



# Minipuberty: Looking Back to Understand Moving Forward

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Hypothalamic-pituitary-gonadal (HPG) axis activation occurs three times in life: the first is during fetal life, and has a crucial role in sex determination, the second time is during the first postnatal months of life, and the third is with the onset of puberty. These windows of activation recall the three windows of the “Developmental Origin of Health and Disease” (DOHaD) paradigm and may play a substantial role in several aspects of human development, such as growth, behavior, and neurodevelopment. From the second trimester of pregnancy there is a peak in gonadotropin levels, followed by a decrease toward term and complete suppression at birth. This is due to the negative feedback of placental estrogens. Studies have shown that in this prenatal HPG axis activation, gonadotropin levels display a sex-related pattern which plays a crucial role in sex differentiation of internal and external genitalia. Soon after birth, there is a new increase in LH, FSH, and sex hormone concentrations, both in males and females, due to HPG re-activation. This postnatal activation is known as “minipuberty.” The HPG axis activity in infancy demonstrates a pulsatile pattern with hormone levels similar to those of true puberty. We review the studies on the changes of these hormones in infancy and their influence on several aspects of future development, from linear growth to fertility and neurobehavior.

**Keywords:** minipuberty, neurobehavior, neonate, gonadotrophins, hypothalamic-pituitary-gonadal axis, hypogonadism

## INTRODUCTION

During embryogenesis, the pituitary gland begins synthesizing both Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH) at around 9 weeks of gestation (1). LH and FSH can be detected in fetal blood from 12 to 14 weeks (2, 3) and start to be GnRH-dependent after 31–32 weeks (4, 5).

Prenatal modulation of the HPG axis activity is also due to placental hormone production. In fact, the structure of hCG is an analog of LH and may bind to the LH receptor, with similar biological effects on gonadal tissues (2, 6). Moreover, the placenta produces Estrogens (E) and Progesterone (P), that rise during the third trimester. This has a negative effect on gonadotropin levels and results in a drop in LH and FSH in cord blood at birth in healthy infants of both sexes (6–8).

After birth, the removal of placental hormones from the neonate’s circulation results in a lack of negative feedback on the GnRH pulse generator and reactivation of the HPG axis. This postnatal activation that starts in the first few days of life is known as “minipuberty” (9).

Studies on healthy term neonates indicate that the rise of LH and FSH begins at around 1 week of age. It achieves a peak, reaching the pubertal range, between 1 and 3 months of life and then declines toward the age of 6 months (6–8, 10–13). These postnatal hormonal changes have different trends in boys and girls. Particularly in males, it seems to be related to the development and maturation of the reproductive system. Furthermore, the impact of gonadotropins and sex-steroid hormones during this first period of life has been studied and relates to many different aspects of infant growth and behavior (14).

The aim of this review is to summarize the current understanding on minipuberty and its role as a temporary window of opportunity for diagnosis and possible treatment in babies with disorders of sex development (DSD). Moreover, we would like to highlight the extent of what happens (or not) during minipuberty in terms of hormonal changes and trends which may influence future neurobehavior.

## INFANTS BORN AT TERM

**Males:** In male neonates, both LH and FSH levels peak between 1 and 3 months of age and then gradually decrease to prepubertal levels at around 6–9 months (9, 12, 14). The LH peak is higher than the FSH level. Testosterone (T) starts to increase 1 week following the LH rise and declines to prepubertal values by 6 months of age (11, 12, 15). T levels, both in cord blood and in serum during the first postnatal months, are higher in boys than in girls (11–13, 15–17).

The number of Leydig cells in both testes increases considerably until the third month of life, which correlates with the T trend and then gradually decreases due to an apoptosis process (18, 19). Sertoli cells also grow during the first postnatal months under the stimulation of FSH (20) but, without the expression of androgen receptors (AR) during infancy, they do not complete their maturation and spermatogenesis does not occur (21). This leads to an increase of testicular volume during the first months after birth, which then gradually decreases until the second year of life due to the halt in cell proliferation, the reduction of AMH production, and the formation of the blood-testicular barrier (22, 23).

All these hormonal changes during the first months of life have a great impact on the urogenital system. This involves not only the testes but also the development and growth of the penis, prostate, and scrotal hair. In fact, the postnatal T surge within the first three months has been associated with penile growth in infancy (24). The increase in androgens has been associated with cutaneous manifestations, such as sebaceous gland hypertrophy and acne (25). There is also a link with the development of transient isolated scrotal hair between 3 and 6 months of life with a spontaneous disappearance within the first year of life (26).

**Females:** In female infants, FSH levels are higher than LH, following a different trend than in males. FSH shows the same gonadotropin peak as in males at 1–3 months of age but can remain elevated up to 3–4 years of life. In contrast, LH levels decrease at the same age as in boys (9, 12, 27).

E levels at birth are high, with similar values in the cord blood of both sexes (28), followed by a gradual decrease during the first days of life and a new increase after the first week only in girls.

E remains high until 6 months with fluctuating levels, probably related to the FSH trend, and decreases toward 2 years of life (29–31). The mammary glands and uterus are certainly E target tissues but evidence of minipuberty effects is not univocal. At birth, most full-term babies of both sexes have palpable breast tissue (32) that probably results from placental E effect. In the following months, breast tissue in females remains larger and persists longer due to HPG axis activity and its consequent E production (30). In contrast, uterine length increases *in utero* but, after birth, there is a steady decrease from day 7 toward the third month, after which the volume remains stable until the second year (30).

With little evidence from few studies, the biological role of minipuberty in girls is still controversial and partially unknown.

**Babies born small for gestational age (SGA):** The HPG axis activation in SGA infants born at term is not well defined and its short-term and long-term effects on growth and development are still controversial. Studies on SGA females found higher postnatal FSH levels compared with neonates born appropriate for gestational age (AGA). This different pattern of secretion in SGA females was also associated with reduced uterine and ovarian size that persisted into young adulthood (33, 34). Moreover, Anti Mullerian Hormone (AMH) levels have been reported to be higher in SGA girls at 2–3 months of life, suggesting possible altered follicular development (35).

In contrast with these findings, other studies have reported higher E in SGA females after the administration of a GnRH stimulation test, although the reported basal levels were not significantly different (35).

In male SGA term neonates, HPG axis activation has been linked both to lower (36) and higher (34) FSH and T (37) levels, with uncertain effects in adult life (38).

Further studies are necessary to clarify the pattern of minipuberty in SGA male and female infants, along with the clinical implications. It is important to bear in mind that SGA neonates are at increased risk of metabolic and endocrinological disorders. These include reduced insulin sensitivity and increased adrenal hyperandrogenism, with consequent precocious pubarche and reduced ovulation rate (39).

## PRETERM INFANTS

Little is known about the influence of prematurity on HPG axis activity and its effects. Fewer studies have investigated this pattern longitudinally in preterm (PT) babies compared with those born full-term (FT). Preterm birth does not seem to influence the postnatal HPG axis activation, as gonadotropin levels begin to rise after birth (whenever that is, as fetal-placental interruption) with the same timing as in FT infants.

Moreover, this hormonal surge might be even stronger and more prolonged than in FT infants (40, 41). However, these data are not univocal (40–42) in either the amplitude or the duration between different sexes. Immaturity of the

hypothalamic feedback has been suggested as a possible mechanism for this strong and prolonged activation, although its biological significance is still not completely understood.

The most recent longitudinal data suggest that minipuberty declines at about the same post-term age in term neonates compared with premature infants, suggesting that the HPG activity is regulated in an evolutionarily way (13). In particular, Kuiri-Hanninen et al. (13) used spot urine samples in order to compare gonadotropin and testosterone levels in a small cohort of FT and PT male neonates with a gestational age (GA) between 24.7 and 36.6 weeks. From day 7 to 14 months of age they measured length, weight, penile length, and testicular volume. They simultaneously collected urine samples to detect urinary gonadotropins and testosterone levels until 6 months of age. Their findings revealed higher hormonal levels in PT babies with a positive association between testosterone levels and penile growth, as well as between FSH levels and testicular growth after birth until 5 months of age, when a subsequent decrease occurred. In addition, studies on PT female infants demonstrated higher gonadotropin levels than those in FT girls with a prolonged duration of the peak (43) but a sharp decrease around term age (30). In these PT girls, an amplified postnatal E surge was observed at around three months of corrected age and there was an association with increased growth of the mammary gland and uterine length. Possible clinical consequences of this intensive stimulation in premature females are evidenced by features of the ovarian hyperstimulation syndrome with edema of the vulva, solitary or multiple cysts in the ovaries on ultrasonography, breast growth, and occasional vaginal bleeding (44, 45).

The hormonal differences between boys and girls during minipuberty appear to be fundamental for later sexual differentiation and development. In particular, we think that increasing knowledge on minipuberty in girls may give us key information about the premature thelarche of girls below 2 years of age, and the early puberty that occurs before 8 years of age. In SGA neonates, results are still controversial and more studies are needed to clarify how gonadotrophins and sexual hormones change according to sex. This may be very useful, considering that SGA-born children may go through early puberty and/or precocious isolated pubarche. In preterm babies, we speculated that the prolonged activation of HPG axis may be one of the factors influencing the early re-activation of the HPG axis before puberty age.

## BABIES WITH DISORDERS OF SEX DEVELOPMENT

The development of internal and external genitalia is a complex balance between gene expression and hormonal influence and an anomaly at each stage can result in 46, XY DSD.

From this point of view, minipuberty can be considered as a window of sensitivity, because it may allow the clinician to come to an early diagnosis and possible treatment opportunity.

Studies on primates testing the effects of a reversible suppression of minipuberty using GnRH agonists or antagonists described lower testis volume and penile length in cases treated

compared with controls (46–48). Male infants with congenital central hypogonadism (CHH) were found to have an absence of both fetal and postnatal FSH, LH, and T surges (49). This lack of postnatal FSH secretion seems to be the main reason for impaired germ cell differentiation with later infertility, especially if associated with cryptorchidism (50, 51). As a result, minipuberty may potentially provide a short window of time to make an early diagnosis and for treatment in male neonates that exhibit a micropenis with or without cryptorchidism (52, 53), improving the outcome of orchidopexy, and also reducing the long-term consequences of an absent minipuberty.

On the other hand, the finding of elevated gonadotropins during minipuberty in a 46, XY male neonate with undetectable testosterone levels may suggest congenital anorchism (vanishing testis or testicular regression syndrome). Infants with complete androgen insensitivity syndrome may present with lower-than-normal postnatal LH and T levels, whereas these hormones may be normal or high in cases of partial androgen insensitivity syndrome (54).

We have a unique opportunity to evaluate the spontaneous function of the HPG hormone axis during minipuberty. It is therefore recommended that serum FSH, LH, and testosterone are measured during the first 6 months of life in infants with DSD or suspected CHH. In particular, we would suggest checking for these hormones at seven days of life, and one, three, and, if possible, 6 months of life, to detect the minipuberty trend. The use of the LH/FSH ratio may provide important information in the workup of infants suspected of DSD, especially regarding the sex specific ratio detected in literature (55).

## MINIPUBERTY: A WINDOW FOR TREATMENT?

Neonates affected by cryptorchidism, micropenis, and CHH must receive timely treatment to optimize genital development. The current recommendation for a micropenis is brief therapy with low-dose testosterone delivered by intramuscular injection or by topical application to induce penile growth. This treatment was also assumed to work for cryptorchidism. However, while exogenous T stimulates penile growth, it does not affect testicular development. In fact, current recommendations advocate surgical correction for undescended testes during the first year of life (56). Nevertheless, there are limitations to this treatment. Small testes augment the risk of testicular trauma and tissue loss during orchidopexy (57), increasing the likelihood of a negative impact on future fertility. Moreover, successful scrotal repositioning of testes does not prevent infertility. However, a normal minipuberty after successful surgery may lead to the presence of Ad spermatogonia (58). The role of Ad spermatogonia is to maintain the supply of stem cells for spermatogenesis. In 178 testicular biopsies after orchidopexy the authors found three groups of high, intermediate, and low risk of infertility depending on the presence of Ad spermatogonia. After puberty, sperm concentrations were analyzed and correlated positively with plasma gonadotropin and testosterone levels. For all these reasons, recreating the hormonal milieu of

minipuberty with gonadotrophin treatment could be beneficial for these patients.

In 2002, Main et al. (59) published the first case of CHH and micropenis treated with short-term recombinant human LH and FSH. The outcome of this case was successful; the penile length increased by 50% and the testicular volume almost tripled. Similar results were described in other recent cases (60, 61).

The REMAP study (62) investigated the use of recombinant LH plus FSH preparations in neonates and infants with a micropenis and/or cryptorchidism due to hypogonadotropic hypogonadism. During therapy, all ten patients increased their height velocity: LH levels increased from undetectable to high-normal; FSH reached supranormal levels; and Inhibin-b, AMH, and T reached normal levels. Penile length normalized among all children and intriguingly confirms the emerging evidence that testicular descent is induced by gonadotrophin treatment (61, 63). Furthermore, in this study the therapy may have induced high/normal activation of Sertoli and Leydig cells, restoring testicular endocrine function and improving future fertility.

Vincel et al. (64) analyzed testicular biopsies before and after orchidopexy or hormonal treatment in patients with isolated bilateral cryptorchidism with a high infertility risk. Their results showed how the number of Ad spermatogonia and the number of germ cells per at least 100 tubular cross-sections increased or decreased post-surgery. Indeed, patients who received hormonal treatment showed an important increase in the number of cells and the complete transition of gonocyte and fetal spermatogonia to Ad spermatogonia. These findings support the hypothesis that GnRH induces LH release; LH increases testosterone levels acting directly on Leydig cells, mimicking minipuberty (50, 65).

Finally, studies have focused on the molecular mechanisms that explain the ability of GnRH to rescue fertility. The analysis demonstrates that several lncRNAs involved in epigenetic programming were responsive to GnRH treatment, helping in the preparation of Ad spermatogonial stem cells for commitment to differentiation. In particular, the authors found that DMRTC2, PAX7, BRACHYURY/T, and TERT were associated with defective minipuberty and were responsive to GnRHa (66). Minipuberty may represent a “window of opportunity” to evaluate the HPG axis by measuring basal hormone concentrations with no need for stimulation tests in infants with suspected reproductive disorders. Minipuberty provides a unique opportunity to evaluate the spontaneous function of the HPG axis which is lost thereafter for approximately another 10 years until the HPG axis is reactivated in puberty (67).

## IS OUR ENVIRONMENT INFLUENCING MINIPUBERTY IN HUMANS AND PREDISPOSING THEM TO DSD?

Endocrine Disruptor Chemicals (EDC) are compounds detectable in every setting of daily life. These chemical compounds are found in a range of products such as those

containing pesticides, metals, additives or food contaminants, and personal care products (68). In fact, EDCs are so common that it is almost impossible for individuals to avoid them during everyday activities. These substances may cause adverse health effects, disrupting endocrine function. In particular, they interfere with the endocrine and reproductive systems through nuclear receptors, non-nuclear steroid hormone receptors, non-steroid receptors, orphan receptors, enzymatic pathways, and other mechanisms (69). Children may be exposed both directly and indirectly to EDCs, especially during the three main temporal windows of the DOHaD paradigm and during breastfeeding (70, 71). Concerning breastfed children, Ortega-Garcia et al. detected a linear positive correlation between anogenital distance (AGD) in male infants and the duration of breastfeeding (72). The results of this study, called MALAMA, suggested breastfeeding to be a protective factor against the reduction of the AGD of 2-year-old boys. The authors hypothesized this could be related to early exposure to EDCs through baby formula milk (72).

Moreover, EDCs may interfere with HPG activation (73), both during fetal life or immediately after birth, throughout minipuberty. EDCs during minipuberty in males could impair testicular descent (74). Focusing on some of the most analyzed compounds in this research area, several studies have demonstrated that Bisphenol A (BPA) has an anti-androgen function, decreasing testosterone levels, an event that impacts sex differentiation during fetal life and modifies the AGD length (75, 76). In particular, Sun et al. showed how maternal exposure to BPA was associated with shortened AGD in boys at 12 months of age, highlighting a gender specific effect (77). Another family of EDCs influencing minipuberty are phthalates. Maternal exposure to phthalates during pregnancy showed a reduced T level in males at minipuberty and, because of the antiandrogenic effect of these compounds, the testosterone-luteinizing hormone ratio (T/LH) is also lower in the same period (78). This is probably due to compensated Leydig cell function, requiring higher levels of LH to maintain the necessary level of T for embryo differentiation. It was demonstrated in animal models that phthalates can inhibit *Insl3* production and consequently modify the gubernaculum growth necessary for the testes' transabdominal descent (79, 80). However, the effects of these compounds on *Insl3* and T in the human testes were less attenuated than in rodents. **Table 1** summarizes the most recent studies on the effect of EDC on HPG axis during minipuberty in humans. The impact of EDCs is not limited to the postnatal period. Indeed, alongside this phase there are two more windows of development: fetal and puberty. In these phases, cells are promptly proliferating, and epigenetic changes are more likely to occur (81). All this may lead to additional effects in later stages of life including delayed or precocious puberty (82–85), small testes and high levels of follicle-stimulating hormone (FSH) (86, 87), polycystic ovary syndrome (88), and breast cancer (89).

Our environment plays a crucial role in developmental programming. The influence of EDCs on minipuberty may predispose an individual to undescended testis, AGD modifications, or reduction of T surge. We should always keep an eye on the appearance of the external genitalia in neonates



**TABLE 1** | Possible effects in humans of the main EDCs on minipuberty and long-term consequences (68–89).

EDCs	Possible mechanism of action	Effects on minipuberty	Future effects
BPA	Increased estrogen receptor Inhibition of apoptotic activity in breast tissue	Lower T level Shortened AGD in boys	Premature thelarche Breast neoplastic transformation Infertility
Phthalates	Reduced T synthesis Modified estrogen activity Antiandrogenic effect InsI3 inhibition	Lower T/LH ratio Undescended testis Hypospadias Shortened AGD	Early puberty Premature thelarche Delayed pubic hairs development Increased breast cells proliferation Less recruitment of primary follicles PCOS Spermatogenic failure and infertility
PBDEs/PBB	Modified estrogen activity Antiandrogenic effect	Undescended testis	Early pubic hair stage (boys) Early/late menarche in breastfed girls Early puberty Anticipated menarche
DDT/DDE	Modified estrogen expression Antiandrogenic effect	Undescended testis Hypospadias	Precocious puberty Anticipated menarche Later onset of puberty Increased risk of breast cancer Testicular cancer
PCBs	Augmented level of FSH and estradiol Antiandrogenic effect	Undescended testis Hypospadias	Anticipated menarche Delayed puberty Augmented adipose tissue in breast Semen alteration

and on the possible maternal exposure to phthalates and BPA through a specific interview.

## HOW MINIPUBERTY INFLUENCES LINEAR GROWTH DURING THE FIRST 6 MONTHS OF LIFE

During minipuberty, the transient HPG axis activation results in a sex steroid surge. Some studies have indicated a higher growth velocity and a faster increase in weight (and lean body mass) associated with somatic changes in boys when compared with girls during the first 6 months of life (90–93). Based on these results, studies have tested the hypothesis of an association with minipuberty, particularly with the peak of testosterone production. Kiviranta et al. (94) evaluated the precise timing and the magnitude of this sexual dimorphism in growth among a large cohort of full-term healthy boys and girls during the first years of life. In a smaller sample of healthy neonates, serial measurements of urinary and blood hormones were assessed. Results from this study demonstrated that linear growth was significantly faster in boys than in girls, especially when comparing the first three months of age. Interestingly, this observation occurred simultaneously with the peak of postnatal gonadal activation and the authors found a positive correlation between T levels and growth velocity in both sexes, elucidating a possible novel biological role of minipuberty as an engine of growth velocity during the first months of life. Differences in sex hormones during minipuberty between boys and girls are important for the sex differentiation in linear growth and body composition, with males having a higher

growth velocity and accumulating more lean mass compared to females.

## HOW MINIPUBERTY MODULATES NEUROBEHAVIORAL DEVELOPMENT

As for sexual development, the human brain is also shaped by a combination of genetic, epigenetic, environmental, and hormonal exposure. Sex steroid hormones are among one of the strongest biological factors influencing neural and behavioral development. Over the past two decades, there has been a growing interest in understanding how sex determination and sexual hormones may affect structural and functional brain development (95, 96). The cellular and molecular mechanisms induced by T (converted to estradiol in the brain) are multifaceted and include neurogenesis, cellular differentiation, axon guidance, synaptic pruning, apoptosis, and phagocytosis. Several studies on mammalian brains, including humans, have demonstrated that early androgen exposure has an influence on sex differences in juvenile behavior (54, 96, 97). Indeed, manipulating androgens prenatally in non-human primates alters brain regions and behaviors (98). Many studies have been performed in girls prenatally exposed to high levels of androgens because of congenital adrenal hyperplasia (CAH) where there is strong evidence of male typical play behavior, suggesting a similar hormonal influence on human brain development (98–104). This influence of androgen levels on the brain has been identified not only among affected girls but also in the general population. Fetal T measured from amniotic fluid positively correlates with male typical play in preschool girls and boys assessed with a standardized questionnaire (105). This prenatal

**TABLE 2 |** Main studies on minipuberty and neurobehavior.

References	Methods	Results	Future prospectives
Lamminmäki et al. (110)	<ul style="list-style-type: none"> <li>– <b>Urinary testosterone</b> at 7 days of age (D7), and months 1, 2, 3, 4, 5 and 6 (M1–M6)</li> <li>– The <b>PSAI</b> is a 24-item, standardized questionnaire designed to discriminate gender related behavior within the sexes, as well as between girls and boys (111). It has been validated in the age-group 2 to 7 years. The questions covers three aspects of behavior: <b>play with sex-typed toys</b> (e.g., dolls, cars), <b>engagement in sex-typed activities</b> (e.g., ballgames, playing at cooking/cleaning) and <b>sex-typed child characteristics</b> (e.g., interest in snakes/spiders/insects, liking pretty things).</li> <li>– <b>The toy preference test:</b> the child was seated in the middle of a semi-circle formed by 9 toys. These toys were selected to be <b>female-preferred</b> (a tea set, a soft doll, and a baby doll and bathtub), <b>male-preferred</b> (a truck, a train, and a parking structure with two motorcycles), or <b>gender neutral</b> (a teddy bear, a soft picture book, and a set of keys). Two toys from the same category were never adjacent to each other. The session of 10 min was videotaped and the score consisted on how long (in seconds) that the child played with each toy, with play defined as the child touching the toy for 1 s or longer.</li> </ul>	<ul style="list-style-type: none"> <li>– In boys, <b>urinary testosterone</b> concentrations peaked at 1 month postnatal and decreased to low levels by the age 6 months.</li> <li>In girls, urinary testosterone concentrations were slightly elevated at D7 and M1, and then decreased to low levels.</li> <li>In the overall population, urinary testosterone was significantly higher in boys than in girls.</li> <li>– In boys, but not in girls, testosterone AUC correlated positively with <b>PSAI</b> scores.</li> <li>– Both boys and girls played significantly more with the same sex-related toy.</li> <li>– Testosterone in boys was negatively related to the female-preferred toy playing, but not in girls. Testosterone in girls was positively correlated with the male-preferred toy playing. A significant negative association between testosterone and time spent playing with the truck and a significant positive association between testosterone and time spent playing with the soft book among boys was detected.</li> </ul>	<p>The study underlined how testosterone may exert organizational effects on neurobehavioral development during early infancy both in girls and in boys.</p> <p>The urinary sampling method could be easier to be used in neonates and infants.</p>
Constantinescu et al. (116)	<p>61 healthy infants (29 males, 32 females) and 59 mothers and 3 fathers.</p> <p><b>Saliva samples of testosterone</b> when infants were 1–2.5 months of age, and <b>mental rotation</b> performance was assessed at 5–6 months of age.</p> <p>Mental rotation ability was assessed using the procedure developed by Constantinescu et al. (116).</p> <p>The stimuli were video representations of dynamic 3D objects, depicted in rotational movement around their vertical axis in 3D space.</p>	<p>Testosterone concentrations were significantly higher in boys than in girls at age 1–2.5 months.</p> <p>In contrast, at age 5–6 months, testosterone concentrations were significantly lower in both sexes than they were at the first visit.</p> <ul style="list-style-type: none"> <li>– male infants spent a significantly longer time looking at the novel stimulus than at the familiar one, and 65% of the male infants preferred the novel stimulus.</li> <li>– In contrast, female infants looked at the familiar and novel test stimuli about equally, and 46% of the female infants preferred the novel stimulus. These findings suggest that more male than female infants had developed an ability for mental rotation at 5–6 months of age.</li> <li>– The male infants' novelty preference was significantly greater than that of the female infants</li> <li>– Testosterone concentrations at 1–2.5 months of age correlated significantly with novelty preference scores on the 3D mental rotation task in 5- to 6-month-old boys but not in girls.</li> </ul>	<ul style="list-style-type: none"> <li>– Testosterone may have organizational influences on mental rotation performance</li> <li>– In girls, mental rotation performance at age 5–6 months correlated negatively with parents' traditional attitudes on gender. This finding suggests that parents could influence their daughters. "mental rotation abilities" beginning very early in life.</li> </ul>

(Continued)

TABLE 2 | Continued

References	Methods	Results	Future prospectives
Kung et al. (114)	<ul style="list-style-type: none"> <li>-Saliva samples for testosterone between 1 and 3 months old.</li> <li>-Between 18 and 30 months, all of the parent participants were invited to complete an online questionnaire assessing the children's expressive vocabulary size</li> <li>-The toddler short form for vocabulary production from the <b>MacArthur Communicative Development Inventory [CDI; (117)]</b> is a parent-report measure designed to assess expressive vocabulary production in toddlers aged 16–30 months</li> </ul>	<ul style="list-style-type: none"> <li>-boys had significantly higher concentrations of testosterone during mini-puberty and significantly lower CDI scores at age 18–30 months than girls</li> <li>- there was a significant negative correlation between concentrations of testosterone during mini-puberty and later CDI scores in boys</li> <li>- Differences were found between boys and girls in salivary testosterone at 1–3 months of age and in expressive vocabulary size at 18–30 months of age. A negative link between testosterone during mini-puberty and expressive vocabulary was found in boys, in girls, and in the entire sample. Results also showed that testosterone accounted for significant additional variance in expressive vocabulary, when other predictors, such as child's age at vocabulary assessment and paternal education were controlled, suggesting that the effects of testosterone are independent from those of other predictors</li> <li>- higher concentrations of salivary testosterone during the peak of mini-puberty at age 1–3 months predicted smaller expressive vocabulary at age 18–30 months in boys and in girls.</li> </ul>	Similar future research might usefully assess the independent contributions of prenatal and postnatal androgen exposure to expressive vocabulary and to other aspects of development that also differ by sex.
Kung et al. (118)	Testosterone in saliva samples collected from children at 1 to 3 months of age (40 boys, 47 girls). When the children reached 18 to 30 months of age, parents completed the <b>Quantitative Checklist for Autism in Toddlers (Q-CHAT)</b> .	Boys had higher concentrations of testosterone postnatally and higher Q-CHAT scores than girls. However, testosterone did not correlate with Q-CHAT scores in boys, girls, or the entire sample. There is no relationship between testosterone exposure during mini-puberty and autistic traits.	This does not preclude effects of mini-puberty on other behaviors (see the gender-typed play behavior). Other studies have hypothesized a correlation between prenatal exposure to testosterone and autistic traits.
Tanja Kuiri-Hanninen et al. (13)	Urinary gonadotropins and testosterone were measured in serial urine samples and compared with testicular and penile growth in preterm (PT) and full term (FT) neonates. Urinary prostate-specific antigen was measured as an androgen biomarker.	LH and testosterone levels were higher in PT boys than FT boys. Compared with FT boys, FSH levels were lower at day 7 but higher from month 1 to month 3 in PT boys. This was associated with significantly faster testicular and penile growth in PT boys compared with FT boys.	Postnatal HPG axis activation in infancy is increased in PT boys and associated with faster testicular and penile growth compared with FT boys. As mentioned in <b>Table 1</b> , there is a possible long-term consequence of hyperandrogenism in PT infant boys warrant further research.
Pasterski et al. (113)	<ul style="list-style-type: none"> <li>- Penile length, Ano-genital-distance (AGD), and body length were among the growth parameters assessed as part of the larger baby growth study. Measurements were taken at birth, and at 3, 12, 18, and 24 months of age in typically developing infants.</li> <li>- Gender-related behavior was measured at 3 to 4 years of age using the Preschool Activities Inventory (PSAI).</li> </ul>	AGD at birth and penile growth during the first three months postnatal independently predicted increased masculine/decreased feminine behavior in boys at 3 to 4 years of age. AGD at birth may be employed as a biomarker of prenatal androgen exposure, while penile growth during mini-puberty may reliably reflect variance in early postnatal androgen exposure.	Future research could use these biomarkers in large-scale population studies to further elucidate neurobehavioral effects of perinatal androgen exposure. Such large-scale investigations could also permit a prospective assessment of other factors known to influence variance in gender-related behavior, such as socialization and cognitive development, along with their interactions with early androgen exposure.

period of HPG axis activation is therefore critical for the sexual differentiation that drives the different organization in circuitry and neuroanatomy between the male and female brain. The early postnatal surge of gonadotropins and T in boys during minipuberty can potentially provide a window of opportunity in understanding the effects of sex steroid hormones on human gender development (106). Emerging evidence suggests that T levels during minipuberty have an influence not only on male genitalia and reproductive function but also on later gender-typical behavior. Indeed, minipuberty occurs during a period of huge and rapid brain development in terms of volume, cortical thickness, surface, and cortical network development (107–109). Lamminmäki et al. (110) found a positive correlation between T levels in FT infants from day 7 to 6 months and future sex-typed behavior at 14 months of life. In this study, the Pre-School Activities Inventory (PSAI) (111, 112) playroom was used during an observation of toy choices. In boys, T levels correlated significantly with PSAI scores and playing with trains. Conversely, playing with dolls was significantly correlated with a negative trend. In addition, Pasterski et al. (113) used AGD at birth and penile growth from birth to 3 months of age to estimate prenatal and postnatal androgen exposure. They re-evaluated children included in the study at 3 to 4 years of age using the PSAI suggesting that T levels in both periods, prenatal and postnatal, are independent contributors to later gender-related behavior. Language development is another area of investigation of a possible correlation with early postnatal HPG axis activation. Results based on small samples suggest a correlation between T levels and a different expressive vocabulary in boys and girls (114, 115). We have summarized some of the clearest studies

of the last decade in **Table 2** in order to better understand the influence of hormonal changes happening during minipuberty on sex-related behavior. All these emerging results may support a role for the imprinting of T during early infancy in human neurobehavioral sexual differentiation, although its effects are still largely unknown in both the short and long-term.

## CONCLUSION

Although further studies are needed, pre- and postnatal activation of the HPG axis could be considered an important window of prediction on how each newborn will grow and develop. Measurement of LH, FSH, and testosterone at 7 days, one and three months, and, when possible, six months, may help the clinician to better understand how minipuberty develops in different neonates. This will give us important information once the baby approaches puberty or when he or she shows impaired linear growth. Moreover, minipuberty must be considered a fundamental moment for possible therapeutic intervention in DSD. Therapeutic interventions may be able to change the natural history of some DSD or, at least, to improve prognosis in terms of fertility and quality of life.

## AUTHOR CONTRIBUTIONS

LL, VT, ABo, and LM analyzed the existing literature and wrote the draft of the review. ABe and LI critically reviewed the manuscript. All authors read and approved the final version of the manuscript. All authors contributed to the article and approved the submitted version.

## REFERENCES

- Hagen C, McNeilly AS. The gonadotrophins and their subunits in foetal pituitary glands and circulation. *J Steroid Biochem.* (1977) 8:537–44. doi: 10.1016/0022-4731(77)90259-X
- Clements JA, Reyes FI, Winter JSD, Faiman C. Studies on human sexual development. III. fetal pituitary and serum, and amniotic fluid concentrations of LH, CG, and FSH. *J Clin Endocrinol Metab.* (1976) 42:9–19. doi: 10.1210/jcem-42-1-9
- Kaplan SL, Grumbach MM. The ontogenesis of human foetal hormones. II. Luteinizing hormone (LH) and follicle stimulating hormone (FSH). *Acta Endocrinologica.* (1976) 81:808–29. doi: 10.1530/acta.0.0810808
- Guimiot F, Chevrier L, Dreux S, Chevenne D, Caraty A, Delezoide AL. Negative fetal FSH/LH regulation in late pregnancy is associated with declined kisspeptin/KISS1R expression in the tuberal hypothalamus. *J Clin Endocrinol Metab.* (2012) 97:E2221–9. doi: 10.1210/jc.2012-2078
- Pilavdzic D, Kovacs K, Asa SL. Pituitary morphology in anencephalic human fetuses. *Neuroendocrinology.* (1997) 65:164–72. doi: 10.1159/000127177
- Varvarigou AA, Liatsis SG, Vassilakos P, Decavalas G, Beratis NG. Effect of maternal smoking on cord blood estriol, placental lactogen, chorionic gonadotropin, FSH, LH, and cortisol. *J Perin Med.* (2009) 37:28. doi: 10.1515/JPM.2009.028
- Winter JSD, Faiman C, Hobson WC, Prasad AV, Reyes FI. Pituitary-Gonadal relations in infancy. I. patterns of serum gonadotropin concentrations from birth to four years of age in man and chimpanzee. *J Clin Endocrinol Metab.* (1975) 40:545–51. doi: 10.1210/jcem-40-4-545
- Winter JSD, Hughes IA, Reyes FI, Faiman C. Pituitary-Gonadal relations in infancy: 2. Patterns of serum gonadal steroid concentrations in man from birth to two years of age. *J Clin Endocrinol Metab.* (1976) 42:679–86. doi: 10.1210/jcem-42-4-679
- Kuiri-Hänninen T, Sankilampi U, Dunkel L. Activation of the hypothalamic-Pituitary-Gonadal axis in infancy: minipuberty. *Horm Res Paediatr.* (2014) 82:73–80. doi: 10.1159/000362414
- Schmidt H, Schwarz H. Serum concentrations of LH and FSH in the healthy newborn. *Eur J Endocrinol.* (2000) 2000:213–5. doi: 10.1530/eje.0.1430213
- Bergadá I, Milani C, Bedecarrás P, Andreone L, Ropelato MG, Gottlieb S et al. Time course of the serum gonadotropin surge, inhibins, and anti-Müllerian hormone in normal newborn males during the first month of life. *J Clin Endocrinol Metab.* (2006) 91:4092–8. doi: 10.1210/jc.2006-1079
- Andersson A-M. Longitudinal reproductive hormone profiles in infants: peak of inhibin b Levels in infant boys exceeds levels in adult men. *J Clin Endocrinol Metab.* (1998) 83:675–81. doi: 10.1210/jc.83.2.675
- Kuiri-Hänninen T, Seuri R, Tyrväinen E, Turpeinen U, Hamalainen E, Stenman U-H et al. Increased activity of the hypothalamic-Pituitary-Testicular axis in infancy results in increased androgen action in premature boys. *J Clin Endocrinol Metab.* (2011) 96:98–105. doi: 10.1210/jc.2010-1359
- Zegher FD, Devlieger H, Veldhuis JD. Pulsatile and sexually dimorphic secretion of luteinizing hormone in the human infant on the day of birth. *Pediatr Res.* (1992) 32:605–7. doi: 10.1203/00006450-199211000-00025
- Forest MG, Cathiard AM, Bertrand JA. Evidence of testicular activity in early infancy. *J Clin Endocrinol Metab.* (1973) 37:148–51. doi: 10.1210/jcem-37-1-148
- Barry JA, Hardiman PJ, Siddiqui MR, Thomas M. Meta-analysis of sex difference in testosterone levels in umbilical cord blood. *J Obst Gyn.* (2011) 31:697–702. doi: 10.3109/01443615.2011.614971
- Garagorri JM, Rodríguez G, Lario-Elboj AJ, Olivares JL, Lario-Muñoz Á, Orden I. Reference levels for 17-hydroxyprogesterone, 11-desoxycortisol,



- cortisol, testosterone, dehydroepiandrosterone sulfate and androstenedione in infants from birth to six months of age. *Eur J Pediatr.* (2008) 167:647–53. doi: 10.1007/s00431-007-0565-1
18. Nistal M, Paniagua R, Regadera J, Santamaria L, Amat P. A quantitative morphological study of human Leydig cells from birth to adulthood. *Cell Tissue Res.* (1986) 246:229–36. doi: 10.1007/BF00215884
  19. Chemes HE. Infancy is not a quiescent period of testicular development. *Int J Androl.* (2001) 24:2–7. doi: 10.1046/j.1365-2605.2001.00260.x
  20. Cortes D, Müller J, Skakkebaek NE. Proliferation of Sertoli cells during development of the human testis assessed by stereological methods. *Int J Androl.* (1987) 10:589–96. doi: 10.1111/j.1365-2605.1987.tb00358.x
  21. Chemes HE, Rey RA, Nistal M, Regadera J, Musse M, Gonzales-Peramato P, et al. Physiological androgen insensitivity of the fetal, neonatal, and early infantile testis is explained by the ontogeny of the androgen receptor expression in Sertoli cells. *J Clin Endocrinol Metab.* (2008) 93:4408–12. doi: 10.1210/jc.2008-0915
  22. Kuijper EAM, van Kooten J, Verbeke JML, van Rooijen M, Lambalk CB. Ultrasonographically measured testicular volumes in 0- to 6-year-old boys. *Hum Reprod.* (2008) 23:792–6. doi: 10.1093/humrep/den021
  23. Cassorla FG, Golden SM, Johnsonbaugh RE, Heroman WM, Loriaux DL, Sherins RJ. Testicular volume during early infancy. *J Pediatr.* (1981) 99:742–3. doi: 10.1016/S0022-3476(81)80398-8
  24. Boas M, Boisen KA, Virtanen HE, Kaleva M, Suomi AM, Schmidt IM, et al. Postnatal penile length and growth rate correlate to serum testosterone levels: a longitudinal study of 1962 normal boys. *Eur J Endocrinol.* (2006) 154:125–9. doi: 10.1530/eje.1.02066
  25. Kuiri-Hänninen T, Haanpää M, Turpeinen U, Hämäläinen E, Dunkel L, Sankilampi U. Transient postnatal secretion of androgen hormones is associated with acne and sebaceous gland hypertrophy in early infancy. *J Clin Endocrinol Metab.* (2013) 98:199–206. doi: 10.1210/jc.2012-2680
  26. Janus D, Wojcik M, Tyrawa K, Starzyk J. Transient isolated scrotal hair development in infancy. *Clin Pediatr.* (2013) 52:628–32. doi: 10.1177/0009922813480845
  27. Kuiri-Hänninen T, Kallio S, Seuri R, Tyväinen E, Liakka A, Tapanainen J, et al. Postnatal developmental changes in the pituitary-ovarian axis in preterm and term infant girls. *J Clin Endocrinol Metab.* (2011) 96:3432–9. doi: 10.1210/jc.2011-1502
  28. Troisi R, Potischman N, Roberts J, Siiteri P, DAftary A, Sims C, et al. Associations of maternal and umbilical cord hormone concentrations with maternal, gestational and neonatal factors (United States). *Cancer Causes Control.* (2003) 14:347–55. doi: 10.1023/a:1023934518975
  29. Schmidt IM, Chellakooty M, Haavisto A-M, Boisen KA, Damgaard IN, Steendahl U, et al. Gender difference in breast tissue size in infancy: correlation with serum estradiol. *Pediatr Res.* (2002) 52:682–6. doi: 10.1203/00006450-200211000-00012
  30. Kuiri-Hänninen T, Haanpää M, Turpeinen U, Hamalainen E, Seuri R, Tyrvainen E, et al. Postnatal ovarian activation has effects in estrogen target tissues in infant girls. *J Clin Endocrinol Metab.* (2013) 98:4709–16. doi: 10.1210/jc.2013-1677
  31. Chellakooty M, Schmidt IM, Haavisto AM, Boisen KA, Damgaard IN, Petersen JH, et al. Inhibin A, inhibin B, follicle-stimulating hormone, luteinizing hormone, estradiol, and sex hormone-binding globulin levels in 473 healthy infant girls. *J Clin Endocrinol Metab.* (2003) 88:3515–20. doi: 10.1210/jc.2002-021468
  32. Jayasinghe Y, Cha R, Horn-Ommen J, O'Brien P, Simmons PS. Establishment of normative data for the amount of breast tissue present in healthy children up to two years of age. *J Pediatr Adolesc Gynecol.* (2010) 23:305–11. doi: 10.1016/j.jpog.2010.03.002
  33. Ibáñez L, Potau N, Enriquez G, Marcos MV, de Zegher F. Hypergonadotrophinaemia with reduced uterine and ovarian size in women born small-for-gestational-age. *Hum Reprod.* (2003) 18:1565–9. doi: 10.1093/humrep/deg351
  34. Ibáñez L, Valls C, Cols M, Ferrer A, Marcos MV, de Zegher F. Hypersecretion of fSH in infant boys and girls born small for gestational age. *J Clin Endocrinol Metab.* (2002) 87:1986–8. doi: 10.1210/jcem.87.5.8459
  35. Sir-Petermann T, Hitchensfeld C, Codner E, Maliqueo M, Iniguez G, Echiburú B, et al. Gonadal function in low birth weight infants: a Pilot study. *J Pediatr Endocrinol Metab.* (2007) 20:405. doi: 10.1515/JPEM.2007.20.3.405
  36. Nagai S, Kawai M, Myowa-Yamakoshi M, Morimoto T, Matsukura T, Heike T. Gonadotropin levels in urine during early postnatal period in small for gestational age preterm male infants with fetal growth restriction. *J Perinatol.* (2017) 37:843–7. doi: 10.1038/jp.2017.55
  37. Forest MG, de Peretti E, Bertrand J. Testicular and adrenal androgens and their binding to plasma proteins in the perinatal period: developmental patterns of plasma testosterone, 4-androstenedione, dehydroepiandrosterone and its sulfate in premature and small for date infants as compared with that of full-term infants. *J Steroid Biochem.* (1980) 12:25–36. doi: 10.1016/0022-4731(80)90247-2
  38. Cicognani A, Alessandrini R, Pasini A, Pirazzoli P, Cassio A, Barbieri E, et al. Low birth weight for gestational age and subsequent male gonadal function. *J Pediatr.* (2002) 141:376–80. doi: 10.1067/mpd.2002.126300
  39. Ibáñez L, de Zegher F. Puberty after prenatal growth restraint. *Horm Res.* (2006) 65(Suppl. 3):112–5. doi: 10.1159/000091515
  40. Tapanainen J, Koivisto M, Vihko R, Huhtaniemi I. Enhanced activity of the pituitary-Gonadal axis in premature human infants. *J Clin Endocrinol Metab.* (1981) 52:235–8. doi: 10.1210/jcem-52-2-235
  41. Shinkawa O, Furuhashi N, Fukaya T, Suzuki M, Kono H, Tachibana Y. Changes of serum gonadotropin levels and sex differences in premature and mature infant during neonatal life. *J Clin Endocrinol Metab.* (1983) 56:1327–31. doi: 10.1210/jcem-56-6-1327
  42. Greaves RF, Hunt RW, Chiriano AS, Zacharin MR. Luteinizing hormone and follicle-stimulating hormone levels in extreme prematurity: development of reference intervals. *Pediatrics.* (2008) 121:e574–e80. doi: 10.1542/peds.2007-1327
  43. de Jong M, Rotteveel J, Heijboer AC, Cranendonk A, Twisk JWR, van Weissenbruch MM. Urine gonadotropin and estradiol levels in female very-low-birth-weight infants. *Early Hum Dev.* (2013) 89:131–5. doi: 10.1016/j.earlhumdev.2012.09.007
  44. Sedin G, Bergquist C, Lindgren PG. Ovarian hyperstimulation syndrome in preterm infants. *Pediatr Res.* (1985) 19:548–52. doi: 10.1203/00006450-198506000-00009
  45. Poon WYS, Tung JYL. Vaginal bleeding in an infant with extreme prematurity. *Case Rep Pediatr.* (2020) 2020:8881634. doi: 10.1155/2020/8881634
  46. Lunn SF, Cowen GM, Fraser HM. Blockade of the neonatal increase in testosterone by a GnRH antagonist: the free androgen index, reproductive capacity and postmortem findings in the male marmoset monkey. *J Endocrinol.* (1997) 154:125–31. doi: 10.1677/joe.0.1540125
  47. Nevison B, Dixon F. Manipulation of postnatal testosterone levels affects phallic and clitoral development in infant rhesus monkeys. *Int J Androl.* (1999) 22:119–28. doi: 10.1046/j.1365-2605.1999.00158.x
  48. Liu L, Cristiano AM, Southerns JL. Effects of pituitary-Testicular axis suppression in utero and during the early neonatal period with a long-acting luteinizing hormone-releasing hormone analog on genital development, somatic growth, and bone density in male cynomolgus monkeys in the first 6 months of life. *J Clin Endocrinol Metab.* (1991) 73:1038–43. doi: 10.1210/jcem-73-5-1038
  49. Grumbach MM. A window of opportunity: the diagnosis of gonadotropin deficiency in the male infant 1. *J Clin Endocrinol Metab.* (2005) 90:3122–7. doi: 10.1210/jc.2004-2465
  50. Hadziselimovic F, Zivkovic D, Bica DTG, Emmons LR. The importance of mini-puberty for fertility in cryptorchidism. *J Urol.* (2005) 174(4 Part 2):1536–9. doi: 10.1097/01.ju.0000181506.97839.b0
  51. Hadziselimovic F, Emmons LR, Buser MW. A diminished postnatal surge of ad spermatogonia in cryptorchid infants is additional evidence for hypogonadotropic hypogonadism. *Swiss Med Wkly.* (2004) 134:381–4.
  52. Quinton R, Mamoojee Y, Jayasena CN, Young J, Howard S, Dunkel L, et al. Society for endocrinology UK guidance on the evaluation of suspected disorders of sexual development: emphasizing the opportunity to predict adolescent pubertal failure through a neonatal diagnosis of absent minipuberty. *Clin Endocrinol.* (2017) 86:305–6. doi: 10.1111/cen.13257
  53. Dwyer AA, Jayasena CN, Quinton R. Congenital hypogonadotropic hypogonadism: implications of absent mini-puberty. *Min Endocrinol.* (2016) 10:10. doi: 10.3389/fendo.2019.00353
  54. Bouvattier C, Carel J-C, Lecointre C, David A, Sultan C, Berrand A-M, et al. Postnatal changes of t, LH, and fSH in 46,XY infants with mutations in the

- AR gene. *J Clin Endocrinol Metab.* (2002) 87:29–32. doi: 10.1210/jcem.87.1.7923
55. Johannsen TH, Main KM, Ljubicic ML, Jensen TK, Andersen HR, Andersen MS, et al. Sex differences in reproductive hormones during mini-Puberty in infants with normal and disordered sex development. *J Clin Endocrinol Metab.* (2018) 103:3028–37. doi: 10.1210/jc.2018-00482
  56. Kolon TF, Herndon CDA, Baker LA, Baskin LS, Baxter CG, Cheng EY, et al. Evaluation and treatment of cryptorchidism: aUA guideline. *J Urol.* (2014) 192:337–45 doi: 10.1016/j.juro.2014.05.005
  57. Bouvattier C, Maione L, Bouligand J, Dodé C, Guiochon-Mantel A, Young J. Neonatal gonadotropin therapy in male congenital hypogonadotropic hypogonadism. *Nat Rev Endocrinol.* (2012) 8:172–82 doi: 10.1038/nrendo.2011.164
  58. Hadziselimovic F, Hoecht B. Testicular histology related to fertility outcome and postpubertal hormone status in cryptorchidism. *Klinische Padiatrie.* (2008) 220:302–7 doi: 10.1055/s-2007-993194
  59. Main KM, Schmidt IM, Toppari J, Skakkebaek NE. Early postnatal treatment of hypogonadotropic hypogonadism with recombinant human FSH and LH. *Eur J Endocrinol.* (2002) 146:75–9 doi: 10.1530/eje.0.1460075
  60. Bougnères P, François M, Pantalone L, Rodrigue D, Bouvattier C, Demesteere E, et al. Effects of an early postnatal treatment of hypogonadotropic hypogonadism with a continuous subcutaneous infusion of recombinant follicle-stimulating hormone and luteinizing hormone. *J Clin Endocrinol Metab.* (2008) 93:2202–5 doi: 10.1210/jc.2008-0121
  61. Stoupa A, Samara-Boustani D, Flechtner I, Pinto G, Jourdon I, González-Briceno L, et al. Efficacy and safety of continuous subcutaneous infusion of recombinant human gonadotropins for congenital micropenis during early infancy. *Horm Res Paediatr.* (2017) 87:103–10 doi: 10.1159/000454861
  62. Papadimitriou DT, Chrysis D, Nyktari G, Zoupanos G, Liakou E, Papadimitriou A, et al. Replacement of male mini-Puberty. *J Endocr Society.* (2019) 3:1275–82 doi: 10.1210/je.2019-00083
  63. Lambert A, Bougnères P. Growth and descent of the testes in infants with hypogonadotropic hypogonadism receiving subcutaneous gonadotropin infusion. *Int J Pediatr Endocrinol.* (2016) 2016:13 doi: 10.1186/s13633-016-0031-9
  64. Vincel B, Verkauskas G, Bilius V, Dasevicius D, Malcius D, Jones B, et al. Gonadotropin-Releasing hormone agonist corrects defective mini-Puberty in boys with cryptorchidism: a Prospective randomized study. *BioMed Research International.* (2018) 46:51218 doi: 10.1155/2018/4651218
  65. Zivkovic D, Bica DTG, Hadziselimovic F. Relationship between adult dark spermatogonia and secretory capacity of Leydig cells in cryptorchidism. *BJU Int.* (2007) 100:1147–9. doi: 10.1111/j.1464-410X.2007.07034.x
  66. Gegenschatz-Schmid K, Verkauskas G, Demougin P, Bilius V, Dasevicius D, Stadler MB, et al. DMRTC2, PAX7, BRACHYURU/T and tert are implicated in male germ cell development following curative hormone treatment for cryptorchidism-induced infertility. *Genes.* (2017) 8:267. doi: 10.3390/genes8100267
  67. Renault CH, Aksglaede L, Wøjdemann D, Hansen AB, Jensen RB, Juul A. Minipuberty of human infancy - a window of opportunity to evaluate hypogonadism and differences of sex development? *Ann Pediatr Endocrinol Metab.* (2020) 25:84–91 doi: 10.6065/apem.2040094.047
  68. <https://www.endocrine.org/advocacy/position-statements/endocrine-disrupting-chemicals> (Accessed September 19, 2020).
  69. Phillips KP, Foster WG. Key developments in endocrine disrupter research and human health. *J Toxicol Environ Health B Crit Rev.* (2008) 11:322–44 doi: 10.1080/10937400701876194
  70. Watkins DJ, Sánchez BN, Téllez-Rojo MM, Lee JM, Mercado-García A, Blank-Goldenberg C, et al. Phthalate and bisphenol a exposure during in utero windows of susceptibility in relation to reproductive hormones and pubertal development in girls. *Environ Res.* (2017) 159:143–51 doi: 10.1016/j.envres.2017.07.051
  71. Bowman JD, Choudhury M. Phthalates in neonatal health: friend or foe? *J Dev Orig Health Dis.* (2016) 7:652–64 doi: 10.1017/S2040174416000349
  72. Ortega-García JA, Olano-Soler HA, Martínez-Alvarez A, Campillo-Lopez F, Gomariz-Penalver V, Mendiola-Olivares J, et al. Breastfeeding duration and anogenital distance in 2-year-old infants. *Breastfeed Med.* (2016) 1:350–5. doi: 10.1089/bfm.2016.0034
  73. Winneke G, Ranft U, Wittsiepe J, Kasper-Sonnenberg M, Furst P, Kramer U, et al. Behavioral sexual dimorphism in school-age children and early developmental exposure to dioxins and PCBs: a follow-up study of the Duisburg cohort. *Environ Health Perspect.* (2014) 122:292–8. doi: 10.1289/ehp.1306533
  74. Ünüvar T, Büyükgöbüz A. Fetal and neonatal endocrine disruptors. *J Clin Res Pediatr Endocrinol.* (2012) 4:51–60 doi: 10.4274/Jcrpe.569
  75. Liu C, Xu X, Zhang Y, Li W, Huo X. Associations between maternal phenolic exposure and cord sex hormones in male newborns. *Hum. Reprod.* (2016) 31:648–56. doi: 10.1093/humrep/dev327
  76. Steckler T, Wang J, Bartol FF, Roy SK, Padmanabhan V. Fetal programming: prenatal testosterone treatment causes intrauterine growth retardation, reduces ovarian reserve and increases ovarian follicular recruitment. *Endocrinology.* (2015) 146:3185–93. doi: 10.1210/en.2004-1444
  77. Sun X, Li D, Liang H, Miao M, Song X, Wang Z, et al. Maternal exposure to biphenol a and anogenital distance throughout infancy: a longitudinal study from Shanghai, China. *Environ Int.* (2018) 121(Pt 1):269–75. doi: 10.1016/j.envint.2018.08.055
  78. Muerkøster A-P, Frederiksen H, Juul A, Andersson A-M, Jensen RC, Glintborg D, et al. Maternal phthalate exposure associated with decreased testosterone/LH ratio in male offspring during mini-puberty. *Odense Child Cohort. Environ Int.* (2020) 144:106025 doi: 10.1016/j.envint.2020.106025
  79. Howdeshell KL, Furr J, Lambright CR, Rider CV, Wilson VS, Gray LE. Cumulative effects of dibutyl phthalate and diethylhexyl phthalate on male rat reproductive tract development: altered fetal steroid hormones and genes. *Toxicological Sciences.* (2007) 99:190–202. doi: 10.1093/toxsci/kfm069
  80. Borch J, Metzдорff SB, Vinggaard AM, Brokken L, Dalgaard M. Mechanisms underlying the anti-androgenic effects of diethylhexyl phthalate in fetal rat testis. *Toxicology.* (2006) 223:144–55 doi: 10.1016/j.tox.2006.03.015
  81. Gore AC, Chappell VA, Fenton SE, Flaws JA, Nadal A, Prins GS, et al. EDC-2: the endocrine society's second scientific statement on endocrine-Disrupting chemicals. *Endocr Rev.* (2015) 36:E1–E50 doi: 10.1210/er.2015-1010
  82. Vasiliu O, Muttineni J, Karmaus W. In utero exposure to organochlorines and age at menarche. *Hum Reprod.* (2004) 19:1506–12 doi: 10.1093/humrep/deh292
  83. Windham GC, Pinney SM, Voss RW, Sjödin A, Biro FM, Greenspan LC, et al. Brominated flame retardants and other persistent organohalogenated compounds in relation to timing of puberty in a longitudinal study of girls. *Environ Health Perspect.* (2015) 123:1046–52 doi: 10.1289/ehp.1408778
  84. Hashemipour M, Kelishadi R, Amin MM, Ebrahim K. Is there any association between phthalate exposure and precocious puberty in girls? *Environ Sci Pollut Res Int.* (2018) 25:13589–96 doi: 10.1007/s11356-018-1567-4
  85. Chen Y, Wang Y, Ding G, Tian Y, Zhou Z, Wang X, et al. Association between bisphenol a exposure and idiopathic central precocious puberty (ICPP) among school-aged girls in Shanghai, China. *Environ Int.* (2018) 115:410–6 doi: 10.1016/j.envint.2018.02.041
  86. Hsu PC, Lai TJ, Guo NW, Lambert GH, Leon Guo Y. Serum hormones in boys prenatally exposed to polychlorinated biphenyls and dibenzofurans. *J Toxicol Environ Health A.* (2005) 68:1447–56. doi: 10.1080/15287390590967360
  87. Yang CY, Yu ML, Guo HR, Lai TJ, Hsu CC, Lambert G, et al. The endocrine and reproductive function of the female yucheng adolescents prenatally exposed to PCBs/PCDFs. *Chemosphere.* (2005) 61:355–6 doi: 10.1016/j.chemosphere.2005.02.089
  88. Palioura E, Diamanti-Kandaraki E. Polycystic ovary syndrome (PCOS) and endocrine disrupting chemicals (EDCs). *Rev Endocr and Met Disorders.* (2016) 16:365–71 doi: 10.1007/s11154-016-9326-7
  89. Burks H, Martin E, McLachlan J, Bunnell B, Burrow M. Endocrine disruptors and the tumor microenvironment: a new paradigm in breast cancer biology. *Mol Cell Endocrinol.* (2017) 457:13–9 doi: 10.1016/j.mce.2016.12.010
  90. Roche AF, Guo S, Moore WM. Weight and recumbent length from 1 to 12 mo of age: reference data for 1-mo increments. *Am J Clin Nutr.* (1989) 49:599–607. doi: 10.1093/ajcn/49.4.599
  91. Rosen O, Cohen A. Analysis of growth curves via mixtures. *Statist Med.* (2003) 22:3641–54. doi: 10.1002/sim.1582
  92. Gasser T, Sheehy AL, Molinari. Sex dimorphism in growth. *Ann Hum Biol.* (2000) 27:187–97. doi: 10.1080/030144600282299

93. Becker M, Oehler K, Partsch C-J, Ulmen U, Schmutzler R, Cammann H, et al. Hormonal 'minipuberty' influences the somatic development of boys but not of girls up to the age of 6 years. *Clin Endocrinol.* (2015) 83:694–701. doi: 10.1111/cen.12827
94. Bizzarri C, Cappa M. Ontogeny of hypothalamus-Pituitary gonadal axis and minipuberty: an ongoing debate? *Front Endocrinol.* (2020) 11:187 doi: 10.3389/fendo.2020.00187
95. Kiviranta P, Kuiri-Hänninen T, Saari A, Lamidi M-L, Dunkel L, Sankilampi U. Transient postnatal gonadal activation and growth velocity in infancy. *Pediatrics.* (2016) 138:e20153561–e20153561. doi: 10.1542/peds.2015-3561
96. Lenroot RK, Gogtay N, Greenstein DK, Molloy Wells E, Wallace GL, Clasen LS, et al. Sexual dimorphism of brain developmental trajectories during childhood and adolescence. *NeuroImage.* (2007) 36:1065–73. doi: 10.1016/j.neuroimage.2007.03.053
97. Goddings A-L, Beltz A, Peper JS, Crone EA, Braams BR. Understanding the role of puberty in structural and functional development of the adolescent brain. *J Res Adolesc.* (2019) 29:32–53. doi: 10.1111/jora.12408
98. Hines M, Brook C, Conway GS. Androgen and psychosexual development: core gender identity, sexual orientation, and recalled childhood gender role behavior in women and men with congenital adrenal hyperplasia (CAH). *J Sex Res.* (2004) 41:75–81. doi: 10.1080/00224490409552215
99. Ehrhardt A, Meyer-Bahlburg H. Effects of prenatal sex hormones on gender-related behavior. *Science.* (1981) 211:1312–8. doi: 10.1126/science.7209510
100. New MI. Diagnosis and management of congenital adrenal hyperplasia. *Annu Rev Med.* (1998) 49:311–28. doi: 10.1146/annurev.med.49.1.311
101. Berenbaum SA, Hines M. Early androgens are related to childhood sex-typed toy preferences. *Psychol Sci.* (1992) 3:203–6. doi: 10.1111/j.1467-9280.1992.tb00028.x
102. Pasterski VL, Geffner ME, Brain C, Hindmarsh P, Brook C, Hines M. Prenatal hormones and postnatal socialization by parents as determinants of male-typical toy play in girls with congenital adrenal hyperplasia. *Child Dev.* (2005) 76:264–78. doi: 10.1111/j.1467-8624.2005.00843.x
103. Pasterski V, Zucker KJ, Hindmarsh PC, Hughes IA, Acerini C, Spencer D, et al. Increased cross-Gender identification independent of gender role behavior in girls with congenital adrenal hyperplasia: results from a standardized assessment of 4- to 11-Year-Old children. *Arch Sex Behav.* (2015) 44:1363–75. doi: 10.1007/s10508-014-0385-0
104. Frisén L, Nordenström A, Falhammar H, Flipsson H, Holmdahl G, Janson PO, et al. Gender role behavior, sexuality, and psychosocial adaptation in women with congenital adrenal hyperplasia due to CYP21A2 deficiency. *J Clin Endocrinol Metab.* (2009) 94:3432–9. doi: 10.1210/jc.2009-0636
105. Auyeung B, Baron-Cohen S, Ashwin E, Knickmeter R, Taylor K, Hackett G, et al. Fetal testosterone predicts sexually differentiated childhood behavior in girls and in boys. *Psychol Sci.* (2009) 20:144–8. doi: 10.1111/j.1467-9280.2009.02279.x
106. Hines M, Spencer D, Kung KT, Browne WV, Constantinescu M, Noorderhaven RM. The early postnatal period, mini-puberty, provides a window on the role of testosterone in human neurobehavioural development. *Curr Opin Neurobiol.* (2016) 38:69–73. doi: 10.1016/j.conb.2016.02.008
107. Knickmeyer RC, Gouttard S, Kang C, Evans D, Wilber K, Smith JK, et al. A structural MRI study of human brain development from birth to 2 years. *J Neurosci.* (2008) 28:12176–82. doi: 10.1523/JNEUROSCI.3479-08.2008
108. Lyall AE, Shi F, Geng X, Woolson S, Wang L, Hamer RM, et al. Dynamic development of regional cortical thickness and surface area in early childhood. *Cereb Cortex.* (2015) 25:2204–12. doi: 10.1093/cercor/bhu027
109. Li G, Lin W, Gilmore JH, Shen D. Spatial patterns, longitudinal development, and hemispheric asymmetries of cortical thickness in infants from birth to 2 years of age. *J Neurosci.* (2015) 35:9150–62. doi: 10.1523/JNEUROSCI.4107-14.2015
110. Lamminmäki A, Hines M, Kuiri-Hänninen T, Kilpeläinen L, Dunkel L, Sankilampi U. Testosterone measured in infancy predicts subsequent sex-typed behavior in boys and in girls. *Horm Behav.* (2012) 61:611–6. doi: 10.1016/j.yhbeh.2012.02.013
111. Golombok S, Rust J. The measurement of gender role behaviour in pre-School children: a research note. *J Child Psychol Psychiatr.* (1993) 34:805–11. doi: 10.1111/j.1469-7610.1993.tb01072.x
112. Golombok S, Rust J, Zervoulis K, Croudace T, Golding J, Hines M. Developmental trajectories of sex-typed behavior in boys and girls: a Longitudinal general population study of children aged 2.5-8 years. *Child Dev.* (2008) 79:1583–93. doi: 10.1111/j.1467-8624.2008.01207.x
113. Pasterski V, Acerini CL, Dunger DB, Ong KK, Hughes IA, Thankamony A, et al. Postnatal penile growth concurrent with mini-puberty predicts later sex-typed play behavior: evidence for neurobehavioral effects of the postnatal androgen surge in typically developing boys. *Horm Behav.* (2015) 69:98–105. doi: 10.1016/j.yhbeh.2015.01.002
114. Kung KTF, Browne WV, Constantinescu M, Noorderhaven RM, Hines M. Early postnatal testosterone predicts sex-related differences in early expressive vocabulary. *Psychoneuroendocrinology.* (2016) 68:111–6. doi: 10.1016/j.psyneuen.2016.03.001
115. Schaadt G, Hesse V, Friederici AD. Sex hormones in early infancy seem to predict aspects of later language development. *Brain Lang.* (2015) 141:70–6. doi: 10.1016/j.bandl.2014.11.015
116. Constantinescu M, Moore DS, Johnson SP, Hines M. Early contributions to infants' mental rotation abilities. *Dev Sci.* (2018) 21:e12613. doi: 10.1111/desc.12613
117. Fenson L, Bates E, Dale P, Goodman J, Reznick JS, Thal D. Measuring variability in early child language: don't shoot the messenger. *Child Dev.* (2000) 71:323–8. doi: 10.1111/1467-8624.00147
118. Kung KT, Constantinescu M, Browne WV, Noorderhaven RM, Hines M. No relationship between early postnatal testosterone concentrations and autistic traits in 18 to 30-month-old children. *Mol Autism.* (2016) 7:15. doi: 10.1186/s13229-016-0078-8

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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