Current Research in Toxicology 2 (2021) 179–191

Contents lists available at ScienceDirect

Current Research in Toxicology

journal homepage: www.elsevier.com/locate/crtox

Endocrine disrupting chemicals in the pathogenesis of hypospadias; developmental and toxicological perspectives

Deidre M. Mattiske *, Andrew J. Pask

School of BioSciences, The University of Melbourne, Victoria 3010, Australia

ARTICLE INFO

Keywords:
Hypospadias
Estrogen
Androgen
Penis
Endocrine disruptors

ABSTRACT

Hypospadias is a defect in penile urethral closure that occurs in approximately 1/150 live male births in developed nations, making it one of the most common congenital abnormalities worldwide. Alarmingly, the frequency of hypospadias has increased rapidly over recent decades and is continuing to rise. Recent research reviewed herein suggests that the rise in hypospadias rates can be directly linked to our increasing exposure to endocrine disrupting chemicals (EDCs), especially those that affect estrogen and androgen signalling. Understanding the mechanistic links between endocrine disruptors and hypospadias requires toxicologists and developmental biologists to define exposures and biological impacts on penis development. In this review we examine recent insights from toxicological, developmental and epidemiological studies on the hormonal control of normal penis development and describe the rationale and evidence for EDC exposures that impact these pathways to cause hypospadias. Continued collaboration across these fields is imperative to understand the full impact of endocrine disrupting chemicals on the increasing rates of hypospadias.

Contents

Abbreviations: BBP, benzyl butyl phthalate; BPA, bisphenol A; DBP, Σdibutyl phthalate; DDT, dichlorodiphenyltrichloroethane; DEHP, Σdi-2(ethylhexyl)-phthalate; DHT, dihydrotestosterone; EDC, endocrine disrupting chemicals; EMT, epithelial to mesenchymal transition; ER, estrogen receptor; GT, genital tubercle; NOAEL, no observed adverse effect level; PBB, polybrominated biphenyl; PBDE, polybrominated diphenyl ether; PCB, polychlorinated biphenyl; PCE, tetrachloroethylene.

* Corresponding author.
E-mail address: deidre.mattiske@unimelb.edu.au (D.M. Mattiske).

https://doi.org/10.1016/j.crtox.2021.03.004
Received 31 October 2020; Revised 24 March 2021; Accepted 25 March 2021
Available online xxxx

Introduction

Endocrine disruptors are "*substances that alter one or more functions of the endocrine system and consequently cause adverse health effects in an intact organism, or its progeny, or (sub)populations*" (WHO, international Programme on Chemical Safety). There is unequivocal data pertaining to worldwide levels of human exposure to a range of endocrine disrupting compounds. Currently there are over 85,000 known chemical products (Street et al., 2018). The vast majority of these have not undergone stringent toxicology screenings to assess their effects on human health. Despite this, already more than 1000 of these chemicals have been demonstrated to be potentially harmful endocrine disrupting chemicals (EDCs) and can disrupt hormone signalling in a wide range of tissues (Street et al., 2018). Most people are exposed to multiple EDCs through the environment, diet and occupational exposures (Parron et al., 2014). Exposure to EDCs can also involve non-monotonic dose–response curves with low doses resulting in significant impacts (Vandenberg et al., 2012). This makes the study of EDCs very complex, particularly when trying to make predictions about their potential health impacts.

EDC exposures are particularly deleterious during embryonic development when the hormonal environment is tightly regulated and when tissues are rapidly growing and patterning. Many EDCs can cross the placenta and interfere with the normal development of the reproductive, nervous, immune and hormonal systems, ultimately altering the normal function of the exposed individual and impacting future generations. The mechanisms through which EDCs exert specific actions are dependent on the cell and tissue type and are influenced by other factors such as developmental stage and sex of the embryo. Recently, an Expert Consensus Statement identified ten key characteristics of EDCs to describe the properties of chemicals that alter hormone action and improve hazard identification (La Merrill et al., 2020). These characteristics recognise not only antagonism of hormones and their receptors, but also alteration of hormone synthesis, transport, metabolism and signal transduction.

Over the last decade, exposure to EDCs has emerged as a global environmental risk and is now considered an urgent threat to public health by organisations such as the Endocrine Society and WHO (Gore et al., 2015a; WHO/UNEP, 2012). Multiple studies have been conducted examining trends in human reproductive health and the impact of EDCs on wildlife reproductive health. These have been conducted alongside *in vitro* and *in vivo* experimental data to further refine our understanding of how these chemicals and exposures impact our development and reproductive function (Bonde et al., 2016).

Development of the human penis and hypospadias

The development of the genitalia is one of the most hormone sensitive processes during sexual development. The external genitalia of both males and females develops from the sexually indifferent genital tubercle (GT) in the early embryo. Outgrowth and development of the GT into a penis in males is triggered by a surge of androgens produced from the fetal testis shortly after sex determination. This results in a structure with an internalised urethra that terminates at the tip of the glans (Carlson, 1994; Hamilton et al., 1972). Hypospadias, a misplacement of the urethra meatus, results from a failure of urethral closure during development and can have both underlying genetic and hormonal causes. Hypospadias requires surgical intervention and individuals and their families often experience significant psychological trauma, resulting in a substantial burden to health care (Attina et al., 2016; Paulozzi et al., 1997). The severity of the hypospadias phenotype varies depending on when in development the closure process is interrupted. The more proximal the urethral opening, the more severe the phenotype. Mild or distal forms of hypospadias are the most common form of the disease (accounting for ~70% of cases) and result from defects in hormonal signalling in the early embryo (Fig. 1) (Kalfa et al., 2010).

Exposures to EDCs are proposed to be a major underlying cause of this developmental defect (Baskin and Ebbers, 2006; Fernandez et al., 2016; Kalfa et al., 2015, 2011b; Wang and Baskin, 2008; Willingham and Baskin, 2007). Alongside increasing EDCs in our environment, rates of hypospadias are increasing across many countries, even when accounting for increased reporting and changes to clinical practise (Nassar et al., 2007; Paulozzi, 1999; Yu et al., 2019). Hypospadias currently impacts ~1/150 live male births in developed countries (Baskin, 2000; Nassar et al., 2007) and is predicted to be increasing still at a rate of 1% PA (Nassar et al., 2007; Yu et al., 2019). Despite the common occurrence of hypospadias worldwide, definitive mechanistic links between EDCs and hypospadias are yet to be defined. This has posed a major barrier to our understanding of the impacts of EDCs to male reproductive health, the development of policies regulating our exposures to these chemicals and the development of methods to mitigate the impacts of EDCs.

The mouse as a model for human penis development and hypospadias

Mice develop a genital tubercle using identical hormonal and genetic pathways to humans (Haraguchi et al., 2000; Phillips et al., 2015). Although the mouse and human penis look morphologically

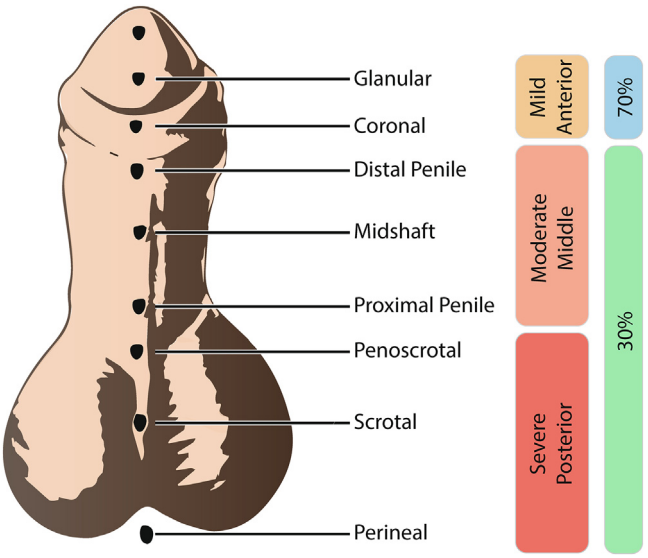

Fig. 1. Classification scheme of hypospadias. Degree of severity of hypospadias relates to the position of the urethra opening, from mild hypospadias in anterior regions to severe hypospadias in scrotal and perineal regions. Mild hypospadias occur in approximately 70% of cases with more moderate and severe cases accounting for approximately 30% of cases.

different, all the structures are homologous (Phillips et al., 2015). Penis outgrowth and urethral closure are primarily androgen driven, and occurs in the early embryo, as in humans. Interestingly, estrogen has recently been shown to play a key role in the urethral closure process in mice, particularly mediating distal urethral closure (Baskin et al., 2001; Cripps et al., 2019; Govers et al., 2019b; Seifert et al., 2009, 2010). As in humans, any disruption to hormonal signalling in early male mice development can cause hypospadias, indicating that urethral closure is a tightly regulated process. The conserved hormonal and molecular mechanisms governing penis development make it susceptible to EDC exposures in all animals, not just mice and humans. Many studies on EDC exposures have occurred in aquatic animals with relatively less in terrestrial mammal species (Bjerregaard et al., 1998; Cook et al., 2020). However, these studies demonstrate that the findings on the impacts of EDCs from rodents and humans are likely to have broad implications for impacts these chemicals may have on all vertebrates.

Establishing the link between EDCs and hypospadias

The possible health threats posed by EDCs were officially outlined by the Endocrine Society (Gore et al., 2015a) and the WHO/UNEP (WHO/UNEP, 2012), with the conclusion that exposure to EDCs is related to a multitude of diseases, including impaired reproduction. However, systemic reviews and meta-analyses focusing on human studies have demonstrated inconsistent results (Kahn et al., 2020; Lee, 2018). While the ability of EDCs to promote adverse modifications of endocrine homeostasis in human and wildlife raises serious concerns about their potential health impact, uncertainty remains around a definitive link between EDCs and reproductive disorders and mechanistic links between the two (Bergman et al., 2013, 2015; Combarnous and Nguyen, 2019; Dietrich et al., 2013; Gore et al., 2013; Kortenkamp et al., 2012; Kumar et al., 2020; Lamb et al., 2015; Rhomberg et al., 2012; Zoeller et al., 2014). Swaen et al (Swaen et al., 2018) argue that profound changes in human reproduction have a major impact on the incidence of reproductive disorders. Increased rates of several reproductive disorders were compared to changes of reproductive factors within a Dutch population from 1955 to 2015. A combination of decrease in parity or family size and an increase in maternal age at first pregnancy accounted for a 34% increase in the prevalence of hypospadias. Although this raises the possibility that changes in human reproductive factors can partly explain fractions of reported increases in hypospadias, there are numerous studies that demonstrate a strong association between increasing rates of hypospadias and exposures to endocrine disruptors. Yu et al examined the rate of hypospadias during 1980–2010 from 27 surveillance programs around the world, analysing changes over time in international total prevalence of hypospadias, prevalence for each specific program and prevalence across different degrees of severity of hypospadias. They concluded a consistency in observed trends across programs and in severity, indicating that the prevalence of hypospadias continues to increase internationally (Yu et al., 2019).

Exogenous estrogens and anti-androgens can adversely impact external genital development in humans (Fernandez et al., 2016; Kalfa et al., 2015) mice (Ma et al., 2009; Yang et al., 2010; Yucel et al., 2003; Zheng et al., 2015) and rats (Christiansen et al., 2009; Lee, 1998; Metzdorff et al., 2007; Sinclair et al., 2017; Vorherr et al., 1979), resulting in hypospadias. Exogenous estrogens and estrogenic EDCs are among the most pervasive chemicals in our environment and, along with anti-androgenic EDCs, are potential causative agents of hypospadias (Fig. 2). Both estrogenic chemicals and those that antagonise the androgen receptor or inhibit fetal testis steroidogenesis, reduce testosterone production and/or its action to cause male reproductive tract malformations, including hypospadias. However, estrogen receptors are also present in the penis and isolated penis cultures have demonstrated that estrogen and estrogenic EDCs can directly activate these to cause hypospadias, independent of altered hormonal outputs from the gonads (Fig. 3) (Govers et al., 2020, 2019b). Thus, the penis also appears to be a direct target of EDCs in the development of hypospadias. Importantly, the ability of EDCs to affect penis development both directly and indirectly highlights the susceptibility of penis development to EDC exposure.

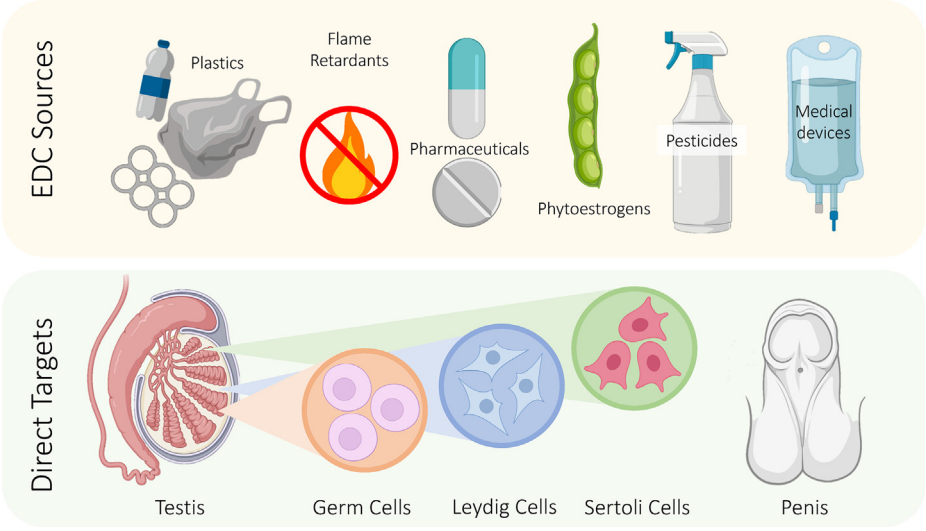

Fig. 2. EDC sources and their direct target tissues in the male. The top panel shows known sources of EDC that impact male reproductive development and cause hypospadias. These include plastics, flame retardants, pharmaceuticals, phytoestrogens, pesticides and medical devices. Many of these chemicals can act as estrogen receptor agonists while others block estrogen action or can have pro or anti androgen functions. The lower panel shows the direct targets of EDCs in the male reproductive system. The testes and in particular the germ cells, Leydig cells and Sertoli cells are all sensitive to the hormonal environment and express both androgen and estrogen receptors. EDCs impact cell fate decision in the developing Sertoli cells, alter steroid output from the Leydig cells and alter the epigenome of germ cells leading to multigenerational and transgenerational transmission of disease. EDCs also directly target the penis. The penis has a broad distribution of androgen and estrogen receptors and both androgen and estrogen levels are critical for normal patterning. Experiments on isolated genital tubercles have shown estrogen can directly target the penis to cause hypospadias.

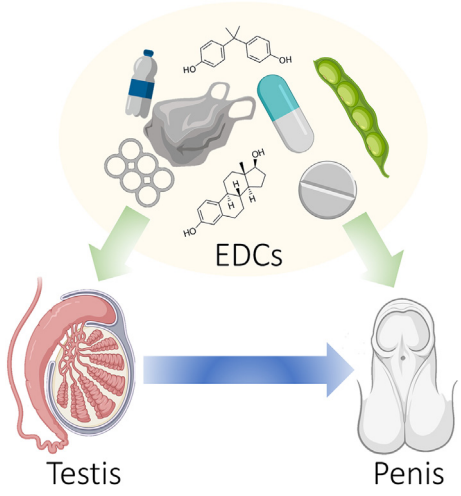

Fig. 3. EDCs impact penis development through direct and indirect mechanisms. EDCs impact development of the testis and many alter its hormonal output (green arrow). In particular, estrogenic EDCs are known to suppress androgen output from the testis. This leads to downstream impacts on the virilization of the penis and can cause hypospadias (blue arrow). In addition, EDCs can directly target the penis to cause hypospadias (green arrow). Thus, the penis is impacted both directly and indirectly (via altered hormonal output from the testis) by EDCs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Interestingly, recent research has shown that a loss of estrogen signalling in the penis can also cause hypospadias (Cripps et al., 2019; Govers et al., 2019b). Distal hypospadias (the most common form seen in humans) was identified in mice deficient for estrogen signalling, with mutations in either *ERα* or the *Cyp19a1* gene (which encodes aromatase). Distal hypospadias are also the most common phenotype caused by exogenous estrogen signalling in mice and humans. Thus, a tightly maintained balance between androgen and estrogen signalling is critical for normal penis development. Any disruption causing either too much or too little estrogen signalling can result in hypospadias. Disruption of this tightly regulated balance can occur from exposure to EDCs that interact with either androgen or estrogen and their associated receptors (Baskin and Ebbers, 2006; Fernandez et al., 2016; Kalfa et al., 2015, 2011b; Wang and Baskin, 2008; Willingham and Baskin, 2007) and most likely contributes to the prevalence of hypospadias.

While many epidemiological studies indicate an increased risk of male reproductive disorders following prenatal and postnatal exposure to EDCs (Bonde et al., 2016; Gore et al., 2015b; Skakkebaek, 2016), the evidence specifically linking hypospadias to EDC exposure is more limited. Bonde et al. (2016) undertook a rigorous, systematic meta-analysis of the epidemiological evidence on prenatal and postnatal EDC exposure and male reproductive disorders. They analysed 33 papers and found a shared prenatal etiology of male reproductive disorders, but also a heterogeneity of EDCs in terms of the potential effects on male reproduction. They also highlighted the limitations of observational epidemiological studies, urging caution be applied to the interpretation of such studies and emphasising the need for high quality epidemiological studies to add weight to the EDC hypothesis. Similarly, Raghavan conducted a literature review and concluded that pharmaceuticals have been associated with hypospadias risk and data on dichlorodiphenyltrichloroethane and hexaclorobenzene pesticides, as well as non-persistent pollutants, particularly phthalates, also suggests associations with hypospadias (Raghavan et al., 2018). However, they also cautioned that improvements in assessment of EDC exposure, timing during development and the use of clear identification and classification schemes are required to increase the evidence of a link between EDC exposure and male reproductive health. It is difficult to establish a cause-effect relationship between EDCs and hypospadias due to a number of confounding factors. EDCs are ubiquitous within our environment but vary in their concentrations and mixes and their subsequent impact, making it difficult to assign phenotypes to an isolated EDC. Within epidemiological studies, control groups are not always available or included, and genetic causes and susceptibilities are not often accounted for. Metabolism, body composition, lifestyle choices such as smoking and genetic susceptibility can all make the effects of EDCs highly variable between individuals (Kalfa et al., 2015). It is clear that there needs to be collaboration between epidemiological, toxicological and developmental studies to establish a definitive causative role and uncover mechanistic links between EDCs and hypospadias.

Impacts of EDCs can differ between occupational and environmental exposures, involving differences in the route, dose, duration and frequency of exposure (Garcia et al., 2017). Limited data exists on the risk of hypospadias with environmental exposures, in contrast to numerous studies evaluating associations between occupational exposure during pregnancy and hypospadias (Estors Sastre et al., 2019; Morales-Suarez-Varela et al., 2011; Nassar et al., 2010). Analysis of the relationship between risk of hypospadias and maternal occupation with regard to EDC exposure found little evidence for a correlation, but the authors cautioned that the exposure classification was crude and warranted further studies (Vrijheid et al., 2003). Kalfa et al. (2015) studied a cohort of isolated hypospadias without genetic confounders and found that occupational and environmental exposures to EDCs during fetal life was a significant risk factor for hypospadias. Detergents, pesticides and cosmetics accounted for 75% of the cases, and maternal exposure was a greater risk factor than paternal exposure. Furthermore, severity of hypospadias and exposure levels were not a linear dose–effect relationship, indicating that even low exposure may have potent effects.

Nassar et al. (2010) assessed occupational exposure to EDCs in both parents in a job-exposure matrix. They demonstrated an association between maternal occupation exposure to heavy metal and phthalates and hypospadias, and an association between paternal occupation exposure to polychlorinated organic and bi-phenolic compounds. Giordano et al. (2010) also confirmed that a risk of hypospadias was associated with perinatal maternal occupational exposures to EDCs. Although the number of cases was below 100, serum from mothers of infants with hypospadias and controls was analysed for dichlorodiphenyldichloroethylene, hexachlorobenzene, and several polychlorinated biphenyl congeners, with evidence for an association between maternal EDC exposure and hypospadias. Furthermore, prevalence rates of hypospadias are significantly greater in agricultural areas with high use of pesticides (Garcia et al., 2017; Kraft et al., 2010; Kristensen et al., 1997; Morales-Suarez-Varela et al., 2011). In a case-control study of over 1600 newborn males, Gaspari et al found male genital malformations, including cryptorchidism, micropenis and hypospadias, were significantly associated with pesticide exposures (Gaspari et al., 2011).

EDCs associated with hypospadias

Over the last 5–10 years there has been an exponential increase in research into the effects of potential EDCs on male reproductive health. Here, our focus is on recent studies of potential associations between exposure to EDCs and hypospadias and investigating the molecular impacts these exposures have on penis development (Table 1).

Pharmaceutical agents

Pharmaceutical EDCs are a heterogeneous group of compounds with mostly estrogenic and anti-androgenic properties. Estrogenic

Table 1
EDCs described in this review and their impacts on hypospadias. EDCs listed represent the most extensively studied EDCs that impact male reproductive development and in particular are associated with increased rates of hypospadias. It should be noted that this is not an exhaustive list and many other EDCs exist with plausible links to hypospadias but require further study to confirm a definitive link. It should also be noted that although chemicals are often classified as either estrogen agonists or anti-androgens, estrogen agonists also suppress androgen output from the testis.

EDCs	Sources	Mechanism	Impact on urethra development	References
Atrazine	Herbicide	ER agonist	Association with hypospadias	(Agopian et al., 2013; Garcia et al., 2017; Govers et al., 2019a; Kraft et al., 2010; Kristensen et al., 1997; Morales-Suarez-Varela et al., 2011)
BPA	Polycarbonate plastics, epoxy resins, plastic toys and bottles, lining of food cans	ER agonist Antiandrogen	Association with hypospadias	(Choi et al., 2012; Fernandez et al., 2016; Liu et al., 2015)
Clomiphene, progestins, oral contraceptives	Pharmaceuticals	ER agonist/ antagonist	Mixed results -Moderate and severe hypospadias increased	(Carmichael et al., 2005; Meijer et al., 2006; van Rooij et al., 2013)
DES	Historical pharmaceutical	ER agonist	Hypospadias	(Kalfa et al., 2011a; Klip et al., 2002; Mahawong et al., 2014; Palmer et al., 2005; Vorherr et al., 1979)
DDT	Contaminated water, soil, fish, crops	ER agonist Antiandrogen	Association with hypospadias	(Bornman et al., 2010; Fernandez et al., 2007)
Genistein	Phytoestrogen in legumes and clover	ER agonist	Hypospadias	(Michikawa et al., 2019; Moule, 1961; North and Golding, 2000; Padilla-Banks et al., 2012; Ross et al., 2011; Vilela et al., 2007)
Parabens	Cosmetics and pharmaceutical products, toothpaste and food preservatives	ER agonist	Possible association with hypospadias	(Fernandez et al., 2016)
PBB, PBDE	Flame retardants	ER agonist/ antagonist Antiandrogen	Association with hypospadias	(Koren et al., 2019; Poon et al., 2018)
Phthalates	Contaminated food, PVC plastics and flooring, personal care, medical devices	ER agonist/ antagonist Antiandrogen	Strong association with hypospadias	(Jiang et al., 2016; Nassar et al., 2010; Ormond et al., 2009; Qian et al., 2020; Raghavan et al., 2018; Sathyanarayana et al., 2017; van den Driesche et al., 2017; Vrijheid et al., 2003; Zhu et al., 2009, 2020)
Vinclozolin	Fungicide	ER agonist Antiandrogen	Hypospadias	(Amato et al., 2018; Gray et al., 1999; Kelce et al., 1994; Vilela et al., 2007; Yang et al., 2019)

pharmaceuticals were some of the first EDCs to be studied in relation to their association with hypospadias. Diethylstilbestrol (DES) is a synthetic non-steroidal estrogen that is associated with an increased risk of reproductive abnormalities, including hypospadias, in males exposed in utero (Klip et al., 2002; Palmer et al., 2005). However, the degree of hypospadias risk is still being investigated. In a small cohort of sons of French men prenatally exposed to DES, cryptorchidism and hypoplasia of the penis was increased, but not hypospadias (Tournaire et al., 2018). Hypospadias has also been reported in rats treated prenatally with DES (Vorherr et al., 1979).

Pharmaceuticals such as clomiphene (Meijer et al., 2006; Sorensen et al., 2005), a selective estrogen receptor modulator, progestins (Carmichael et al., 2005; Given et al., 2016) and oral contraceptives (Carmichael et al., 2005; Norgaard et al., 2009; van Rooij et al., 2013; Wogelius et al., 2006), along with a range of non-steroidal drugs (Blotiere et al., 2019; Bouty et al., 2015; Raghavan et al., 2018) have all been implicated in increasing hypospadias risk, although data is not consistent. In rats, the non-steroidal anti-androgen flutamide causes hypospadias consistent with perturbation of processes in both epithelial and mesenchymal tissue involved in normal penile development (Sinclair et al., 2017). Similarly, prenatal exposure of rats to the anti-androgen finasteride, used in various treatments including hair loss and benign prostatic hyperplasia in men, specifically inhibited DHT-mediated development, resulting in multiple reproductive malformations including hypospadias (Bowman et al., 2003). Heterogeneity between studies is often due to confounding elements such as genetic susceptibility, that make it difficult to confirm associations between pharmaceutical use in pregnancy and hypospadias (Raghavan et al., 2018). Ibuprofen, an antiandrogen, causes transcriptional repression in the human fetal testes, resulting in the clinical condition compensated hypogonadism (Kristensen et al., 2018). Ibuprofen and other mild analgesics administered during pregnancy are also associated with shorter anogenital distance (AGD) in male offspring which, in turn, is associated with genital malformations such as cryptorchidism and hypospadias (Lind et al., 2017).

Genistein

Genistein is an isoflavone phytoestrogen that is commonly found in all legumes, with specifically high levels in soybeans and their products (Fukutake et al., 1996). Along with other isoflavones, genistein is also abundant in a key feed crop for ruminant animals; subterranean clover (Curnow, 1954; Wang et al., 2008). Genistein is able to cross the placenta (Balakrishnan et al., 2010), has a similar structure to human estrogen (Barnes, 2004) and has a high relative affinity for ERα, 150–300 fold higher to ERα than other isoflavones (Scippo et al., 2004). Genistein consumption has been associated with increased rates of hypospadias in humans (Michikawa et al., 2019; North and Golding, 2000), mice (Padilla-Banks et al., 2012; Ross et al., 2011; Vilela et al., 2007) and in sheep (Moule, 1961). As the consumption of soy products increases in western populations, understanding the role of genistein in inducing hypospadias becomes even more important (Huang et al., 2016).

Vegetarian and vegan mothers, whose intake of genistein is higher than the general population, have a 4-fold elevated risk that their sons would be born with hypospadias (North and Golding, 2000). However, Carmichael et al. associated an increased soy intake with a decrease in hypospadias risk (Carmichael et al., 2013a). A prospective birth cohort study of Japanese mothers found an association between low genistein intake and hypospadias (Michikawa et al., 2019). However, neither of these studies included first degree 'mild' hypospadias, which account for a significant majority of phenotypes. Several smaller studies have concluded that maternal ingestion of either legumes or soy based products was not associated with either an increased or decreased risk of hypospadias (Giordano et al., 2008; Pierik et al., 2004), however both of these studies were limited in their heterogeneous EDC exposure and retrospective self-reporting of genistein consumption. *In utero* exposure to genistein induces hypospadias and other genital abnormalities in mouse models (Padilla-Banks et al., 2012; Ross et al., 2011; Vilela et al., 2007). Hypospadias was observed in approximately 25% mice that were fed a diet comprising genistein but was not observed in a

control group with a soya-free diet (Vilela et al., 2007). Genistein exposure contributes to hypospadias in mice by altering pathways of tissue morphogenesis, cell proliferation and cell survival (MAPK, TGF-beta, FOXO, HOX and ER) (Ross et al., 2011).

Bisphenol A (BPA)

One of the most common EDCs that humans are exposed to is the plasticiser Bisphenol A (BPA). BPA binds to both ERα and Erβ (Acconcia et al., 2015), along with several residue binding sites for AR and PGR (Rehan et al., 2015). Gestational exposure to BPA has been associated with a wide range of developmental abnormalities, including male reproductive tract abnormalities and fertility (Meli et al., 2020; Santoro et al., 2019). While only a few studies have analysed the association of BPA exposure with hypospadias in humans, these indicate that mothers with high concentrations of BPA in urine, blood or placenta have male offspring with significantly higher risks of developing hypospadias (Choi et al., 2012; Fernandez et al., 2016). A genome-wide screen using human foreskin fibroblast cells derived from hypospadic patients identified inhibition of a specific matrix metallopeptidase *MMP11*, a known effector of development (Qin et al., 2012). Exposure to BPA during sexual differentiation in male mice induced hypospadias along with reduced AGD, reduced testicular weight, decreased testosterone levels and elevate estradiol. This was combined with decreased expression of androgen dependent genes related to male sexual development - *WT1*, *LHR*, *17BHSD3* and *SRD5A2* (Liu et al., 2015). Gestational exposure to BPA in rats did not induce hypospadias, although this was only at relatively low doses (2–200 ug/kg/day) (Howdeshell et al., 2008). Indeed, studies into the effect of low dose BPA exposures on reproductive health show inconsistent results and require further investigation (Hong and Yang, 2017).

Phthalates

Phthalates belong to a group of compounds known as non-persistent organic pollutants, EDCs that have a short residence time in the environment and short half-lives in the human body (Gregoraszczuk and Ptak, 2013). They are ubiquitous in synthetic products as they are widely used as plasticisers and are one of the most widely studied EDCs in relation to male reproductive health effects (Czubacka et al., 2021; Foster et al., 2017). The primary phthalate pollutants in the environment are Σdi-2(ethylhexyl)-phthalate (DEHP) and Σdibutyl phthalate (DBP) (Gao and Wen, 2016; Szewczynska et al., 2020).

Phthalates accumulate within the body and their metabolites are capable of crossing the placenta, affecting embryo development (Qian et al., 2020). Phthalate metabolites activate ERα, ERβ and AR, making them prime candidates for disrupting penile development (Engel et al., 2017; Takeuchi et al., 2005). Many reproductive abnormalities may be induced by phthalate exposure during pregnancy, including shortened anogenital distance, cryptorchidism and hypospadias (Czubacka et al., 2021; Qian et al., 2020; Skakkebaek et al., 2016). Phthalate exposure disrupts testicular androgen production in mice, lowering sperm quality and reducing fertility (Barakat et al., 2017, 2019). Multiple studies in humans have linked maternal blood concentrations of DEHP and the metabolite MEHP to higher incidences of male offspring with reduced anogenital distance and reduced overall penis size, and a reduced volume of the penis at birth, similar in nature to BPA (Bustamante-Montes et al., 2013; Suzuki et al., 2012). Replication of these studies in rats also indicated that maternal exposure of DEHP over the critical window of hormone dependent development of the GT had a higher instance of undescended testis and reduced anogenital distance (Ema and Miyawaki, 2001).

Occupational exposure studies have indicated an association between exposure to estrogenic or anti-androgenic phthalates and hypospadias. A high rate of hypospadias was observed in children whose mothers were exposed to phthalates, particularly mothers working in the hairdressing, beauty, or cleaning industries, during pregnancy (Nassar et al., 2010; Ormond et al., 2009; Vrijheid et al., 2003).

Cryptorchidism and hypospadias are associated with increased amniotic concentration of *INSL3*, a testis specific biomarker impacted by phthalate endocrine disruptors, during gestation (Anand-Ivell et al., 2018). The authors conclude that phthalates may be altering the dynamics of testicular development and hormone production, leading to hypospadias. Sathyanarayana et al also examined the relationship between phthalates and estrogen and testosterone levels during early fetal development (Sathyanarayana et al., 2017). They found that DEHP induced lower testosterone levels resulted in a higher prevalence of male genital abnormalities, including hypospadias. Similarly, hypospadias were also observed after DBP-induced androgen deprivation during the masculinizing window in rats (van den Driesche et al., 2017). Although these studies only addressed the indirect effects of EDC exposure on the penis, the estrogenic and antiandrogenic properties of phthalates also have the potential to directly impact development of the GT leading to a hypospadias phenotype (Govers et al., 2019b).

More recently, studies have started to uncover the mechanism behind phthalate induced hypospadias. In prenatally DBP exposed rats, Jiang et al found a potential correlation between decreased testosterone levels, reduced *AR* and *Srd5a2* and hypospadias (Jiang et al., 2016). Significantly decreased expression of key signalling molecules in GT formation was observed in DBP induced hypospadias in newborn rats, including sonic hedgehog, fibroblast growth factor and transforming growth factor-beta family members (Zhu et al., 2009). Furthermore, in rats, DBP causes hypospadias via its oxidative stress effect, significantly increasing calcium concentrations and inhibiting epithelial to mesenchymal transition (EMT) in urethral epithelial cells, blocking the fusion process of the urethral groove (Zhu et al., 2020). Prenatal exposure to DBP induces abnormal hedgehog signalling and autophagy in uroepithelial cells which may have an important role in hypospadias development (Li et al., 2017; Zhao et al., 2018, 2019). In mice, an association between miRNAs and mice has been uncovered. Male mice with hypospadias have significantly higher miR-494 and lower *Nedd4L* expression, a target gene of miR-494. Down regulation of miR-494 exerted protective effects on urethral epithelial cells by impeding cell proliferation and inducing cell apoptosis, indicating that downregulation of miR-494 inhibits TGF-β1/Smad signalling, preventing the development of hypospadias through upregulation of *Nedd4L* (Tian et al., 2020).

Interestingly, postnatal exposure to DEHP from birth in rats led to the complete failure of preputial separation, where the glans of the penis remained fully attached to the prepuce in the dorsal region, forming a 'hood' at adulthood (Moore et al., 2001). This 'hood' foreskin phenotype is consistent with the known effects of *in vivo* estrogen exposure on male embryos in both mice and humans (Phillips et al., 2015; Zheng et al., 2015).

Parabens

Parabens are another group of well described estrogen agonists (Lemini et al., 2003; Routledge et al., 1998) that also act as anti-androgens and can induce a variety of reproductive effects *in vivo*, in both male and female offspring during developmental exposure (Boberg et al., 2020). An association between exposure to parabens and both cryptorchidism and hypospadias has also been implicated (Fernandez et al., 2016). It should be noted that there were confounding factors identified in this study and no other studies have assessed a direct association between hypospadias and parabens. However, a number of earlier studies indicated no significant effects were seen on male reproductive outcomes following exposure to parabens (Janjua et al., 2007; Meeker et al., 2011). Interestingly, in animal

studies, delayed preputial separation was observed in rats exposed to butylparaben, a paraben that has more than one modality, including estrogenic and anti-androgenic actions (Boberg et al., 2020; Maske et al., 2020; Zhang et al., 2014). An early disruption in preputial separation and mild hypospadias was also observed in aromatase knockout mice, demonstrating a requirement for correct estrogen signalling for normal preputial development and urethral closure (Cripps et al., 2019). Exposure to butylparaben in rats also resulted in delayed testicular descent, and decreased sperm count, motility and daily sperm production (Maske et al., 2020). Significantly perturbed expression of a number of testicular genes was also observed, along with increased testosterone and LH levels, resulting in subfertility (Maske et al., 2020). Numerous studies in rats and mice have also shown effects of parabens on spermatogenesis (Guerra et al., 2017; Oishi, 2001, 2002; Zhang et al., 2014) and a reduced AGD (Boberg et al., 2016; Kang et al., 2002; Yang et al., 2016; Zhang et al., 2014). Therefore, the estrogenic/anti-androgenic actions of parabens observed in rats and mice, in combination with the suggested association with hypospadias (Fernandez et al., 2016), points to a need for further animal studies to understand their impacts on male reproduction, including development of the penis.

Flame retardants

Accumulating evidence suggests that exposure to flame retardants, such as polybrominated biphenyl (PBB), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), during critical windows of development may be associated with impacts on male reproduction and fertility (Hales and Robaire, 2020). PBDEs persistence in the environment and ability to bioaccumulate, along with their adverse impacts on human health, led governments to restrict their use worldwide (Covaci et al., 2011). Gestational exposure to eight PBDEs was measured in mothers of boys diagnosed with hypospadias. Total PBDE levels were significantly higher (up to 48%) among mothers of infants with hypospadias than among controls (Koren et al., 2019; Poon et al., 2018). A similar study found that maternal PBDE levels were not significantly different between women with infants with hypospadias and women with unaffected infants, however only 20 hypospadias infants were included in this study (Carmichael et al., 2010). Similarly, despite effects on other male genitourinary conditions, no significant association was seen between PBB exposure and either cryptorchidism and hypospadias in humans (Small et al., 2009). A novel study that used a combination of epidemiological and *in vitro* studies showed that PBB153 exposure alters the epigenome by disrupting methyltransferase activity leading to defects in imprint establishment causing altered gene expression, therefore having the potential to contribute to reproductive, developmental and endocrine issues in offspring (Greeson et al., 2020). Inconsistencies between epidemiological studies into flame retardants as outlined above point to a need for further studies combining animal experimental models with epidemiological studies to define their mechanistic link with hypospadias.

Solvents

Tetrachloroethylene (PCE) is a solvent used in dry cleaning, textile processing, and metal degreasing (Aschengrau et al., 2008). Studies of residents in the USA exposed to PCE contaminated drinking water showed that mothers with high levels of PCE exposure had increased risk of having a child with hypospadias (Aschengrau et al., 2018). These findings support previous studies that found an increased risk of birth defects following occupational prenatal exposure to solvents (Chevrier et al., 2006; Desrosiers et al., 2012) and highlight the risk of environmental contaminants inducing birth defects, including hypospadias, over several decades.

Agricultural chemicals

Atrazine is a commonly used pesticide throughout Australia and the USA and is a persistent organic pollutant that has a long half-life in the environment, up to 228 days in soil (Solomon et al., 2008). Due to the widespread use of atrazine, several lakes, subterranean water stores and human drinking supplies have become contaminated (Benotti et al., 2009; Solomon et al., 2008). A higher prevalence and risk of hypospadias was observed in children of parents in areas of high pesticide exposure (Garcia et al., 2017), confirming previous studies that identified an association between prenatal pesticide exposure and hypospadias prevalence (Kraft et al., 2010; Kristensen et al., 1997; Morales-Suarez-Varela et al., 2011).

The endocrine disrupting effects of atrazine have been well documented across amphibians (Hayes et al., 2011), fish (Van Der Kraak et al., 2014), reptiles (Solomon et al., 2008) rodents (Cook et al., 2019; Jin et al., 2013) and mammals (Cook et al., 2020). Atrazine can have a wide range of effects resulting in a disruption in the fine balance of hormones in the reproductive tract. Atrazine increases the expression of *Cyp19a1*, leading to an increase in expression of the enzyme aromatase which is responsible for the conversion of testosterone to oestradiol (Holloway et al., 2008). Atrazine also suppresses *Srd5a2* in rats leading to a reduction in the conversion of testosterone into dihydrotestosterone (DHT) (Holloway et al., 2008; Jin et al., 2013; Kniewald et al., 1995). Atrazine exposure during pregnancy induces a shortening of penis structures and increases the incidence of hypospadias in mice (Govers et al., 2019a). An association between hypospadias and estimated medium levels of maternal atrazine exposure has been identified in humans, particularly for more severe, posterior hypospadias (Agopian et al., 2013). However, a subsequent study showed only a very weak association between drinking water atrazine and hypospadias (Winston et al., 2016). Both of these studies highlight the need for further research using improved exposure characterization to confirm an association and define a mechanism of atrazine action.

Exposures to antiandrogens such as vinclozolin, a fungicide, also cause hypospadias in both mice (Vilela et al., 2007) and rats (Gray et al., 1999; Kelce et al., 1994). Vinclozolin is regularly used to induce hypospadias in mouse models to investigate the mechanism of antiandrogen action on the development of the urethra (Amato et al., 2018; Yang et al., 2019). Vinclozolin also affects the sperm epigenome in rats, resulting in long lasting impacts on spermatogenesis across multiple generations (Beck et al., 2017; Ben Maamar et al., 2018; Nilsson et al., 2018; Schuster et al., 2016; Skinner et al., 2019). However, there is still no definitive evidence for vinclozolin induced hypospadias in humans.

Dichlorodiphenyltrichloroethane (DDT) is a well known and controversial pesticide originally used worldwide. Mothers exposed to household DDT spraying had a 33% greater chance of having a son with urogenital malformations, with 11% of boys developing either hypospadias or cryptorchidism (Bornman et al., 2010). In a case control study in Spain, Fernandez et al found an association between high pesticide use, including DDT, and male urogenital malformations, including hypospadias (Fernandez et al., 2007).

Although risk assessments of pesticides are typically based on the no observed adverse effect levels (NOAELs) for single chemicals, exposures are more often to a mixture of several EDCs (Blount et al., 2000). Exposure of rats to a mixture of low doses of five pesticides caused nipple retention and increased incidence and severity of genital malformations, including hypospadias, in male offspring. These effects were severe at dose levels where the individual pesticides caused smaller or no effects when administered alone (Hass et al., 2012).

EDC mixtures

Typically, EDC exposures In humans occur to more than one chemical at any given time and several anti-androgenic chemicals have been

identified as being present as mixtures (MIX) in humans (Blount et al., 2000; Swan et al., 2005), making it crucial to understand the effects of exposure to combined EDCs. Rats exposed to anti-androgens vinclozolin, flutamide and procymidone in a MIX had an increased rate of hypospadias at doses where each of the individual chemicals caused no observable effects (Christiansen et al., 2008). Similarly, the effect of exposure to a MIX of four anti-androgens - DEHP, vinclozolin, prochloraz and finasteride - was synergistic in male rats, resulting in malformation of external sex organs (Christiansen et al., 2009). These studies confirm that anti-androgens act in an additive way with the ability to cause a very high frequency of male reproductive malformations, including hypospadias, when interacting with other similarly acting anti-androgens (Hass et al., 2007; Metzdorff et al., 2007). These MIX findings highlight that risk assessments based on NOAELs for single anti-androgens underestimate the risk of hypospadias.

Anti-androgenic chemicals have the capacity to alter the androgen signalling pathway via multiple mechanisms. *In utero* exposure of rats to DEHP and DBP, both of which inhibit fetal testis hormone production, caused additive effects on differentiation of the male reproductive tract, including hypospadias (Howdeshell et al., 2007). Mixtures of benzyl butyl phthalate (BBP) and linuron (Hotchkiss et al., 2004), or procymidone and DBP (Gray et al., 2004), anti-androgens which act through different mechanisms, also induced hypospadias in a dose-additive manner, indicating that the effect on hypospadias was dose additive, even though the mechanisms of action were different. Similarly, a mixture of seven anti-androgens that alter androgen signalling through diverse mechanisms caused hypospadias in a cumulative dose-additive manner (Rider et al., 2008). In an even more complex study, a mixture of ten chemicals (four pesticides and six phthalates) that elicit their anti-androgenic effects through AR antagonism or inhibition of androgen synthesis, caused hypospadias in a cumulative dose-additive model (Rider et al., 2010). Taken together, these studies suggest that the effects of EDCs on development of the male reproductive tract must take into consideration both exposures to individual chemicals and the cumulative effect of EDCs that are present during the critical period of urethral closure.

Transgenerational impacts of EDC exposures

EDCs impact not only the individual exposed but have the potential to impact the germ line causing effects on subsequent generations. These effects are mediated through epigenetic mechanisms and are termed epigenetic transgenerational inheritance. Epigenetic alterations induced by EDCs include DNA methylation, non-coding RNAs and histone modifications, which together alter the chromatin structure. Transgenerational impacts on male reproductive health following EDC exposure have been identified (Anway et al., 2006; Anway and Skinner, 2008; Guerrero-Bosagna et al., 2012; Nilsson et al., 2018). These findings raise the concern that certain environmental exposures may not only induce hypospadias in the exposed individual, but also create long-lasting epigenetic changes that continue to increase the hypospadias risk for generations to come.

Several studies in both humans and mice have observed an association between maternal exposure to DES, a synthetic estrogen, and increased rates of hypospadias in the next two generations (Kalfa et al., 2011a; Mahawong et al., 2014). This demonstrated that estrogenic exposures can alter the epigenome to impact male reproductive health in subsequent generations. However, no data yet exists on the potential for these effects to be transgenerational. Transgenerational, effects must persist into the F3 generation, since a single exposure to EDCs in utero exposes the fetus and the germ cells which give rise to the F2. As yet, few studies have addressed the question of transgenerational inheritance of hypospadias and the potential for EDC exposure to create persistent epigenetic changes in multiple generations.

DES interferes with histone methylation (Baccarelli and Bollati, 2009), thus suggesting a potential epigenetic mechanism for the action of DES (Bredfeldt et al., 2010). Estrogen receptor activation is also well known to alter the epigenetic landscape (Wong and Walker, 2013), suggesting that estrogenic compounds known to cause hypospadias are equally likely to be making long-lasting changes to the transcriptional environment. Importantly, DNA methylation changes have been identified in hypospadias patients in the androgen receptor (Vottero et al., 2011), along with *SCARB1* and *MYBPH* (Choudhry et al., 2012). A further 25 CpGs were identified in a epigenome-wide association study of 45 hypospadias cases and these were associated with germ layer differentiation, beta-catenin signaling, androgens and reproductive traits (Richard et al., 2020). Epigenetic mechanisms of transgenerational impacts of vinclozolin exposure on spermatogenesis have been extensively studied in rats. Epimutations in DNA methylation, non-coding RNA expression and histone retention have all been observed in germ cells of F3 males following an ancestral exposure to vinclozolin (Beck et al., 2017; Ben Maamar et al., 2018; Nilsson et al., 2018; Schuster et al., 2016; Skinner et al., 2019). Evidence that both vinclozolin, an anti-androgen, and DES, a synthetic estrogen, cause epigenetic changes implies a broad range of EDCs can impact the germ line to cause ongoing health impacts long after the chemical exposure.

Genetic pathways altered by EDCs

Understanding the impacts that EDCs have on genetic pathways regulating urethral closure has also proven challenging. However, some critical penis development genes have been identified which seem to be especially sensitive to the impacts of hormonal disruptions. Among these are the hedgehog family members *SHH* and *IHH*. HH signalling is critical for penis development and patterning (Perriton et al., 2002). Mutations in *SHH* and its downstream signalling pathway mediators are associated with hypospadias and genital abnormalities in humans (Carmichael et al., 2013b) and mice (Perriton et al., 2002). Hormonal manipulations in wallabies and mice impact *SHH* expression and demonstrate that it is under control from both androgen and estrogen signalling. SHH is supressed by androgens during normal penis development (Chen et al., 2018c) and is upregulated by estrogens (Cripps et al., 2019). Similarly IHH is a downstream target of androgen signalling in the development of the penis (Zheng et al., 2015). Given the fundamental role of HH signalling in the development and outgrowth of the penis, it is likely that this pathway is a major target of both estrogenic and ant-androgenic EDCs in the development of hypospadias.

The WNT, FGF and BMP signalling pathways play fundamental roles in the development and patterning of many tissues across the body, including the penis. These pathways are interconnected with HH signalling and there is considerable crosstalk between their gene networks. Not surprisingly then, several members of the WNT, FGF and BMP signalling pathways are sensitive to hormonal perturbations during development and are known to be associated with hypospadias in both humans and mouse models (Fig. 4) (Beleza-Meireles et al., 2007; Haraguchi et al., 2000; Li et al., 2006; Person et al., 2010; Petiot et al., 2005; Yamaguchi et al., 1999). Hormonal manipulation in the wallaby has shown that, like HH signalling, these pathways can be regulated by both androgen and estrogen signalling (Chen et al., 2018b) again highlighting the range of EDCs which can then impact these pathways during development. Among the most impacted genes are *Wnt4* which is activated by androgens, *Wnt5a* which is supressed by androgens, while *Wif1* and *Wnt7a* are supressed by exogenous estrogen in the developing penis (Chen et al., 2018b). Interestingly *Fgfr2* appears to be supressed by endogenous estrogens during normal penis development in mice, and was activated in the development of distal hypospadias in the estrogen receptor alpha

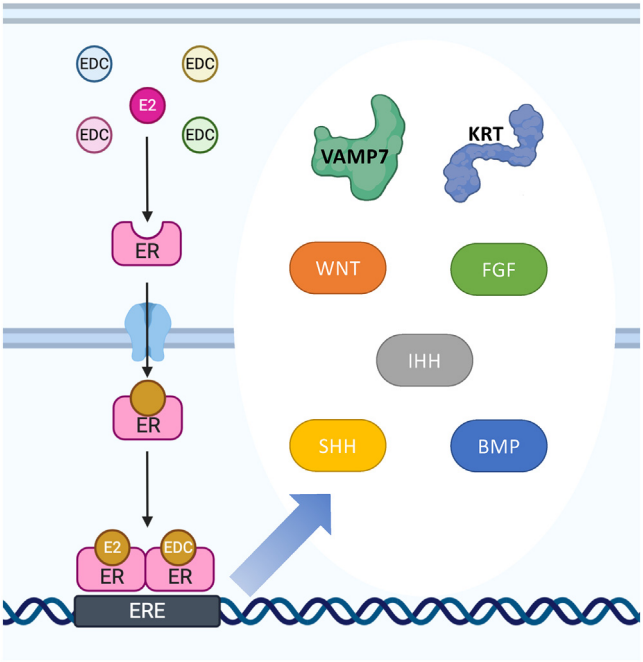

Fig. 4. EDCs impact hormonal signaling to alter key patterning pathways and genes mediating penis development. The figure shows the estrogen signaling pathways which are best studies with regards to penis development and the impact of EDCs. EDCs or native ligands (estradiol - E2) bind to the estrogen receptor (ER) in the cytoplasm of target cells. This complex then translocates into the nucleus and dimerizes. Estrogen receptor diamers can bind to estrogen response elements (EREs) contained within the genome to alter gene expression (blue arrow). Estrogenic EDS in particular are known to impact key patterning pathways including FGF, BMP, WNT and HH as well as VAMP7 and keratin genes ultimately leading to defective penis development and hypospadias. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

knockout mouse (Cripps et al., 2019). Exogenous estrogen also supresses *Bmp5*, and its neighbouring lnc-BMP5 during penis development in the tammar wallaby suggesting some of the impacts of estrogen might be mediated by regulatory lncRNAs (Chen et al., 2018a).

Several other genes have been identified that have key roles in penis development and are also hormonally responsive, and sit outside of the HH, FGF and WNT signalling pathways. These are *VAMP7* and the keratin genes (Fig. 4). *VAMP7* copy number variation is associated with hypospadias in humans (Chavez-Lopez et al., 2020; Tannour-Louet et al., 2014) and gene duplications cause an upregulation of estrogen receptor alpha which in turn causes an upregulation of estrogen-responsive genes (Tannour-Louet et al., 2014). Similar to *FgfR2*, endogenous estrogen in normal male penis development appears to supress *Vamp7* levels, while in the absence of estrogen signalling, *Vamp7* was significantly increased. Together, these studies illustrate a potential feedback mechanism between VAMP7 and estrogen receptor levels which is required for normal penis development. It also appears that endogenous estrogen in the developing penis is required to regulate keratin gene expression, with both *Krt6a* and *Krt8* significantly impacted by a loss of estrogen signalling in the developing penis. In mice, this leads to a shift in the developmental timing of critical delamination events in the developing penis and an eventual distal hypospadias phenotype (Cripps et al., 2019).

Conclusions

Development of the male urethra and patterning of the penis is an exquisitely hormonally sensitive process. The identification of hormonally regulated genes has been critical for our understanding of normal penis development as well as the manifestation of hypospadias. Many of these hormonally sensitive genes sit in fundamental patterning pathways and appear to be regulated by both androgen and estrogen signalling. Perhaps then it is not so surprising that chemicals that interfere with androgen and estrogen levels during development can easily result in hypospadias, providing a plausible causative agent for the incidence and increasing levels of this disease over recent decades. Hypospadias would be expected to result from estrogen agonists as well as antagonists, and androgen agonists as well as antagonists. Alarmingly, these EDCs also have the ability to alter the epigenetic landscape, resulting in hypospadias in successive, unexposed generations. As the number of potential EDCs continues to increase there is an urgent need to improve assessment of exposures, define critical exposure windows and use consistent methodologies and hypospadias classification schemes. The collaboration of toxicological, developmental and epidemiological scientists is crucial to allow the collation of data across studies and enable the identification of clear dose-response relationships.

CRediT authorship contribution statement

Deidre M. Mattiske: Writing - original draft, Writing - review & editing. **Andrew J. Pask:** Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This work was supported by the U.S. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Grant R01DK096263 (to A.J.P.).

References

Acconcia, F., Pallottini, V., Marino, M., 2015. Molecular Mechanisms of Action of BPA. Dose Response 13, 1559325815610582.
Agopian, A.J., Lupo, P.J., Canfield, M.A., Langlois, P.H., 2013. Case-control study of maternal residential atrazine exposure and male genital malformations. Am. J. Med. Genet. A 161A, 977–982.
Amato, C.M., Boyd, M., Yang, J., McCoy, K.A., 2018. Organizational effects of the antiandrogen, vinclozolin, on penis development in the mouse. Biol. Reprod. 99, 639–649.
Anand-Ivell, R., Cohen, A., Norgaard-Pedersen, B., Jonsson, B.A.G., Bonde, J.P., Hougaard, D.M., Lindh, C.H., Toft, G., Lindhard, M.S., Ivell, R., 2018. Amniotic fluid INSL3 measured during the critical time window in human pregnancy relates to cryptorchidism, hypospadias, and phthalate load: a large case-control study. Front. Physiol. 9, 406.
Anway, M.D., Memon, M.A., Uzumcu, M., Skinner, M.K., 2006. Transgenerational effect of the endocrine disruptor vinclozolin on male spermatogenesis. J. Androl. 27, 868–879.
Anway, M.D., Skinner, M.K., 2008. Transgenerational effects of the endocrine disruptor vinclozolin on the prostate transcriptome and adult onset disease. Prostate 68, 517–529.
Aschengrau, A., Gallagher, L.G., Winter, M., Butler, L., Patricia Fabian, M., Vieira, V.M., 2018. Modeled exposure to tetrachloroethylene-contaminated drinking water and the occurrence of birth defects: a case-control study from Massachusetts and Rhode Island. Environ. Health 17, 75.
Aschengrau, A., Weinberg, J., Rogers, S., Gallagher, L., Winter, M., Vieira, V., Webster, T., Ozonoff, D., 2008. Prenatal exposure to tetrachloroethylene-contaminated drinking water and the risk of adverse birth outcomes. Environ. Health Perspect. 116, 814–820.
Attina, T.M., Hauser, R., Sathyanarayana, S., Hunt, P.A., Bourguignon, J.P., Myers, J.P., DiGangi, J., Zoeller, R.T., Trasande, L., 2016. Exposure to endocrine-disrupting chemicals in the USA: a population-based disease burden and cost analysis. Lancet Diabetes Endocrinol. 4, 996–1003.
Baccarelli, A., Bollati, V., 2009. Epigenetics and environmental chemicals. Curr. Opin. Pediatr. 21, 243–251.

Balakrishnan, B., Thorstensen, E.B., Ponnampalam, A.P., Mitchell, M.D., 2010. Transplacental transfer and biotransformation of genistein in human placenta. Placenta 31, 506–511.
Barakat, R., Lin, P.P., Rattan, S., Brehm, E., Canisso, I.F., Abosalum, M.E., Flaws, J.A., Hess, R., Ko, C., 2017. Prenatal exposure to DEHP induces premature reproductive senescence in male mice. Toxicol. Sci. 156, 96–108.
Barakat, R., Seymore, T., Lin, P.P., Park, C.J., Ko, C.J., 2019. Prenatal exposure to an environmentally relevant phthalate mixture disrupts testicular steroidogenesis in adult male mice. Environ. Res. 172, 194–201.
Barnes, S., 2004. Soy isoflavones–phytoestrogens and what else?. J. Nutr. 134, 1225S–1228S.
Baskin, L., 2000. Hypospadias and urethral development. J. Urol. 163, 951–960.
Baskin, L.S., Ebbers, M.B., 2006. Hypospadias: anatomy, etiology, and technique. J. Pediatr. Surg. 41, 463–472.
Baskin, L.S., Erol, A., Jegatheesan, P., Li, Y., Liu, W., Cunha, G.R., 2001. Urethral seam formation and hypospadias. Cell Tissue Res. 305, 379–387.
Beck, D., Sadler-Riggleman, I., Skinner, M.K., 2017. Generational comparisons (F1 versus F3) of vinclozolin induced epigenetic transgenerational inheritance of sperm differential DNA methylation regions (epimutations) using MeDIP-Seq. Environ. Epigenet. 3.
Beleza-Meireles, A., Lundberg, F., Lagerstedt, K., Zhou, X., Omrani, D., Frisen, L., Nordenskjold, A., 2007. FGFR2, FGF8, FGF10 and BMP7 as candidate genes for hypospadias. Eur. J. Hum. Genet. 15, 405–410.
Ben Maamar, M., Sadler-Riggleman, I., Beck, D., McBirney, M., Nilsson, E., Klukovich, R., Xie, Y., Tang, C., Yan, W., Skinner, M.K., 2018. Alterations in sperm DNA methylation, non-coding RNA expression, and histone retention mediate vinclozolin-induced epigenetic transgenerational inheritance of disease. Environ. Epigenet. 4. dvy010.
Benotti, M.J., Trenholm, R.A., Vanderford, B.J., Holady, J.C., Stanford, B.D., Snyder, S. A., 2009. Pharmaceuticals and endocrine disrupting compounds in U.S. drinking water. Environ. Sci. Technol. 43, 597–603.
Bergman, A., Andersson, A.M., Becher, G., van den Berg, M., Blumberg, B., Bjerregaard, P., Bornehag, C.G., Bornman, R., Brandt, I., Brian, J.V., Casey, S.C., Fowler, P.A., Frouin, H., Giudice, L.C., Iguchi, T., Hass, U., Jobling, S., Juul, A., Kidd, K.A., Kortenkamp, A., Lind, M., Martin, O.V., Muir, D., Ochieng, R., Olea, N., Norrgren, L., Ropstad, E., Ross, P.S., Ruden, C., Scheringer, M., Skakkebaek, N.E., Soder, O., Sonnenschein, C., Soto, A., Swan, S., Toppari, J., Tyler, C.R., Vandenberg, L.N., Vinggaard, A.M., Wiberg, K., Zoeller, R.T., 2013. Science and policy on endocrine disrupters must not be mixed: a reply to a “common sense” intervention by toxicology journal editors. Environ. Health 12, 69.
Bergman, A., Becher, G., Blumberg, B., Bjerregaard, P., Bornman, R., Brandt, I., Casey, S. C., Frouin, H., Giudice, L.C., Heindel, J.J., Iguchi, T., Jobling, S., Kidd, K.A., Kortenkamp, A., Lind, P.M., Muir, D., Ochieng, R., Ropstad, E., Ross, P.S., Skakkebaek, N.E., Toppari, J., Vandenberg, L.N., Woodruff, T.J., Zoeller, R.T., 2015. Manufacturing doubt about endocrine disrupter science–A rebuttal of industry-sponsored critical comments on the UNEP/WHO report “State of the Science of Endocrine Disrupting Chemicals 2012”. Regul. Toxicol. Pharmacol. 73, 1007–1017.
Bjerregaard, P., Korsgaard, B., Christiansen, L.B., Pedersen, K.L., Christensen, L.J., Pedersen, S.N., Horn, P., 1998. Monitoring and risk assessment for endocrine disruptors in the aquatic environment: a biomarker approach. Arch. Toxicol. Suppl. 20, 97–107.
Blotiere, P.O., Raguideau, F., Weill, A., Elefant, E., Perthus, I., Goulet, V., Rouget, F., Zureik, M., Coste, J., Dray-Spira, R., 2019. Risks of 23 specific malformations associated with prenatal exposure to 10 antiepileptic drugs. Neurology 93, e167–e180.
Blount, B.C., Silva, M.J., Caudill, S.P., Needham, L.L., Pirkle, J.L., Sampson, E.J., Lucier, G.W., Jackson, R.J., Brock, J.W., 2000. Levels of seven urinary phthalate metabolites in a human reference population. Environ. Health Perspect. 108, 979–982.
Boberg, J., Axelstad, M., Svingen, T., Mandrup, K., Christiansen, S., Vinggaard, A.M., Hass, U., 2016. Multiple endocrine disrupting effects in rats perinatally exposed to butylparaben. Toxicol. Sci. 152, 244–256.
Boberg, J., Johansson, H.K.L., Axelstad, M., Olsen, G.P.M., Johansen, M., Holmboe, S.A., Andersson, A.M., Svingen, T., 2020. Using assessment criteria for pesticides to evaluate the endocrine disrupting potential of non-pesticide chemicals: case butylparaben. Environ. Int. 144, 105996.
Bonde, J.P., Flachs, E.M., Rimborg, S., Glazer, C.H., Giwercman, A., Ramlau-Hansen, C. H., Hougaard, K.S., Hoyer, B.B., Haervig, K.K., Petersen, S.B., Rylander, L., Specht, I. O., Toft, G., Brauner, E.V., 2016. The epidemiologic evidence linking prenatal and postnatal exposure to endocrine disrupting chemicals with male reproductive disorders: a systematic review and meta-analysis. Hum. Reprod. Update 23, 104–125.
Bornman, R., de Jager, C., Worku, Z., Farias, P., Reif, S., 2010. DDT and urogenital malformations in newborn boys in a malarial area. BJU Int. 106, 405–411.
Bouty, A., Ayers, K.L., Pask, A., Heloury, Y., Sinclair, A.H., 2015. The genetic and environmental factors underlying hypospadias. Sex Dev. 9, 239–259.
Bowman, C.J., Barlow, N.J., Turner, K.J., Wallace, D.G., Foster, P.M., 2003. Effects of in utero exposure to finasteride on androgen-dependent reproductive development in the male rat. Toxicol. Sci. 74, 393–406.
Bredfeldt, T.G., Greathouse, K.L., Safe, S.H., Hung, M.C., Bedford, M.T., Walker, C.L., 2010. Xenoestrogen-induced regulation of EZH2 and histone methylation via estrogen receptor signaling to PI3K/AKT. Mol. Endocrinol. 24, 993–1006.
Bustamante-Montes, L.P., Hernandez-Valero, M.A., Flores-Pimentel, D., Garcia-Fabila, M., Amaya-Chavez, A., Barr, D.B., Borja-Aburto, V.H., 2013. Prenatal exposure to phthalates is associated with decreased anogenital distance and penile size in male newborns. J. Dev. Orig. Health Dis. 4, 300–306.
Carlson, B.M., 1994. Human Embryology and Developmental Biology. Mosby, St. Louis.
Carmichael, S.L., Cogswell, M.E., Ma, C., Gonzalez-Feliciano, A., Olney, R.S., Correa, A., Shaw, G.M., National Birth Defects Prevention, S., 2013a. Hypospadias and maternal intake of phytoestrogens. Am J Epidemiol 178, 434–440.
Carmichael, S.L., Herring, A.H., Sjodin, A., Jones, R., Needham, L., Ma, C., Ding, K., Shaw, G.M., 2010. Hypospadias and halogenated organic pollutant levels in maternal mid-pregnancy serum samples. Chemosphere 80, 641–646.
Carmichael, S.L., Ma, C., Choudhry, S., Lammer, E.J., Witte, J.S., Shaw, G.M., 2013b. Hypospadias and genes related to genital tubercle and early urethral development. J. Urol. 190, 1884–1892.
Carmichael, S.L., Shaw, G.M., Laurent, C., Croughan, M.S., Olney, R.S., Lammer, E.J., 2005. Maternal progestin intake and risk of hypospadias. Arch. Pediatr. Adolesc. Med. 159, 957–962.
Chavez-Lopez, S., de Jesus Lugo-Trampe, J., Ibarra-Ramirez, M., Calvo-Anguiano, G., Martinez-de-Villarreal, L.E., Campos-Acevedo, L.D., 2020. A case series of infants with increased VAMP7 gene dosage at birth and virilization defects. J. Pediatr. Urol. 16, 423 e421–423 e426.
Chen, Y., Kuroki, Y., Shaw, G., Pask, A.J., Yu, H., Toyoda, A., Fujiyama, A., Renfree, M. B., 2018a. Androgen and oestrogen affect the expression of long non-coding RNAs during phallus development in a marsupial. Noncoding RNA 5.
Chen, Y., Yu, H., Pask, A.J., Fujiyama, A., Suzuki, Y., Sugano, S., Shaw, G., Renfree, M.B., 2018b. Hormone-responsive genes in the SHH and WNT/beta-catenin signaling pathways influence urethral closure and phallus growth. Biol. Reprod. 99, 806–816.
Chen, Y., Yu, H., Pask, A.J., Fujiyama, A., Suzuki, Y., Sugano, S., Shaw, G., Renfree, M.B., 2018c. Hormone-responsive genes in the SHH and WNT/beta-catenin signaling pathways influence urethral closure and phallus growth. Biol. Reprod..
Chevrier, C., Dananche, B., Bahuau, M., Nelva, A., Herman, C., Francannet, C., Robert-Gnansia, E., Cordier, S., 2006. Occupational exposure to organic solvent mixtures during pregnancy and the risk of non-syndromic oral clefts. Occup. Environ. Med. 63, 617–623.
Choi, H., Kim, J., Im, Y., Lee, S., Kim, Y., 2012. The association between some endocrine disruptors and hypospadias in biological samples. J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng. 47, 2173–2179.
Choudhry, S., Deshpande, A., Qiao, L., Beckman, K., Sen, S., Baskin, L.S., 2012. Genome-wide DNA methylation profiling of CpG islands in hypospadias. J. Urol. 188, 1450–1455.
Christiansen, S., Scholze, M., Axelstad, M., Boberg, J., Kortenkamp, A., Hass, U., 2008. Combined exposure to anti-androgens causes markedly increased frequencies of hypospadias in the rat. Int. J. Androl. 31, 241–248.
Christiansen, S., Scholze, M., Dalgaard, M., Vinggaard, A.M., Axelstad, M., Kortenkamp, A., Hass, U., 2009. Synergistic disruption of external male sex organ development by a mixture of four antiandrogens. Environ. Health Perspect. 117, 1839–1846.
Combarnous, Y., Nguyen, T.M.D., 2019. Comparative overview of the mechanisms of action of hormones and endocrine disruptor compounds. Toxics 7.
Cook, L.E., Chen, Y., Renfree, M., Pask, A.j., 2020. Long-term maternal exposure to atrazine in the drinking water reduces penis length in the tammar wallaby. Reprod. Fertil. Dev. 32, 1099–1107.
Cook, L.E., Finger, B.J., Green, M.P., Pask, A.J., 2019. Exposure to atrazine during puberty reduces sperm viability, increases weight gain and alters the expression of key metabolic genes in the liver of male mice. Reprod. Fertil. Dev. 31, 920–931.
Covaci, A., Harrad, S., Abdallah, M.A., Ali, N., Law, R.J., Herzke, D., de Wit, C.A., 2011. Novel brominated flame retardants: a review of their analysis, environmental fate and behaviour. Environ. Int. 37, 532–556.
Cripps, S.M., Mattiske, D.M., Black, J.R., Risbridger, G.P., Govers, L.C., Phillips, T.R., Pask, A.J., 2019. A loss of estrogen signaling in the aromatase deficient mouse penis results in mild hypospadias. Differentiation 109, 42–52.
Curnow, D.H., 1954. Oestrogenic activity of subterranean clover. II. The isolation of genistein from subterranean clover and methods of quantitative estimation. Biochem. J. 58, 283–287.
Czubacka, E., Czerczak, S., Kupczewska-Dobecka, M.M., 2021. The overview of current evidence on the reproductive toxicity of dibutyl phthalate. Int. J. Occup. Med. Environ. Health 34, 15–37.
Desrosiers, T.A., Herring, A.H., Shapira, S.K., Hooiveld, M., Luben, T.J., Herdt-Losavio, M.L., Lin, S., Olshan, A.F., National Birth Defects Prevention, S., 2012. Paternal occupation and birth defects: findings from the National Birth Defects Prevention Study. Occup. Environ. Med. 69, 534–542.
Dietrich, D.R., von Aulock, S., Marquardt, H., Blaauboer, B., Dekant, W., Kehrer, J., Hengstler, J., Collier, A., Gori, G.B., Pelkonen, O.P., Lang, F., Nijkamp, F.P., Stemmer, K., Li, A., Savolainen, K., Hayes, A., Gooderham, N., Harvey, A., 2013. Scientifically unfounded precaution drives European Commission's recommendations on EDC regulation, while defying common sense, well-established science and risk assessment principles. Toxicol. In Vitro 27, 2110–2114.
Ema, M., Miyawaki, E., 2001. Adverse effects on development of the reproductive system in male offspring of rats given monobutyl phthalate, a metabolite of dibutyl phthalate, during late pregnancy. Reprod. Toxicol. 15, 189–194.
Engel, A., Buhrke, T., Imber, F., Jessel, S., Seidel, A., Volkel, W., Lampen, A., 2017. Agonistic and antagonistic effects of phthalates and their urinary metabolites on the steroid hormone receptors ERalpha, ERbeta, and AR. Toxicol. Lett. 277, 54–63.
Estors Sastre, B., Campillo Artero, C., Gonzalez Ruiz, Y., Fernandez Atuan, R.L., Bragagnini Rodriguez, P., Frontera Juan, G., Gracia Romero, J., 2019. Occupational exposure to endocrine-disrupting chemicals and other parental risk factors in hypospadias and cryptorchidism development: a case-control study. J. Pediatr. Urol. 15, 520 e521–520 e528.

Fernandez, M.F., Arrebola, J.P., Jimenez-Diaz, I., Saenz, J.M., Molina-Molina, J.M., Ballesteros, O., Kortenkamp, A., Olea, N., 2016. Bisphenol A and other phenols in human placenta from children with cryptorchidism or hypospadias. Reprod. Toxicol. 59, 89–95.

Fernandez, M.F., Olmos, B., Granada, A., Lopez-Espinosa, M.J., Molina-Molina, J.M., Fernandez, J.M., Cruz, M., Olea-Serrano, F., Olea, N., 2007. Human exposure to endocrine-disrupting chemicals and prenatal risk factors for cryptorchidism and hypospadias: a nested case-control study. Environ. Health Perspect. 115 (Suppl 1), 8–14.

Foster, W.G., Evans, J.A., Little, J., Arbour, L., Moore, A., Sauve, R., Andres Leon, J., Luo, W., 2017. Human exposure to environmental contaminants and congenital anomalies: a critical review. Crit. Rev. Toxicol. 47, 59–84.

Fukutake, M., Takahashi, M., Ishida, K., Kawamura, H., Sugimura, T., Wakabayashi, K., 1996. Quantification of genistein and genistin in soybeans and soybean products. Food Chem. Toxicol. 34, 457–461.

Gao, D.W., Wen, Z.D., 2016. Phthalate esters in the environment: a critical review of their occurrence, biodegradation, and removal during wastewater treatment processes. Sci. Total Environ. 541, 986–1001.

Garcia, J., Ventura, M.I., Requena, M., Hernandez, A.F., Parron, T., Alarcon, R., 2017. Association of reproductive disorders and male congenital anomalies with environmental exposure to endocrine active pesticides. Reprod. Toxicol. 71, 95–100.

Gaspari, L., Paris, F., Jandel, C., Kalfa, N., Orsini, M., Daures, J.P., Sultan, C., 2011. Prenatal environmental risk factors for genital malformations in a population of 1442 French male newborns: a nested case-control study. Hum. Reprod. 26, 3155–3162.

Giordano, F., Abballe, A., De Felip, E., di Domenico, A., Ferro, F., Grammatico, P., Ingelido, A.M., Marra, V., Marrocco, G., Vallasciani, S., Figa-Talamanca, I., 2010. Maternal exposures to endocrine disrupting chemicals and hypospadias in offspring. Birth Defects Res. A Clin. Mol. Teratol. 88, 241–250.

Giordano, F., Carbone, P., Nori, F., Mantovani, A., Taruscio, D., Figa-Talamanca, I., 2008. Maternal diet and the risk of hypospadias and cryptorchidism in the offspring. Paediatr. Perinat. Epidemiol. 22, 249–260.

Given, J.E., Loane, M., Luteijn, J.M., Morris, J.K., de Jong van den Berg, L.T., Garne, E., Addor, M.C., Barisic, I., de Walle, H., Gatt, M., Klungsoyr, K., Khoshnood, B., Latos-Bielenska, A., Nelen, V., Neville, A.J., O'Mahony, M., Pierini, A., Tucker, D., Wiesel, A., Dolk, H., 2016. EUROmediCAT signal detection: an evaluation of selected congenital anomaly-medication associations. Br. J. Clin. Pharmacol. 82, 1094–1109.

Gore, A.C., Balthazart, J., Bikle, D., Carpenter, D.O., Crews, D., Czernichow, P., Diamanti-Kandarakis, E., Dores, R.M., Grattan, D., Hof, P.R., Hollenberg, A.N., Lange, C., Lee, A.V., Levine, J.E., Millar, R.P., Nelson, R.J., Porta, M., Poth, M., Power, D.M., Prins, G.S., Ridgway, E.C., Rissman, E.F., Romijn, J.A., Sawchenko, P. E., Sly, P.D., Soder, O., Taylor, H.S., Tena-Sempere, M., Vaudry, H., Wallen, K., Wang, Z., Wartofsky, L., Watson, C.S., 2013. Policy decisions on endocrine disruptors should be based on science across disciplines: a response to Dietrich et al. Eur. J. Endocrinol. 169, E1-4.

Gore, A.C., Chappell, V.A., Fenton, S.E., Flaws, J.A., Nadal, A., Prins, G.S., Toppari, J., Zoeller, R.T., 2015a. EDC-2: the endocrine society's second scientific statement on endocrine-disrupting chemicals. Endocr. Rev. 36, E1–E150.

Gore, A.C., Chappell, V.A., Fenton, S.E., Flaws, J.A., Nadal, A., Prins, G.S., Toppari, J., Zoeller, R.T., 2015b. Executive summary to EDC-2: The Endocrine Society's second scientific statement on endocrine-disrupting chemicals. Endocr. Rev. 36, 593–602.

Govers, L.C., Harper, A.P., Finger, B.J., Mattiske, D.M., Pask, A.J., Green, M.P., 2019a. Atrazine induces penis abnormalities including hypospadias in mice. J. Dev. Orig. Health Dis., 1–4.

Govers, L.C., Harper, A.P., Finger, B.J., Mattiske, D.M., Pask, A.J., Green, M.P., 2020. Atrazine induces penis abnormalities including hypospadias in mice. J. Dev. Orig. Health Dis. 11, 246–249.

Govers, L.C., Phillips, T.R., Mattiske, D.M., Rashoo, N., Black, J.R., Sinclair, A., Baskin, L. S., Risbridger, G.P., Pask, A.J., 2019b. A critical role for estrogen signaling in penis development. FASEB J. 33, 10383–10392.

Gray Jr., L.E., Ostby, J., Furr, J., Wolf, C., Lambright, C., Wilson, V., Noriega, N., 2004. Toxicant-induced hypospadias in the male rat. Adv. Exp. Med. Biol. 545, 217–241.

Gray Jr., L.E., Ostby, J., Monosson, E., Kelce, W.R., 1999. Environmental antiandrogens: low doses of the fungicide vinclozolin alter sexual differentiation of the male rat. Toxicol. Ind. Health 15, 48–64.

Greeson, K.W., Fowler, K.L., Estave, P.M., Kate Thompson, S., Wagner, C., Clayton Edenfield, R., Symosko, K.M., Steves, A.N., Marder, E.M., Terrell, M.L., Barton, H., Koval, M., Marcus, M., Easley, C.A.t., 2020. Detrimental effects of flame retardant, PBB153, exposure on sperm and future generations. Sci. Rep. 10, 8567.

Gregoraszczuk, E.L., Ptak, A., 2013. Endocrine-disrupting chemicals: some actions of POPs on female reproduction. Int. J. Endocrinol. 2013, 828532.

Guerra, M.T., Sanabria, M., Leite, G.A., Borges, C.S., Cucielo, M.S., Anselmo-Franci, J.A., Foster, W.G., Kempinas, W.G., 2017. Maternal exposure to butyl paraben impairs testicular structure and sperm quality on male rats. Environ. Toxicol. 32, 1273–1289.

Guerrero-Bosagna, C., Covert, T.R., Haque, M.M., Settles, M., Nilsson, E.E., Anway, M.D., Skinner, M.K., 2012. Epigenetic transgenerational inheritance of vinclozolin induced mouse adult onset disease and associated sperm epigenome biomarkers. Reprod. Toxicol. 34, 694–707.

Hales, B.F., Robaire, B., 2020. Effects of brominated and organophosphate ester flame retardants on male reproduction. Andrology 8, 915–923.

Hamilton, W.J., Boyd, J.D., Mossman, H.W., 1972. Human Embryology. Williams & Wilkins, Baltimore.

Haraguchi, R., Suzuki, K., Murakami, R., Sakai, M., Kamikawa, M., Kengaku, M., Sekine, K., Kawano, H., Kato, S., Ueno, N., Yamada, G., 2000. Molecular analysis of external genitalia formation: the role of fibroblast growth factor (Fgf) genes during genital tubercle formation. Development 127, 2471–2479.

Hass, U., Boberg, J., Christiansen, S., Jacobsen, P.R., Vinggaard, A.M., Taxvig, C., Poulsen, M.E., Herrmann, S.S., Jensen, B.H., Petersen, A., Clemmensen, L.H., Axelstad, M., 2012. Adverse effects on sexual development in rat offspring after low dose exposure to a mixture of endocrine disrupting pesticides. Reprod. Toxicol. 34, 261–274.

Hass, U., Scholze, M., Christiansen, S., Dalgaard, M., Vinggaard, A.M., Axelstad, M., Metzdorff, S.B., Kortenkamp, A., 2007. Combined exposure to anti-androgens exacerbates disruption of sexual differentiation in the rat. Environ. Health Perspect. 115 (Suppl 1), 122–128.

Hayes, T.B., Anderson, L.L., Beasley, V.R., de Solla, S.R., Iguchi, T., Ingraham, H., Kestemont, P., Kniewald, J., Kniewald, Z., Langlois, V.S., Luque, E.H., McCoy, K.A., Munoz-de-Toro, M., Oka, T., Oliveira, C.A., Orton, F., Ruby, S., Suzawa, M., Tavera-Mendoza, L.E., Trudeau, V.L., Victor-Costa, A.B., Willingham, E., 2011. Demasculinization and feminization of male gonads by atrazine: consistent effects across vertebrate classes. J. Steroid. Biochem. Mol. Biol. 127, 64–73.

Holloway, A.C., Anger, D.A., Crankshaw, D.J., Wu, M., Foster, W.G., 2008. Atrazine-induced changes in aromatase activity in estrogen sensitive target tissues. J. Appl. Toxicol. 28, 260–270.

Hong, Y.-P., Yang, Y.-J., 2017. Low-dose exposure to bisphenol a in early life. In: Erkekoglu, P., Kocer-Gumusel, B. (Eds.), Bisphenol A Exposure and Health Risks. IntechOpen.

Hotchkiss, A.K., Parks-Saldutti, L.G., Ostby, J.S., Lambright, C., Furr, J., Vandenbergh, J. G., Gray Jr., L.E., 2004. A mixture of the "antiandrogens" linuron and butyl benzyl phthalate alters sexual differentiation of the male rat in a cumulative fashion. Biol. Reprod. 71, 1852–1861.

Howdeshell, K.L., Furr, J., Lambright, C.R., Rider, C.V., Wilson, V.S., Gray Jr., L.E., 2007. Cumulative effects of dibutyl phthalate and diethylhexyl phthalate on male rat reproductive tract development: altered fetal steroid hormones and genes. Toxicol. Sci. 99, 190–202.

Howdeshell, K.L., Furr, J., Lambright, C.R., Wilson, V.S., Ryan, B.C., Gray Jr., L.E., 2008. Gestational and lactational exposure to ethinyl estradiol, but not bisphenol A, decreases androgen-dependent reproductive organ weights and epididymal sperm abundance in the male long evans hooded rat. Toxicol. Sci. 102, 371–382.

Huang, H., Krishnan, H.B., Pham, Q., Yu, L.L., Wang, T.T., 2016. Soy and gut microbiota: interaction and implication for human health. J. Agric. Food Chem. 64, 8695–8709.

Janjua, N.R., Mortensen, G.K., Andersson, A.M., Kongshoj, B., Skakkebaek, N.E., Wulf, H.C., 2007. Systemic uptake of diethyl phthalate, dibutyl phthalate, and butyl paraben following whole-body topical application and reproductive and thyroid hormone levels in humans. Environ. Sci. Technol. 41, 5564–5570.

Jiang, J.T., Zhong, C., Zhu, Y.P., Xu, D.L., Wood, K., Sun, W.L., Li, E.H., Liu, Z.H., Zhao, W., Ruan, Y., Xia, S.J., 2016. Prenatal exposure to di-n-butyl phthalate (DBP) differentially alters androgen cascade in undeformed versus hypospadiac male rat offspring. Reprod. Toxicol. 61, 75–81.

Jin, Y., Wang, L., Fu, Z., 2013. Oral exposure to atrazine modulates hormone synthesis and the transcription of steroidogenic genes in male peripubertal mice. Gen. Comp. Endocrinol. 184, 120–127.

Kahn, L.G., Philippat, C., Nakayama, S.F., Slama, R., Trasande, L., 2020. Endocrine-disrupting chemicals: implications for human health. Lancet Diabetes Endocrinol. 8, 703–718.

Kalfa, N., Paris, F., Philibert, P., Orsini, M., Broussous, S., Fauconnet-Servant, N., Audran, F., Gaspari, L., Lehors, H., Haddad, M., Guys, J.M., Reynaud, R., Alessandrini, P., Merrot, T., Wagner, K., Kurzenne, J.Y., Bastiani, F., Breaud, J., Valla, J.S., Lacombe, G.M., Dobremez, E., Zahhaf, A., Daures, J.P., Sultan, C., 2015. Is hypospadias associated with prenatal exposure to endocrine disruptors? a french collaborative controlled study of a cohort of 300 consecutive children without genetic defect. Eur. Urol. 68, 1023–1030.

Kalfa, N., Paris, F., Soyer-Gobillard, M.O., Daures, J.P., Sultan, C., 2011a. Prevalence of hypospadias in grandsons of women exposed to diethylstilbestrol during pregnancy: a multigenerational national cohort study. Fertil. Steril. 95, 2574–2577.

Kalfa, N., Philibert, P., Baskin, L.S., Sultan, C., 2011b. Hypospadias: interactions between environment and genetics. Mol. Cell. Endocrinol. 335, 89–95.

Kalfa, N., Sultan, C., Baskin, L.S., 2010. Hypospadias: etiology and current research. Urol. Clin. North Am. 37, 159–166.

Kang, K.S., Che, J.H., Ryu, D.Y., Kim, T.W., Li, G.X., Lee, Y.S., 2002. Decreased sperm number and motile activity on the F1 offspring maternally exposed to butyl p-hydroxybenzoic acid (butyl paraben). J Vet Med Sci 64, 227–235.

Kelce, W.R., Monosson, E., Gamcsik, M.P., Laws, S.C., Gray Jr., L.E., 1994. Environmental hormone disruptors: evidence that vinclozolin developmental toxicity is mediated by antiandrogenic metabolites. Toxicol. Appl. Pharmacol. 126, 276–285.

Klip, H., Verloop, J., van Gool, J.D., Koster, M.E., Burger, C.W., van Leeuwen, F.E., Group, O.P., 2002. Hypospadias in sons of women exposed to diethylstilbestrol in utero: a cohort study. Lancet 359, 1102–1107.

Kniewald, J., Osredecki, V., Gojmerac, T., Zechner, V., Kniewald, Z., 1995. Effect of s-triazine compounds on testosterone metabolism in the rat prostate. J. Appl. Toxicol. 15, 215–218.

Koren, G., Carnevale, A., Ling, J., Ozsarfati, J., Kapur, B., Bagli, D., 2019. Fetal exposure to polybrominated diphenyl ethers and the risk of hypospadias: focus on the congeners involved. J. Pediatr. Urol. 15, 405 e401–405 e406.

Kortenkamp, A., Martin, O., Evans, R., Orton, F., McKinlay, R., Rosivatz, E., Faust, M., 2012. Response to A critique of the European Commission Document, "State of the Art Assessment of Endocrine Disrupters" by Rhomberg and colleagues–letter to the editor. Crit. Rev. Toxicol. 42, 787-789; author reply 790-781.

Kraft, K.H., Shukla, A.R., Canning, D.A., 2010. Hypospadias. Urol. Clin. North. Am. 37, 167–181.
Kristensen, D.M., Desdoits-Lethimonier, C., Mackey, A.L., Dalgaard, M.D., De Masi, F., Munkbol, C.H., Styrishave, B., Antignac, J.P., Le Bizec, B., Platel, C., Hay-Schmidt, A., Jensen, T.K., Lesne, L., Mazaud-Guittot, S., Kristiansen, K., Brunak, S., Kjaer, M., Juul, A., Jegou, B., 2018. Ibuprofen alters human testicular physiology to produce a state of compensated hypogonadism. Proc. Natl. Acad. Sci. U.S.A. 115, E715–E724.
Kristensen, P., Irgens, L.M., Andersen, A., Bye, A.S., Sundheim, L., 1997. Birth defects among offspring of Norwegian farmers, 1967–1991. Epidemiology 8, 537–544.
Kumar, M., Sarma, D.K., Shubham, S., Kumawat, M., Verma, V., Prakash, A., Tiwari, R., 2020. Environmental endocrine-disrupting chemical exposure: role in non-communicable diseases. Front. Public Health 8, 553850.
La Merrill, M.A., Vandenberg, L.N., Smith, M.T., Goodson, W., Browne, P., Patisaul, H.B., Guyton, K.Z., Kortenkamp, A., Cogliano, V.J., Woodruff, T.J., Rieswijk, L., Sone, H., Korach, K.S., Gore, A.C., Zeise, L., Zoeller, R.T., 2020. Consensus on the key characteristics of endocrine-disrupting chemicals as a basis for hazard identification. Nat. Rev. Endocrinol. 16, 45–57.
Lamb, J.C.t., Boffetta, P., Foster, W.G., Goodman, J.E., Hentz, K.L., Rhomberg, L.R., Staveley, J., Swaen, G., Van Der Kraak, G., Williams, A.L., 2015. Comments on the opinions published by Bergman et al. (2015) on Critical Comments on the WHO-UNEP State of the Science of Endocrine Disrupting Chemicals (Lamb et al., 2014). Regul. Toxicol. Pharmacol. 73, 754–757.
Lee, D.H., 2018. Evidence of the Possible Harm of Endocrine-Disrupting Chemicals in Humans: Ongoing Debates and Key Issues. Endocrinol. Metab. (Seoul.) 33, 44–52.
Lee, P.C., 1998. Disruption of male reproductive tract development by administration of the xenoestrogen, nonylphenol, to male newborn rats. Endocrine 9, 105–111.
Lemini, C., Jaimez, R., Avila, M.E., Franco, Y., Larrea, F., Lemus, A.E., 2003. In vivo and in vitro estrogen bioactivities of alkyl parabens. Toxicol. Ind. Health 19, 69–79.
Li, J., Willingham, E., Baskin, L.S., 2006. Gene expression profiles in mouse urethral development. BJU Int. 98, 880–885.
Li, X., Li, J., Zhang, Y., Zhou, Y., 2017. Di-n-butyl phthalate induced hypospadias relates to autophagy in genital tubercle via the PI3K/Akt/mTOR pathway. J. Occup. Health 59, 8–16.
Lind, D.V., Main, K.M., Kyhl, H.B., Kristensen, D.M., Toppari, J., Andersen, H.R., Andersen, M.S., Skakkebaek, N.E., Jensen, T.K., 2017. Maternal use of mild analgesics during pregnancy associated with reduced anogenital distance in sons: a cohort study of 1027 mother-child pairs. Hum. Reprod. 32, 223–231.
Liu, D., Shen, L., Tao, Y., Kuang, Y., Cai, L., Wang, D., He, M., Tong, X., Zhou, S., Sun, J., Shi, C., Wang, C., Wu, Y., 2015. Alterations in gene expression during sexual differentiation in androgen receptor knockout mice induced by environmental endocrine disruptors. Int. J. Mol. Med. 35, 399–404.
Ma, L.M., Wang, Z., Wang, H., Li, R.S., Zhou, J., Liu, B.C., Baskin, L.S., 2009. Estrogen effects on fetal penile and urethral development in organotypic mouse genital tubercle culture. J Urol 182, 2511–2517.
Mahawong, P., Sinclair, A., Li, Y., Schlomer, B., Rodriguez Jr., E., Ferretti, M.M., Liu, B., Baskin, L.S., Cunha, G.R., 2014. Prenatal diethylstilbestrol induces malformation of the external genitalia of male and female mice and persistent second-generation developmental abnormalities of the external genitalia in two mouse strains. Differentiation 88, 51–69.
Maske, P., Dighe, V., Mote, C., Vanage, G., 2020. n-Butylparaben exposure through gestation and lactation impairs spermatogenesis and steroidogenesis causing reduced fertility in the F1 generation male rats. Environ. Pollut. 256, 112957.
Meeker, J.D., Yang, T., Ye, X., Calafat, A.M., Hauser, R., 2011. Urinary concentrations of parabens and serum hormone levels, semen quality parameters, and sperm DNA damage. Environ. Health Perspect. 119, 252–257.
Meijer, W.M., de Jong-Van den Berg, L.T., van den Berg, M.D., Verheij, J.B., de Walle, H. E., 2006. Clomiphene and hypospadias on a detailed level: signal or chance? Birth Defects Res. A Clin. Mol. Teratol. 76, 249–252.
Meli, R., Monnolo, A., Annunziata, C., Pirozzi, C., Ferrante, M.C., 2020. Oxidative Stress and BPA Toxicity: An Antioxidant Approach for Male and Female Reproductive Dysfunction. Antioxidants (Basel) 9.
Metzdorff, S.B., Dalgaard, M., Christiansen, S., Axelstad, M., Hass, U., Kiersgaard, M.K., Scholze, M., Kortenkamp, A., Vinggaard, A.M., 2007. Dysgenesis and histological changes of genitals and perturbations of gene expression in male rats after in utero exposure to antiandrogen mixtures. Toxicol. Sci. 98, 87–98.
Michikawa, T., Yamazaki, S., Ono, M., Kuroda, T., Nakayama, S.F., Suda, E., Isobe, T., Iwai-Shimada, M., Kobayashi, Y., Yonemoto, J., Tamura, K., Kawamoto, T., Nitta, H., Japan, E., Children's Study, G., 2019. Isoflavone Intake in Early Pregnancy and Hypospadias in the Japan Environment and Children's Study. Urology 124, 229–236.
Moore, R.W., Rudy, T.A., Lin, T.M., Ko, K., Peterson, R.E., 2001. Abnormalities of sexual development in male rats with in utero and lactational exposure to the antiandrogenic plasticizer Di(2-ethylhexyl) phthalate. Environ. Health Perspect. 109, 229–237.
Morales-Suarez-Varela, M.M., Toft, G.V., Jensen, M.S., Ramlau-Hansen, C., Kaerlev, L., Thulstrup, A.M., Llopis-Gonzalez, A., Olsen, J., Bonde, J.P., 2011. Parental occupational exposure to endocrine disrupting chemicals and male genital malformations: a study in the Danish National Birth Cohort study. Environ. Health 10, 3.
Moule, G.R., 1961. The fertility of sheep grazing oestrogenic pastures. Aust. Vet. J. 37, 109–114.
Nassar, N., Abeywardana, P., Barker, A., Bower, C., 2010. Parental occupational exposure to potential endocrine disrupting chemicals and risk of hypospadias in infants. Occup. Environ. Med. 67, 585–589.
Nassar, N., Bower, C., Barker, A., 2007. Increasing prevalence of hypospadias in Western Australia, 1980–2000. Arch. Dis. Child. 92, 580–584.
Nilsson, E., King, S.E., McBirney, M., Kubsad, D., Pappalardo, M., Beck, D., Sadler-Riggleman, I., Skinner, M.K., 2018. Vinclozolin induced epigenetic transgenerational inheritance of pathologies and sperm epimutation biomarkers for specific diseases. PLoS ONE 13, e0202662.
Norgaard, M., Wogelius, P., Pedersen, L., Rothman, K.J., Sorensen, H.T., 2009. Maternal use of oral contraceptives during early pregnancy and risk of hypospadias in male offspring. Urology 74, 583–587.
North, K., Golding, J., 2000. A maternal vegetarian diet in pregnancy is associated with hypospadias. The ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. BJU Int. 85, 107–113.
Oishi, S., 2001. Effects of butylparaben on the male reproductive system in rats. Toxicol. Ind. Health 17, 31–39.
Oishi, S., 2002. Effects of butyl paraben on the male reproductive system in mice. Arch. Toxicol. 76, 423–429.
Ormond, G., Nieuwenhuijsen, M.J., Nelson, P., Toledano, M.B., Iszatt, N., Geneletti, S., Elliott, P., 2009. Endocrine disruptors in the workplace, hair spray, folate supplementation, and risk of hypospadias: case-control study. Environ. Health Perspect. 117, 303–307.
Padilla-Banks, E., Jefferson, W.N., Myers, P.H., Goulding, D.R., Williams, C.J., 2012. Neonatal phytoestrogen exposure causes hypospadias in female mice. Mol. Reprod. Dev. 79, 3.
Palmer, J.R., Wise, L.A., Robboy, S.J., Titus-Ernstoff, L., Noller, K.L., Herbst, A.L., Troisi, R., Hoover, R.N., 2005. Hypospadias in sons of women exposed to diethylstilbestrol in utero. Epidemiology 16, 583–586.
Parron, T., Requena, M., Hernandez, A.F., Alarcon, R., 2014. Environmental exposure to pesticides and cancer risk in multiple human organ systems. Toxicol. Lett. 230, 157–165.
Paulozzi, L.J., 1999. International trends in rates of hypospadias and cryptorchidism. Environ. Health Perspect. 107, 297–302.
Paulozzi, L.J., Erickson, J.D., Jackson, R.J., 1997. Hypospadias trends in two US surveillance systems. Pediatrics 100, 831–834.
Perriton, C.L., Powles, N., Chiang, C., Maconochie, M.K., Cohn, M.J., 2002. Sonic hedgehog signaling from the urethral epithelium controls external genital development. Dev. Biol. 247, 26–46.
Person, A.D., Beiraghi, S., Sieben, C.M., Hermanson, S., Neumann, A.N., Robu, M.E., Schleiffarth, J.R., Billington Jr., C.J., van Bokhoven, H., Hoogeboom, J.M., Mazzeu, J.F., Petryk, A., Schimmenti, L.A., Brunner, H.G., Ekker, S.C., Lohr, J.L., 2010. WNT5A mutations in patients with autosomal dominant Robinow syndrome. Dev. Dyn. 239, 327–337.
Petiot, A., Perriton, C.L., Dickson, C., Cohn, M.J., 2005. Development of the mammalian urethra is controlled by Fgfr2-IIIb. Development 132, 2441–2450.
Phillips, T.R., Wright, D.K., Gradie, P.E., Johnston, L.A., Pask, A.J., 2015. A comprehensive atlas of the adult mouse penis. Sex Dev. 9, 162–172.
Pierik, F.H., Burdorf, A., Deddens, J.A., Juttmann, R.E., Weber, R.F., 2004. Maternal and paternal risk factors for cryptorchidism and hypospadias: a case-control study in newborn boys. Environ. Health Perspect. 112, 1570–1576.
Poon, S., Koren, G., Carnevale, A., Aleksa, K., Ling, J., Ozsarfati, J., Kapur, B.M., Bagli, D., 2018. Association of in utero exposure to polybrominated diphenyl ethers with the risk of hypospadias. JAMA Pediatr. 172, 851–856.
Qian, Y., Shao, H., Ying, X., Huang, W., Hua, Y., 2020. The endocrine disruption of prenatal phthalate exposure in mother and offspring. Front. Public Health 8, 366.
Qin, X.Y., Kojima, Y., Mizuno, K., Ueoka, K., Muroya, K., Miyado, M., Zaha, H., Akanuma, H., Zeng, Q., Fukuda, T., Yoshinaga, J., Yonemoto, J., Kohri, K., Hayashi, Y., Fukami, M., Ogata, T., Sone, H., 2012. Identification of novel low-dose bisphenol a targets in human foreskin fibroblast cells derived from hypospadias patients. PLoS ONE 7, e36711.
Raghavan, R., Romano, M.E., Karagas, M.R., Penna, F.J., 2018. Pharmacologic and environmental endocrine disruptors in the pathogenesis of hypospadias: a review. Curr. Environ. Health Rep. 5, 499–511.
Rehan, M., Ahmad, E., Sheikh, I.A., Abuzenadah, A.M., Damanhouri, G.A., Bajouh, O.S., AlBasri, S.F., Assiri, M.M., Beg, M.A., 2015. Androgen and progesterone receptors are targets for bisphenol A (BPA), 4-methyl-2,4-bis-(P-hydroxyphenyl)pent-1-ene–a potent metabolite of BPA, and 4-tert-octylphenol: a computational insight. PLoS ONE 10, e0138438.
Rhomberg, L.R., Goodman, J.E., Foster, W.G., Borgert, C.J., Van Der Kraak, G., 2012. A critique of the European Commission document, “State of the Art Assessment of Endocrine Disrupters”. Crit. Rev. Toxicol 42, 465–473.
Richard, M.A., Sok, P., Canon, S., Nembhard, W.N., Brown, A.L., Peckham-Gregory, E.C., Ton, M., Ehli, E.A., Kallsen, N.A., Peyton, S.A., Davies, G.E., Patel, A., Zamilpa, I., Hobbs, C.A., Scheurer, M.E., Lupo, P.J., 2020. Altered mechanisms of genital development identified through integration of DNA methylation and genomic measures in hypospadias. Sci. Rep. 10, 12715.
Rider, C.V., Furr, J., Wilson, V.S., Gray Jr., L.E., 2008. A mixture of seven antiandrogens induces reproductive malformations in rats. Int. J. Androl. 31, 249–262.
Rider, C.V., Furr, J.R., Wilson, V.S., Gray Jr., L.E., 2010. Cumulative effects of in utero administration of mixtures of reproductive toxicants that disrupt common target tissues via diverse mechanisms of toxicity. Int. J. Androl 33, 443–462.
Ross, A.E., Marchionni, L., Phillips, T.M., Miller, R.M., Hurley, P.J., Simons, B.W., Salmasi, A.H., Schaeffer, A.J., Gearhart, J.P., Schaeffer, E.M., 2011. Molecular effects of genistein on male urethral development. J. Urol. 185, 1894–1898.
Routledge, E.J., Parker, J., Odum, J., Ashby, J., Sumpter, J.P., 1998. Some alkyl hydroxy benzoate preservatives (parabens) are estrogenic. Toxicol. Appl. Pharmacol. 153, 12–19.
Santoro, A., Chianese, R., Troisi, J., Richards, S., Nori, S.L., Fasano, S., Guida, M., Plunk, E., Viggiano, A., Pierantoni, R., Meccariello, R., 2019. Neuro-toxic and Reproductive Effects of BPA. Curr. Neuropharmacol. 17, 1109–1132.

Sathyanarayana, S., Butts, S., Wang, C., Barrett, E., Nguyen, R., Schwartz, S.M., Haaland, W., Swan, S.H., Team, T., 2017. Early prenatal phthalate exposure, sex steroid hormones, and birth outcomes. J. Clin. Endocrinol. Metab. 102, 1870–1878.
Schuster, A., Skinner, M.K., Yan, W., 2016. Ancestral vinclozolin exposure alters the epigenetic transgenerational inheritance of sperm small noncoding RNAs. Environ. Epigenet. 2.
Scippo, M.L., Argiris, C., Van De Weerdt, C., Muller, M., Willemsen, P., Martial, J., Maghuin-Rogister, G., 2004. Recombinant human estrogen, androgen and progesterone receptors for detection of potential endocrine disruptors. Anal. Bioanal. Chem. 378, 664–669.
Seifert, A.W., Bouldin, C.M., Choi, K.S., Harfe, B.D., Cohn, M.J., 2009. Multiphasic and tissue-specific roles of sonic hedgehog in cloacal septation and external genitalia development. Development 136, 3949–3957.
Seifert, A.W., Zheng, Z., Ormerod, B.K., Cohn, M.J., 2010. Sonic hedgehog controls growth of external genitalia by regulating cell cycle kinetics. Nat. Commun. 1, 23.
Sinclair, A.W., Cao, M., Pask, A., Baskin, L., Cunha, G.R., 2017. Flutamide-induced hypospadias in rats: A critical assessment. Differentiation 94, 37–57.
Skakkebaek, N.E., 2016. A brief review of the link between environment and male reproductive health: lessons from studies of testicular germ cell cancer. Horm. Res. Paediatr. 86, 240–246.
Skakkebaek, N.E., Rajpert-De Meyts, E., Buck Louis, G.M., Toppari, J., Andersson, A.M., Eisenberg, M.L., Jensen, T.K., Jorgensen, N., Swan, S.H., Sapra, K.J., Ziebe, S., Priskorn, L., Juul, A., 2016. Male reproductive disorders and fertility trends: influences of environment and genetic susceptibility. Physiol. Rev. 96, 55–97.
Skinner, M.K., Nilsson, E., Sadler-Riggleman, I., Beck, D., Ben Maamar, M., McCarrey, J. R., 2019. Transgenerational sperm DNA methylation epimutation developmental origins following ancestral vinclozolin exposure. Epigenetics 14, 721–739.
Small, C.M., DeCaro, J.J., Terrell, M.L., Dominguez, C., Cameron, L.L., Wirth, J., Marcus, M., 2009. Maternal exposure to a brominated flame retardant and genitourinary conditions in male offspring. Environ. Health Perspect. 117, 1175–1179.
Solomon, K.R., Carr, J.A., Du Preez, L.H., Giesy, J.P., Kendall, R.J., Smith, E.E., Van Der Kraak, G.J., 2008. Effects of atrazine on fish, amphibians, and aquatic reptiles: a critical review. Crit. Rev. Toxicol. 38, 721–772.
Sorensen, H.T., Pedersen, L., Skriver, M.V., Norgaard, M., Norgard, B., Hatch, E.E., 2005. Use of clomifene during early pregnancy and risk of hypospadias: population based case-control study. BMJ 330, 126–127.
Street, M.E., Angelini, S., Bernasconi, S., Burgio, E., Cassio, A., Catellani, C., Cirillo, F., Deodati, A., Fabbrizi, E., Fanos, V., Gargano, G., Grossi, E., Iughetti, L., Lazzeroni, P., Mantovani, A., Migliore, L., Palanza, P., Panzica, G., Papini, A.M., Parmigiani, S., Predieri, B., Sartori, C., Tridenti, G., Amarri, S., 2018. Current Knowledge on Endocrine Disrupting Chemicals (EDCs) from Animal Biology to Humans, from Pregnancy to Adulthood: Highlights from a National Italian Meeting. Int. J. Mol. Sci. 19.
Suzuki, Y., Yoshinaga, J., Mizumoto, Y., Serizawa, S., Shiraishi, H., 2012. Foetal exposure to phthalate esters and anogenital distance in male newborns. Int. J. Androl. 35, 236–244.
Swaen, G.M.H., Boffetta, P., Zeegers, M., 2018. Impact of changes in human reproduction on the incidence of endocrine-related diseases. Crit. Rev. Toxicol. 48, 789–795.
Swan, S.H., Main, K.M., Liu, F., Stewart, S.L., Kruse, R.L., Calafat, A.M., Mao, C.S., Redmon, J.B., Ternand, C.L., Sullivan, S., Teague, J.L., Study for Future Families Research, T., 2005. Decrease in anogenital distance among male infants with prenatal phthalate exposure. Environ. Health Perspect. 113, 1056–1061.
Szewczynska, M., Dobrzynska, E., Posniak, M., 2020. Determination of phthalates in particulate matter and gaseous phase emitted in indoor air of offices. Environ. Sci. Pollut. Res. Int..
Takeuchi, S., Iida, M., Kobayashi, S., Jin, K., Matsuda, T., Kojima, H., 2005. Differential effects of phthalate esters on transcriptional activities via human estrogen receptors alpha and beta, and androgen receptor. Toxicology 210, 223–233.
Tannour-Louet, M., Han, S., Louet, J.F., Zhang, B., Romero, K., Addai, J., Sahin, A., Cheung, S.W., Lamb, D.J., 2014. Increased gene copy number of VAMP7 disrupts human male urogenital development through altered estrogen action. Nat. Med. 20, 715–724.
Tian, R.H., Guo, K.M., Han, G.H., Bai, Y., 2020. Downregulation of MicroRNA-494 inhibits the TGF-beta1/Smads signaling pathway and prevents the development of hypospadias through upregulating Nedd4L. Exp. Mol. Pathol. 115, 104452.
Tournaire, M., Devouche, E., Epelboin, S., Cabau, A., Dunbavand, A., Levadou, A., 2018. Birth defects in children of men exposed in utero to diethylstilbestrol (DES). Therapie 73, 399–407.
van den Driesche, S., Kilcoyne, K.R., Wagner, I., Rebourcet, D., Boyle, A., Mitchell, R., McKinnell, C., Macpherson, S., Donat, R., Shukla, C.J., Jorgensen, A., Meyts, E.R., Skakkebaek, N.E., Sharpe, R.M., 2017. Experimentally induced testicular dysgenesis syndrome originates in the masculinization programming window. JCI Insight 2, e91204.
Van Der Kraak, G.J., Hosmer, A.J., Hanson, M.L., Kloas, W., Solomon, K.R., 2014. Effects of atrazine in fish, amphibians, and reptiles: an analysis based on quantitative weight of evidence. Crit. Rev. Toxicol. 44 (Suppl 5), 1–66.
van Rooij, I.A., van der Zanden, L.F., Brouwers, M.M., Knoers, N.V., Feitz, W.F., Roeleveld, N., 2013. Risk factors for different phenotypes of hypospadias: results from a Dutch case-control study. BJU Int. 112, 121–128.
Vandenberg, L.N., Colborn, T., Hayes, T.B., Heindel, J.J., Jacobs Jr., D.R., Lee, D.H., Shioda, T., Soto, A.M., vom Saal, F.S., Welshons, W.V., Zoeller, R.T., Myers, J.P., 2012. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocr. Rev. 33, 378–455.
Vilela, M.L., Willingham, E., Buckley, J., Liu, B.C., Agras, K., Shiroyanagi, Y., Baskin, L. S., 2007. Endocrine disruptors and hypospadias: role of genistein and the fungicide vinclozolin. Urology 70, 618–621.
Vorherr, H., Messer, R.H., Vorherr, U.F., Jordan, S.W., Kornfeld, M., 1979. Teratogenesis and carcinogenesis in rat offspring after transplacental and transmammary exposure to diethylstilbestrol. Biochem. Pharmacol. 28, 1865–1877.
Vottero, A., Minari, R., Viani, I., Tassi, F., Bonatti, F., Neri, T.M., Bertolini, L., Bernasconi, S., Ghizzoni, L., 2011. Evidence for epigenetic abnormalities of the androgen receptor gene in foreskin from children with hypospadias. J. Clin. Endocrinol. Metab. 96, E1953–1962.
Vrijheid, M., Armstrong, B., Dolk, H., van Tongeren, M., Botting, B., 2003. Risk of hypospadias in relation to maternal occupational exposure to potential endocrine disrupting chemicals. Occup. Environ. Med. 60, 543–550.
Wang, M.H., Baskin, L.S., 2008. Endocrine disruptors, genital development, and hypospadias. J. Androl. 29, 499–505.
Wang, S.W., Chen, Y., Joseph, T., Hu, M., 2008. Variable isoflavone content of red clover products affects intestinal disposition of biochanin A, formononetin, genistein, and daidzein. J. Altern. Complement Med. 14, 287–297.
WHO/UNEP, 2012. State of the science of endocrine disrupting chemicals - 2012. https://www.who.int/ceh/publications/endocrine/en/.
Willingham, E., Baskin, L.S., 2007. Candidate genes and their response to environmental agents in the etiology of hypospadias. Nat. Clin. Pract. Urol. 4, 270–279.
Winston, J.J., Emch, M., Meyer, R.E., Langlois, P., Weyer, P., Mosley, B., Olshan, A.F., Band, L.E., Luben, T.J., National Birth Defects Prevention, S., 2016. Hypospadias and maternal exposure to atrazine via drinking water in the National Birth Defects Prevention study. Environ. Health 15, 76.
Wogelius, P., Horvath-Puho, E., Pedersen, L., Norgaard, M., Czeizel, A.E., Sorensen, H.T., 2006. Maternal use of oral contraceptives and risk of hypospadias – a population-based case-control study. Eur. J. Epidemiol. 21, 777–781.
Wong, R.L., Walker, C.L., 2013. Molecular pathways: environmental estrogens activate nongenomic signaling to developmentally reprogram the epigenome. Clin. Cancer Res. 19, 3732–3737.
Yamaguchi, T.P., Bradley, A., McMahon, A.P., Jones, S., 1999. A Wnt5a pathway underlies outgrowth of multiple structures in the vertebrate embryo. Development 126, 1211–1223.
Yang, J., Yin, H., Zhang, Y., Yan, Z., Fu, H., Zhu, Y., 2019. Role of Hippo signaling pathway in mice hypospadias induced by vinclozolin. Wei Sheng Yan Jiu 48, 615–620.
Yang, J.H., Menshenina, J., Cunha, G.R., Place, N., Baskin, L.S., 2010. Morphology of mouse external genitalia: implications for a role of estrogen in sexual dimorphism of the mouse genital tubercle. J. Urol. 184, 1604–1609.
Yang, Y.J., Hong, Y.P., Chae, S.A., 2016. Reduction in semen quality after mixed exposure to bisphenol A and isobutylparaben in utero and during lactation periods. Hum. Exp. Toxicol. 35, 902–911.
Yu, X., Nassar, N., Mastroiacovo, P., Canfield, M., Groisman, B., Bermejo-Sanchez, E., Ritvanen, A., Kiuru-Kuhlefelt, S., Benavides, A., Sipek, A., Pierini, A., Bianchi, F., Kallen, K., Gatt, M., Morgan, M., Tucker, D., Canessa, M.A., Gajardo, R., Mutchinick, O.M., Szabova, E., Csaky-Szunyogh, M., Tagliabue, G., Cragan, J.D., Nembhard, W. N., Rissmann, A., Goetz, D., Bower, C., Baynam, G., Lowry, R.B., Leon, J.A., Luo, W., Rouleau, J., Zarante, I., Fernandez, N., Amar, E., Dastgiri, S., Contiero, P., Martinez-de-Villarreal, L.E., Borman, B., Bergman, J.E.H., de Walle, H.E.K., Hobbs, C.A., Nance, A.E., Agopian, A.J., 2019. Hypospadias prevalence and trends in international birth defect surveillance systems, 1980–2010. Eur. Urol. 76, 482–490.
Yucel, S., Cavalcanti, A.G., Desouza, A., Wang, Z., Baskin, L.S., 2003. The effect of oestrogen and testosterone on the urethral seam of the developing male mouse genital tubercle. BJU Int. 92, 1016–1021.
Zhang, L., Dong, L., Ding, S., Qiao, P., Wang, C., Zhang, M., Zhang, L., Du, Q., Li, Y., Tang, N., Chang, B., 2014. Effects of n-butylparaben on steroidogenesis and spermatogenesis through changed E(2) levels in male rat offspring. Environ. Toxicol. Pharmacol. 37, 705–717.
Zhao, S., Li, D., Bei, X.Y., Zhu, Y.P., Sun, W.L., Shen, C., Wood, K., Han, B.M., Jiang, J.T., 2018. Maternal exposure to di-n-butyl phthalate (DBP) promotes epithelial-mesenchymal transition via regulation of autophagy in uroepithelial cell. Toxicology 406–407, 114–122.
Zhao, S., Pan, L., Chen, M., Zhu, Y.P., Han, B.M., Xia, S.J., Jiang, J.T., 2019. Di-n-butyl phthalate induced autophagy of uroepithelial cells via inhibition of hedgehog signaling in newborn male hypospadias rats. Toxicology 428, 152300.
Zheng, Z., Armfield, B.A., Cohn, M.J., 2015. Timing of androgen receptor disruption and estrogen exposure underlies a spectrum of congenital penile anomalies. Proc. Natl. Acad. Sci. U.S.A. 112, E7194–7203.
Zhu, Y.J., Jiang, J.T., Ma, L., Zhang, J., Hong, Y., Liao, K., Liu, Q., Liu, G.H., 2009. Molecular and toxicologic research in newborn hypospadiac male rats following in utero exposure to di-n-butyl phthalate (DBP). Toxicology 260, 120–125.
Zhu, Y.P., Zhao, W., Sun, W.L., Zhao, S., Chen, M., Pan, L., Zhou, Z., Xia, S.J., Jiang, J.T., 2020. Di-n-butyl phthalate (DBP) reduces epithelial-mesenchymal transition via IP3R in hypospadias during maternal exposure. Ecotoxicol. Environ. Saf. 192, 110201.
Zoeller, R.T., Bergman, A., Becher, G., Bjerregaard, P., Bornman, R., Brandt, I., Iguchi, T., Jobling, S., Kidd, K.A., Kortenkamp, A., Skakkebaek, N.E., Toppari, J., Vandenberg, L.N., 2014. A path forward in the debate over health impacts of endocrine disrupting chemicals. Environ. Health 13, 118.