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Review

Cryptorchidism and endocrine disrupting chemicals

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ABSTRACT

Prospective clinical studies have suggested that the rate of congenital cryptorchidism has increased since the 1950s. It has been hypothesized that this may be related to environmental factors. Testicular descent occurs in two phases controlled by Leydig cell-derived hormones insulin-like peptide 3 (INSL3) and testosterone. Disorders in fetal androgen production/action or suppression of *Insl3* are mechanisms causing cryptorchidism in rodents. In humans, prenatal exposure to potent estrogen diethylstilbestrol (DES) has been associated with increased risk of cryptorchidism. In addition, epidemiological studies have suggested that exposure to pesticides may also be associated with cryptorchidism. Some case–control studies analyzing environmental chemical levels in maternal breast milk samples have reported associations between cryptorchidism and chemical levels. Furthermore, it has been suggested that exposure levels of some chemicals may be associated with infant reproductive hormone levels.

Contents

Abbreviations: AGD, anogenital distance; AGI, anogenital index; AhR, aryl hydrocarbon receptor; AMH, anti-Müllerian hormone; AR, androgen receptor; ARNT, aryl hydrocarbon receptor nuclear translocator; BBP, benzyl butyl phthalate; CGRP, calcitonin gene-related peptide; *cis*-HE, *cis*-heptachloroepoxide; CSL, cranial suspensory ligament; DBP, di-*n*-butyl phthalate; DDT, 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane; DEHP, di-2-ethylhexyl phthalate; DES, diethylstilbestrol; E_2B, estradiol benzoate; ED, embryonic day; EDC, endocrine disrupting chemical; ERα, estrogen receptor α; FSH, follicle-stimulating hormone; GREAT, G protein-coupled receptor affecting testicular descent; HCB, heptachlorobenzene; HCE, heptachloroepoxide; HCH, hexachlorocyclohexane; β-HCH, β-hexachlorocyclohexane; Insl3, insulin-like peptide 3; LGR8, leucine-rich repeat-containing G protein-coupled receptor 8; LH, luteinizing hormone; M1, 2-[[(3,5-dichlorophenyl)-carbamoyl]oxy]-2-methyl-3-butenoic acid; M2, 3′,5′-dichloro-2hydroxy-2-methylbut-3-enanilide; mBP, mono-*n*-butyl phthalate; mBzP, mono-benzyl phthalate; mCPP, mono-3-carboxypropyl phthalate; mEHHP, mono-2-ethyl-5-hydroxyhexyl phthalate; mEHP, mono-2-ethylhexyl phthalate; mEOHP, mono-2-ethyl-5-oxohexyl phthalate; mEP, mono-ethyl phthalate; miBP, mono-isobutyl phthalate; miNP, mono-isononyl phthalate; mMP, mono-methyl phthalate; PBB, polybrominated biphenyl; PBDE, polybrominated diphenyl ether; PCB, polychlorinated biphenyl; PCDD, polychlorinated dibenzo-*p*-dioxin; PCDF, polychlorinated dibenzofuran; PE, phthalate ester; *p,p′*-DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; PVC, polyvinyl chloride; RXFP2, relaxin-family peptide receptor 2; T, testosterone; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TDS, testicular dysgenesis syndrome; SHBG, sex hormone-binding globulin.

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1. Background

Cryptorchidism, i.e. undescended testis, is one of the most common urogenital abnormalities in newborn boys. During the last two decades the incidence of congenital cryptorchidism in boys with birth weight $\geqslant$2500 g has varied between 1% and 8% in prospective clinical studies using similar clearly defined criteria (Acerini et al., 2009; Boisen et al., 2004; Gaspari et al., 2011; Preiksa et al., 2005; Thong et al., 1998). Some of the clinical studies have suggested an increase in the incidence in Denmark and in Great Britain since the 1950s (Acerini et al., 2009; Boisen et al., 2004; Buemann et al., 1961; Group, 1992; Scorer, 1964). Due to the rapid pace of the increase it has been speculated that environmental factors rather than genetic factors may have a role in the increase (Boisen et al., 2004; Skakkebaek et al., 2001). It has been hypothesized that cryptorchidism, hypospadias, testicular cancer and poor semen quality may all be symptoms of an entity called the testicular dysgenesis syndrome (TDS), which has its origin in fetal life (Skakkebaek et al., 2001). Testicular dysgenesis may be caused by genetic and environmental factors, lifestyle factors or by combination of these (Sharpe and Skakkebaek, 2008; Skakkebaek et al., 2001). Environmental factors include among others environmental chemicals with endocrine disrupting properties, i.e. endocrine disrupters, which are "exogenous substances or mixtures that alter function(s) of the endocrine system and consequently cause adverse health effects in an intact organism, or its progeny or (sub)populations" (IPCS, 2002). This review will concentrate on the association between exposure to endocrine disrupting chemicals and cryptorchidism.

2. Testicular descent

Testicular descent has been described to occur in two phases (Hutson, 1985). The first, i.e. transabdominal, phase is, at least in mice, dependent on Leydig cell-derived hormone insulin-like peptide 3 (Insl3)-mediated male-like development of the gubernaculum (Gorlov et al., 2002; Nef and Parada, 1999; Overbeek et al., 2001; Zimmermann et al., 1999). The gubernaculum is a caudal ligament, and its enlargement anchors the testis into the inguinal area keeping the testes there as the fetal lumbar region grows (Shono et al., 1994). Anti-Müllerian hormone (AMH) may also have a role in the swelling of the gubernaculum in man: The gubernaculum has been reported to be feminized in boys with persistent Müllerian duct syndrome, which is due to abnormality of AMH or its receptor (Clarnette et al., 1997). In humans the testis has been described to glide over the genital ducts, dip into swollen gubernaculum and approach the inner inguinal ring by 20th week of gestation (Barteczko and Jacob, 2000).

Also the cranial suspensory ligament (CSL) regresses in male mice and this regression is dependent on androgens (Zimmermann et al., 1999; Hutson, 1986). However, male mice with testicular feminization (mutant for the androgen receptor gene) show normal transabdominal testicular descent, but the inguinoscrotal phase is disturbed (Hutson, 1986).

In humans the transabdominal phase of testicular descent is rarely disrupted, and only a few percentage of cryptorchid patients show intra-abdominal testis in operation (Beltran-Brown and Villegas-Alvarez, 1988; Cendron et al., 1993). Accordingly, although several sequence variants in *INSL3* gene or in the gene of its receptor, relaxin-family peptide receptor 2 (RXFP2) [previously called as leucine-rich repeat-containing G protein-coupled receptor 8 (LGR8) and G protein-coupled receptor affecting testicular descent (GREAT)] have been described (reviewed in Feng et al., 2009; Foresta et al., 2008), only T222P RXFP2 variant (Bogatcheva et al., 2007; Ferlin et al., 2003, 2008; Gorlov et al., 2002) was thought to be strongly associated with cryptorchidism. However, the T222P variant has more recently been described also in control populations (Ars et al., 2011; El Houate et al., 2008; Nuti et al., 2008).

Anand-Ivell et al. and Bay et al. have measured amniotic fluid INSL3 levels (Anand-Ivell et al., 2008; Bay et al., 2008): INSL3 was measurable only in pregnancies with male fetus and the levels were highest at 15–18 weeks of gestation, and declined thereafter to undetectable levels. Only one sample representing earlier gestational weeks was analyzed and the authors discussed that the levels may have been even higher during earlier weeks of gestation (Anand-Ivell et al., 2008).

In the latter phase of testicular descent, i.e. inguinoscrotal phase, the testis and the epididymis pass the inguinal canal and migrate from the inguinal area into the scrotum and the gubernaculum shrinks (Amann and Veeramachaneni, 2007; Barteczko and Jacob, 2000; Heyns, 1987). These events are preceded by the expansion of the gubernaculum, which dilates the inguinal canal, and extension of the gubernaculum and processus vaginalis distally through the external inguinal ring (Barteczko and Jacob, 2000; Amann and Veeramachaneni, 2007). It has been suggested, that in humans intra-abdominal pressure may help the testis and epididymis into the scrotum via inguinal canal widened by the gubernaculum (Barteczko and Jacob, 2000; Heyns, 1987). The inguinoscrotal phase starts around 23rd week of gestation in humans (Heyns, 1987; Rotondi et al., 2001; Sampaio and Favorito, 1998) and by the 35th week of gestation the testes have usually descended into the scrotum (Achiron et al., 1998; Rotondi et al., 2001; Sampaio and Favorito, 1998), but the inguinoscrotal phase of testicular descent occurs only postnatally in rodents (Amann and Veeramachaneni, 2007; Shono et al., 1994). The inguinoscrotal phase is dependent on androgens (Hutson, 1986), and according to animal studies it seems that proper androgen action must occur already before this phase rather than during it (Amann and Veeramachaneni, 2007; Spencer et al., 1991). A few human patients with androgen insensitivity have testes in their labia, which may be due to intra-abdominal pressure or partial androgen effect (Hutson, 1986). In rodents, androgens have been suggested to affect the gubernacular migration and growth via or together with genitofemoral nerve and its neurotransmitter calcitonin gene-related peptide (CGRP) (Beasley and Hutson, 1987; Ng et al., 2005; Park and Hutson, 1991; Shenker et al., 2006), but the role of the genitofemoral nerve and CGRP in humans is unclear (Hutson et al., 2010). It has been hypothesized that during migration the gubernaculum has similar properties as a developing embryonic limb bud (Huynh et al., 2007; Na et al., 2007; Nightingale et al., 2008). In addition to androgens, also INSL3/RXFP2 signalling have been suggested to have a role in the inguinoscrotal descent of the testis (Bay and Andersson, 2011; Yuan et al., 2010). The inguinoscrotal phase of testicular descent has also been described as two separate phases, i.e. quick transinguinal phase and then final inguinoscrotal phase (Amann and Veeramachaneni, 2007; Heyns, 1987).

Although the inguinoscrotal phase of testicular descent is dependent on androgens, mutations in androgen receptor (AR) have been rarely found in cryptorchid patients (Ferlin et al., 2008). Furthermore, albeit cryptorchidism is common e.g. in hypogonadotropic hypogonadism and in some syndromes, in most cases the etiology of cryptorchidism is unknown (Foresta et al., 2008). The etiology of isolated cryptorchidism is likely to be complex and multifactorial (Bay et al., 2011).

Congenital cryptorchidism may resolve spontaneously in up to 75% of cases (Boisen et al., 2004). These cases and mild high scrotal cases requiring no treatment are not included in studies based on orchiopexy figures (Main et al., 2010). Studies based on malformation register may also have classification pitfalls due to

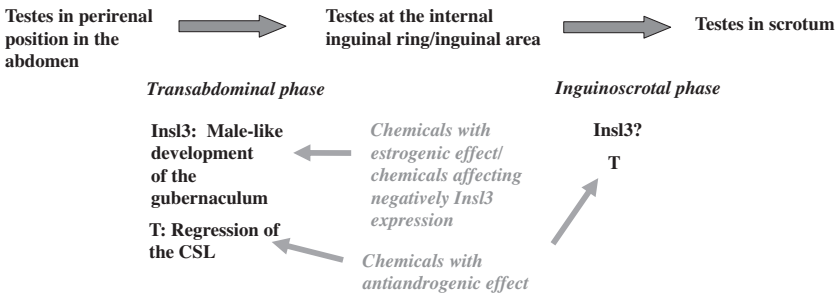

Fig. 1. Schematic presentation of the two phases of testicular descent as possible targets for chemicals with endocrine disrupting properties. Based on rodent studies (see text for references). CSL: cranial suspensory ligament Insl3: insulin-like peptide 3 T: testosterone.

underreporting of cases into registers (Toppari et al., 2001). Furthermore, orchiopexy figures include also cases with acquired cryptorchidism, i.e. boys whose testes were normally descended at birth, but ascended later on during childhood (Acerini et al., 2009; Hack et al., 2003; Wohlfahrt-Veje et al., 2009). In prospective clinical studies on congenital cryptorchidism, cases and controls can be clearly classified.

3. Animal studies

3.1. Estrogenic compounds

Several studies in laboratory animals have shown that developmental exposure to high levels of estrogenic chemicals can cause cryptorchidism and thereby affect reproductive function of males (Shono et al., 1996; McLachlan et al., 1975; Nomura and Kanzaki, 1977).

One widely investigated compound is diethylstilbestrol (DES), a synthetic nonsteroidal estrogen that causes broad spectrum of reproductive malformations in male offspring (McLachlan et al., 1975; Newbold et al., 1987; Yamamoto et al., 2005). Maternal exposure to DES has been shown to cause among others maldevelopment of the gubernaculum and cryptorchidism (Nomura and Kanzaki, 1977; Emmen et al., 2000). The mechanism underlying these effects seems to be disrupted *Insl3* expression: male mouse fetuses exposed *in utero* to DES had 3-fold decrease in testicular Insl3 mRNA expression levels compared with the control treatment (Emmen et al., 2000) (Fig. 1).

The ability of DES to cross the placental barrier is due to its low binding capacity to alpha fetoprotein and sex hormone-binding globulins (SHBGs) (Sheehan and Young, 1979; Savu et al., 1981), proteins that normally protect the fetus from maternal steroid hormones. DES has a high affinity to estrogen receptor binding sites (Kuiper et al., 1997). Experimental studies suggests that gonadal abnormalities in DES exposed male offspring result from estrogen receptor alpha (ERα) activation in the tissues (Couse and Korach, 2004; Cederroth et al., 2007; Prins et al., 2001). However, disrupted normal estrogen-androgen balance is suggested to be even more important factor in fetal environment rather than estrogenic effect alone (McKinnell et al., 2001; Rivas et al., 2002). McKinnell et al. (2001)) have also clearly demonstrated how neonatal exposure to 10 μg/kg of DES suppressed AR in reproductive tract tissues. The mechanism via which this suppression is induced is not clear; it may be more indicative of an effect of DES on AR gene expression or protein production or metabolism (Williams et al., 2001). Nowadays DES is widely used as a model compound for assessing estrogenic effects of wide variety of chemicals in reproductive toxicity studies.

In addition to DES, estradiol benzoate (E_2B) *in utero* causes maldevelopment of the gubernaculum and thereby inhibition of transabdominal testicular descent in fetal male mice analyzed by scanning electron microscope (Shono et al., 1996).

3.2. Antiandrogenic compounds

Flutamide is an antiandrogenic drug that has been used to treat androgen dependent prostate cancer (Brogden and Chrisp, 1991). It is a non-steroidal chemical that acts as an AR antagonist by competing with endogenous androgens for binding to the receptor. Male offspring exposed *in utero* to flutamide suffer from reproductive tract abnormalities: reduced anogenital distance (AGD), cryptorchidism (usually ectopic testes, disrupted inguinoscrotal phase of testicular descent) (Fig. 1) and also retained nipples are typical manifestations for chemical antiandrogenic effects (Miyata et al., 2002; McIntyre et al., 2001; Imperato-McGinley et al., 1992). In rats, androgen blockage has been shown to be most effective when exposure to flutamide is applied throughout the period of gubernacular outgrowth that is from embryonic days (EDs) 16–19 (Spencer et al., 1991). However, similar degree of inhibition of testicular descent has been shown to occur when exposure to flutamide is further restricted to the earliest phase of gubernacular outgrowth and transabdominal testicular descent that occurs between EDs 15.5 and 17 (Spencer et al., 1991). Masculinization of male reproductive tract tissues is programmed by androgen action during a common early programming window that is prior to ED 19.5 in rat (Welsh et al., 2008). The factors determining the masculinization programming window are unknown, but they are likely to involve regulation of the onset of AR expression; reproductive tract masculinization involves maximal AR activation (Welsh et al., 2008). Due to the well-known antiandrogenic properties, flutamide is used as a positive control in many bioassays (e.g. Hershberger and Enhanced OECD Test Guidline no 407) to assess chemicals with androgenic or antiandrogenic characteristics (Toyoda et al., 2000; Yamada et al., 2000).

Phthalate esters (PEs) are mainly used as plasticizers to enhance the flexibility of polyvinyl chloride (PVC) plastics. PEs are used in many industrial and consumer products such as in food wrappings, toys, medical devices, building materials and personal care products (Schettler, 2006). PEs are not persistent in the environment; they are rapidly metabolized and excreted mainly into urine but also into feces (Kluwe, 1982). The monoester metabolites are believed to be the toxic species for male reproductive system (Li et al., 1998; Ema and Miyawaki, 2001; Gray and Gangolli, 1986). Some PEs pose endocrine activity and they act as antiandrogens without interacting directly with AR contrary to flutamide (Mylchreest et al., 1999; Parks et al., 2000). It has been shown that PEs inhibit *Insl3* expression and testosterone production by fetal Leydig cells in rodent (Parks et al., 2000; Borch et al., 2004; Barlow et al., 2003; Wilson et al., 2007, 2004; Lehmann et al., 2004) (Fig. 1). Di-*n*-butyl phthalate (DBP) is one of the phthalates that causes disorders in male external genitalia at a high dose level. Maternal exposure to DBP 500 mg/kg

has been shown to cause small intra-abdominal testes in male rat offspring (Mylchreest et al., 1999, 2000; McKinnell et al., 2005; Gray et al., 1999b). However, in the study by McKinnell et al. (2005) in which rats were exposed *in utero* to DBP 500 mg/kg, the authors could not find any consistent correlation between the suppression of Insl3 immunoexpression and the occurrence of abnormally located testes in individual fetal male rat. Due to the ability of DBP to cause common reproductive tract malformations in male rat offspring similar to the ones reported in human TDS, it has been suggested that DBP could serve as a model compound to delineate the cellular pathways in TDS (Fisher et al., 2003). Other phthalates, di-2-ethylhexyl phthalate (DEHP) and benzyl butyl phthalate (BBP) have also harmful effects on male reproductive health. Testicular malformations of a different grade can be induced by maternal and neonatal exposure to these compounds. For example nondescent of the testis associated with abnormalities of the gubernaculum or the presence of CSLs have been observed (Gray et al., 2000). Some of these undescended testes have been reported to be free floating, completely lacking a gubernaculum, while others have been reported to be attached to a thin elongated gubernaculum. In addition, some scrotal testes lacked any gubernacular tissue, indicating that abdominal pressure and muscle tone around the inguinal ring were sufficient to induce scrotal retention of the testes (Gray et al., 2000).

Vinclozolin is a dicarboximide fungicide used to protect fruits and vegetables. It is well known for its antiandrogenic properties. The metabolites of vinclozolin 2-[[(3,5-dichlorophenyl)-carbamoyl]oxy]-2-methyl-3-butenoic acid (M1) and 3′,5′-dichloro-2hydroxy-2-methylbut-3-enanilide (M2) have been shown to act as pure competitive antagonists of AR *in vitro* (Kelce et al., 1994; Wong et al., 1995). Maternal and lactational exposure to vinclozolin produces remarkable profile of malformations in male offspring including vaginal pouches, nipple retention, shortened anogenital distance and undescended ectopic scrota/testes and a very small or absent prostate (Shono et al., 2004a; Gray et al., 1994, 1999a; Metzdorff et al., 2007). Gray et al. (1994) have demonstrated that approximately 45% of the males treated with vinclozolin during the critical period of androgen-mediated differentiation had undescended testes located within an ectopic cremaster sac anterior to the abdominal musculature. Migration of the gubernaculum (Shono et al., 2004a) and the development of the genitofemoral nerve (Shono et al., 2004b) have also been reported to be affected by developmental vinclozolin treatment (dose of 200 mg/kg). Also a rather low-dose exposure (already at dose of 3.125 and 6.25 mg/kg of vinclozolin) has the potency to induce alterations in male reproductive tract (Gray et al., 1999a) although the severity of abnormality increases with the dose.

Procymidone, another dicarboximide fungicide has also antiandrogenic properties; it acts as an AR antagonist (Ostby et al., 1999). Reproductive tract abnormalities have been described in male offspring of procymidone-treated rat dams (Gray et al., 1999b; Metzdorff et al., 2007; Ostby et al., 1999) including ectopic undescended testes (Ostby et al., 1999). However, procymidone has been shown to be about 2.5-fold less potent than vinclozolin (Ostby et al., 1999).

3.3. Other compounds having demasculinizing effects

Synthetic pesticide DDT [1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane] has been used for over 60 years to control agricultural and forest pests and to protect against malaria and other vector-borne diseases (Rogan and Chen, 2005; Turusov et al., 2002). Its use was banned in developed countries in the 1970s due to its harmful environmental effects in wildlife; DDT is highly lipophilic and metabolically resistant (Rogan and Chen, 2005). It bioaccumulates in body fat with age and biomagnifies along the food chain. DDT is a mixture of 20% *o,p′*-DDT, a substance with estrogenic activity, and 80% *p,p′*-DDT (Vidaeff and Sever, 2005), which is metabolized to *p,p′*-DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene], that has been reported to be antiandrogenic (Kelce et al., 1995). Endocrine disrupting effects of DDT and its metabolites are well-described in Lake Apopka alligators and fish-eating and raptorial birds (Guillette et al., 1994; Hickey and Anderson, 1968). In a study by Veeramachaneni et al. (2007) in which rabbits were exposed *in utero* and through lactation to *p,p′*-DDT (low-DDT, 8.85 mg/kg or high-DDT 88.5 mg/kg) testicular descent was impaired in some pups of DDT-exposed groups. These animals were unilaterally or bilaterally cryptorchid and the animals having abdominal testes had also grossly underdeveloped gubernaculum.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most toxic dioxin congener. In general, the term "dioxin" refers to a heterogenous mixture of polyhalogenated (usually polychlorinated) dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (PCBs). PCDD/Fs are the by-products of diverse industrial processes (e.g. manufacturing of chlorinated chemicals and paper bleaching) and incomplete burning. Dioxins are highly lipophilic and persistent chemicals that biomagnify along the food chain. Several studies on laboratory animals have shown that *in utero* and lactational exposure to TCDD affects reproductive system of male offspring; such manifestations of TCDD exposure include among others reduced ejaculated sperm counts, delayed puberty, reduced size of accessory sex organs and decreased anogenital distance (Ohsako et al., 2001; Simanainen et al., 2004; Mably et al., 1992; Gray et al., 1995). Delayed testicular descent but also cryptorchidism and Wolffian duct anomalies have been reported in animals following fetal exposure to TCDD (Mably et al., 1992; Barthold et al., 1999).

Most of the toxic effects of TCDD are mediated through binding to aryl hydrocarbon receptor (AhR) that acts as a multifunctional molecular switch regulating endo- and xenobiotic metabolism, cell growth and proliferation (Bock and Kohle, 2006). The mechanisms underlying TCDD-induced reproductive disorders have been speculated to involve reduced expression of sex steroid and LH receptors (Ohsako et al., 2001; Tian et al., 1998; Fukuzawa et al., 2004) by promoting ubiquitination and degradation of receptor protein (Ohtake et al., 2007), altered steroidogenesis (Mutoh et al., 2006; Myllymaki et al., 2005), modulating of ER signaling by a co-regulatory-like function of activated AhR/aryl hydrocarbon receptor nuclear translocator (ARNT) (Ohtake et al., 2003) and/or induction of cytochrome P450 1 family enzymes resulting in enhanced inactivation of steroid hormones (Badawi et al., 2000).

3.4. Chemical mixtures

Recent data have shown that mixtures of endocrine disrupting chemicals with diverse modes of action can display cumulative, dose additive or synergistic effects on male reproductive tract development (Metzdorff et al., 2007; Veeramachaneni et al., 2007; Hass et al., 2007; Christiansen et al., 2009; Rider et al., 2008; Jacobsen et al., 2010). Pregnant rabbits exposed to a low or a high dose mixture of vinclozolin and DDT (low-mix, high-mix; 4.425 or 44.25 mg/kg DDT and 3.6 or 36 mg/kg vinclozolin) produced a spectrum of perturbations combing those that the chemicals caused individually, such as cryptorchidism (Veeramachaneni et al., 2007). In rats, developmental exposure to mixture of seven antiandrogens containing vinclozolin, promycidone, linuron, prochloraz, BBP, DBP and DEHP at four dilutions affected all androgen-dependent endpoints in a dose-additive manner, including cryptorchidism in male offspring (Rider et al., 2008). The incidence of gubernacular agenesis was 10% and the incidence of cryptorchidism was 80% in the high dose mixture. The toxic equivalency and dose addition models provided the best fits to the response of cryptorchidism in the high dose group (Rider et al., 2008).

4. Human studies

4.1. Exposure to estrogens/estrogenic agents

It was originally proposed in the so called estrogen hypothesis, that increased *in utero* exposure to estrogens may be related to the increasing incidence of disorders of male reproductive health (Sharpe and Skakkebaek, 1993). Martin et al. performed a quantitative meta-analysis on the association between symptoms of TDS (cryptorchidism, hypospadias and testicular cancer) and prenatal exposure to estrogenic agents (Martin et al., 2008). Of the 28 studies evaluating the association between cryptorchidism and exposure to estrogenic agents only six were eligible for the final analysis on the basis of inclusion and exclusion criteria. The authors concluded that there was no evidence of an association between cryptorchidism and *in utero* exposure to other estrogens than DES (Martin et al., 2008). Also in a previous meta-analysis, which evaluated association between first trimester exposure to sex hormones excluding DES and external genital malformations in boys, no significant relationship was found (Raman-Wilms et al., 1995). Studies on exposure to phytoestrogens and to a group of compounds with unspecified mode of effect, like pesticides, and studies on maternal endogenous hormones were not included in the analysis by Martin et al. (2008).

Results from the meta-analysis by Martin et al. suggested a doubling of the risk of cryptorchidism after *in utero* exposure to DES (Martin et al., 2008). Palmer et al. published recently results from a follow-up study on three US cohorts of men exposed to DES *in utero* (Palmer et al., 2009). Data on cryptorchidism was based on self-reports. Prenatal DES exposure was associated with almost 2-fold risk of cryptorchidism, and a stronger association was observed for exposures starting before the 11th week of pregnancy (Palmer et al., 2009).

In a review concerning the association between fetal estrogen exposure and cryptorchidism or other symptoms of TDS, Storgaard et al. evaluated the possible role of several aspects: Maternal estradiol levels during pregnancy, exposure to DES, accidental oral contraceptive use during pregnancy, environmental exposures and conditions possibly related with altered estrogen levels, i.e. obesity, twin pregnancy, hyperemesis gravidarum, first pregnancy, and pre-eclampsia (Storgaard et al., 2006). Conclusion was that no consistent evidence for a link between prenatal exposure to estrogens and cryptorchidism was found (Storgaard et al., 2006). As a part of a systematic review on association between prenatal exposure to environmental estrogens and male reproductive health, Vidaeff et al. reviewed the association between pesticide exposure and cryptorchidism (Vidaeff and Sever, 2005). They found no certain support to the hypothesis that environmental estrogens would have contributed to the increase in disorders of male reproductive health or reason to reject the hypothesis (Vidaeff and Sever, 2005). However, some pesticides, e.g. vinclozolin and DDE, have anti-androgenic effects and it was hypothesized in 1996 that exposure to other hormonally active environmental chemicals (e.g. antiandrogens) during development may have a role in the adverse trends in male reproductive health (Toppari et al., 1996).

In the following studies evaluating the association between human cryptorchidism and exposure to pesticides (including 5 studies reviewed already by Vidaeff and Sever (2005) and other quite recent studies) or to other environmental chemicals with possible antiandrogenic/mixture effects, will be reviewed (Table 1).

4.2. Pesticides

Ecological studies have evaluated the association between the rate of cryptorchidism and the use of pesticides in the geographical area. In the Spanish study the frequency of orchiopexy tended to increase together with higher levels of pesticide use in the area except for level 0 (Garcia-Rodriguez et al., 1996). Boys aged 1–16 years were included in the analysis. When compared to areas with the lowest level of pesticide use, the odds ratio for cryptorchidism (orchiopexy) was significantly increased in areas with the highest level of pesticide use (Garcia-Rodriguez et al., 1996). In the Italian study from Sicily, data on cryptorchidism in boys born 1998–2002 was obtained from records of local pediatric services and only operated cases or cases with operation scheduled were included (Carbone et al., 2006). An increasing rate of cryptorchidism in relation to increasing pesticide impact of the area was found, but the trend was statistically significant only when cryptorchidism and hypospadias were pooled together (Carbone et al., 2006).

Several epidemiological studies have evaluated the role of parental occupation and possible pesticide exposure as a risk factor for cryptorchidism in the offspring. In a case–control study nested within a study on adverse reproductive outcomes in floriculture workers in Colombia, case and referent status was verified via medical examination and records (Restrepo et al., 1990). A significant association between mother's exposure to pesticides during pregnancy and all congenital malformations (especially hemangiomas) was found, but the association with cryptorchidism was not statistically significant (Restrepo et al., 1990). In a Norwegian Medical Birth Registry-based study, there was no significant difference in the rate of cryptorchidism between farmers' and non-farmers' offsprings (Kristensen et al., 1997). Pesticide purchase on the farm was associated with cryptorchidism, but only for births after 1971, and not during the first years of the study when the use of organochlorine compounds was more extensive (Kristensen et al., 1997).

Two Danish studies have evaluated the role of parental occupation in farming or gardening as a risk factor for cryptorchidism. In the first register based case–control study, the risk of cryptorchidism was significantly increased in sons of women working in farming or gardening (when looking separately, significant risk was found only in gardening), but paternal occupation in farming or gardening was not associated with a significantly increased risk of cryptorchidism (Weidner et al., 1998). In a more recent prospective study based on the Danish National Birth Cohort, pregnancies of gardeners and pregnancies of farmers were compared to pregnancies of other workers (Zhu et al., 2006). Diagnoses of cryptorchidism were obtained from linkage to National Hospital Register. No cryptorchid cases were found in the sons of gardeners in this study and the rate of cryptorchidism was not increased in the group of farmers. Furthermore, no significant association between pesticide exposure and congenital malformations was found (Zhu et al., 2006). A third Danish study reported prevalence of cryptorchidism in sons of pregnant women employed in Danish greenhouses (Andersen et al., 2008). The prevalence of cryptorchidism at 3 months of age was 6.2%, which is significantly higher than previously described from Copenhagen area using the same examination technique, i.e. 1.9% (Andersen et al., 2008; Boisen et al., 2004). There was no significant difference in the rate of cryptorchidism between the sons of non-exposed and exposed mothers, although all cases were born to pesticide exposed mothers (Andersen et al., 2008). However, the sons of pesticide exposed mothers had reduced penile length, testicular volume and testosterone (T) and inhibin B levels and increased levels of follicle-stimulating hormone (FSH), SHBG and increased luteinizing hormone: testosterone ratio (LH/T). A joint-multivariate test showed that the difference between exposed and non-exposed group was statistically significant, suggesting that fetal exposure to pesticides has an adverse effect on Leydig cells and Sertoli cells (Andersen et al., 2008). The authors report that the pesticides used in the working areas had low biological persistence (Andersen et al., 2008), which is in contrast to organochlorine pesticides, which are persistent chemicals.

Table 1
Reviewed studies evaluating possible association between environmental chemical exposure and cryptorchidism.

Chemical group	Study type	References	Study size: number of cryptorchid cases/number of cases and controls or all boys	Association with cryptorchidism
Pesticides	Ecological studies evaluating association with pesticide use in the area	Garcia-Rodriguez et al. (1996)	270 boys/502321 person years	+
		Carbone et al. (2006)	60/8199	(+)
	Epidemiological studies evaluating association with parental occupation/ possible occupational exposure to pesticides	Restrepo et al. (1990)	16/222	(+) (maternal exposure during pregnancy)
		Kristensen et al. (1997)	251/130375	+ (pesticide purchase on the farm, for births after 1971)
		Weidner et al. (1998)	6177/29450	+ (maternal work in farming or gardening)
		Zhu et al. (2006)	470/30983	–
		Andersen et al. (2008)	7/113	+ (maternal work in greenhouse), also hormonal association with exposure to pesticides
		Pierik et al. (2004)	78/391	+ (paternal exposure to pesticides)
		Carbone et al. (2007)	48/251	(+) (maternal work in agriculture, probable exposure to pesticides, paternal indirect contact with agricultural products)
		Biggs et al. (2002)	2395/11975	(+) (with maternal occupation in farming or horticulture)
		Kurahashi et al. (2005)	96/212	–
		Morales-Suarez-Varela et al. (2011)	1002/45341	–
	Studies based on measurement of pesticide levels in biological matrices	Hosie et al. (2000)	18/48	fat: + (HCE and HCB)
		Damgaard et al. (2006)	62/130	milk: + (combined analysis of 8 pesticides)
		Brucker-Davis et al. (2008)	56/125 67/151	milk: (+) (DDE) Cord serum: –
		Bhatia et al. (2005)	75/358	Maternal serum: –
		Longnecker et al. (2002)	219/771	Maternal serum: +–
		Pierik et al. (2007)	214–219/768–783	maternal serum: +–
PCBs	Studies based on measurement of PCB levels in biological matrices	Hosie et al. (2000)	18/48	fat: –
		Brucker-Davis et al. (2008)	56/125 67/151	milk: + (sum of 7 PCBs) cord serum: –
		McGlynn et al. (2009)	230/823	maternal serum: –
		Virtanen et al. (in press)	56/112 (FIN) 39/168 (DK)	placenta: – (hormonal association) placenta: –
Dioxins	Epidemiological study evaluating association with paternal occupational exposure to dioxin contaminated chlorophenate	Dimich-Ward et al (1996)	57/NA	(+) (cumulative paternal exposure through the entire period of pregnancy)
	Study based on measurement of dioxin levels in biological matrices	Virtanen et al. (in press)	56/112 (FIN)	placenta: –
			39/168 (DK)	placenta: –
Flame retardants	Studies based on measurement of flame retardant levels in biological matrices	Main et al. (2007)	62/130	milk: + (sum of 7 PBDEs), also hormonal association
			95/280	placenta: –
		Small et al. (2009)	9/459	maternal serum: –
Phthalates	Studies based on measurement of phthalate levels in biological matrices	Main et al. (2006)	62/130	milk: –, hormonal association
		Brucker-Davis et al. (2008)	31/71 36/85	milk: (+) cord serum: –

(*continued on next page*)

Table 1 (*continued*)

Chemical group	Study type	References	Study size: number of cryptorchid cases/number of cases and controls or all boys	Association with cryptorchidism
		Swan et al. (2005)	(NA/85)	(maternal urine: inverse association with AGI)
	Epidemiological studies evaluating association with parental occupational exposure to phthalates	Morales-Suarez-Varela et al. (2011)	1002/45341	–
		Wagner-Mahler et al. (2011)	95/283	+

+ = significant positive association with cryptorchidism.
(+) = tendency to positive association/non-significant positive association.
+– = inconclusive results.
– = no significant association/reduced risk.
NA = not available.

Other case–control studies have also evaluated the association between cryptorchidism and parental occupation in farming or horticulture and possible occupational exposure to pesticides. In the Dutch case–control study nested within a cohort of male births, boys were examined for cryptorchidism at a median age of 34 days (Pierik et al., 2004). Paternal, but not maternal, probable occupational exposure to pesticides [based on job-exposure matrix for potential endocrine disrupting chemicals (EDCs) (Van Tongeren et al., 2002)] was associated with an increased risk of cryptorchidism (Pierik et al., 2004). However, the study included several nationalities and the association was not statistically significant when including only Dutch subjects (Pierik et al., 2004). In a population based Italian case–control study from Sicily, data on cryptorchidism was obtained from records of local pediatric services, and only operated cases or cases with operation scheduled were included (Carbone et al., 2007). An increased risk of cryptorchidism was observed for maternal work in agriculture during pregnancy, probable maternal exposure to pesticides and paternal occupations with indirect contact with agricultural products, but the associations were not statistically significant (Carbone et al., 2007). In an US population-based case–control study, cryptorchid cases were defined as boys with a diagnosis of cryptorchidism on birth hospitalization record (Biggs et al., 2002). Mothers working in farming or horticulture had an increased risk of giving birth to a boy with undescended testis. However, the risk was not statistically significant (Biggs et al., 2002). In the Japanese case–control study, operated cryptorchid cases were compared to boys with no genitourinary malformations (Kurahashi et al., 2005). Parental occupation as farmer was not associated with an increased risk of cryptorchidism, but the proportion of farmers in the study was small (Kurahashi et al., 2005).

Besides ecological and epidemiological studies, several case–control studies have evaluated the association between pesticide exposure and cryptorchidism by measuring pesticide levels in different biological matrices. In a German study, pesticide levels in fat samples taken from boys undergoing orhiopexy (cases) or another surgical procedure (controls) were analyzed (Hosie et al., 2000). The levels of pesticides heptachloroepoxide (HCE) and hexachlorobenzene (HCB) were statistically significantly higher in the group of cryptorchid boys, but no significant difference was found in the levels other chlorinated cyclodienes or benzenes, DDT or its metabolites, toxaphenes or hexachlorocyclohexane (HCH). However, the boys were 0–15 years old and thus the chemical levels reflect also postnatal exposure for several years (Hosie et al., 2000), and are therefore difficult to interpret.

Two case–control studies based on prospective cohort studies on congenital cryptorchidism have evaluated the link between prenatal exposure to pesticides and cryptorchidism by analyzing pesticide levels in maternal breast milk samples (Brucker-Davis et al., 2008; Damgaard et al., 2006). In both studies boys were examined for cryptorchidism at birth and at 3 months. In the Danish–Finnish study, breast milk samples were collected between the ages of 1 and 3 months and they were analyzed for the levels of 27 organochlorine pesticides (Damgaard et al., 2006). Eight pesticides (*p,p′*-DDT, *p,p′*-DDE, β-HCH, HCB, α-endosulfan, *cis*-heptachloroepoxide (*cis*-HCE), oxychlordane, dieldrin) were measurable in all samples, and the median levels tended to be higher in the group of cryptorchid boys, although none of them reached statistical significance. However, combined statistical analysis of these eight chemicals showed that maternal breast milk pesticide levels were significantly higher in the group of cryptorchid boys than in controls (Damgaard et al., 2006). In the French study, maternal colostrum levels of DDE were analyzed (Brucker-Davis et al., 2008). DDE-levels tended to be higher in the group of cryptorchid boys, and cryptorchid boys tended to have a higher individual exposure score for DDE than control boys, but the differences were not quite statistically significant (Brucker-Davis et al., 2008).

Brucker-Davis et al. measured also the cord serum levels of DDE, and they found no difference between cases and controls (Brucker-Davis et al., 2008). Three studies analyzed historical maternal serum samples from the 1960s, when DDE levels were higher (Bhatia et al., 2005; Longnecker et al., 2002; Pierik et al., 2007). In a Californian case–control study, nested within an US longitudinal cohort study of pregnant women and their children (Child Health and Development Studies, CHDS), maternal serum samples taken during pregnancy or after delivery were analyzed (Bhatia et al., 2005). Children were followed up in the study until at least 2 years of age. No significant association between maternal DDE levels and cryptorchidism persisting until 2 years of age was found (Bhatia et al., 2005). Bhatia et al. analyzed also the levels of estrogenic DDT, and this data were included in the previously mentioned meta-analysis (Martin et al., 2008); no association with cryptorchidism was found (Bhatia et al., 2005). Two case–control studies have evaluated third trimester maternal serum sample data from another US birth cohort study (Collaborative Perinatal Project, CPP) (Longnecker et al., 2002; Pierik et al., 2007). In CPP, children were followed up until the age of 7 years. In the case–control studies, boys classified as having undescended testis at any time during the first year of life were considered as cryptorchid cases. The study by Longnecker et al. analyzed DDE levels in maternal serum samples (Longnecker et al., 2002). Boys in the highest DDE exposure category had an increased risk of cryptorchidism as compared to the boys in the lowest exposure category, but the confidence intervals were wide and the results were inconclusive (Longnecker et al., 2002). Pierik et al. analysed maternal levels of seven organochlorine pesticides i.e. HCE, HCB, β-HCH, DDE, *p,p′*-DDT, oxychlordane and dieldrin (Pierik et al., 2007). When comparing the highest exposure category with the lowest exposure cate-

gory of each pesticide, no statistically significant associations with cryptorchidism were found for HCE and HCB. For β-HCH, statistically significant association with cryptorchidism was found for maternal levels between 50th and 90th percentiles, but there was no statistically significant trend over exposure categories. No significant association was observed between the levels of four other organochlorines and congenital cryptorchidism (Pierik et al., 2007).

4.3. PCBs

PCBs are persistent environmental chemicals which have been used for instance as lubricants for machinery, dielectric fluids in transformers and fire retardants (Guo et al., 2004). PCBs may have estrogenic, anti-estrogenic and anti-androgenic properties (Schrader and Cooke, 2003; Wolff et al., 1997). In addition to pesticide levels, Hosie et al. examined also the levels of 6 PCB congeners (PCB 28, 52, 101, 138, 153 and 180) and their sum in fat samples taken from boys undergoing orchiopexy (cases) or some other surgical procedure (controls) (Hosie et al., 2000). There were no statistically significant differences between the case and control groups in the levels of PCBs (Hosie et al., 2000). In a previously mentioned case–control study Brucker-Davis et al. analyzed the levels of seven non-planar PCBs (PCB 28, 52, 101, 118, 138, 153 and 180) in maternal colostrum and cord serum samples (Brucker-Davis et al., 2008). In breast milk, but not in cord serum, median PCB level tended to be higher in the group of cryptorchid boys than in the control group, though not statistically significantly. Boys belonging to the highest exposure class of ∑PCBs in colostrum had a significantly increased risk of cryptorchidism (Brucker-Davis et al., 2008). Brucker-Davis et al. evaluated also the composite score of exposure in milk by adding the DDE and ∑PCB scores (describing exposure category of individual chemicals). Cryptorchid boys showed a tendency to be classified more likely into the most contaminated group for composite score ∑PCB + DDE (Brucker-Davis et al., 2008). Another case–control study, nested within a previously mentioned US birth cohort study (Collaborative Perinatal Project, CPP) analyzed the levels of 11 PCB congeners (PCB 28, 52, 74, 105, 118, 138, 153, 170, 180, 194 and 203) in third trimester maternal serum samples from the 1960s (McGlynn et al., 2009). Boys with a diagnosis of undescended testes during the first year of life were defined as cases in this study. No statistically significant association between maternal PCB levels and cryptorchidism in their sons was observed (McGlynn et al., 2009).

In a clinical study evaluating the association between prenatal PCB exposure and pubertal development in boys belonging to the Faroese birth cohort, Mol et al. found no difference in umbilical cord levels of PCBs (sum of PCBs 138, 153 and 180) between boys with abnormalities in testicular development and other boys (Mol et al., 2002). However, the group of boys with abnormalities in testicular development included besides boys with cryptorchidism also one case with testis torsion (Mol et al., 2002). In a case–control study based on the previously mentioned Danish–Finnish prospective cohort study on congenital cryptorchidism, levels of 37 PCBs (including 12 dioxin-like PCBs) in placenta were analyzed (Virtanen et al., in press). No significant differences between cases and controls in the sum of PCBs or in the PCB WHO-TEq level in placenta were observed in either country. In the Finnish material, placenta PCB WHO-TEq levels associated positively with infant LH levels at 3 months of age (Virtanen et al., in press).

4.4. Dioxins

The above mentioned Danish–Finnish case–control study also evaluated the levels of 17 toxic PCDD/Fs in placenta (Virtanen et al., in press). No significant differences between cryptorchid cases and controls were observed in either country in the sum of 17 dioxins or in the dioxin WHO-TEq level in placenta (Virtanen et al., in press). In a case–control study nested in a cohort of British Columbia sawmill workers, a positive association between paternal cumulative exposure to dioxin-contaminated chlorophenates during pregnancy and congenital anomalies of the genital organs in the offspring was reported (Dimich-Ward et al., 1996). However, the association between cumulative paternal exposure to chlorophenates during pregnancy and cryptorchidism in the son was only borderline statistically significant. Diagnosis of cryptorchidism was based on British Columbia Health Surveillance Registry and estimates of cumulative exposures were based on job titles and time spent in a particular job (Dimich-Ward et al., 1996).

4.5. Flame retardants

Polybrominated biphenyls (PBBs) and polybrominated diphenyl ethers (PBDEs) are persistent chemicals which have been used as flame retardants. They are suspected to have endocrine disrupting activities, and thus two studies have evaluated whether *in utero* exposure to them is associated with the risk of cryptorchidism (Main et al., 2007; Small et al., 2009). Some PBDEs have anti-androgenic activities (Stoker et al., 2005). PBBs may have estrogenic, anti-estrogenic or anti-androgenic activities (McCormack et al., 1979; Nakari and Pessala, 2005; Newton et al., 1982; Small et al., 2009). In the previously mentioned Danish–Finnish case–control study, the levels of 14 PBDEs (PBDEs 28, 75, 71, 47, 66, 77, 100, 119, 99, 85, 154, 153, 138 and 183) in maternal breast milk samples and placentas were analyzed (Main et al., 2007). Seven PBDEs were measurable in all milk samples and their sum was significantly higher in the group of Danish cryptorchid boys than in the Danish controls. In the Finnish material, the sum of seven PBDEs in breast milk correlated positively with infant LH levels at 3 months. In placentas, the levels were generally lower than in breast milk. Only five PBDEs were measurable in all placenta samples and there was no significant association between their sum and congenital cryptorchidism or infant hormone levels at 3 months of age (Main et al., 2007). In an US cohort study of sons born to women accidentally exposed to PBBs in 1973–1974, maternal serum PBB levels at the time of enrollment into the study were analyzed (Small et al., 2009). Quantitation of PBBs was based on congener PBB-153. An increasing trend for reporting hernia or hydrocele was found in the sons belonging to the highest exposure class, but no association between cryptorchidism and PBB exposure was found (Small et al., 2009).

4.6. Phthalates

In the case–control study based on the Danish–Finnish cohort study on cryptorchidism, also the levels of six phthalate monoesters (mono-methyl phthalate (mMP), mono-ethyl phthalate (mEP), mono-*n*-butyl phthalate (mBP), mono-benzyl phthalate (mBzP), mono-2-ethylhexyl phthalate (mEHP), mono-isononyl phthalate (miNP)) in maternal breast milk samples were analyzed (Main et al., 2006). There was no association between breast milk phthalate levels and congenital cryptorchidism. However, levels of phthalate monoesters (mEP, mBP, mMP or miNP) showed anti-androgen like associations with infant sex hormone levels at 3 months; phthalate levels correlated positively with SHBG and LH levels and LH/free testosterone ratio, and negatively with free testosterone levels, suggesting an adverse effect on Leydig cells (Main et al., 2006). Brucker-Davis et al. analyzed in the previously mentioned case–control study from Nice area monobutylphthalate (mBP) levels in maternal colostrum and cord serum samples (Brucker-Davis et al., 2008). In colostrum, but not in the cord serum, the median mBP level tended to be higher in the group of cryptorchid boys, but the difference was not statistically significant (Brucker-

Davis et al., 2008). In an American study, the association between prenatal phthalate exposure and AGD in infant boys was evaluated (Swan et al., 2005). AGD was measured when the boys were 2–36 months old. Maternal urine samples taken during pregnancy were analyzed in the study for nine phthalate metabolites (mBP, mBzP, mono-3-carboxypropyl phthalate (mCPP), mEP, mono-isobutyl phthalate (miBP), mMP, mono-2-ethyl-5-hydroxyhexyl phthalate (mEHHP), MEHP and mono-2-ethyl-5-oxohexyl phthalate (mEOHP)). Maternal urine levels of four phthalate metabolites (mEP, mBP, mBzP and miBP) were inversely related to son's anogenital index (AGI), which is calculated by dividing AGD with weight. Summary phthalate score of the four phthalate metabolites was negatively associated with age-adjusted AGI. AGI in turn was associated with the rate of cryptorchidism, so that cryptorchidism was significantly more common in boys with short AGI (AGI below 25th percentile for age) than in boys with long or intermediate AGI (Swan et al., 2005).

Two recently published studies have evaluated associations between cryptorchidism and parental exposure to several classes of endocrine disrupters (Morales-Suarez-Varela et al., 2011; Wagner-Mahler et al., 2011). The Danish follow-up study on boys belonging to the Danish National Birth Cohort (1997–2009) (Morales-Suarez-Varela et al., 2011) evaluated possible or probable parental occupational exposure to different groups of EDCs using a job exposure matrix (Van Tongeren et al., 2002). Diagnosis of cryptorchidism was based on the Medical Birth and National Hospital Discharge Register and both congenital and acquired cases were included (Morales-Suarez-Varela et al., 2011). Maternal potential exposure to EDCs in general or to any of the seven different groups of EDCs, i.e. pesticides, polychlorinated organic compounds, phthalate esters, alkylphenols, bis-phenols, heavy metals (cadmium, lead, mercury) or other hormone disrupting chemicals, was not associated with a significantly increased risk of cryptorchidism, but father's probable exposure to heavy metals was associated with an increased risk of cryptorchidism (Morales-Suarez-Varela et al., 2011). A recent French case–control study also evaluated the association between parental occupational exposures to several classes of endocrine disrupting chemicals and cryptorchidism (Wagner-Mahler et al., 2011). The study was based on a prospective clinical study on the incidence of cryptorchidism in newborn boys, and data on parental occupational exposures to EDCs was based on a questionnaire data (Wagner-Mahler et al., 2011). Maternal self-reported occupational exposure to anti-rust products and phthalates correlated positively with cryptorchidism and PCBs exhibited a similar trend, but the correlations were based on small numbers (Wagner-Mahler et al., 2011). Also the previously mentioned studies by Pierik et al. (2004) and Carbone et al. (2007) evaluated the association between cryptorchidism and probable parental exposure to EDCs in general (based on job-exposure matrix (Van Tongeren et al., 2002)), but no statistically significant association was found (Pierik et al., 2004; Carbone et al., 2007).

However, as mentioned at the beginning of the review, not only environmental factors, but also lifestyle and genetic factors may affect testicular development and descent and cause testicular dysgenesis and cryptorchidism (Skakkebaek et al., 2001; Toppari et al., 2010). Some studies have for instance suggested an association between cryptorchidism and maternal smoking (Akre et al., 1999; Biggs et al., 2002; Jensen et al., 2007; McBride et al., 1991). Maternal smoking is also a well-known risk factor for reduced intrauterine growth, and being born as small for gestational age is one of the risk factors of cryptorchidism (Akre et al., 1999; Berkowitz et al., 1993; Biggs et al., 2002; Boisen et al., 2004; Ghirri et al., 2002). It has also been suggested, that use of mild analgesics during pregnancy, especially during the first and second trimester, may be associated with an increased risk of cryptorchidism (Jensen et al., 2010; Kristensen et al., 2011). In rats, fetal exposure to paracetamol has been associated with reduced anogenital distance, and paracetamol and acetylsalisylic acid reduced testosterone secretion of fetal rat testes in *ex vivo* system (Kristensen et al., 2011).

5. Conclusions

Various xenobiotics have been found to disrupt the endocrine system in animals. Reduction in the dominance of androgens to estrogens, and interference with androgen or Insl3 production or action during fetal life, are apparent mechanisms causing cryptorchidism in animals. When evaluating associations between fetal exposure to estrogenic agents and cryptorchidism in humans, exposure to DES was associated with an increased risk of cryptorchidism. Studies evaluating pesticide use in a geographical area or parental possible occupational exposure to pesticides, have suggested that also exposure to them may be associated with an increased risk of cryptorchidism in boys. Some case–control studies evaluating maternal breast milk levels of chemicals have reported associations between congenital cryptorchidism and the levels of environmental chemicals with possible endocrine disrupting activities. No clear positive association was reported in studies evaluating levels of endocrine disrupting chemicals in placenta, cord serum or maternal serum. Maternal breast milk phthalate and PBDE levels have shown anti-androgen-like associations with infant reproductive hormone levels. More studies are needed to confirm the observed associations and to evaluate associations between cryptorchidism and combined exposures.

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References

Acerini, C.L., Miles, H.L., Dunger, D.B., Ong, K.K., Hughes, I.A., 2009. The descriptive epidemiology of congenital and acquired cryptorchidism in a UK infant cohort. Arch. Dis. Child. 94, 868–872.
Achiron, R., Pinhas-Hamiel, O., Zalel, Y., Rotstein, Z., Lipitz, S., 1998. Development of fetal male gender: prenatal sonographic measurement of the scrotum and evaluation of testicular descent. Ultrasound Obstet. Gynecol. 11, 242–245.
Akre, O., Lipworth, L., Cnattingius, S., Sparen, P., Ekbom, A., 1999. Risk factor patterns for cryptorchidism and hypospadias. Epidemiology 10, 364–369.
Amann, R.P., Veeramachaneni, D.N., 2007. Cryptorchidism in common eutherian mammals. Reproduction 133, 541–561.
Anand-Ivell, R., Ivell, R., Driscoll, D., Manson, J., 2008. Insulin-like factor 3 levels in amniotic fluid of human male fetuses. Hum. Reprod. 23, 1180–1186.
Andersen, H.R., Schmidt, I.M., Grandjean, P., Jensen, T.K., Budtz-Jorgensen, E., Kjaerstad, M.B., Baelum, J., Nielsen, J.B., Skakkebaek, N.E., Main, K.M., 2008. Impaired reproductive development in sons of women occupationally exposed to pesticides during pregnancy. Environ. Health Perspect. 116, 566–572.
Ars, E., Lo Giacco, D., Bassas, L., Nuti, F., Rajmil, O., Ruiz, P., Garat, J.M., Ruiz-Castane, E., Krausz, C., 2011. Further insights into the role of T222P variant of RXFP2 in non-syndromic cryptorchidism in two Mediterranean populations. Int. J. Androl. 34, 333–338.
Badawi, A.F., Cavalieri, E.L., Rogan, E.G., 2000. Effect of chlorinated hydrocarbons on expression of cytochrome P450 1A1, 1A2 and 1B1 and 2- and 4-hydroxylation of 17beta-estradiol in female Sprague-Dawley rats. Carcinogenesis 21, 1593–1599.
Barlow, N.J., Phillips, S.L., Wallace, D.G., Sar, M., Gaido, K.W., Foster, P.M., 2003. Quantitative changes in gene expression in fetal rat testes following exposure to di(*n*-butyl)phthalate. Toxicol. Sci. 73, 431–441.
Barthold, J.S., Kryger, J.V., Derusha, A.M., Duel, B.P., Jednak, R., Skafar, D.F., 1999. Effects of an environmental endocrine disruptor on fetal development, estrogen receptor(alpha) and epidermal growth factor receptor expression in the porcine male genital tract. J. Urol. 162, 864–871.
Barteczko, K.J., Jacob, M.I., 2000. The testicular descent in human. Origin, development and fate of the gubernaculum Hunteri, processus vaginalis peritonei, and gonadal ligaments. Adv. Anat. Embryol. Cell Biol. 156, 1–98.
Bay, K., Cohen, A.S., Jorgensen, F.S., Jorgensen, C., Lind, A.M., Skakkebaek, N.E., Andersson, A.M., 2008. Insulin-like factor 3 levels in second-trimester amniotic fluid. J. Clin. Endocrinol. Metab. 93, 4048–4051.
Bay, K., Andersson, A.M., 2011. Human testicular insulin-like factor 3: in relation to development, reproductive hormones and andrological disorders. Int. J. Androl. 34, 97–109.

Bay, K., Main, K.M., Toppari, J., Skakkebaek, N.E., 2011. Testicular descent: INSL3, testosterone, genes and the intrauterine milieu. Nat. Rev. Urol. 8, 187–196.
Beasley, S.W., Hutson, J.M., 1987. Effect of division of genitofemoral nerve on testicular descent in the rat. Aust. N.Z. J. Surg. 57, 49–51.
Beltran-Brown, F., Villegas-Alvarez, F., 1988. Clinical classification for undescended testes: experience in 1010 orchidopexies. J. Pediatr. Surg. 23, 444–447.
Berkowitz, G.S., Lapinski, R.H., Dolgin, S.E., Gazella, J.G., Bodian, C.A., Holzman, I.R., 1993. Prevalence and natural history of cryptorchidism. Pediatrics 92, 44–49.
Bhatia, R., Shiau, R., Petreas, M., Weintraub, J.M., Farhang, L., Eskenazi, B., 2005. Organochlorine pesticides and male genital anomalies in the child health and development studies. Environ. Health Perspect. 113, 220–224.
Biggs, M.L., Baer, A., Critchlow, C.W., 2002. Maternal, delivery, and perinatal characteristics associated with cryptorchidism: a population-based case–control study among births in Washington State. Epidemiology 13, 197–204.
Bock, K.W., Kohle, C., 2006. Ah receptor: dioxin-mediated toxic responses as hints to deregulated physiologic functions. Biochem. Pharmacol. 72, 393–404.
Bogatcheva, N.V., Ferlin, A., Feng, S., Truong, A., Gianesello, L., Foresta, C., Agoulnik, A.I., 2007. T222P mutation of the insulin-like 3 hormone receptor LGR8 is associated with testicular maldescent and hinders receptor expression on the cell surface membrane. Am. J. Physiol. Endocrinol. Metab. 292, E138–144.
Boisen, K.A., Kaleva, M., Main, K.M., Virtanen, H.E., Haavisto, A.M., Schmidt, I.M., Chellakooty, M., Damgaard, I.N., Mau, C., Reunanen, M., Skakkebaek, N.E., Toppari, J., 2004. Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries. Lancet 363, 1264–1269.
Borch, J., Ladefoged, O., Hass, U., Vinggaard, A.M., 2004. Steroidogenesis in fetal male rats is reduced by DEHP and DINP, but endocrine effects of DEHP are not modulated by DEHA in fetal, prepubertal and adult male rats. Reprod. Toxicol. 18, 53–61.
Brogden, R.N., Chrisp, P., 1991. Flutamide. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in advanced prostatic cancer. Drugs Aging 1, 104–115.
Brucker-Davis, F., Wagner-Mahler, K., Delattre, I., Ducot, B., Ferrari, P., Bongain, A., Kurzenne, J.Y., Mas, J.C., Fenichel, P., 2008. Cryptorchidism at birth in Nice area (France) is associated with higher prenatal exposure to PCBs and DDE, as assessed by colostrum concentrations. Hum. Reprod. 23, 1708–1718.
Buemann, B., Henriksen, H., Villumsen, A.L., Westh, A., Zachau-Christiansen, B., 1961. Incidence of undescended testis in the newborn. Acta Chir. Scand. Suppl. 283, 289–293.
Carbone, P., Giordano, F., Nori, F., Mantovani, A., Taruscio, D., Lauria, L., Figa-Talamanca, I., 2006. Cryptorchidism and hypospadias in the Sicilian district of Ragusa and the use of pesticides. Reprod. Toxicol. 22, 8–12.
Carbone, P., Giordano, F., Nori, F., Mantovani, A., Taruscio, D., Lauria, L., Figa-Talamanca, I., 2007. The possible role of endocrine disrupting chemicals in the aetiology of cryptorchidism and hypospadias: a population-based case–control study in rural Sicily. Int. J. Androl. 30, 3–13.
Cederroth, C.R., Schaad, O., Descombes, P., Chambon, P., Vassalli, J.D., Nef, S., 2007. Estrogen receptor alpha is a major contributor to estrogen-mediated fetal testis dysgenesis and cryptorchidism. Endocrinology 148, 5507–5519.
Cendron, M., Huff, D.S., Keating, M.A., Snyder 3rd, H.M., Duckett, J.W., 1993. Anatomical, morphological and volumetric analysis: a review of 759 cases of testicular maldescent. J. Urol. 149, 570–573.
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.
Clarnette, T.D., Sugita, Y., Hutson, J.M., 1997. Genital anomalies in human and animal models reveal the mechanisms and hormones governing testicular descent. Br. J. Urol. 79, 99–112.
Couse, J.F., Korach, K.S., 2004. Estrogen receptor-alpha mediates the detrimental effects of neonatal diethylstilbestrol (DES) exposure in the murine reproductive tract. Toxicology 205, 55–63.
Damgaard, I.N., Skakkebaek, N.E., Toppari, J., Virtanen, H.E., Shen, H., Schramm, K.W., Petersen, J.H., Jensen, T.K., Main, K.M., 2006. Persistent pesticides in human breast milk and cryptorchidism. Environ. Health Perspect. 114, 1133–1138.
Dimich-Ward, H., Hertzman, C., Teschke, K., Hershler, R., Marion, S.A., Ostry, A., Kelly, S., 1996. Reproductive effects of paternal exposure to chlorophenate wood preservatives in the sawmill industry. Scand. J. Work Environ. Health 22, 267–273.
El Houate, B., Rouba, H., Imken, L., Sibai, H., Chafik, A., Boulouiz, R., Chadli, E., Hassar, M., McElreavey, K., Barakat, A., 2008. No association between T222P/LGR8 mutation and cryptorchidism in the Moroccan population. Horm. Res. 70, 236–239.
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.
Emmen, J.M., McLuskey, A., Adham, I.M., Engel, W., Verhoef-Post, M., Themmen, A.P., Grootegoed, J.A., Brinkmann, A.O., 2000. Involvement of insulin-like factor 3 (Insl3) in diethylstilbestrol-induced cryptorchidism. Endocrinology 141, 846–849.
Feng, S., Ferlin, A., Truong, A., Bathgate, R., Wade, J.D., Corbett, S., Han, S., Tannour-Louet, M., Lamb, D.J., Foresta, C., Agoulnik, A.I., 2009. INSL3/RXFP2 signaling in testicular descent. Ann. N.Y. Acad. Sci. 1160, 197–204.
Ferlin, A., Simonato, M., Bartoloni, L., Rizzo, G., Bettella, A., Dottorini, T., Dallapiccola, B., Foresta, C., 2003. The INSL3-LGR8/GREAT ligand–receptor pair in human cryptorchidism. J. Clin. Endocrinol. Metab. 88, 4273–4279.
Ferlin, A., Zuccarello, D., Zuccarello, B., Chirico, M.R., Zanon, G.F., Foresta, C., 2008. Genetic alterations associated with cryptorchidism. JAMA 300, 2271–2276.
Fisher, J.S., Macpherson, S., Marchetti, N., Sharpe, R.M., 2003. Human 'testicular dysgenesis syndrome': a possible model using in-utero exposure of the rat to dibutyl phthalate. Hum. Reprod. 18, 1383–1394.
Foresta, C., Zuccarello, D., Garolla, A., Ferlin, A., 2008. Role of hormones, genes, and environment in human cryptorchidism. Endocr. Rev. 29, 560–580.
Fukuzawa, N.H., Ohsako, S., Wu, Q., Sakaue, M., Fujii-Kuriyama, Y., Baba, T., Tohyama, C., 2004. Testicular cytochrome P450scc and LHR as possible targets of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the mouse. Mol. Cell. Endocrinol. 221, 87–96.
Garcia-Rodriguez, J., Garcia-Martin, M., Nogueras-Ocana, M., de Dios Luna-del-Castillo, J., Espigares Garcia, M., Olea, N., Lardelli-Claret, P., 1996. Exposure to pesticides and cryptorchidism: geographical evidence of a possible association. Environ. Health Perspect. 104, 1090–1095.
Gaspari, L., Paris, F., Jandel, C., Kalfa, N., Orsini, M., Daurès, 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.
Ghirri, P., Ciulli, C., Vuerich, M., Cuttano, A., Faraoni, M., Guerrini, L., Spinelli, C., Tognetti, S., Boldrini, A., 2002. Incidence at birth and natural history of cryptorchidism: a study of 10,730 consecutive male infants. J. Endocrinol. Invest. 25, 709–715.
Gorlov, I.P., Kamat, A., Bogatcheva, N.V., Jones, E., Lamb, D.J., Truong, A., Bishop, C.E., McElreavey, K., Agoulnik, A.I., 2002. Mutations of the GREAT gene cause cryptorchidism. Hum. Mol. Genet. 11, 2309–2318.
Gray Jr., L.E., Ostby, J.S., Kelce, W.R., 1994. Developmental effects of an environmental antiandrogen: the fungicide vinclozolin alters sex differentiation of the male rat. Toxicol. Appl. Pharmacol. 129, 46–52.
Gray Jr., L.E., Kelce, W.R., Monosson, E., Ostby, J.S., Birnbaum, L.S., 1995. Exposure to TCDD during development permanently alters reproductive function in male Long Evans rats and hamsters: reduced ejaculated and epididymal sperm numbers and sex accessory gland weights in offspring with normal androgenic status. Toxicol. Appl. Pharmacol. 131, 108–118.
Gray Jr., L.E., Ostby, J., Monosson, E., Kelce, W.R., 1999a. Environmental antiandrogens: low doses of the fungicide vinclozolin alter sexual differentiation of the male rat. Toxicol. Ind. Health 15, 48–64.
Gray Jr., L.E., Wolf, C., Lambright, C., Mann, P., Price, M., Cooper, R.L., Ostby, J., 1999b. Administration of potentially antiandrogenic pesticides (procymidone, linuron, iprodione, chlozolinate, *p,p'*-DDE, and ketoconazole) and toxic substances (dibutyl- and diethylhexyl phthalate, PCB 169, and ethane dimethane sulphonate) during sexual differentiation produces diverse profiles of reproductive malformations in the male rat. Toxicol. Ind. Health 15, 94–118.
Gray Jr., L.E., Ostby, J., Furr, J., Price, M., Veeramachaneni, D.N., Parks, L., 2000. Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. Toxicol. Sci. 58, 350–365.
Gray, T.J., Gangolli, S.D., 1986. Aspects of the testicular toxicity of phthalate esters. Environ. Health Perspect. 65, 229–235.
John Radcliffe Hospital Cryptorchidism Study Group, 1992. Cryptorchidism: a prospective study of 7500 consecutive male births, 1984–8. Arch. Dis. Child. 67, 892–899.
Guillette Jr., L.J., Gross, T.S., Masson, G.R., Matter, J.M., Percival, H.F., Woodward, A.R., 1994. Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. Environ. Health Perspect. 102, 680–688.
Guo, Y.L., Lambert, G.H., Hsu, C.C., Hsu, M.M., 2004. Yucheng: health effects of prenatal exposure to polychlorinated biphenyls and dibenzofurans. Int. Arch. Occup. Environ. Health 77, 153–158.
Hack, W.W., Meijer, R.W., Van Der Voort-Doedens, L.M., Bos, S.D., De Kok, M.E., 2003. Previous testicular position in boys referred for an undescended testis: further explanation of the late orchidopexy enigma? BJU Int. 92, 293–296.
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.
Heyns, C.F., 1987. The gubernaculum during testicular descent in the human fetus. J. Anat. 153, 93–112.
Hickey, J.J., Anderson, D.W., 1968. Chlorinated hydrocarbons and eggshell changes in raptorial and fish-eating birds. Science 162, 271–273.
Hosie, S., Loff, S., Witt, K., Niessen, K., Waag, K.L., 2000. Is there a correlation between organochlorine compounds and undescended testes? Eur. J. Pediatr. Surg. 10, 304–309.
Hutson, J.M., 1985. A biphasic model for the hormonal control of testicular descent. Lancet 2, 419–421.
Hutson, J.M., 1986. Testicular feminization: a model for testicular descent in mice and men. J. Pediatr. Surg. 21, 195–198.
Hutson, J.M., Balic, A., Nation, T., Southwell, B., 2010. Cryptorchidism. Semin. Pediatr. Surg. 19, 215–224.
Huynh, J., Shenker, N.S., Nightingale, S., Hutson, J.M., 2007. Signalling molecules: clues from development of the limb bud for cryptorchidism? Pediatr. Surg. Int. 23, 617–624.
Imperato-McGinley, J., Sanchez, R.S., Spencer, J.R., Yee, B., Vaughan, E.D., 1992. Comparison of the effects of the 5 alpha-reductase inhibitor finasteride and the antiandrogen flutamide on prostate and genital differentiation: dose–response studies. Endocrinology 131, 1149–1156.

IPCS International Programme on Chemical Safety. Available from: <http://whqlibdoc.who.int/hq/2002/a76784_ch1.pdf> (accessed 10.08.2011).
Jacobsen, P.R., Christiansen, S., Boberg, J., Nellemann, C., Hass, U., 2010. Combined exposure to endocrine disrupting pesticides impairs parturition, causes pup mortality and affects sexual differentiation in rats. Int. J. Androl. 33, 434–442.
Jensen, M.S., Toft, G., Thulstrup, A.M., Bonde, J.P., Olsen, J., 2007. Cryptorchidism according to maternal gestational smoking. Epidemiology 18, 220–225.
Jensen, M.S., Rebordosa, C., Thulstrup, A.M., Toft, G., Sorensen, H.T., Bonde, J.P., Henriksen, T.B., Olsen, J., 2010. Maternal use of acetaminophen, ibuprofen, and acetylsalicylic acid during pregnancy and risk of cryptorchidism. Epidemiology 21, 779–785.
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.
Kelce, W.R., Stone, C.R., Laws, S.C., Gray, L.E., Kemppainen, J.A., Wilson, E.M., 1995. Persistent DDT metabolite *p,p′*-DDE is a potent androgen receptor antagonist. Nature 375, 581–585.
Kluwe, W.M., 1982. Overview of phthalate ester pharmacokinetics in mammalian species. Environ. Health Perspect. 45, 3–9.
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.
Kristensen, D.M., Hass, U., Lesne, L., Lottrup, G., Jacobsen, P.R., Desdoits-Lethimonier, C., Boberg, J., Petersen, J.H., Toppari, J., Jensen, T.K., Brunak, S., Skakkebaek, N.E., Nellemann, C., Main, K.M., Jégou, B., Leffers, H., 2011. Intrauterine exposure to mild analgesics is a risk factor for development of male reproductive disorders in human and rat. Hum. Reprod. 26, 235–244.
Kuiper, G.G., Carlsson, B., Grandien, K., Enmark, E., Haggblad, J., Nilsson, S., Gustafsson, J.A., 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology 138, 863–870.
Kurahashi, N., Kasai, S., Shibata, T., Kakizaki, H., Nonomura, K., Sata, F., Kishi, R., 2005. Parental and neonatal risk factors for cryptorchidism. Med. Sci. Monit. 11, CR274–CR283.
Lehmann, K.P., Phillips, S., Sar, M., Foster, P.M., Gaido, K.W., 2004. Dose-dependent alterations in gene expression and testosterone synthesis in the fetal testes of male rats exposed to di(*n*-butyl)phthalate. Toxicol. Sci. 81, 60–68.
Li, L.H., Jester Jr., W.F., Orth, J.M., 1998. Effects of relatively low levels of mono-(2-ethylhexyl) phthalate on cocultured Sertoli cells and gonocytes from neonatal rats. Toxicol. Appl. Pharmacol. 153, 258–265.
Longnecker, M.P., Klebanoff, M.A., Brock, J.W., Zhou, H., Gray, K.A., Needham, L.L., Wilcox, A.J., 2002. Maternal serum level of 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene and risk of cryptorchidism, hypospadias, and polythelia among male offspring. Am. J. Epidemiol. 155, 313–322.
Mably, T.A., Moore, R.W., Peterson, R.E., 1992. In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. 1. Effects on androgenic status. Toxicol. Appl. Pharmacol. 114, 97–107.
Main, K.M., Mortensen, G.K., Kaleva, M.M., Boisen, K.A., Damgaard, I.N., Chellakooty, M., Schmidt, I.M., Suomi, A.M., Virtanen, H.E., Petersen, D.V., Andersson, A.M., Toppari, J., Skakkebaek, N.E., 2006. Human breast milk contamination with phthalates and alterations of endogenous reproductive hormones in infants three months of age. Environ. Health Perspect. 114, 270–276.
Main, K.M., Kiviranta, H., Virtanen, H.E., Sundqvist, E., Tuomisto, J.T., Tuomisto, J., Vartiainen, T., Skakkebaek, N.E., Toppari, J., 2007. Flame retardants in placenta and breast milk and cryptorchidism in newborn boys. Environ. Health Perspect. 115, 1519–1526.
Main, K.M., Skakkebaek, N.E., Virtanen, H.E., Toppari, J., 2010. Genital anomalies in boys and the environment. Best Pract. Res. Clin. Endocrinol. Metab. 24, 279–289.
Martin, O.V., Shialis, T., Lester, J.N., Scrimshaw, M.D., Boobis, A.R., Voulvoulis, N., 2008. Testicular dysgenesis syndrome and the estrogen hypothesis: a quantitative meta-analysis. Environ. Health Perspect. 116, 149–157.
McBride, M.L., Van den Steen, N., Lamb, C.W., Gallagher, R.P., 1991. Maternal and gestational factors in cryptorchidism. Int. J. Epidemiol. 20, 964–970.
McCormack, K.M., Arneric, S.P., Hook, J.B., 1979. Action of exogenously administered steroid hormones following perinatal exposure to polybrominated biphenyls. J. Toxicol. Environ. Health 5, 1085–1094.
McGlynn, K.A., Guo, X., Graubard, B.I., Brock, J.W., Klebanoff, M.A., Longnecker, M.P., 2009. Maternal pregnancy levels of polychlorinated biphenyls and risk of hypospadias and cryptorchidism in male offspring. Environ. Health Perspect. 117, 1472–1476.
McIntyre, B.S., Barlow, N.J., Foster, P.M., 2001. Androgen-mediated development in male rat offspring exposed to flutamide in utero: permanence and correlation of early postnatal changes in anogenital distance and nipple retention with malformations in androgen-dependent tissues. Toxicol. Sci. 62, 236–249.
McKinnell, C., Atanassova, N., Williams, K., Fisher, J.S., Walker, M., Turner, K.J., Saunders, T.K., Sharpe, R.M., 2001. Suppression of androgen action and the induction of gross abnormalities of the reproductive tract in male rats treated neonatally with diethylstilbestrol. J. Androl. 22, 323–338.
McKinnell, C., Sharpe, R.M., Mahood, K., Hallmark, N., Scott, H., Ivell, R., Staub, C., Jegou, B., Haag, F., Koch-Nolte, F., Hartung, S., 2005. Expression of insulin-like factor 3 protein in the rat testis during fetal and postnatal development and in relation to cryptorchidism induced by in utero exposure to di(*n*-butyl)phthalate. Endocrinology 146, 4536–4544.
McLachlan, J.A., Newbold, R.R., Bullock, B., 1975. Reproductive tract lesions in male mice exposed prenatally to diethylstilbestrol. Science 190, 991–992.
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.
Miyata, K., Yabushita, S., Sukata, T., Sano, M., Yoshino, H., Nakanishi, T., Okuno, Y., Matsuo, M., 2002. Effects of perinatal exposure to flutamide on sex hormones and androgen-dependent organs in F1 male rats. J. Toxicol. Sci. 27, 19–33.
Mol, N.M., Sørensen, N., Weihe, P., Andersson, A.-M., Jørgensen, N., Skakkebaek, N.E., Keiding, N., Grandjean, P., 2002. Spermaturia and serum hormone concentrations at the age of puberty in booys prenatally exposed to polychlorinated biphenyls. Eur. J. Endocrinol. 146, 357–363.
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.
Mutoh, J., Taketoh, J., Okamura, K., Kagawa, T., Ishida, T., Ishii, Y., Yamada, H., 2006. Fetal pituitary gonadotropin as an initial target of dioxin in its impairment of cholesterol transportation and steroidogenesis in rats. Endocrinology 147, 927–936.
Mylchreest, E., Sar, M., Cattley, R.C., Foster, P.M., 1999. Disruption of androgen-regulated male reproductive development by di(*n*-butyl)phthalate during late gestation in rats is different from flutamide. Toxicol. Appl. Pharmacol. 156, 81–95.
Mylchreest, E., Wallace, D.G., Cattley, R.C., Foster, P.M., 2000. Dose-dependent alterations in androgen-regulated male reproductive development in rats exposed to di(*n*-butyl)phthalate during late gestation. Toxicol. Sci. 55, 143–151.
Myllymaki, S.A., Haavisto, T.E., Brokken, L.J., Viluksela, M., Toppari, J., Paranko, J., 2005. In utero and lactational exposure to TCDD; steroidogenic outcomes differ in male and female rat pups. Toxicol. Sci. 88, 534–544.
Na, A.F., Harnaen, E.J., Farmer, P.J., Sourial, M., Southwell, B.R., Hutson, J.M., 2007. Cell membrane and mitotic markers show that the neonatal rat gubernaculum grows in a similar way to an embryonic limb bud. J. Pediatr. Surg. 42, 1566–1573.
Nakari, T., Pessala, P., 2005. In vitro estrogenicity of polybrominated flame retardants. Aquat. Toxicol. 74, 272–279.
Nef, S., Parada, L.F., 1999. Cryptorchidism in mice mutant for Insl3. Nat. Genet. 22, 295–299.
Newbold, R.R., Bullock, B.C., McLachlan, J.A., 1987. Testicular tumors in mice exposed in utero to diethylstilbestrol. J. Urol. 138, 1446–1450.
Newton, J.F., Braselton Jr., W.E., Lepper, L.F., McCormack, K.M., Hook, J.B., 1982. Effects of polybrominated biphenyls on metabolism of testosterone by rat hepatic microsomes. Toxicol. Appl. Pharmacol. 63, 142–149.
Ng, S.L., Bidarkar, S.S., Sourial, M., Farmer, P.J., Donath, S., Hutson, J.M., 2005. Gubernacular cell division in different rodent models of cryptorchidism supports indirect androgenic action via the genitofemoral nerve. J. Pediatr. Surg. 40, 434–441.
Nightingale, S.S., Western, P., Hutson, J.M., 2008. The migrating gubernaculum grows like a "limb bud". J. Pediatr. Surg. 43, 387–390.
Nomura, T., Kanzaki, T., 1977. Induction of urogenital anomalies and some tumors in the progeny of mice receiving diethylstilbestrol during pregnancy. Cancer Res. 37, 1099–1104.
Nuti, F., Marinari, E., Erdei, E., El-Hamshari, M., Echavarria, M.G., Ars, E., Balercia, G., Merksz, M., Giachini, C., Shaeer, K.Z., Forti, G., Ruiz-Castane, E., Krausz, C., 2008. The leucine-rich repeat-containing G protein-coupled receptor 8 gene T222P mutation does not cause cryptorchidism. J. Clin. Endocrinol. Metab. 93, 1072–1076.
Ohsako, S., Miyabara, Y., Nishimura, N., Kurosawa, S., Sakaue, M., Ishimura, R., Sato, M., Takeda, K., Aoki, Y., Sone, H., Tohyama, C., Yonemoto, J., 2001. Maternal exposure to a low dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) suppressed the development of reproductive organs of male rats: dose-dependent increase of mRNA levels of 5alpha-reductase type 2 in contrast to decrease of androgen receptor in the pubertal ventral prostate. Toxicol. Sci. 60, 132–143.
Ohtake, F., Takeyama, K., Matsumoto, T., Kitagawa, H., Yamamoto, Y., Nohara, K., Tohyama, C., Krust, A., Mimura, J., Chambon, P., Yanagisawa, J., Fujii-Kuriyama, Y., Kato, S., 2003. Modulation of oestrogen receptor signalling by association with the activated dioxin receptor. Nature 423, 545–550.
Ohtake, F., Baba, A., Takada, I., Okada, M., Iwasaki, K., Miki, H., Takahashi, S., Kouzmenko, A., Nohara, K., Chiba, T., Fujii-Kuriyama, Y., Kato, S., 2007. Dioxin receptor is a ligand-dependent E3 ubiquitin ligase. Nature 446, 562–566.
Ostby, J., Kelce, W.R., Lambright, C., Wolf, C.J., Mann, P., Gray Jr., L.E., 1999. The fungicide procymidone alters sexual differentiation in the male rat by acting as an androgen-receptor antagonist in vivo and in vitro. Toxicol. Ind. Health 15, 80–93.
Overbeek, P.A., Gorlov, I.P., Sutherland, R.W., Houston, J.B., Harrison, W.R., Boettger-Tong, H.L., Bishop, C.E., Agoulnik, A.I., 2001. A transgenic insertion causing cryptorchidism in mice. Genesis 30, 26–35.
Palmer, J.R., Herbst, A.L., Noller, K.L., Boggs, D.A., Troisi, R., Titus-Ernstoff, L., Hatch, E.E., Wise, L.A., Strohsnitter, W.C., Hoover, R.N., 2009. Urogenital abnormalities in men exposed to diethylstilbestrol in utero: a cohort study. Environ. Health 8, 37.
Park, W.H., Hutson, J.M., 1991. The gubernaculum shows rhythmic contractility and active movement during testicular descent. J. Pediatr. Surg. 26, 615–617.

Parks, L.G., Ostby, J.S., Lambright, C.R., Abbott, B.D., Klinefelter, G.R., Barlow, N.J., Gray Jr., L.E., 2000. The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat. Toxicol. Sci. 58, 339–349.
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.
Pierik, F.H., Klebanoff, M.A., Brock, J.W., Longnecker, M.P., 2007. Maternal pregnancy serum level of heptachlor epoxide, hexachlorobenzene, and beta-hexachlorocyclohexane and risk of cryptorchidism in offspring. Environ. Res. 105, 364–369.
Preiksa, R.T., Zilaitiene, B., Matulevicius, V., Skakkebaek, N.E., Petersen, J.H., Jørgensen, N., Toppari, J., 2005. Higher than expected prevalence of congenital cryptorchidism in Lithuania: a study of 1204 boys at birth and 1 year follow-up. Hum. Reprod. 20, 1928–1932.
Prins, G.S., Birch, L., Couse, J.F., Choi, I., Katzenellenbogen, B., Korach, K.S., 2001. Estrogen imprinting of the developing prostate gland is mediated through stromal estrogen receptor alpha: studies with alphaERKO and betaERKO mice. Cancer Res. 61, 6089–6097.
Raman-Wilms, L., Tseng, A.L., Wighardt, S., Einarson, T.R., Koren, G., 1995. Fetal genital effects of first-trimester sex hormone exposure: a meta-analysis. Obstet. Gynecol. 85, 141–149.
Restrepo, M., Munoz, N., Day, N., Parra, J.E., Hernandez, C., Blettner, M., Giraldo, A., 1990. Birth defects among children born to a population occupationally exposed to pesticides in Colombia. Scand. J. Work Environ. Health 16, 239–246.
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.
Rivas, A., Fisher, J.S., McKinnell, C., Atanassova, N., Sharpe, R.M., 2002. Induction of reproductive tract developmental abnormalities in the male rat by lowering androgen production or action in combination with a low dose of diethylstilbestrol: evidence for importance of the androgen–estrogen balance. Endocrinology 143, 4797–4808.
Rogan, W.J., Chen, A., 2005. Health risks and benefits of bis(4-chlorophenyl)-1,1,1-trichloroethane (DDT). Lancet 366, 763–773.
Rotondi, M., Valenzano, F., Bilancioni, E., Spano, G., Giorlandino, C., 2001. Prenatal measurement of testicular diameter by ultrasonography: development of fetal male gender and evaluation of testicular descent. Prenat. Diagn. 21, 112–115.
Sampaio, F.J., Favorito, L.A., 1998. Analysis of testicular migration during the fetal period in humans. J. Urol. 159, 540–542.
Savu, L., Benassayag, C., Vallette, G., Christeff, N., Nunez, E., 1981. Mouse alpha 1-fetoprotein and albumin. A comparison of their binding properties with estrogen and fatty acid ligands. J. Biol. Chem. 256, 9414–9418.
Schettler, T., 2006. Human exposure to phthalates via consumer products. Int. J. Androl. 29, 134–139, discussion 181–5.
Schrader, T.J., Cooke, G.M., 2003. Effects of Aroclors and individual PCB congeners on activation of the human androgen receptor in vitro. Reprod. Toxicol. 17, 15–23.
Scorer, C.G., 1964. The descent of the testis. Arch. Dis. Child. 39, 605–609.
Sharpe, R.M., Skakkebaek, N.E., 1993. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? Lancet 341, 1392–1395.
Sharpe, R.M., Skakkebaek, N.E., 2008. Testicular dysgenesis syndrome: mechanistic insights and potential new downstream effects. Fertil. Steril. 89, e33–38.
Sheehan, D.M., Young, M., 1979. Diethylstilbestrol and estradiol binding to serum albumin and pregnancy plasma of rat and human. Endocrinology 104, 1442–1446.
Shenker, N.S., Huynh, J., Farmer, P.J., Hutson, J.M., 2006. A new role for androgen in testicular descent: permitting gubernacular cell proliferation in response to the neuropeptide, calcitonin gene-related peptide. J. Pediatr. Surg. 41, 407–412.
Shono, T., Ramm-Anderson, S., Hutson, J.M., 1994. Transabdominal testicular descent is really ovarian ascent. J. Urol. 152, 781–784.
Shono, T., Hutson, J.M., Watts, L., Goh, D.W., Momose, Y., Middlesworth, B., Zhou, B., Ramm-Anderson, S., 1996. Scanning electron microscopy shows inhibited gubernacular development in relation to undescended testes in oestrogen-treated mice. Int. J. Androl. 19, 263–270.
Shono, T., Suita, S., Kai, H., Yamaguchi, Y., 2004a. The effect of a prenatal androgen disruptor, vinclozolin, on gubernacular migration and testicular descent in rats. J. Pediatr. Surg. 39, 213–216.
Shono, T., Suita, S., Kai, H., Yamaguchi, Y., 2004b. Short-time exposure to vinclozolin in utero induces testicular maldescent associated with a spinal nucleus alteration of the genitofemoral nerve in rats. J. Pediatr. Surg. 39, 217–219, discussion 217–9.
Simanainen, U., Haavisto, T., Tuomisto, J.T., Paranko, J., Toppari, J., Tuomisto, J., Peterson, R.E., Viluksela, M., 2004. Pattern of male reproductive system effects after in utero and lactational 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposure in three differentially TCDD-sensitive rat lines. Toxicol. Sci. 80, 101–108.
Skakkebaek, N.E., Rajpert-De Meyts, E., Main, K.M., 2001. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum. Reprod. 16, 972–978.
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.
Spencer, J.R., Torrado, T., Sanchez, R.S., Vaughan Jr., E.D., Imperato-McGinley, J., 1991. Effects of flutamide and finasteride on rat testicular descent. Endocrinology 129, 741–748.
Stoker, T.E., Cooper, R.L., Lambright, C.S., Wilson, V.S., Furr, J., Gray, L.E., 2005. In vivo and in vitro anti-androgenic effects of DE-71, a commercial polybrominated diphenyl ether (PBDE) mixture. Toxicol. Appl. Pharmacol. 207, 78–88.
Storgaard, L., Bonde, J.P., Olsen, J., 2006. Male reproductive disorders in humans and prenatal indicators of estrogen exposure. A review of published epidemiological studies. Reprod. Toxicol. 21, 4–15.
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., 2005. Decrease in anogenital distance among male infants with prenatal phthalate exposure. Environ. Health Perspect. 113, 1056–1061.
Thong, M., Lim, C., Fatimah, H., 1998. Undescended testes: incidence in 1002 consecutive male infants and outcome at 1 year of age. Pediatr. Surg. Int. 13, 37–41.
Tian, Y., Ke, S., Thomas, T., Meeker, R.J., Gallo, M.A., 1998. Transcriptional suppression of estrogen receptor gene expression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). J. Steroid Biochem. Mol. Biol. 67, 17–24.
Toppari, J., Larsen, J.C., Christiansen, P., Giwercman, A., Grandjean, P., Guillette Jr., L.J., Jegou, B., Jensen, T.K., Jouannet, P., Keiding, N., Leffers, H., McLachlan, J.A., Meyer, O., Muller, J., Rajpert-De Meyts, E., Scheike, T., Sharpe, R., Sumpter, J., Skakkebaek, N.E., 1996. Male reproductive health and environmental xenoestrogens. Environ. Health Perspect. 104 (Suppl. 4), 741–803.
Toppari, J., Kaleva, M., Virtanen, H.E., 2001. Trends in the incidence of cryptorchidism and hypospadias, and methodological limitations of registry-based data. Hum. Reprod. Update 7, 282–286.
Toppari, J., Virtanen, H.E., Main, K.M., Skakkebaek, N.E., 2010. Cryptorchidism and hypospadias as a sign of testicular dysgenesis syndrome (TDS): environmental connection. Birth Defects Res. A Clin. Mol. Teratol. 88, 910–919.
Toyoda, K., Shibutani, M., Tamura, T., Koujitani, T., Uneyama, C., Hirose, M., 2000. Repeated dose (28 days) oral toxicity study of flutamide in rats, based on the draft protocol for the 'Enhanced OECD Test Guideline 407' for screening for endocrine-disrupting chemicals. Arch. Toxicol. 74, 127–132.
Turusov, V., Rakitsky, V., Tomatis, L., 2002. Dichlorodiphenyltrichloroethane (DDT): ubiquity, persistence, and risks. Environ. Health Perspect. 110, 125–128.
Van Tongeren, M., Nieuwenhuijsen, M.J., Gardiner, K., Armstrong, B., Vrijheid, M., Dolk, H., Botting, B., 2002. A job-exposure matrix for potential endocrine-disrupting chemicals developed for a study into the association between maternal occupational exposure and hypospadias. Ann. Occup. Hyg. 46, 465–477.
Veeramachaneni, D.N., Palmer, J.S., Amann, R.P., Pau, K.Y., 2007. Sequelae in male rabbits following developmental exposure to *p,p′*-DDT or a mixture of *p,p′*-DDT and vinclozolin: cryptorchidism, germ cell atypia, and sexual dysfunction. Reprod. Toxicol. 23, 353–365.
Vidaeff, A.C., Sever, L.E., 2005. In utero exposure to environmental estrogens and male reproductive health: a systematic review of biological and epidemiologic evidence. Reprod. Toxicol. 20, 5–20.
Virtanen, H.E., Koskenniemi, J.J., Sundqvist, E., Main, K.M., Kiviranta, H., Tuomisto, J.T., Tuomisto, J., Viluksela, M., Vartiainen, T., Skakkebaek, N.E., Toppari, J., in press. Associations between congenital cryptorchidism in newborn boys and levels of dioxins and PCBs in placenta. Int. J. Androl. doi:10.1111/j.1365-2605.2011.01233.x.
Wagner-Mahler, K., Kurzenne, J.Y., Delattre, I., Bérard, E., Mas, J.C., Bornebush, L., Tommasi, C., Boda-Buccino, M., Ducot, B., Boullé, C., Ferrari, P., Azuar, P., Bongain, A., Fénichel, P., Brucker-Davis, F., 2011. Prospective study on the prevalence and associated risk factors of cryptorchidism in 6246 newborn boys from Nice area, France. Int. J. Androl. doi: 10.1111/j.1365-2605.2011.01211.x. [Epub ahead of print].
Weidner, I.S., Moller, H., Jensen, T.K., Skakkebaek, N.E., 1998. Cryptorchidism and hypospadias in sons of gardeners and farmers. Environ. Health Perspect. 106, 793–796.
Welsh, M., Saunders, P.T., Fisken, M., Scott, H.M., Hutchison, G.R., Smith, L.B., Sharpe, R.M., 2008. Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism. J. Clin. Invest. 118, 1479–1490.
Williams, K., McKinnell, C., Saunders, P.T., Walker, M., Fisher, J.S., Turner, K.J., Atanassova, N., Sharpe, M., 2001. Neonatal exposure to potent and environmental oestrogens and abnormalities of the male reproductive system in the rat: evidence for importance of the androgen–oestrogen balance and assessment of the relevance to man. Hum. Reprod. Update 7, 236–247.
Wilson, V.S., Lambright, C., Furr, J., Ostby, J., Wood, C., Held, G., Gray Jr., L.E., 2004. Phthalate ester-induced gubernacular lesions are associated with reduced insl3 gene expression in the fetal rat testis. Toxicol. Lett. 146, 207–215.
Wilson, V.S., Howdeshell, K.L., Lambright, C.S., Furr, J., Earl Gray Jr., L., 2007. Differential expression of the phthalate syndrome in male Sprague-Dawley and Wistar rats after in utero DEHP exposure. Toxicol. Lett. 170, 177–184.
Wohlfahrt-Veje, C., Boisen, K.A., Boas, M., Damgaard, I.N., Kai, C.M., Schmidt, I.M., Chellakooty, M., Suomi, A.M., Toppari, J., Skakkebaek, N.E., Main, K.M., 2009. Acquired cryptorchidism is frequent in infancy and childhood. Int. J. Androl. 32, 423–428.
Wolff, M.S., Camann, D., Gammon, M., Stellman, S.D., 1997. Proposed PCB congener groupings for epidemiological studies. Environ. Health Perspect. 105, 13–14.
Wong, C., Kelce, W.R., Sar, M., Wilson, E.M., 1995. Androgen receptor antagonist versus agonist activities of the fungicide vinclozolin relative to hydroxyflutamide. J. Biol. Chem. 270, 19998–20003.
Yamada, T., Kunimatsu, T., Sako, H., Yabushita, S., Sukata, T., Okuno, Y., Matsuo, M., 2000. Comparative evaluation of a 5-day Hershberger assay utilizing mature male rats and a pubertal male assay for detection of flutamide's antiandrogenic activity. Toxicol. Sci. 53, 289–296.

Yamamoto, M., Shirai, M., Tamura, A., Kobayashi, T., Kohara, S., Murakami, M., Arishima, K., 2005. Effects of maternal exposure to a low dose of diethylstilbestrol on sexual dimorphic nucleus volume and male reproductive system in rat offspring. J. Toxicol. Sci. 30, 7–18.

Yuan, F.P., Li, X., Lin, J., Schwabe, C., Bullesbach, E.E., Rao, C.V., Lei, Z.M., 2010. The role of RXFP2 in mediating androgen-induced inguinoscrotal testis descent in LH receptor knockout mice. Reproduction 139, 759–769.

Zhu, J.L., Hjollund, N.H., Andersen, A.M., Olsen, J., 2006. Occupational exposure to pesticides and pregnancy outcomes in gardeners and farmers: a study within the Danish National Birth Cohort. J. Occup. Environ. Med. 48, 347–352.

Zimmermann, S., Steding, G., Emmen, J.M., Brinkmann, A.O., Nayernia, K., Holstein, A.F., Engel, W., Adham, I.M., 1999. Targeted disruption of the Insl3 gene causes bilateral cryptorchidism. Mol. Endocrinol. 13, 681–691.