *Sex-Based Analysis: Moderated Moderation Model With Paternal Age at Conception as the Predictor and Baseline Cortisol and Sex as the Moderators*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a,b]	0.3608	0.0018	0.7083	-
WISC Verbal [a,b]	0.2530	0.0049	0.5688	0.5688
WISC Fluid [a,c]	0.1980	0.0149	0.3398	0.6796
SDQ Total Difficulties [a,d]	0.3268	0.0002	0.8999	-
SDQ Prosocial [a,d]	0.2283	0.0327	0.1518	0.4554
SDQ Internalizing [a,d,e]	0.3257	0.0024	0.6797	0.6797
SDQ Externalizing [a,c,d]	0.3864	0.0064	0.4778	0.7167

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: maternal age [a], parental education [b], paternal education [c], Beck Depression Inventory (mom) [d], Beck Anxiety Inventory (mom) [e].

Table A7. *Sex-Based Analysis: Moderated Moderation Model With Paternal Age at Conception as the Predictor and Baseline DHEA/Cortisol and Sex as the Moderators*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a,b]	0.3071	0.0050	0.5521	-
WISC Verbal [a,b]	0.2763	0.0046	0.5739	1
WISC Fluid [a,c]	0.1565	0.0025	0.6995	0.6995
SDQ Total Difficulties [a,d]	0.3992	0.0066	0.4615	-
SDQ Prosocial [a,d]	0.1513	0.0028	0.6875	0.6875
SDQ Internalizing [a,d,e]	0.3698	0.0026	0.6534	0.9801
SDQ Externalizing [a,c,d]	0.4225	0.0096	0.3718	1

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: maternal age [a], parental education [b], paternal education [c], Beck Depression Inventory (mom) [d], Beck Anxiety Inventory (mom) [e].

Table A8. *Sex-Based Analysis: Moderated Moderation Model With Paternal Age at Conception as the Predictor and Baseline Androstenedione/Cortisol and Sex as the Moderators*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a,b]	0.3228	0.0010	0.7876	-
WISC Verbal [a,b]	0.2601	0.0105	0.4130	0.8260
WISC Fluid [a,c]	0.2230	0.0009	0.8170	0.8170
SDQ Total Difficulties [a,d]	0.4383	0.0029	0.6229	-
SDQ Prosocial [a,d]	0.1701	0.0033	0.6665	1
SDQ Internalizing [a,d,e]	0.4091	0.0016	0.7265	1
SDQ Externalizing [a,c,d]	0.4359	0.0000	0.9532	0.9532

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: maternal age [a], parental education [b], paternal education [c], Beck Depression Inventory (mom) [d], Beck Anxiety Inventory (mom) [e].

Table A9. *Sex-Based Analysis: Moderated Moderation Model With Paternal Age at Conception as the Predictor and Baseline Testosterone/Cortisol and Sex as the Moderators*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a,b]	0.3459	0.0159	0.2755	-
WISC Verbal [a,b]	0.2434	0.0001	0.9539	0.9539
WISC Fluid [a,c]	0.1907	0.0244	0.2255	0.4510
SDQ Total Difficulties [a,d]	0.4182	0.0199	0.1966	-
SDQ Prosocial [a,d]	0.2155	0.0191	0.2748	0.4122
SDQ Internalizing [a,d,e]	0.3457	0.0001	0.9161	0.9161
SDQ Externalizing [a,c,d]	0.4407	0.0436	0.0564	0.1692

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: maternal age [a], parental education [b], paternal education [c], Beck Depression Inventory (mom) [d], Beck Anxiety Inventory (mom) [e].

Table A10. *Sex-Based Analysis: Moderated Moderation Model With Paternal Age at Conception as the Predictor and Baseline DHEA/Androstenedione and Sex as the Moderators*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a,b]	0.3510	0.0273	0.1621	-
WISC Verbal [a,b]	0.2974	0.0004	0.8654	0.8654
WISC Fluid [a,c]	0.1637	0.0107	0.4368	0.8736
SDQ Total Difficulties [a,d]	0.3970	0.0086	0.4111	-
SDQ Prosocial [a,d]	0.1721	0.0003	0.8957	0.8957
SDQ Internalizing [a,d,e]	0.3191	0.0060	0.5236	0.7854
SDQ Externalizing [a,c,d]	0.4788	0.0215	0.1705	0.5115

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: maternal age [a], parental education [b], paternal education [c], Beck Depression Inventory (mom) [d], Beck Anxiety Inventory (mom) [e].

Table A11. *Sex-Based Analysis: Moderated Moderation Model With Paternal Age at Conception as the Predictor and Baseline DHEA/Testosterone and Sex as the Moderators*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a,b]	0.3140	0.0000	0.9682	-
WISC Verbal [a,b]	0.3319	0.0201	0.2260	0.4520
WISC Fluid [a,c]	0.1676	0.0034	0.6534	0.6534
SDQ Total Difficulties [a,d]	0.3639	0.0047	0.5444	-
SDQ Prosocial [a,d]	0.1452	0.0015	0.7681	0.7681
SDQ Internalizing [a,d,e]	0.3788	0.0040	0.5747	0.8621
SDQ Externalizing [a,c,d]	0.4043	0.0065	0.4680	1

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: maternal age [a], parental education [b], paternal education [c], Beck Depression Inventory (mom) [d], Beck Anxiety Inventory (mom) [e].

Table A12. *Sex-Based Analysis: Moderated Moderation Model With Paternal Age at Conception as the Predictor and Baseline Androstenedione/Testosterone and Sex as the Moderators*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a,b]	0.3262	0.0032	0.6332	-
WISC Verbal [a,b]	0.2713	0.0118	0.3817	0.7634
WISC Fluid [a,c]	0.1625	0.0091	0.4735	0.4735
SDQ Total Difficulties [a,d]	0.3166	0.0000	0.9927	-
SDQ Prosocial [a,d]	0.1672	0.0055	0.5773	0.8660
SDQ Internalizing [a,d,e]	0.3479	0.0163	0.2833	0.8499
SDQ Externalizing [a,c,d]	0.3671	0.0012	0.7662	0.7662

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: maternal age [a], parental education [b], paternal education [c], Beck Depression Inventory (mom) [d], Beck Anxiety Inventory (mom) [e].

Table A13. *Sex-Based Analysis: Moderated Moderation Model With Age Gap as the Predictor and Baseline DHEA and Sex as the Moderators*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a]	0.3238	0.0283	0.1501	-
WISC Verbal [a]	0.2744	0.0162	0.2906	0.5812
WISC Fluid [b]	0.1694	0.0014	0.7668	0.7668
SDQ Total Difficulties [c]	0.4641	0.0015	0.7070	-
SDQ Prosocial [c]	0.1329	0.0264	0.2185	0.6555
SDQ Internalizing [c,d]	0.3835	0.0000	0.9715	0.9715
SDQ Externalizing [b,c]	0.4907	0.0033	0.5724	0.8586

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: parental education [a], paternal education [b], Beck Depression Inventory (mom) [c], Beck Anxiety Inventory (mom) [d].

Table A14. *Sex-Based Analysis: Moderated Moderation Model With Age Gap as the Predictor and Baseline Androstenedione and Sex as the Moderators*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a]	0.3264	0.0001	0.9388	-
WISC Verbal [a]	0.2534	0.0004	0.8728	0.8728
WISC Fluid [b]	0.1868	0.0283	0.1977	0.3954
SDQ Total Difficulties [c]	0.4429	0.0001	0.9128	-
SDQ Prosocial [c]	0.1600	0.0447	0.1129	0.3387
SDQ Internalizing [c,d]	0.4160	0.0103	0.3627	0.5441
SDQ Externalizing [b,c]	0.4456	0.0011	0.7539	0.7539

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: parental education [a], paternal education [b], Beck Depression Inventory (mom) [c], Beck Anxiety Inventory (mom) [d].

Table A15. *Sex-Based Analysis: Moderated Moderation Model With Age Gap as the Predictor and Baseline Testosterone and Sex as the Moderators*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a]	0.3788	0.0010	0.7734	-
WISC Verbal [a]	0.2834	0.0143	0.3179	0.3179
WISC Fluid [b]	0.2018	0.0170	0.3020	0.6040
SDQ Total Difficulties [c]	0.5059	0.0119	0.2728	-
SDQ Prosocial [c]	0.1412	0.0374	0.1421	0.4263
SDQ Internalizing [c,d]	0.3991	0.0117	0.3293	0.5000
SDQ Externalizing [b,c]	0.4701	0.0049	0.5012	0.5012

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: parental education [a], paternal education [b], Beck Depression Inventory (mom) [c], Beck Anxiety Inventory (mom) [d].

Table A16. *Sex-Based Analysis: Moderated Moderation Model With Age Gap as the Predictor and Baseline Cortisol and Sex as the Moderators*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a]	0.3154	0.0103	0.3845	-
WISC Verbal [a]	0.2673	0.0186	0.2600	0.5200
WISC Fluid [b]	0.1806	0.0013	0.7763	0.7763
SDQ Total Difficulties [c]	0.3158	0.0020	0.7002	-
SDQ Prosocial [c]	0.2104	0.0911	**0.0189***	0.0567
SDQ Internalizing [c,d]	0.3379	0.0003	0.8717	0.8717
SDQ Externalizing [b,c]	0.3584	0.0009	0.7964	1

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: parental education [a], paternal education [b], Beck Depression Inventory (mom) [c], Beck Anxiety Inventory (mom) [d].

Table A17. *Sex-Based Analysis: Moderated Moderation Model With Age Gap as the Predictor and Baseline DHEA/Cortisol and Sex as the Moderators*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a]	0.3012	0.0254	0.1796	-
WISC Verbal [a]	0.2821	0.0008	0.8102	1
WISC Fluid [b]	0.1725	0.0006	0.8488	0.8488
SDQ Total Difficulties [c]	0.3904	0.0067	0.4587	-
SDQ Prosocial [c]	0.1165	0.0160	0.3407	1
SDQ Internalizing [c,d]	0.3675	0.0054	0.5148	0.5148
SDQ Externalizing [b,c]	0.4276	0.0077	0.4149	0.6224

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: parental education [a], paternal education [b], Beck Depression Inventory (mom) [c], Beck Anxiety Inventory (mom) [d].

Table A18. *Sex-Based Analysis: Moderated Moderation Model With Age Gap as the Predictor and Baseline Androstenedione/Cortisol and Sex as the Moderators*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a]	0.2837	0.0000	0.9973	-
WISC Verbal [a]	0.2856	0.0006	0.8388	0.8388
WISC Fluid [b]	0.2225	0.0203	0.2631	0.5262
SDQ Total Difficulties [c]	0.4059	0.0204	0.2010	-
SDQ Prosocial [c]	0.1277	0.0039	0.6407	0.6407
SDQ Internalizing [c,d]	0.4111	0.0309	0.1188	0.3564
SDQ Externalizing [b,c]	0.3999	0.0028	0.6395	0.9592

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: parental education [a], paternal education [b], Beck Depression Inventory (mom) [c], Beck Anxiety Inventory (mom) [d].

Table A19. *Sex-Based Analysis: Moderated Moderation Model With Age Gap as the Predictor and Baseline Testosterone/Cortisol and Sex as the Moderators*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a]	0.3059	0.0078	0.4524	-
WISC Verbal [a]	0.2853	0.0029	0.6513	0.6513
WISC Fluid [b]	0.1721	0.0081	0.4822	0.9644
SDQ Total Difficulties [c]	0.4015	0.0021	0.6770	-
SDQ Prosocial [c]	0.1210	0.0001	0.9439	0.9439
SDQ Internalizing [c,d]	0.3660	0.0065	0.4786	1
SDQ Externalizing [b,c]	0.3886	0.0002	0.9053	1

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: parental education [a], paternal education [b], Beck Depression Inventory (mom) [c], Beck Anxiety Inventory (mom) [d].

Table A20. *Sex-Based Analysis: Moderated Moderation Model With Age Gap as the Predictor and Baseline DHEA/Androstenedione and Sex as the Moderators*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a]	0.3113	0.0206	0.2315	-
WISC Verbal [a]	0.3136	0.0057	0.5280	1
WISC Fluid [b]	0.1730	0.0006	0.8487	0.8487
SDQ Total Difficulties [c]	0.4273	0.0247	0.1520	-
SDQ Prosocial [c]	0.1340	0.0114	0.4248	0.6372
SDQ Internalizing [c,d]	0.3149	0.0016	0.7429	0.7429
SDQ Externalizing [b,c]	0.5291	0.0251	0.1165	0.3495

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: parental education [a], paternal education [b], Beck Depression Inventory (mom) [c], Beck Anxiety Inventory (mom) [d].

Table A21. *Sex-Based Analysis: Moderated Moderation Model With Age Gap as the Predictor and Baseline DHEA/Testosterone and Sex as the Moderators*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a]	0.2745	0.0029	0.6515	-
WISC Verbal [a]	0.3321	0.0090	0.4097	0.8194
WISC Fluid [b]	0.1811	0.0043	0.6051	0.6051
SDQ Total Difficulties [c]	0.3648	0.0109	0.3536	-
SDQ Prosocial [c]	0.0996	0.0175	0.3248	0.4872
SDQ Internalizing [c,d]	0.3991	0.0198	0.2050	0.6150
SDQ Externalizing [b,c]	0.4154	0.0079	0.4140	0.4140

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: parental education [a], paternal education [b], Beck Depression Inventory (mom) [c], Beck Anxiety Inventory (mom) [d].

Table A22. *Sex-Based Analysis: Moderated Moderation Model With Age Gap as the Predictor and Baseline Androstenedione/Testosterone and Sex as the Moderators*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a]	0.3099	0.0102	0.3990	-
WISC Verbal [a]	0.3222	0.0193	0.2434	0.4868
WISC Fluid [b]	0.1615	0.0052	0.5830	0.5830
SDQ Total Difficulties [c]	0.2954	0.0000	0.9666	-
SDQ Prosocial [c]	0.1253	0.0058	0.5713	0.8570
SDQ Internalizing [c,d]	0.3538	0.0209	0.2192	0.6576
SDQ Externalizing [b,c]	0.3632	0.0003	0.8784	0.8784

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: parental education [a], paternal education [b], Beck Depression Inventory (mom) [c], Beck Anxiety Inventory (mom) [d].

Table A23. *Moderation Model With Paternal Age at Conception as the Predictor and Baseline DHEA as the Moderator*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a,b]	0.3090	0.0050	0.5326	-
WISC Verbal [a,b]	0.2302	0.0008	0.8155	0.8155
WISC Fluid [a,c]	0.1604	0.0304	0.1675	0.3350
SDQ Total Difficulties [a,d]	0.2299	0.0051	0.5534	-
SDQ Prosocial [a,d]	0.1378	0.0022	0.7130	1
SDQ Internalizing [a,d,e]	0.2971	0.0001	0.9480	0.9480
SDQ Externalizing [a,c,d]	0.2750	0.0129	0.3358	1

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: maternal age [a], parental education [b], paternal education [c], Beck Depression Inventory (mom) [d], Beck Anxiety Inventory (mom) [e].

Table A24. *Moderation Model With Paternal Age at Conception as the Predictor and Baseline Androstenedione as the Moderator*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a,b]	0.3059	0.0030	0.6400	-
WISC Verbal [a,b]	0.2337	0.0005	0.8588	0.8588
WISC Fluid [a,c]	0.1516	0.0253	0.2183	0.4366
SDQ Total Difficulties [a,d]	0.2450	0.0052	0.5536	-
SDQ Prosocial [a,d]	0.1338	0.0000	0.9623	0.9623
SDQ Internalizing [a,d,e]	0.3280	0.0001	0.9411	1
SDQ Externalizing [a,c,d]	0.2719	0.0045	0.5780	1

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: maternal age [a], parental education [b], paternal education [c], Beck Depression Inventory (mom) [d], Beck Anxiety Inventory (mom) [e].

Table A25. *Moderation Model With Paternal Age at Conception as the Predictor and Baseline Testosterone as the Moderator*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a,b]	0.3084	0.0011	0.7717	-
WISC Verbal [a,b]	0.2294	0.0002	0.9003	0.9003
WISC Fluid [a,c]	0.1517	0.0212	0.2500	0.5000
SDQ Total Difficulties [a,d]	0.2477	0.0125	0.3475	-
SDQ Prosocial [a,d]	0.1373	0.0029	0.6717	0.6717
SDQ Internalizing [a,d,e]	0.3027	0.0038	0.5914	1
SDQ Externalizing [a,c,d]	0.2659	0.0038	0.6031	0.9047

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: maternal age [a], parental education [b], paternal education [c], Beck Depression Inventory (mom) [d], Beck Anxiety Inventory (mom) [e].

Table A26. *Moderation Model With Paternal Age at Conception as the Predictor and Baseline Cortisol as the Moderator*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a,b]	0.3320	0.0274	0.1422	-
WISC Verbal [a,b]	0.2392	0.0100	0.4027	0.4027
WISC Fluid [a,c]	0.1771	0.0246	0.2097	0.4194
SDQ Total Difficulties [a,d]	0.2210	0.0077	0.4671	-
SDQ Prosocial [a,d]	0.1598	0.0273	0.1912	0.2868
SDQ Internalizing [a,d,e]	0.2462	0.0046	0.5704	0.5704
SDQ Externalizing [a,c,d]	0.3034	0.0413	0.0820	0.2460

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: maternal age [a], parental education [b], paternal education [c], Beck Depression Inventory (mom) [d], Beck Anxiety Inventory (mom) [e].

Table A27. *Moderation Model With Paternal Age at Conception as the Predictor and Baseline DHEA/Cortisol as the Moderator*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a,b]	0.2859	0.0000	0.9908	-
WISC Verbal [a,b]	0.2416	0.0016	0.7398	0.7398
WISC Fluid [a,c]	0.1467	0.0051	0.5720	1
SDQ Total Difficulties [a,d]	0.2291	0.0106	0.3916	-
SDQ Prosocial [a,d]	0.1392	0.0001	0.9232	0.9232
SDQ Internalizing [a,d,e]	0.3037	0.0037	0.5957	0.8936
SDQ Externalizing [a,c,d]	0.3049	0.0388	0.0914	0.2742

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: maternal age [a], parental education [b], paternal education [c], Beck Depression Inventory (mom) [d], Beck Anxiety Inventory (mom) [e].

Table A28. *Moderation Model With Paternal Age at Conception as the Predictor and Baseline Testosterone/Cortisol as the Moderator*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a,b]	0.2891	0.0010	0.7846	-
WISC Verbal [a,b]	0.2335	0.0025	0.6779	0.6779
WISC Fluid [a,c]	0.1402	0.0091	0.4541	0.9082
SDQ Total Difficulties [a,d]	0.2416	0.0192	0.2470	-
SDQ Prosocial [a,d]	0.1340	0.0007	0.8321	0.8321
SDQ Internalizing [a,d,e]	0.2962	0.0064	0.4901	0.7352
SDQ Externalizing [a,c,d]	0.2802	0.0134	0.3258	0.9774

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: maternal age [a], parental education [b], paternal education [c], Beck Depression Inventory (mom) [d], Beck Anxiety Inventory (mom) [e].

Table A29. *Moderation Model With Paternal Age at Conception as the Predictor and Baseline DHEA/Androstenedione as the Moderator*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a,b]	0.2978	0.0117	0.3554	-
WISC Verbal [a,b]	0.2671	0.0186	0.2565	0.5130
WISC Fluid [a,c]	0.1428	0.0142	0.3580	0.3580
SDQ Total Difficulties [a,d]	0.2191	0.0044	0.5896	-
SDQ Prosocial [a,d]	0.1457	0.0000	0.9764	0.9764
SDQ Internalizing [a,d,e]	0.2498	0.0006	0.8393	1
SDQ Externalizing [a,c,d]	0.3187	0.0220	0.2055	0.6165

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: maternal age [a], parental education [b], paternal education [c], Beck Depression Inventory (mom) [d], Beck Anxiety Inventory (mom) [e].

Table A30. *Moderation Model With Paternal Age at Conception as the Predictor and Baseline DHEA/Testosterone as the Moderator*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a,b]	0.2860	0.0002	0.8954	-
WISC Verbal [a,b]	0.2412	0.0003	0.8864	0.8864
WISC Fluid [a,c]	0.1410	0.0008	0.8263	1
SDQ Total Difficulties [a,d]	0.2157	0.0017	0.7358	-
SDQ Prosocial [a,d]	0.1400	0.0013	0.7733	0.7733
SDQ Internalizing [a,d,e]	0.2840	0.0135	0.3214	0.4821
SDQ Externalizing [a,c,d]	0.2888	0.0253	0.1758	0.5274

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: maternal age [a], parental education [b], paternal education [c], Beck Depression Inventory (mom) [d], Beck Anxiety Inventory (mom) [e].

Table A31. *Moderation Model With Paternal Age at Conception as the Predictor and Baseline Androstenedione/Testosterone as the Moderator*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a,b]	0.2992	0.0006	0.8317	-
WISC Verbal [a,b]	0.2359	0.0020	0.7130	1
WISC Fluid [a,c]	0.1372	0.0000	0.9851	0.9851
SDQ Total Difficulties [a,d]	0.2113	0.0021	0.7114	-
SDQ Prosocial [a,d]	0.1337	0.0015	0.7638	1
SDQ Internalizing [a,d,e]	0.2464	0.0122	0.3675	1
SDQ Externalizing [a,c,d]	0.2762	0.0010	0.7954	0.7954

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: maternal age [a], parental education [b], paternal education [c], Beck Depression Inventory (mom) [d], Beck Anxiety Inventory (mom) [e].

Table A32. *Moderation Model With Age Gap as the Predictor and Baseline DHEA as the Moderator*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a]	0.2373	0.0090	0.4234	-
WISC Verbal [a]	0.1642	0.0104	0.4127	0.4127
WISC Fluid [b]	0.1478	0.0243	0.2161	0.4322
SDQ Total Difficulties [c]	0.2566	0.0329	0.1244	-
SDQ Prosocial [c]	0.0785	0.0021	0.7216	1
SDQ Internalizing [c,d]	0.2961	0.0001	0.9286	0.9286
SDQ Externalizing [b,c]	0.2729	0.0490	0.0617	0.1851

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: parental education [a], paternal education [b], Beck Depression Inventory (mom) [c], Beck Anxiety Inventory (mom) [d].

Table A33. *Moderation Model With Age Gap as the Predictor and Baseline Androstenedione as the Moderator*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a]	0.2491	0.0034	0.6259	-
WISC Verbal [a]	0.1619	0.0073	0.5009	1
WISC Fluid [b]	0.1295	0.0074	0.5056	0.5056
SDQ Total Difficulties [c]	0.2903	0.0506	0.0573	-
SDQ Prosocial [c]	0.1112	0.0189	0.2934	0.4401
SDQ Internalizing [c,d]	0.3288	0.0118	0.3444	0.3444
SDQ Externalizing [b,c]	0.2734	0.0404	0.0951	0.2853

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: parental education [a], paternal education [b], Beck Depression Inventory (mom) [c], Beck Anxiety Inventory (mom) [d].

Table A34. *Moderation Model With Age Gap as the Predictor and Baseline Cortisol as the Moderator*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a]	0.2513	0.0118	0.3561	-
WISC Verbal [a]	0.1596	0.0046	0.5843	0.5843
WISC Fluid [b]	0.1644	0.0169	0.2965	0.5930
SDQ Total Difficulties [c]	0.2120	0.0001	0.9310	-
SDQ Prosocial [c]	0.0907	0.0188	0.2908	0.4362
SDQ Internalizing [c,d]	0.2570	0.0161	0.2835	0.8505
SDQ Externalizing [b,c]	0.2384	0.0146	0.3141	0.3141

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: parental education [a], paternal education [b], Beck Depression Inventory (mom) [c], Beck Anxiety Inventory (mom) [d].

Table A35. *Moderation Model With Age Gap as the Predictor and Baseline Testosterone/Cortisol as the Moderator*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a]	0.2189	0.0001	0.9412	-
WISC Verbal [a]	0.1595	0.0065	0.5175	1
WISC Fluid [b]	0.1249	0.0020	0.7270	0.7270
SDQ Total Difficulties [c]	0.2488	0.0271	0.1648	-
SDQ Prosocial [c]	0.0828	0.0065	0.5363	0.5363
SDQ Internalizing [c,d]	0.3051	0.0179	0.2431	0.7293
SDQ Externalizing [b,c]	0.2391	0.0139	0.3245	0.4868

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: parental education [a], paternal education [b], Beck Depression Inventory (mom) [c], Beck Anxiety Inventory (mom) [d].

Table A36. *Moderation Model With Age Gap as the Predictor and Baseline DHEA/Androstenedione as the Moderator*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a]	0.2430	0.0231	0.2090	-
WISC Verbal [a]	0.2134	0.0251	0.1995	0.1995
WISC Fluid [b]	0.1550	0.0301	0.1754	0.3508
SDQ Total Difficulties [c]	0.2171	0.0026	0.6758	-
SDQ Prosocial [c]	0.0872	0.0001	0.9512	0.9512
SDQ Internalizing [c,d]	0.2547	0.0055	0.5374	0.8061
SDQ Externalizing [b,c]	0.2910	0.0201	0.2305	0.6915

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: parental education [a], paternal education [b], Beck Depression Inventory (mom) [c], Beck Anxiety Inventory (mom) [d].

Table A37. *Moderation Model With Age Gap as the Predictor and Baseline DHEA/Testosterone as the Moderator*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a]	0.2301	0.0143	0.3173	-
WISC Verbal [a]	0.1840	0.0055	0.5447	0.5447
WISC Fluid [b]	0.1659	0.0295	0.1686	0.3372
SDQ Total Difficulties [c]	0.2255	0.0131	0.3393	-
SDQ Prosocial [c]	0.0752	0.0013	0.7795	0.7795
SDQ Internalizing [c,d]	0.2749	0.0045	0.5658	0.8487
SDQ Externalizing [b,c]	0.2763	0.0481	0.0636	0.1908

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: parental education [a], paternal education [b], Beck Depression Inventory (mom) [c], Beck Anxiety Inventory (mom) [d].

Table A38. *Moderation Model With Age Gap as the Predictor and Baseline Androstenedione/Testosterone as the Moderator*

Outcome	Model R^2	Moderation ΔR^2	p	Benjamini-Hochberg Adjusted p
WISC Full Scale [a]	0.2273	0.0000	0.9643	-
WISC Verbal [a]	0.1532	0.0003	0.8932	0.8932
WISC Fluid [b]	0.1321	0.0003	0.8914	1
SDQ Total Difficulties [c]	0.2079	0.0000	0.9922	-
SDQ Prosocial [c]	0.0857	0.0022	0.7239	0.7239
SDQ Internalizing [c,d]	0.2444	0.0108	0.3935	1
SDQ Externalizing [b,c]	0.2405	0.0057	0.5350	0.8025

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire. Control variables: parental education [a], paternal education [b], Beck Depression Inventory (mom) [c], Beck Anxiety Inventory (mom) [d].

Table A39. *Pearson Correlation Matrix Between Potential Covariates and Baseline Square Root/Log Transformed Androgens*

	Paternal Age	DHEA[a]	A[a]	T[a]	Cortisol[a]	DHEA/C[b]	A/C[b]	T/C[b]	DHEA/A	DHEA/T[b]	A/T[b]
Paternal Age	-	-0.058	-0.257	-0.165	-0.218	0.035	-0.162	-0.081	0.082	0.101	-0.047
Maternal Age	**0.666****	0.013	-0.211	-0.122	-0.058	0.084	-0.242	-0.083	0.258	0.165	0.122
Age Gap	**0.659****	-0.090	-0.132	-0.096	-0.232	-0.038	0.025	-0.025	-0.148	-0.033	0.058
Gestational Weeks	0.061	**-0.282***	-0.211	-0.210	-0.047	-0.150	-0.145	-0.133	-0.221	-0.108	0.037
Maternal Education	**0.437****	-0.117	-0.172	0.007	0.077	-0.105	**-0.329***	-0.107	-0.064	-0.067	-0.165
Paternal Education	**0.266***	0.039	-0.030	0.127	-0.073	0.123	-0.070	0.152	0.147	0.060	**-0.274***
Income	**0.374****	-0.136	-0.129	-0.001	-0.016	-0.081	-0.204	0.008	-0.138	-0.111	-0.206
Maternal Ethnicity 0: Other, 1: White	-0.170	-0.045	-0.180	-0.142	-0.046	0.057	-0.102	0.020	0.126	0.061	-0.125
Paternal Ethnicity 0: Other, 1: White	-0.129	-0.088	-0.185	-0.147	-0.085	-0.086	-0.106	-0.123	-0.012	-0.031	0.065
BDI Mom	0.040	0.127	0.076	0.220	0.143	0.063	-0.114	0.076	0.044	0.032	-0.206
BAI Mom	-0.102	-0.011	-0.011	0.044	0.199	-0.008	-0.167	-0.076	-0.004	0.040	-0.049
BDI Dad	-0.093	-0.110	-0.132	-0.123	-0.063	-0.019	-0.068	-0.007	-0.004	-0.019	-0.058
BAI Dad	-0.078	0.185	0.180	0.135	0.222	0.102	-0.025	-0.018	0.080	0.139	0.011

Season 1:Spring, 2:Summer, 3:Fall, 4:Winter	-0.021	-0.036	0.081	0.053	-0.081	0.149	0.238	**0.267***	-0.111	0.018	-0.131
Sex 0: Males, 1: Females	-0.037	-0.017	-0.006	0.115	-0.222	0.056	0.222	**0.284***	-0.033	-0.115	-0.171
Prenatal Paternal Stress/ Depression/ Anxiety											
PSS	-0.104	-0.093	-0.003	-0.136	-0.172	-0.134	0.217	-0.102	-0.008	-0.106	**0.353****
CESD	-0.075	-0.105	-0.038	-0.134	-0.205	-0.055	0.171	-0.018	-0.043	-0.059	0.190
STR	0.106	-0.225	-0.238	**-0.362****	-0.228	-0.227	-0.080	**-0.344****	-0.033	-0.067	**0.410****
Paternal BMI	0.042	0.139	0.101	-0.009	0.100	0.174	0.131	0.111	0.093	0.151	-0.073
Preconception Paternal Alcohol Consumption	0.140	0.063	0.072	0.189	0.160	0.121	**-0.126**	0.033	-0.096	0.136	-0.151
Preconception Paternal Smoking											
Days/Week Smoked	**0.262***	-0.134	-0.255	**-0.268***	-0.194	-0.146	-0.236	**-0.301***	-0.017	0.009	0.190
# Cigarettes/ Day Smoked	0.180	-0.203	**-0.311***	**-0.319***	-0.111	**-0.271***	**-0.431****	**-0.444****	-0.102	-0.059	0.200
PACOTIS (3-month postpartum)	0.124	0.158	-0.084	-0.020	-0.100	0.156	-0.042	0.037	0.228	0.176	-0.097

***p <0.05, **p <0.01, [a]Square root transformed, [b]Log transformed**

Note. DHEA: Dehydroepiandrosterone; A: Androstenedione; T: Testosterone; C: Cortisol; BDI: Beck Depression Inventory; BAI: Beck Anxiety Inventory; PSS: Perceived Stress Scale; CESD: Centre for Epidemiological Studies Depression Scale; STR: Anxiety Inventory; BMI: Body Mass Index; PACOTIS: Parental Cognitions and Conduct Toward the Infant Scale.

Table A40. *Pearson Correlation Matrix Between Baseline Square Root/Log Transformed Androgens*

	DHEA[a]	A[a]	T[a]	Cortisol[a]	DHEA/C[b]	A/C[b]	T/C[b]	DHEA/A	DHEA/T[b]	A/T[b]
DHEA[a]	-									
A[a]	**0.779****	-								
T[a]	**0.777****	**0.846****	-							
Cortisol[a]	**0.377****	**0.616****	**0.547****	-						
DHEA/C[b]	**0.795****	**0.526****	**0.581****	0.073	-					
A/C[b]	**0.467****	**0.530****	**0.393****	-0.241	**0.594****	-				
T/C[b]	**0.511****	**0.438****	**0.643****	-0.111	**0.663****	**0.671****	-			
DHEA/A	**0.705****	0.204	**0.331***	-0.041	**0.711****	**0.269***	**0.415****	-		
DHEA/T[b]	**0.700****	**0.398****	**0.333****	0.170	**0.868****	**0.332***	0.204	**0.656****	-	
A/T[b]	-0.250	-0.093	**-0.499****	-0.078	**-0.341****	0.034	**-0.718****	**-0.307***	0.032	-

***p <0.05, **p <0.01, [a]Square root transformed, [b]Log transformed**

Note. DHEA: Dehydroepiandrosterone; A: Androstenedione; T: Testosterone; C: Cortisol**.**

Table A41. *Pearson Correlation Matrix Between WISC and SDQ*

	WISC Full Scale	WISC Verbal	WISC Fluid	SDQ Total Difficulties	SDQ Prosocial	SDQ Internal.	SDQ Emotion	SDQ Peer	SDQ External.	SDQ Conduct	SDQ Hyper/I
WISC Full Scale	-										
WISC Verbal	**0.719****	-									
WISC Fluid	**0.799****	**0.362****	-								
SDQ Total Difficulties	-0.240	-0.143	-0.160	-							
SDQ Prosocial	0.147	0.198	-0.021	**-0.478****	-						
SDQ Internalizing	0.119	0.140	0.140	**0.681****	**-0.407****	-					
SDQ Emotion	0.128	0.031	0.182	**0.597****	**-0.361****	**0.889***	-				
SDQ Peer	0.039	0.246	-0.007	**0.449****	**-0.260***	**0.638****	0.214	-			
SDQ Externalizing	**-0.376****	**-0.261***	**-0.284***	**0.915****	**-0.392****	**0.326***	**0.279***	0.227	-		
SDQ Conduct	**-0.314***	**-0.257***	-0.194	**0.810****	**-0.488****	**0.373****	**0.401****	0.121	**0.839****	-	
SDQ Hyper/I	**-0.354****	-0.221	**-0.295***	**0.832****	**-0.267***	0.244	0.157	**0.257***	**0.939****	**0.601****	-

***p <0.05, **p <0.01.**

Note. WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire; SDQ Hyper/I: SDQ Hyperactivity/Inattention subscale.

Table A42. *Pearson Correlation Matrix Between Baseline Square Root/Log Transformed Androgens and WISC/SDQ*

	DHEA[a]	A[a]	T[a]	Cortisol[a]	DHEA/C[b]	T/C[b]	A/C[b]	A/T[b]	DHEA/T[b]	DHEA/A
WISC Full Scale	0.100	0.125	0.241	0.169	-0.002	0.101	-0.120	-0.249	-0.070	-0.062
WISC Verbal	-0.058	-0.004	0.099	0.028	-0.126	0.001	-0.139	-0.136	-0.165	-0.180
WISC Fluid	0.047	0.014	0.112	0.129	-0.097	0.000	-0.223	-0.208	-0.126	-0.036
SDQ Total Difficulties	-0.069	-0.158	-0.056	0.056	-0.055	-0.064	-0.190	-0.094	-0.029	0.072
SDQ Prosocial	0.056	0.091	0.039	-0.038	0.068	0.046	0.138	0.076	0.059	0.073
SDQ Internalizing	-0.248	**-0.282***	-0.161	0.043	-0.252	-0.195	**-0.351****	-0.066	-0.200	-0.164
SDQ Emotion	-0.219	-0.208	-0.145	0.087	**-0.293***	-0.208	**-0.328***	-0.019	-0.245	-0.220
SDQ Peer	-0.161	-0.254	-0.099	-0.056	-0.045	-0.065	-0.200	-0.110	-0.015	0.020
SDQ Externalizing	0.049	-0.047	0.017	0.049	0.069	0.025	-0.050	-0.085	0.073	0.183
SDQ Conduct	-0.009	-0.040	0.007	0.168	-0.035	-0.035	-0.165	-0.110	-0.023	0.045
SDQ Hyper/I	0.077	-0.044	0.021	-0.034	0.123	0.058	0.031	-0.055	0.122	0.242

***p <0.05, **p <0.01, [a]Square root transformed, [b]Log transformed**

Note. DHEA: Dehydroepiandrosterone; A: Androstenedione; T: Testosterone; C: Cortisol; WISC: Weschler Intelligence Scale for Children; SDQ: Strengths and Difficulties Questionnaire; SDQ Hyper/I: SDQ Hyperactivity/Inattention subscale.

Figure A1. *Diagram of the Conceptual Model of the Moderated Moderation With Child's Sex as a Secondary Moderator*

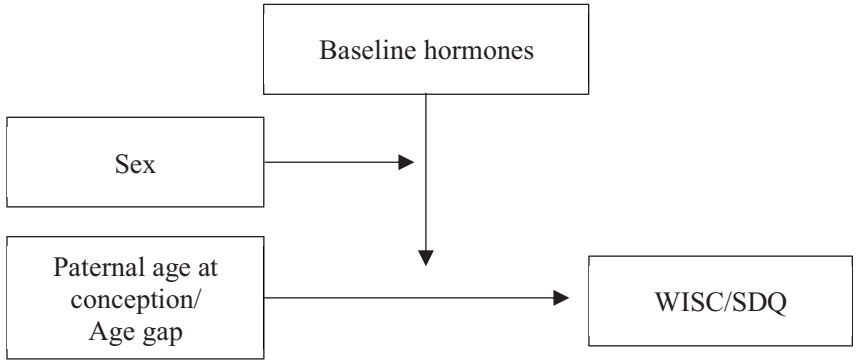


Figure A2. *Scatter Plots of the Main Predictors*

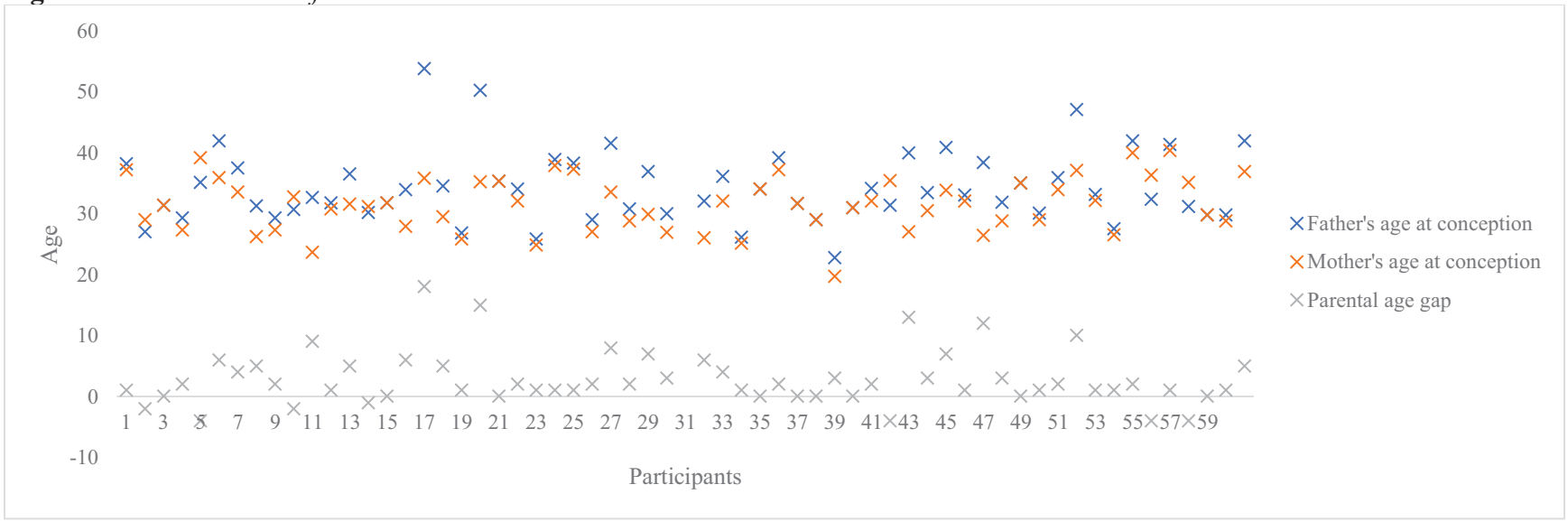
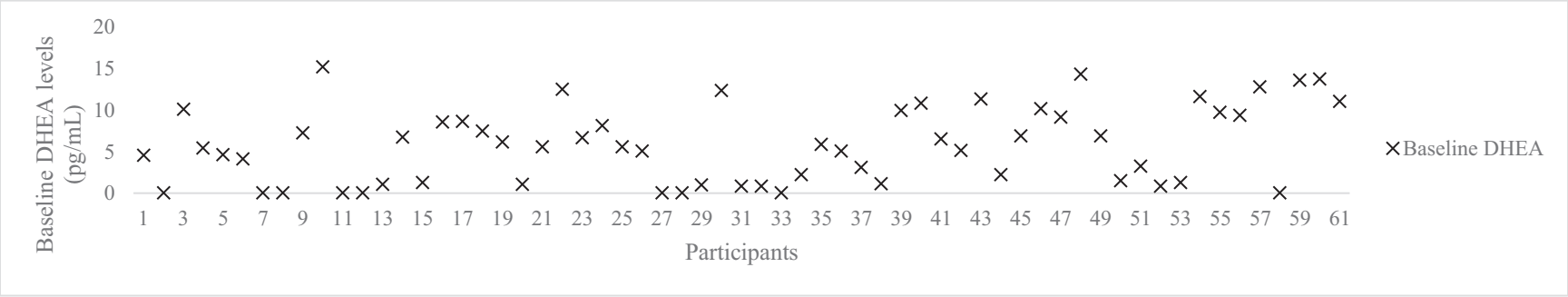


Figure A3. *Scatter Plots of Baseline DHEA (Imputed, Square Root Transformed)*



Note. DHEA: Dehydroepiandrosterone.

Figure A4. *Scatter Plots of Baseline Androstenedione (Imputed, Square Root Transformed)*

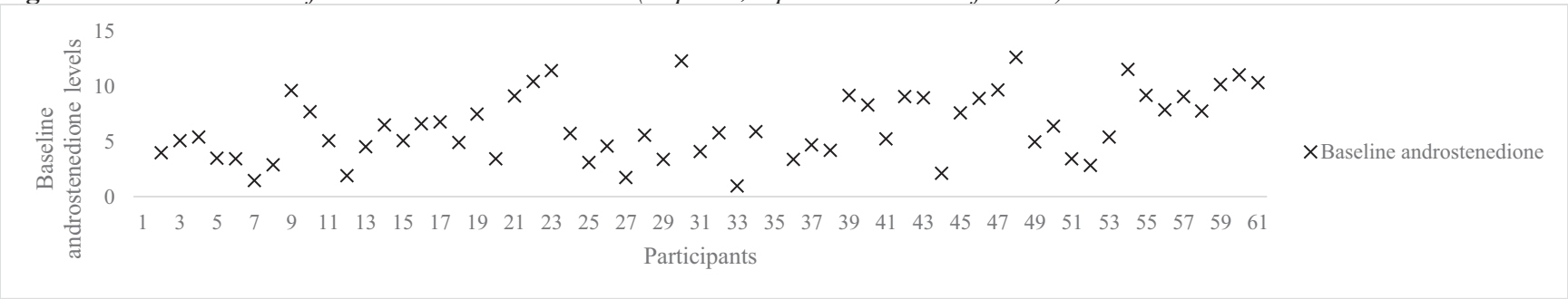


Figure A5. *Scatter Plots of Baseline Testosterone (Imputed, Square Root Transformed)*

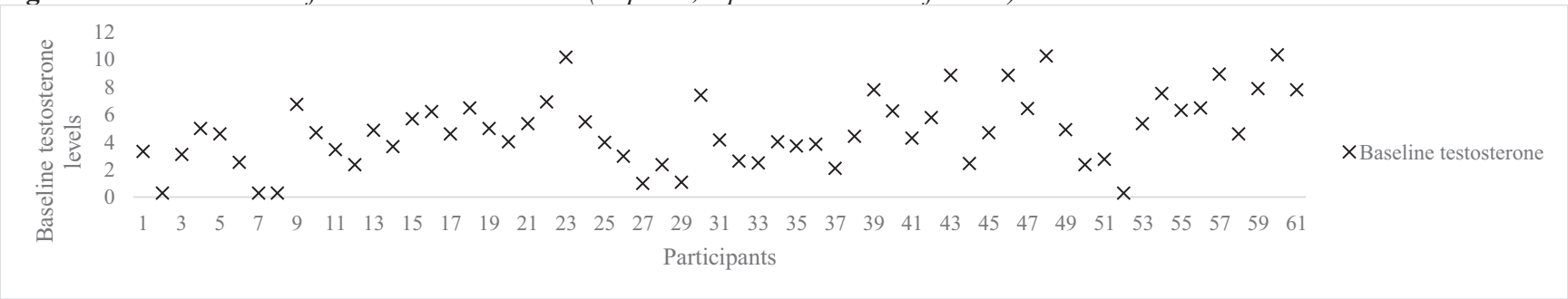


Figure A6. *Scatter Plots of Baseline Cortisol (Imputed, Square Root Transformed)*

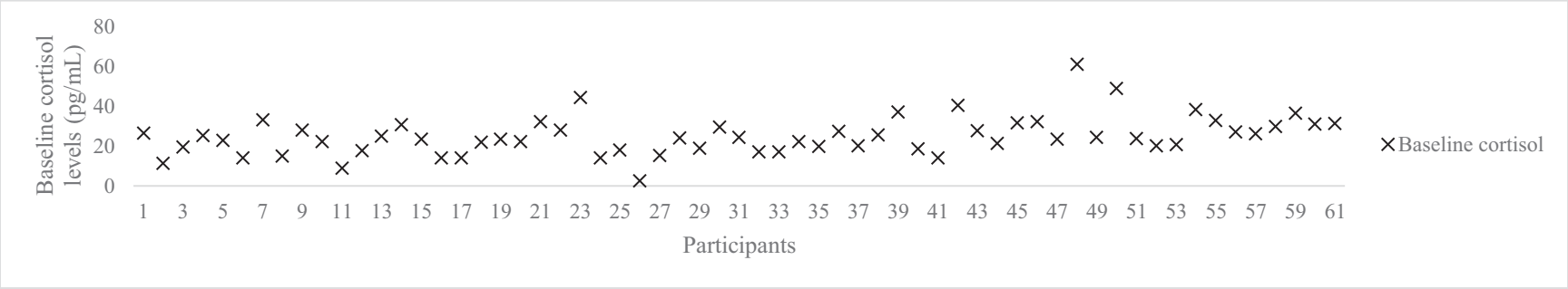
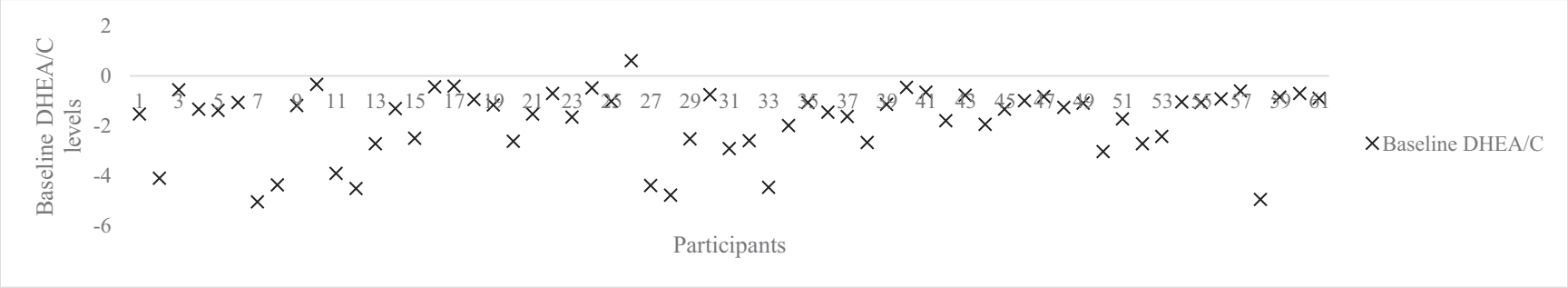
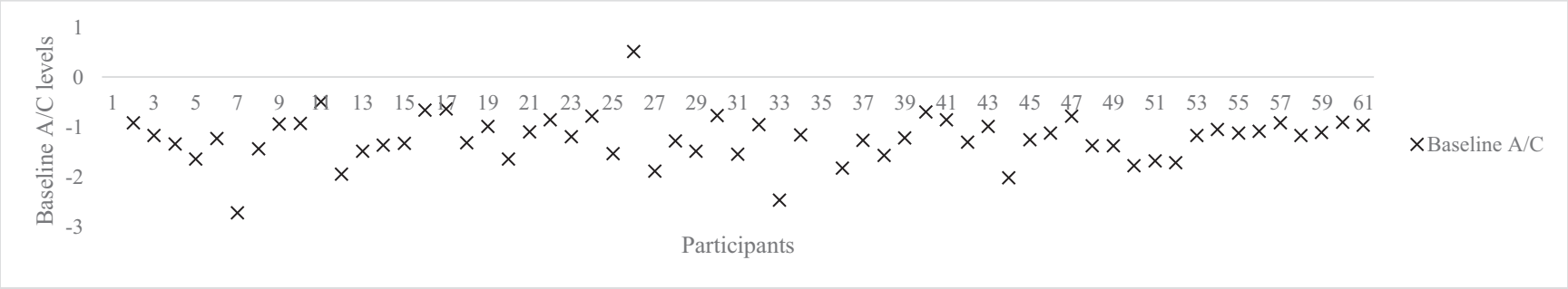


Figure A7. *Scatter Plots of Baseline DHEA/Cortisol (Imputed, Log Transformed)*



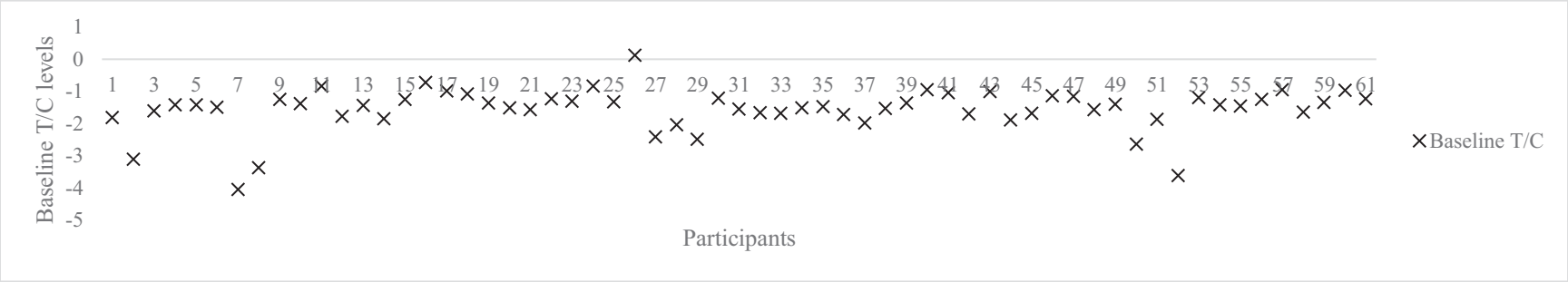
Note. DHEA/C: Dehydroepiandrosterone/cortisol.

Figure A8. *Scatter Plots of Baseline Androstenedione/Cortisol (Imputed, Log Transformed)*



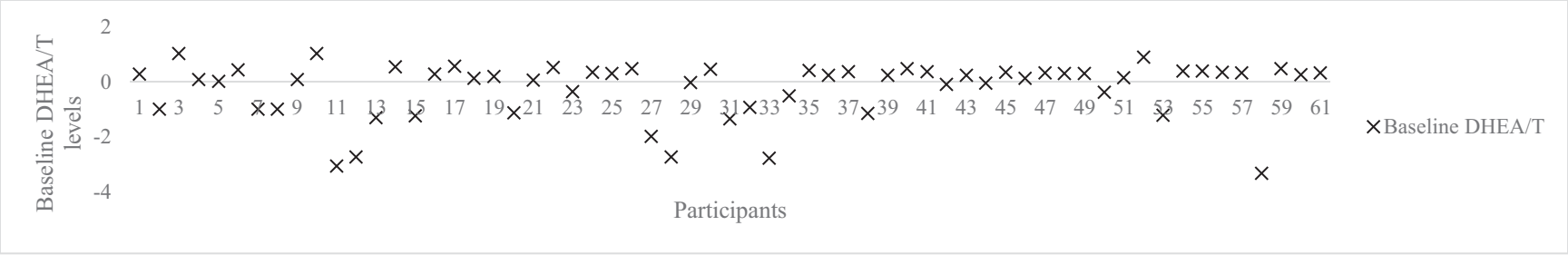
Note. A/C: Androstenedione/cortisol.

Figure A9. *Scatter Plots of Baseline Testosterone/Cortisol (Imputed, Log Transformed)*



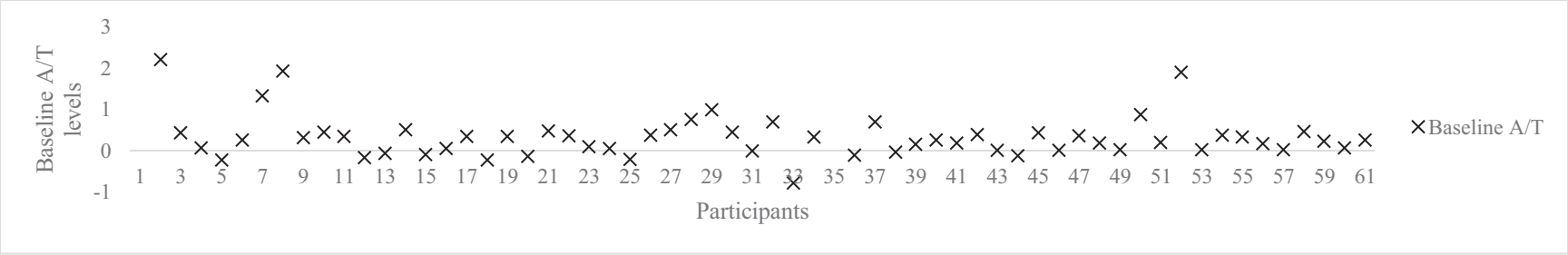
Note. T/C: Testosterone/cortisol.

Figure A10. *Scatter Plots of Baseline DHEA/Testosterone (Imputed, Log Transformed)*



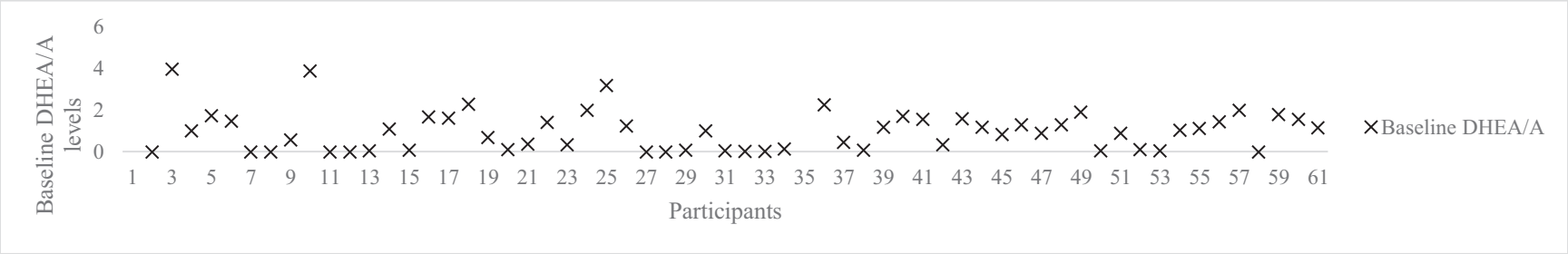
Note. DHEA/T: Dehydroepiandrosterone/testosterone.

Figure A11. *Scatter Plots of Baseline Androstenedione/Testosterone (Imputed, Log Transformed)*



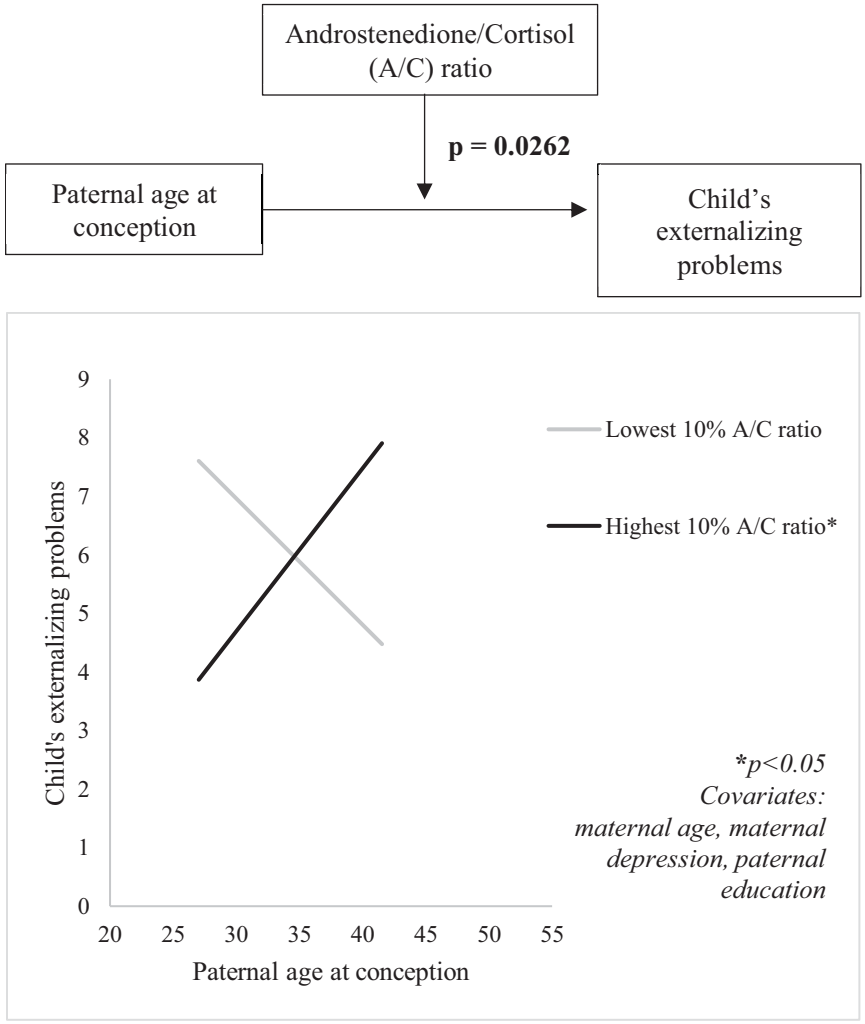
Note. A/T: Androstenedione/testosterone.

Figure A12. *Scatter Plots of Baseline DHEA/Androstenedione (Imputed)*



Note. DHEA/A: Dehydroepiandrosterone/androstenedione.

Figure A13. *Moderation Model With Paternal Age as the Predictor*



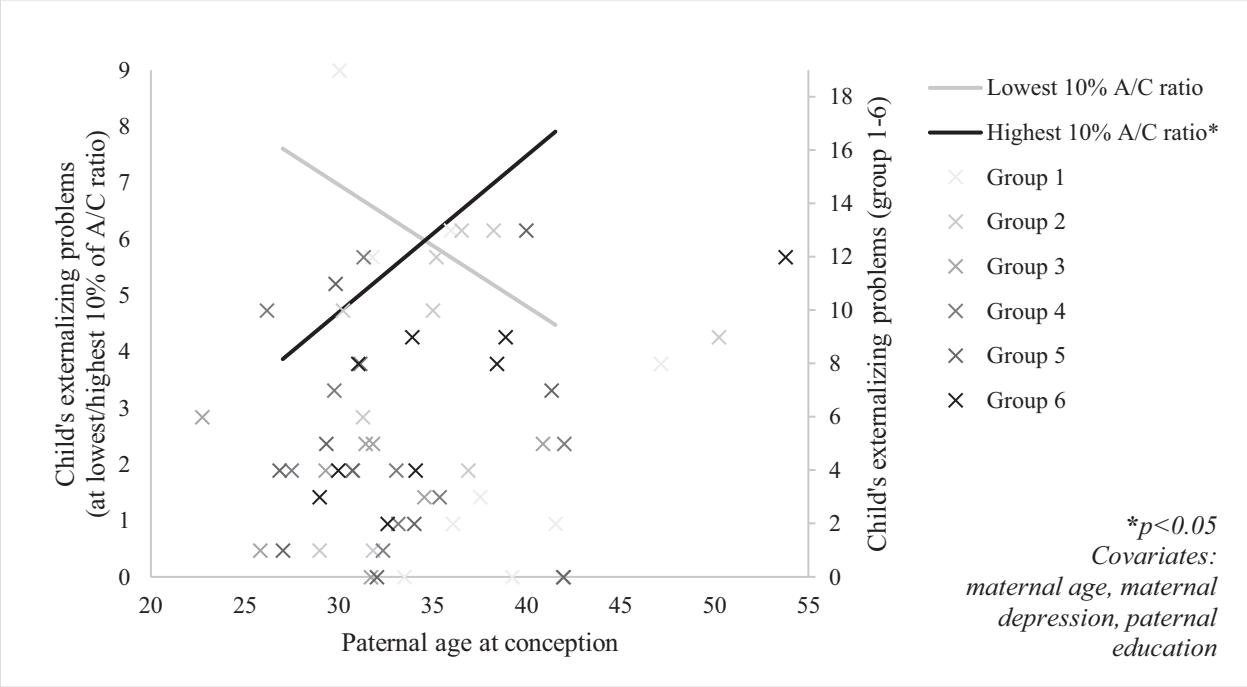
Baseline androstenedione/cortisol (A/C) moderated the relationship between paternal age at conception and child's externalizing problems as measured by Strength and Difficulties Questionnaire at 6-8 years of age (B= 0.4425, SE= 0.1932, p= 0.0262).

The association between paternal age and child's externalizing problems at different levels of baseline androstenedione/cortisol ratio is shown in the figure. The regression lines represent the top and bottom 10th percentiles of A/C as an example of the conditional effect of paternal age on externalizing problems.

At higher A/C ratio (highest 10%; black line), paternal age is positively associated with the offspring's externalizing problems such that older paternal age is associated with higher externalizing problems in the child (conditional effect of paternal age on externalizing problems at highest 10% A/C ratio: B= 0.2783, SE= 0.1365, p< 0.05). Of note, no significant association was found between A/C and scores on child's externalizing problems across father's age (i.e., between 10th and 90th percentiles).

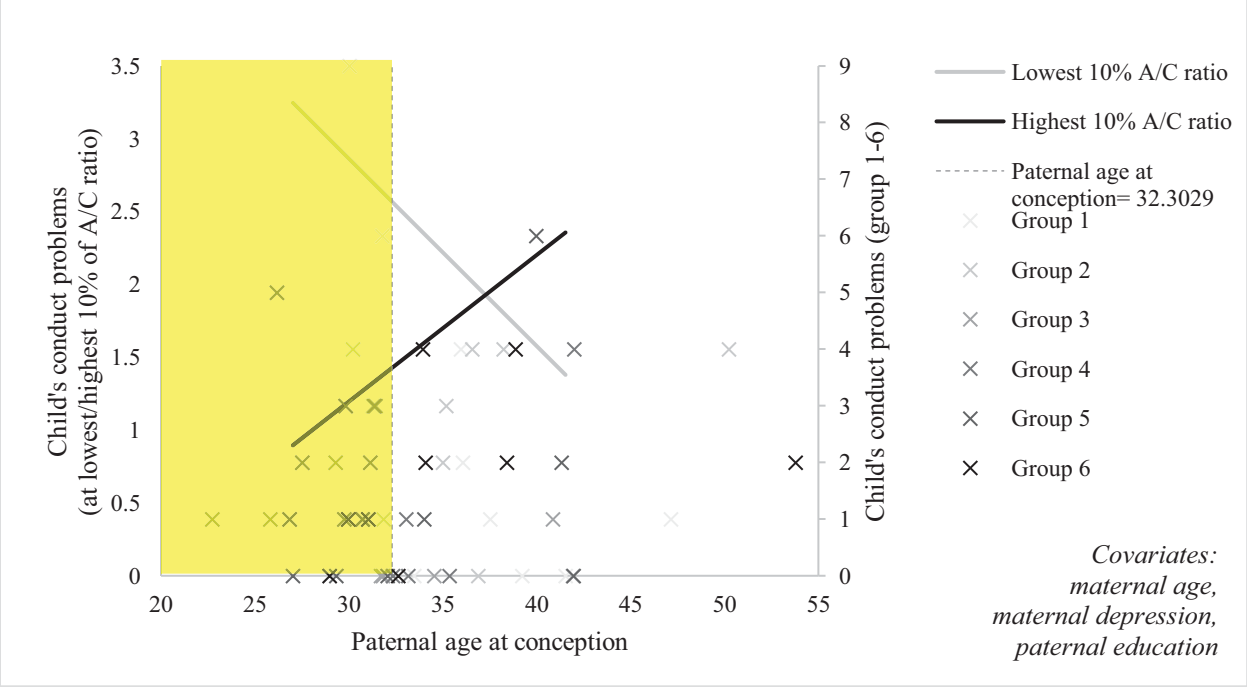
Comparison of this figure and the figure 3 in the proposal denoted that the effects of externalizing problems were primarily driven by its subscale, conduct problems. As described in section 4.2.3, A/C ratio has been log transformed.

Figure A14. *Copy of Figure A13 With Individual Data Points Added for Visualization*



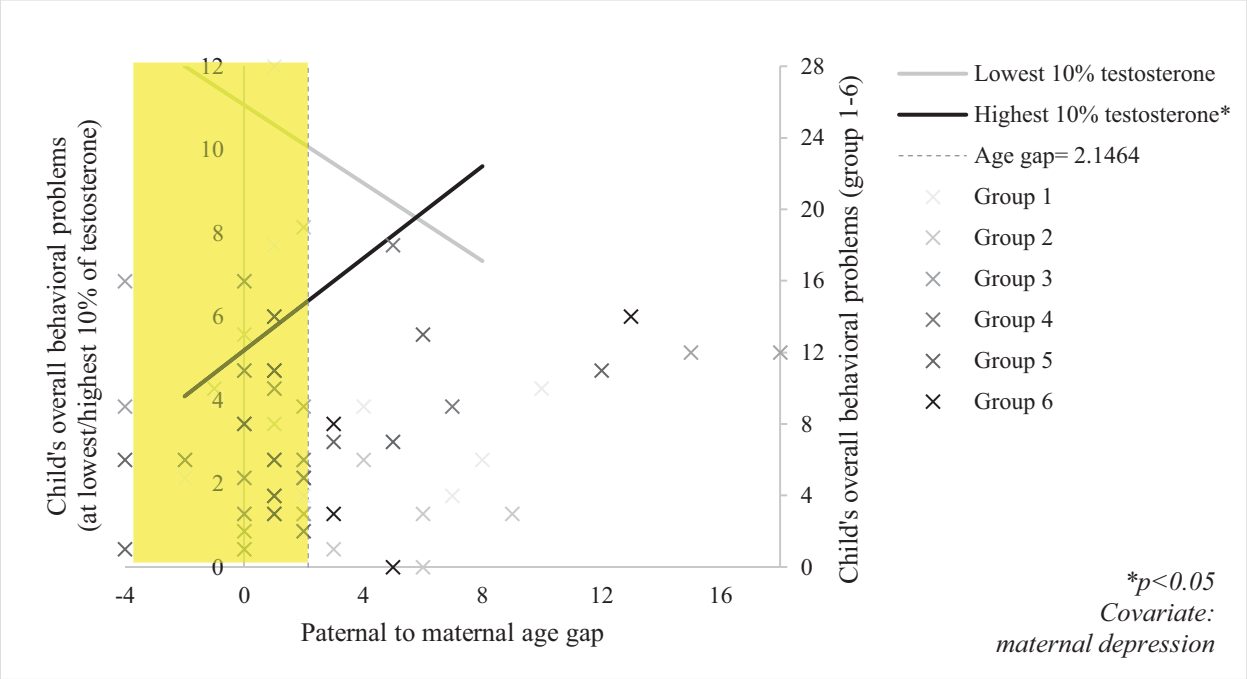
Group 1-6 each consists of 9-10 participants, sorted by ascending levels of androstenedione/ cortisol (A/C). For each participant, their respective data point is presented.

Figure A15. *Copy of Figure 2 With Individual Data Points Added for Visualization*



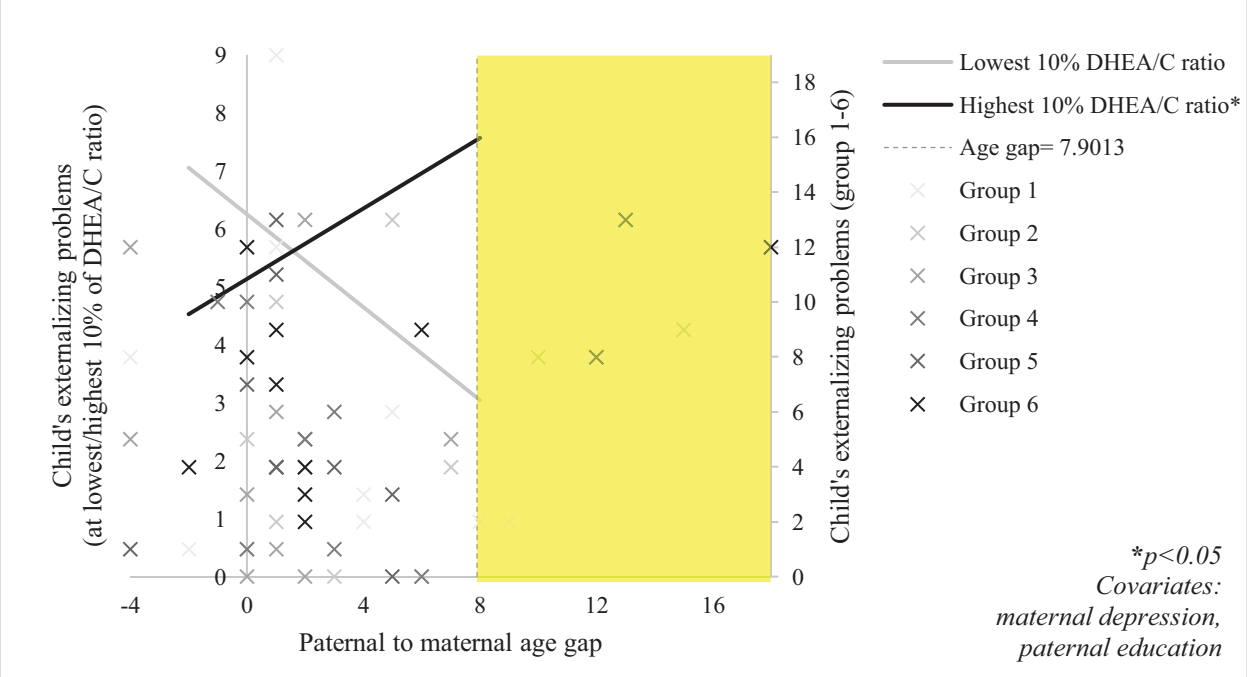
Group 1-6 each consists of 9-10 participants, sorted by ascending levels of androstenedione/ cortisol (A/C). For each participant, their respective data point is presented. As per Figure 2, the region of significance is depicted in yellow.

Figure A16. *Copy of Figure 3 With Individual Data Points Added for Visualization*



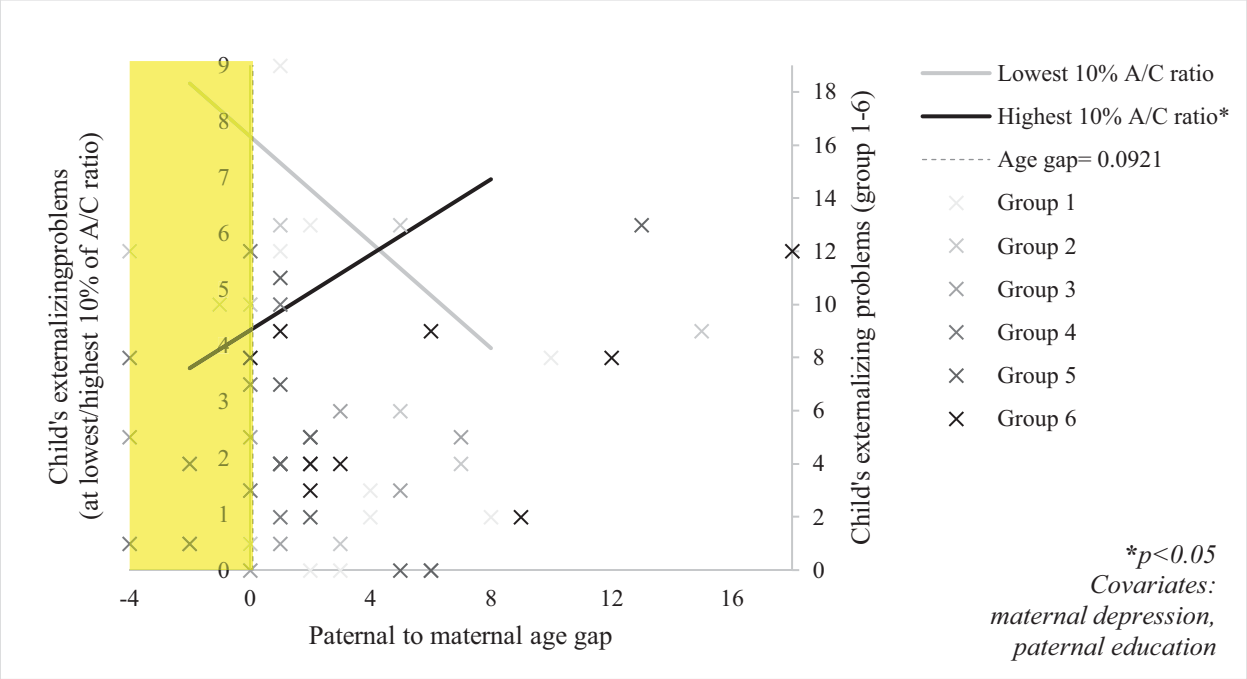
Group 1-6 each consists of 10 participants, sorted by ascending levels of testosterone. For each participant, their respective data point is presented. As per Figure 3, the region of significance is depicted in yellow.

Figure A17. *Copy of Figure 4 With Individual Data Points Added for Visualization*



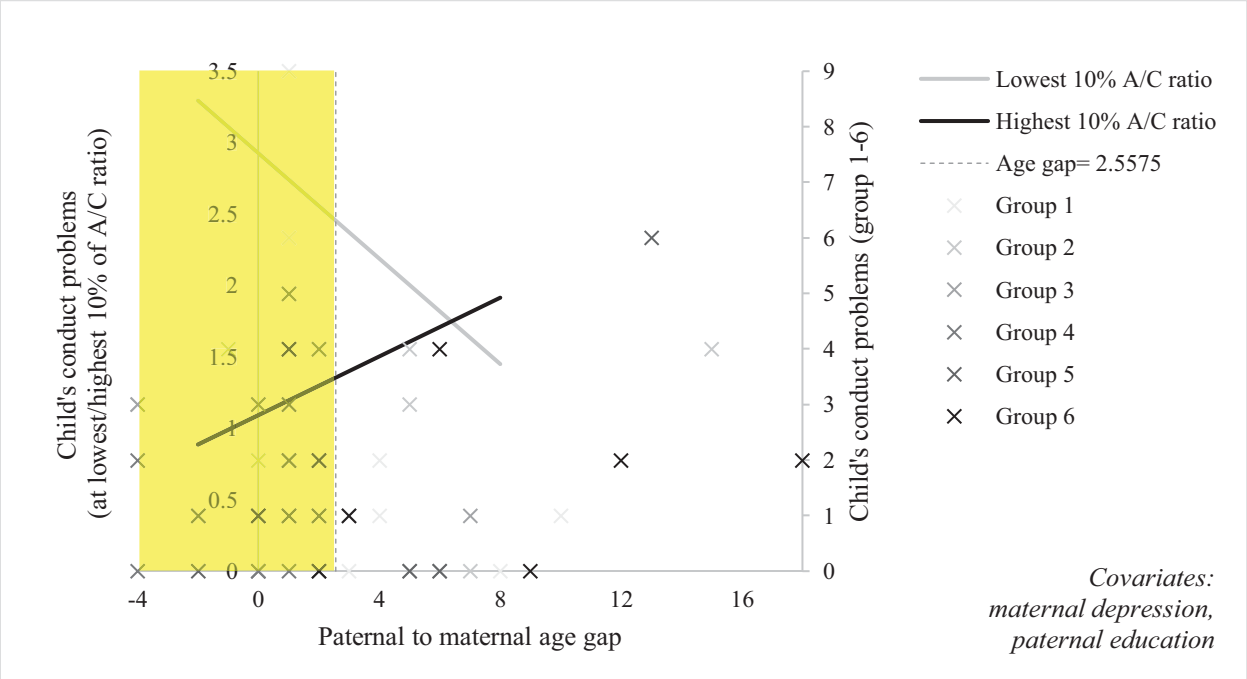
Group 1-6 each consists of 10 participants, sorted by ascending levels of dehydroepiandrosterone/cortisol (DHEA/C). For each participant, their respective data point is presented. As per Figure 4, the region of significance is depicted in yellow.

Figure A18. *Copy of Figure 5A With Individual Data Points Added for Visualization*



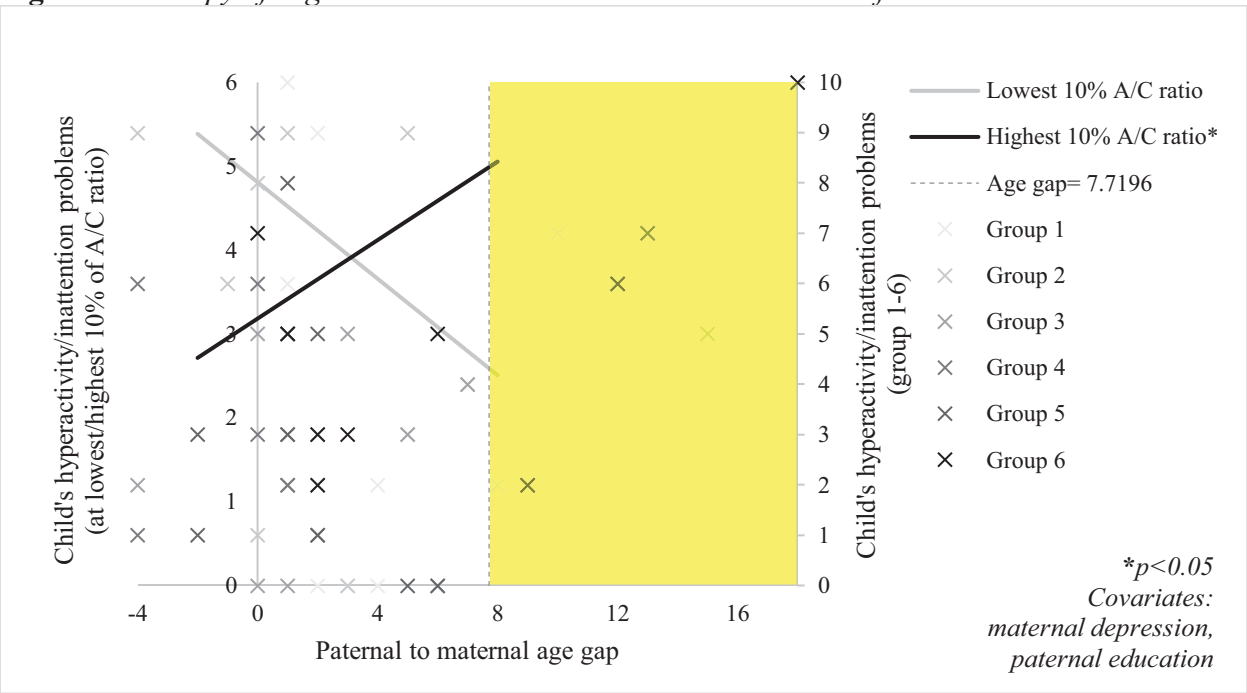
Group 1-6 each consists of 9-10 participants, sorted by ascending levels of Androstenedione/ Cortisol (A/C). For each participant, their respective data point is presented. As per Figure 5A, the region of significance is depicted in yellow.

Figure A19. *Copy of Figure 5B With Individual Data Points Added for Visualization*



Group 1-6 each consists of 9-10 participants, sorted by ascending levels of Androstenedione/ Cortisol (A/C). For each participant, their respective data point is presented. As per Figure 5B, the region of significance is depicted in yellow.

Figure A20. *Copy of Figure 5C With Individual Data Points Added for Visualization*



Group 1-6 each consists of 9-10 participants, sorted by ascending levels of Androstenedione/ Cortisol (A/C). For each participant, their respective data point is presented. As per Figure 5C, the region of significance is depicted in yellow.