

Mini review:

ENDOCRINE DISRUPTING COMPOUNDS EXPOSURE AND TESTIS DEVELOPMENT IN MAMMALS

Biola F. Egbowon^a and Olajide A Mustapha

^a School of Science and Technology, Erasmus Darwin Building ERD 200, Nottingham Trent University, Clifton Lane, NG11 8NS, UK

* corresponding author: ebunfunmi@yahoo.com

ABSTRACT

In the last few decades, there is substantial evidence that male reproductive function is deteriorating in humans and wildlife and this is associated with unintentional exposure to widely used synthetic chemicals. Subsequently, much has been done to show that certain chemicals in the environment adversely interfere with the developing fetal gonads of the laboratory animals. Some *in vitro* studies have demonstrated treatment-induced reproductive problems in offspring exposed to endocrine disrupting compounds (EDC) which are similar to those observed in wildlife and human population. Few EDC studies have demonstrated that there are certain periods of gestation when the developing fetus is highly sensitive and at risk of small endocrine changes. Similar observations have been made in the sewage sludge model, however, while animal studies have been insightful in providing valuable information about the range of effects that can be attributed to *in utero* exposure to EDCs, varying levels of maternal doses administered in different studies exaggerated extrapolation of these results to human. Thus the EDC concentration representative of fetal exposure levels is uncertain because of the complexities of its nature. So far, the level of fetal exposure can only be roughly estimated. There is substantial evidence from animal data to prove that EDCs can adversely affect reproductive development and function in male and more has accumulated on the mechanisms by which they exert their effects. This paper therefore, reviews previous studies to highlight the extent to which testis development can be disrupted during fetal life.

Keywords: endocrine disrupting compound, human direct exposure, humans, wildlife and male reproductive

INTRODUCTION

The adverse effects of environmental disrupting compound (EDC) exposure on various aspects of human health and, especially, reproductive development during fetal and early post-natal life, have been a growing concern in most parts of the world. This is particularly evident from the emerging trends in human reproductive health such as testicular cancer, decreasing sperm counts and or hypospadias/cryptorchidism, which are collectively 'termed testicular dysgenesis syndrome' (TDS), over the last few decades. Clinical and epidemiological

evidence have shown declines in human semen quality during the last 5-6 decades (Leto and Frensilli, 1981; Bostofte et al., 1983). Several studies have reported diverse trends in male reproductive health, including increasing incidence of testicular cancer (Forman and Moller, 1994), declining semen quality (Andersen et al., 2000). Reports have shown significant decreases in sperm concentration (113 million/ml vs. 66 million/ml) and semen volume (3.40 ml vs. 2.75 ml) over the period between 1938 and 1990 (Carlsen et al., 1992). A number of reports on the available data from cancer registries have been reviewed (Toppari et

al., 1996) with evidence of an increase in testicular cancer in many countries including England and Wales (Pike et al., 1987), Scotland (Hakulinen et al., 1986), the Nordic and Baltic countries (Adami et al., 1994; Stone et al., 1991), Australia (Pearce et al., 1987), New Zealand (Wilkinson et al., 1992; Spitz et al., 1986), and the United States (Harris and Steinberg, 1954).

Studies on wildlife species have revealed various effects of environmental compounds (Toppari et al., 1996). These include some key observations in gastropods, fish, reptiles, and mammals (Table 2A, B). Generally, some of the reproductive failures reported in wildlife include decreased fertility, decreased hatching success, birth deformities, metabolic abnormalities, behavioural abnormalities, demasculinisation/feminisation of males, and defeminisation/masculinisation of females (Hollander, 1997). A number of reproductive dysfunctions which were reported in male offspring from animal studies have been associated with maternal EDC exposure (Sweeney and Brooks 1996; Imajima et al., 1997). Experimental evidence from many animal studies has also explained some of the anatomical and physiological changes which may occur as a result of EDC exposed in humans. These includes oviductal malformations (Newbold et al., 1983) and a high incidence of uterine fibroids (Baird and Newbold, 2005; Cook et al., 2005).

Environmental disrupting compounds (EDCs)

Environmental disrupting compounds (EDCs) constitute a diverse range of anthropogenic compounds, including organochlorine pesticides, polychlorinated bisphenyls (PCB), alkylphenol polyethoxylates, phytoestrogens, bisphenol-A, phthalates, dioxins, polybrominated diphenyl ethers and heavy metals (Rhind et al., 2002) (Table 1). They are ubiquitous, persist in the environment at low concentrations, however, they appear to exert a range of adverse

effects, which include growth inhibition, reproductive dysfunction and immune system impairment (Rhind, 2002) on many animal species including ruminants and humans. They are often referred to as endocrine disruptors; indicating their potential ability as either hormone agonists, or antagonists of the endogenous compounds.

Their adverse effects may include impaired testosterone secretion, (certain phthalates), altered metabolism (PCBs, polychlorinated bisphenyls hydrocarbons), blockage of hormone action (pesticides) or direct activation of androgen or estrogen receptors (several EDCs) (Rhind, 2002). Animals may be exposed to relatively high concentrations of EDCs mostly through feeding and water; they could be stored and concentrated mainly in the fat tissues (Ekelund et al., 1990; Ahel et al., 1993; Pojana et al., 2007). The accumulated fats may be utilised in periods of pregnancy and lactation when the animals' energy requirements are particularly very high. This could exert endocrine disrupting effects on the animals (Biggsby et al., 1997) thus exposing their embryos and neonates to relatively high concentrations of EDCs. Detectable concentrations of EDCs were reported in body tissues from adults, young children and fetuses (Fowler et al., 2008, 2009; Choi et al., 2008), following absorption of the EDCs from the environment.

Table 1: Common environmental contaminants, sources and health effects from developmental and adult exposures (animal and human data)

Contaminant	Sources	Selected health effects with postnatal exposure	Selected health effects with prenatal exposure
Bisphenol A (BPA)	Industrial chemical and building block for polycarbonate plastic and epoxy resins, lining of metal food and drink cans, plastic bottles, baby toys, dental sealant, cell phones etc.	Oocyte chromosome abnormalities, recurrent miscarriage, ↓ semen quality (Hunt et al., 2003)	Altered puberty onset, altered prostate development, ↓ semen quality, hormonal changes (Herath et al., 2004)
Pesticides in general	Many classes of insecticides, fungicides, herbicides, rodenticides and fumigants. Exposure can occur through food, drinking water or simply from domestic applications	Menstrual irregularities, ↓ fertility, ↓ semen, quality, premature birth, sperm chromosome abnormalities, hormonal changes (Farr et al., 2004)	Altered sex ratio, altered puberty onset, malformation of reproductive tract, ↓ fertility, impaired fetal growth, (IUGR) (Gray et al., 2001)
Phthalates	Plasticizers (added to soften plastics like PVC), cosmetics, perfumes, toys, pharmaceuticals, medical devices, lubricants and wood finishers	Altered (earlier) menarche onset, altered oestrus cycle, ovulatory irregularities, ↓ semen quality, reduced fertility, fetal loss, endometriosis (Cobellis et al., 2003)	Shortened anogenital distance, malformations of reproductive tract, hormonal changes, ↓ semen quality (Couse and Korach, 2004)
Chlorinated hydrocarbons	PCBs, DDT, dioxins/furans	Menstrual irregularities, endometriosis, reduced fertility, fetal loss, ↓ semen quality, altered puberty onset, altered menarche onset (Venners et al., 2005)	Malformations of the reproductive tract, altered oestrus cycle, reduced fertility altered sex ratio, altered puberty onset, ↓ semen quality, delayed time to pregnancy (Miller et al., 2004; Denham et al., 2005)
Pharmaceuticals	DES, ethynylestradiol	-	Malformations of reproductive tract, altered hormone response, menstrual irregularities, reduced fertility, uterine fibroids, miscarriage, hormonal changes, reduced birth weight, fetal loss (Lau et al., 2004)

Wildlife, laboratory and human studies

Reproductive and developmental abnormalities associated with EDC exposures have been well documented in wildlife. These include birds, frogs, seals, polar bears, marine mollusks, and many other wildlife species. For example, alligators from Lake Apopka in Florida, which was highly polluted due to extensive farming activities around the lake, the presence of a sewage treatment plant, and the past spills of organochlorine pesticides, were reported to have been feminized (Hood, 2005; Milnes et al., 2008). Over time, many of the adverse effects observed in wildlife populations have been induced in laboratory animals, supporting the role of EDCs in their

occurrence. For instance, reduced testosterone synthesis, plasma steroid concentrations and male phallus size were reported in juvenile alligators from seven Florida lakes following EDC exposure (Guillette et al., 1999). The mechanism by which Di(*n*-butyl)phthalate (DBP) caused reduced testosterone levels was through decreased production of androgen and the associated sex steroids by the fetal Leydig cells (Lambright et al., 2003). Exposure of rat testes on gestation days 12-21 caused downregulation of mRNA expression for SRB1, StAR, P450scc, 3β-HSD, P450C17 and c-kit and upregulation of mRNA expression for TRPM-2 (Barlow et al., 2003).

Many studies in a variety of species have shown that there is a tendency for reproductive success to be jeopardized not only by direct effects of pollutants on reproductive pathways but also by adversely affecting the general health status of the individual. EDCs are known to have the potential to disrupt processes as diverse as immune function (Markman et al., 2008), thyroid function (Langer et al., 1998; Newbold et al., 2007), bone structure (Fox et al., 2008), mammary structure and function (Moral et al., 2008), cardiovascular function (Ha et al., 2007) and social behaviours (Markman et al., 2008). Perturbation of any one of these systems has the potential to adversely affect an animal's reproductive success (Rhind, 2009). The complexity of EDCs mechanism could be implicated in processes ranging from the increased incidence of breast cancer (Kortenkamp 2006) and a variety of male reproductive effects (Sharpe and Skakkebaek, 2003) to metabolic disturbances. A number of developmental abnormalities from laboratory studies have been associated with EDC exposures. Many chemicals have been associated with the aetiology of various reproductive disorders which are thought to originate in fetal life (Sharpe and Skakkebaek 2003; Skakkebaek et al., 2001) and can be induced in animal models by fetal exposure to environmental chemicals (Fisher et al., 2003; Mylchreest et al., 1999; Parks et al., 2000). Prolonged exposure of rats to Di-2-ethylhexyl phthalate (DEHP) caused reduced testosterone production (Akingbemi et al., 2004). There is also evidence of obstruct gonocyte development in rats exposed to DBP or DEHP leading to significant reduction in the number of germ cells per Sertoli cell at age 25 days compare with controls, as well as some evidence of altered Sertoli cell function and proliferation after such exposures (Hutchison et al., 2008). DBP exposed rats at days 4-6 postnatally showed reduced numbers of spermatogonia and their resumption of proliferation was delayed. Another study reported increased damage to the DNA in the rats spermatozoa as a result of monoethylphthalate (MEP)

exposure (Duty et al., 2005). In addition, some phthalates such as DEHP can inhibit aromatase activities (Davis et al., 1994) and may thus interfere with estrogen production in fetal testis. During gestation, exposed human maternal-fetal unit and maternal tissues contain levels of EDCs that are associated with many *in utero* effects (Ikezuki et al., 2002; Younglai et al., 2002; Tsutsumi, 2005; Barr et al., 2007; Chao et al., 2007; Huang et al., 2007; Thundiyil et al., 2007). Testes exposed to dieldrin caused a reduction in LH-induced testosterone secretion, tissue protein concentrations of LH receptor, steroid acute regulatory protein and induced proteins associated with cancer and apoptosis (Fowler et al., 2007). In addition, protein expression of WNT-2B in the Sertoli and Leydig cells was significantly reduced. The 'anti-androgenicity' of DBP was shown in the suppression of fetal Leydig cell androgen production and consequently the occurrence of cryptorchidism and hypospadias (Johnston et al., 2004). Studies have shown that Polychlorinated bisphenyls (PCBs) can alter estrogen levels in the body and contribute to reproduction problems such as feminisation of males and intersex (Venners et al., 2005).

EDC exposure and testis development

Testis development is accompanied by the expression and activation of a large number of genes. Perturbation of some of these genes has been reported following *in utero* exposure of developing fetuses to EDCs. The chief of these is sex-determining gene located on the Y chromosome (*Sry* gene), which dictates the extent of sex determination and favours testis development. Sheep *Sry* transcripts persist after the full differentiation of the testis as opposed to what happens in mice, (disappears after differentiation), indicating that its role is not limited to initiating sex determination and Sertoli cell differentiation (Payen et al., 1996). In addition, some other genes are involved in mammalian sex determination including Wilms' tumour gene (*WT-1*), Steroidogenic factor gene (*SF-1*), and some growth factors such as anti-

Mullerian hormone (AMH). Interference with the expression of these genes might result in impaired testis development (Yao et al., 2002).

In humans, disrupted *DHH* gene is also correlated with gonadal dysgenesis (Canto et al., 2004). Insulin-like factor 3 (*INSL3*) acts through the receptor *LGR8* to mediate testicular descent, enhancing the growth gubernaculum's primordia and caudal genitourinary ligament. Mutation of the two genes is associated with failure of testicular descent in developing males (Ferlin et al., 2008; Adham and Agoulnik, 2004). Several

studies have provided more evidence for testosterone and/or dihydrotestosterone (DHT) playing a role in testis descent, this include extensive expression of AR in the gubernaculum (Staub et al., 2005), the expression of 5 α reductase (George, 1989), and interaction of *Insl3* and androgens *in vitro* to regulate gubernaculum growth (Emmen et al., 2000). Exposure of rats to flutamide a synthetic antiandrogen between ER 15.5 and 18.5 caused incomplete inguino-scrotal descent (Mylchreest et al., 1999; Amann and Veeramachaneni, 2007).

Table 2A: Examples of reproductive and developmental abnormalities attributed to endocrine disruption in mammals

Species	Location	Observation	Contaminant	References
Mammals				
Humans	Finland, Denmark	Oligospermia, impotence, hypogonadism, decrease libido, reduced sperm dysfunction, menstrual cycle irregularities Infertility	DDT, kepone, oral contraceptive, stilbene derivatives Coumestrol Isoflavonoids PCBs	Degen and Bolt (2000) Hughes (1988) Tyler et al. (1998)
Cattle Sheep	Detroit, Michigan, USA	Infertility, dystocia Impaired reproductive functions	PCBs, dioxins Isoflavonoids DDE	Safe et al. (2000) Puga et al. (2009) Kung et al. (2009)
Seals Mink Panthers	Wadden sea U.S Columbia River System & Canadian Fraser River System	Population decline, developmental toxicity, hormonal alterations Infertility, ovulation failure, implantation failure Infertility	Isoflavonoids, coumestans	Clarke et al. (2000) Hughes (1988)
Rabbits Guniea pigs Mice	Florida	Low ejaculates volume, low sperm concentrations, poor sperm motility, a very high proportion of sperm with morphological abnormalities	DES, isoflavonoids	Hughes (1988) Newbold et al. (2000)

Table 2B: Examples of reproductive and developmental abnormalities attributed to endocrine disruption in birds, reptiles and fish

Species	Location	Observation	Contaminant	References
Birds Japanese quail Gulls Waterbirds	Pacific coast of the USA	Proliferative lesions, reproductive tract tumours, infertility, inhibition of oestrus, inhibition of ovulation	DDT DDT DDE, PCBs	Newbold et al. (2000) Bryan et al. (1989) Safe et al. (2000)
Reptiles Alligators Red-eared slider turtle	Lake Apopka, Florida, USA	Abnormal reproductive behaviour Feminisation of male embryos Egg shell thinning, developmental abnormalities, growth retardation Abnormal gonads, decreased phallus size, altered sex hormone levels	DDT, DDE, dicofol	Guillette et al. (1994), Guillette et al. (1999) Willingham and Crews (1999)
Fish Mosquito fish	St. Lawrence River	Abnormal reproductive development Abnormal expression of secondary sex characteristics, masculinisation failure Hermaphroditism, vitellogenin in males, altered testes development Early mortality, organs deformities Reduced sex steroid levels, delayed sexual maturity, reduced gonad size Decreased hormone levels, reduced ovarian development, reduced egg/larvae viability	trans-Nonachlor, cis-Nonachlor, arochlor, DDE, chlordane Androstenedione	Howell et al. (1980) Safe et al. (2000)

EDC health risk from animal perspective

There are numerous studies on trends of changes in human reproductive health, evident in declining male fertility and associated developmental disorders (Skakkebaek et al., 2001). However, there appears to be less concern about the risks associated with exposure of farm animals to EDCs as there is little evidence of a defined risk to farm animal fertility as a result of exposure to EDC, however, emerging preliminary observations are suggesting that risks may yet become apparent (Rhind, 2005). Rhind (2009) reported that subtle perturbation of underlying processes associated with EDC exposure is not significantly apparent under field observations however, it may be devastating to animal health or reproductive performance. The possibility that the increases in the incidence of poor performance and ill health may become significant in future is not negligible, particularly if exposure to EDCs is increased (Rhind, 2005). For instance, reduced immune cell

numbers in fetal tissues was evident in the sewage sludge model, suggesting that effects are present and that animal performance may be influenced by small, but potentially economically-significant reduction in immune capacity. The subtle nature of the effects of EDC exposure in farm animals may take a long time to establish whether there are measurable consequences associated with it. There is a need for more evidence of subtle differences in performance due to EDC exposure.

Impacts and limitations of epidemiology and laboratory studies

Substantial evidence accumulated from epidemiological studies to support the human health risk related with exposure to endocrine disruptors. Most studies on the effects of EDCs were based on rodent models, and the impacts of such studies have led to the understanding of potential effects and the mechanisms of actions of individual EDCs from such studies. However, such

studies are of limited empirical value in the assessment of health risks in human development because the patterns of experimental exposure are not representative of normal human exposure. The majority of the laboratory studies have used a number of selected chemicals, which were administered at doses higher than environmental levels (Parks et al., 2000; Gray et al., 2001; Hayes et al., 2002). Nevertheless, the importance of environmental relevance of these compounds to exert reproductive abnormalities in mammals remains. This will certainly involve the study of the effects of multi-component mixtures of compounds to determine the effects on mammalian reproduction.

- **Origin of testicular cells target for EDC effects**

In mammals, gonad formation is initiated by the migration of primordial germ cells from the proximal epiblast of the extraembryonic mesoderm, via the hindgut into the gonad (Adams and McLaren, 2002). The somatic cells which constitute about 80 % of the testicular cells originate from either the coelomic epithelium or mesonephros adjacent to the gonad. However, the origin of both fetal and adult Leydig cells remains controversial and recent studies have suggested that these cells do not have a common source. However, it is not unlikely that both the coelomic epithelium and mesonephros have contributed to the fetal Leydig cell population.

- **Sertoli cells (SC)**

Sertoli cells main functions during testis development include cord formation and secretion of anti-Mullerian hormone (AMH) during fetal life (Mackay, 2000), and provide the germ cells with the physical and metabolic support needed for spermatogenesis during postnatal life (Sharpe, 1994). The pre-Sertoli cells populations are derived from the coelomic epithelium and are stimulated by Sry to differentiate into Sertoli cells. Sertoli cells express a number of genes that play important role in testis development which include *SOX9*, *WT1*,

DAX1, *FGF9*, *DHH* and *DMRT1*. Many other genes are being expressed by the Sertoli cells including *WNT-4*, *LHX-1*, *EMX-1*, *LHX-9* and *SF1* (Wilhelm et al., 2007).

Sertoli cell proliferation forms essential part of testis development as there is finite number of germ cells which each of the Sertoli cells can support through spermatogenesis in adulthood and consequently, the number of Sertoli cells per testis determines both the testis size and maximum number of sperm that it can produce (Sharpe and Skakkebaek, 2003). These cells are no longer mitotic after puberty, so the population at that time is definitive.

Proliferation of Sertoli cells occurs during two periods of life: fetal or neonatal and peripubertal, which is morphologically indifferent in most species. In sheep, sexual differentiation and postnatal testicular growth are separated by a period of at least five months giving a more precise analysis (Hochereau-de Reviers et al., 1995). In sheep there are two periods of mitotic activity of Sertoli cells, first after sexual differentiation and second after birth. By contrast, in the fetal rat testis, mitotic activity of Sertoli cells is maximal just before birth, while gonocytes divide earlier (Hilscher et al., 1972), making the two periods overlap (Sharpe and Skakkebaek, 2003). Only one peak of DNA synthesis in rat Sertoli cells has been observed at the end of gestation (Orth, 1982).

Mitotic divisions of Sertoli cells are more numerous before birth but functional maturation of Sertoli cells occurs around the onset of puberty (Sharpe and Skakkebaek, 2003), coinciding with the time they exit the cell cycle (Gondos and Berndston, 1993). They undergo morphological changes which include enlargement of nucleus to become tripartite and nucleolus to become more distinct (Sharpe and Skakkebaek, 2003) and between each Sertoli cell the formation of specialized tight junctions to create the blood-testis barrier (Gondos and Berndston, 1993).

FSH has been shown to stimulate Sertoli cell proliferation in both around and after birth in rat testis (Orth and Boehm,

1990). However, very few FSH binding sites per Sertoli cell were present in the postnatal testis (Barenton et al., 1983). Furthermore, Sertoli cell number has been examined in fetal and postnatal hypophysectomy sheep and hypogonadal (*hpg*) mice, lacking GnRH and therefore no circulating gonadotrophins (FSH and LH). Fetal Sertoli cell proliferation is independent of gonadotrophins, whereas postnatal Sertoli cell proliferation requires gonadotrophins (Hochereau-de Reviers et al., 1995; Baker and O'Shaughnessy, 2001).

Androgens also play an important role in Sertoli cell proliferation in the fetal testis which may be direct or indirect (Johnston et al., 2004). In addition to the endocrine functions, some growth factors have been found to support Sertoli cell proliferation. This includes epidermal growth factor (EGF) and transforming growth factor- α (TGF- α) which stimulate postnatal Sertoli cell proliferation, at least in culture (Petersen et al., 2001) and testis organ cultures (Cupp and Skinner, 2001). Furthermore, TGF and EGFR expression can be regulated through growth stimulatory hormones such as FSH and testosterone (Cupp and Skinner, 2001). While FSH and androgens were reported to have stimulatory effects on Sertoli cell proliferation, thyroid hormone has been reported to have inhibitory effect on the Sertoli cell proliferation in rodents as hypothyroidism prolonged Sertoli cell proliferation (Van Haaster et al., 1992), and conversely, shortens the period of Sertoli cell proliferation in rodents (van Haaster et al., 1993). In addition, thyroid hormone was found to inhibit Sertoli cell proliferation by increasing the expression of the cyclin-dependent kinase inhibitors (CDKIs), P27kip and P21cip, which disrupt the advancement of the Sertoli cell through the cell cycle and consequently result into premature maturation (Holsberger et al., 2003).

- ***Peritubular myoid (PTM) cells***

The peritubular myoid cells are flat, smooth-muscle-like cells that migrate into the gonad from the mesonephros and contribute to testis cord formation by surround-

ing the clusters of Sertoli cells enclosing germ cells, and cooperating with the Sertoli cells to form the base lamina membrane (Martineau et al., 1997). They proliferate during fetal life but decline rapidly after birth (Palombi et al., 1992). The age at which peritubular myoid cells differentiate is not clear but it was reported that these cells do not differentiate until around puberty when they become flatter and longer (Skinner, 1991). Peritubular myoid cells express all known markers of differentiation including desmin, α -smooth muscle actin (SMA) and alkaline phosphatase, shortly after birth (Palombi et al., 1992). SMA expression has long been reported in the peritubular myoid cells of ER19 fetal rat testes (Fisher et al., 2003). Expression of *dhh* and Protein patched homolog 1 (*ptch1*) is important in peritubular myoid cells differentiation and consequently cord formation as inhibition of the *dhh/ptch1* signaling pathway results in disrupted cord formation (Yao et al., 2002) and mice with a null mutation for *dhh* have impaired differentiation of the peritubular myoid cells (Clarke et al., 2000). This is suggesting that DHH/PTCH1 transduction between SC and PTM cells may play a role in peritubular myoid cell migration and differentiation. Similarly, PTM cells have also been shown to express the androgen receptor during fetal and postnatal life (You and Sar, 1998); hence, they may likely be involved in testicular development (Anderson et al., 2002).

- ***Primordial germ cells (PGC)***

Primordial germ cells are the embryonic precursors of the gametes which are responsible for the transfer of genetic information between generations. Unlike in somatic cells of the testis, PGC neither arise from the coelomic epithelium nor mesonephros, but emerge from a small population of epiblast cells, which are derived from the embryonic endoderm lining the yolk sac. The yolk sac situated on the ventral surface of the embryo forms the developmental circulatory system during early embryo development. It provides nourish-

ment and protection for the developing embryo (Ginsburg et al., 1990).

PGCs migrate along the hindgut, before colonizing the genital ridge. These cells were first thought to migrate to the genital ridge independently (De Felici and Dolci, 1987), however, studies have shown that they relate with one another through cytoplasmic processes called filopodia, which form extensive networks interlinking the cells (Bendel-Stenzel et al., 2000). Cadherins, a family of cell adhesion molecules were shown to be involved in the association of the primordial germ cells during the migration since the absence of E-cadherin has caused an increase in the number of ectopic primordial germ cells (Bendel-Stenzel et al., 2000). The PGCs proliferate rapidly during their migration to the genital ridge (Tam and Snow, 1981). After colonizing the genital ridge, they undergo the process of maturation which involves sequence of transition into fetal germ cells and express germ cell nuclear antigen-1 protein (GCNA1 protein), a marker of post-migratory germ cells (Enders and May, 1994). The germ cells at this stage are bipotential, capable of developing as primary meiotic oocytes or mitotic spermatocytes. Whether they become oocytes or spermatocytes, is dependent upon the sex of the gonad (Ford et al., 1975) that is their surrounding environment rather than the chromosomal sex of the germ cells themselves. For instance, XY germ cells can develop as oocytes in a phenotypically female chimaeric embryo and XX germ cells can develop as spermatocytes in a phenotypically male chimaeric embryo (Palmer and Burgoyne, 1991; Kocer et al., 2009). In other word entry into meiosis is inherently based on a cell-autonomous programme (McLaren and Southee, 1997). The embryonic testis was thought to produce an unknown factor that inhibits entry into meiosis and rather initiates mitotic arrest, therefore establishing a spermatogenic fate (McLaren and Southee, 1997). Retinoic acid is usually produced by the mesonephros of the bipotential gonad (Bowles et al., 2006) inducing germ cells to enter meiosis and establish the fate of fe-

male embryo but the male germ cells have been said to be protected from retinoic acid effect by the Sertoli cells, which express P45026, an enzyme that catabolises retinoic acid and consequently acts as a male-specific meiosis-inhibiting factor (Bowles et al., 2006). Li et al. 2009 also reported that P45026 in Sertoli cells acts as a masculinising factor to arrest male germ cells in the G0 phase of the cell cycle and prevents them from entering meiosis. The gonocytes remain mitotically arrested until after birth when they resume proliferation (McLaren, 1984). They then migrate from the centre of the cord to the basement membrane and start to differentiate into spermatogonia (Boulogne et al., 2003).

- **Leydig cells (LC)**

The primary function of Leydig cell in the testis includes steroid synthesis. Two types of Leydig cells have been identified, both of which are responsible for steroidogenesis; the fetal Leydig cells and adult Leydig cells, arising from different cell lines (Habert et al., 2001; Kerr and Knell, 1988). Both the coelomic epithelium (Yao et al., 2002; Luo et al., 1994) and mesonephros (Buehr and McLaren, 1993; Nishino et al., 2001) were suggested to contribute to the precursors of Leydig cells as the coelomic epithelium is known to give rise partly to the Sertoli cells and a significant number of interstitial cells (Karl and Capel, 1998). These two cell lines are structurally similar, but their functional properties differ considerably (Huhtaniemi, 1989). The main origin of fetal Leydig cells remains controversial as many studies have been involved in this subject and no definitive evidence was shown. They were thought to differentiate from mesenchyme-like stem cells (Byskov, 1986). In addition, neural crest has been suggested to contribute to the fetal Leydig cell population, as these cells express the neural cell adhesion molecule (NCAM) (Mayerhofer et al., 1996). The primary role of the fetal Leydig cells is to produce androgens for masculinisation of the embryo and to secrete insulin-like growth factor 3 (Insl3), which, com-

bine with androgen action to induce testicular descent (Klonisch et al., 2004). The adult Leydig cells differentiate after birth, and are thought to evolve from undifferentiated precursor cells from the mesenchymal cells of the interstitial compartment (Hardy et al., 1993). However, Brennan et al. (2003) reported that coelomic epithelium is not the main origin of Leydig cells although; some of them may differentiate from the coelomic epithelium.

Fetal Leydig cell differentiation and development requires activation of *sfl* and *dhh* (Park et al., 2007). Fetal *DHH* gene could be a target for EDCs' action during testis development. Maternal smoking during pregnancy specifically reduced human fetal *DHH* expression during testis development (Fowler et al., 2008). Sertoli cells, peritubular myoid cells, endothelial cells and interstitial fibroblasts are also involved in Leydig cell differentiation. Sertoli cells activate *dhh* and *pdgf-α* that promote Leydig cell differentiation while others express the X-linked aristaless related homeobox gene (*arx*), which has been shown to contribute to Leydig cell development (Kitamura et al., 2002). The fetal Leydig cells are more active and steroid production per cell is much greater than in the adult Leydig cells (Huhtaniemi et al., 1982). This may be because of the non-regulatory nature of the testosterone synthesis from the fetal Leydig cells. In sheep, mitotic activity of Leydig cells is similar to that of Sertoli cells; at least, seven and six mitotic divisions were reported before birth and before day 110 of gestation respectively (Hochereau-de Reviers et al. 1995). However, Leydig cells decrease in size with increasing fetal age, indicating likely decrease in their secretion during the last month of gestation. Adult Leydig cell steroidogenesis is regulated by luteinizing hormone (LH), component of the hypothalamus pituitary gonadal axis (HPG-axis). Perinatal regulation of steroidogenesis requires LH (O'Shaughnessy et al., 1998) indicating, there must be a switch, around the time of birth, from gonadotrophins independent to gonadotro-

phins dependent steroidogenesis (O'Shaughnessy et al., 1998).

CONCLUSIONS

This review has shown various reproductive health risks associated with EDC exposure. Testicular cells can be a target for EDC adverse effects and EDC exposure responses maybe different among testicular cells. The EDC effects can be directly on the cells number or shape through several other mechanisms which can influence cell function. There is need to investigate what other mechanism plays a role in disrupting the development and functions of the two primary cells in the testis with respect to EDC exposure.

REFERENCES

- Adami HO, Bergström R, Möhner M, Zatoński W, Storm H, Ekblom A et al. Testicular cancer in nine northern European countries. *Int J Cancer* 1994;59:33–8.
- Adams IR, McLaren A. Sexually dimorphic development of mouse primordial germ cells: switching from oogenesis to spermatogenesis. *Development* 2002;129:1155–64.
- Adham IM, Agoulnik AI. Insulin-like 3 signaling in testicular descent. *Int J Androl* 2004;27:257–65.
- Ahel M, McEvoy J, Giger W. Bioaccumulation of the lipophilic metabolites of non-ionic surfactants in freshwater organisms. *Environ Pollut* 1993;79:243–8.
- Akingbemi BT, Sottas CM, Koulova AI, Klinefelter GR, Hardy MP. Inhibition of testicular steroidogenesis by the xenoestrogen bisphenol A is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat Leydig cells. *Endocrinology* 2004;145:592–603.

- Amann RP, Veeramachaneni DN. Cryptorchidism in common eutherian mammals. *Reproduction* 2007;133:541-61.
- Andersen AG, Jensen TK, Carlsen E, Jørgensen N, Andersson AM, Krarup T et al. High frequency of sub-optimal semen quality in an unselected population of young men. *Hum Reprod* 2000;15:366-72.
- Anderson RA, Cambray N, Hartley PS, McNeilly AS. Expression and localization of inhibin alpha, inhibin/activin betaA and betaB and the activin type II and inhibin beta-glycan receptors in the developing human testis. *Reproduction* 2002;123:779-88.
- Baird DD, Newbold R. Prenatal diethylstilbestrol (DES) exposure is associated with uterine leiomyoma development. *Reprod Toxicol* 2005;20:81-4.
- Baker PJ, O-Shaughnessy PJ. Role of gonadotrophins in regulating numbers of Leydig and Sertoli cells during fetal and postnatal development in mice. *Reproduction* 2001;122:227-34.
- Barenton B, Hochereau-de Reviers MT, Perreau C, Saumande J. Changes in testicular gonadotrophin receptors and steroid content through postnatal development until puberty in the lamb. *Endocrinology* 1983;112:1447-53.
- Barlow NJ, Phillips SL, Wallace DG, Sar M, Gaido KW, Foster PM. Quantitative changes in gene expression in fetal rat testes following exposure to di(n-butyl) phthalate. *Toxicol Sci* 2003;73:431-41.
- Barr DB, Bishop A, Needham LL. Concentrations of xenobiotic chemicals in the maternal-fetal unit. *Reprod Toxicol* 2007;23:260-6.
- Bendel-Stenzel MR, Gomperts M, Anderson R, Heasman J, Wylie C. The role of cadherins during primordial germ cell migration and early gonad formation in the mouse. *Mech Dev* 2000;91:143-52.
- Bigsby RM, Caperell-Grant A, Madhukar BV. Xenobiotics released from fat during fasting produce estrogenic effects in ovariectomized mice. *Cancer Res* 1997;57:865-9.
- Bostofte E, Serup J, Rebbe H. Has the fertility of Danish men declined through the years in terms of semen quality? A comparison of semen qualities between 1952 and 1972. *Int J Fertil* 1983;28:91-5.
- Boulogne B, Habert R, Levacher C. Regulation of the proliferation of cocultured gonocytes and Sertoli cells by retinoids, triiodothyronine, and intracellular signaling factors: differences between fetal and neonatal cells. *Mol Reprod Dev* 2003;65:194-203.
- Bowles J, Knight D, Smith C, Wilhelm D, Richman J, Mamiya S et al. Retinoid signaling determines germ cell fate in mice. *Science* 2006;312:596-600.
- Brennan J, Tilmann C, Capel B. Pdgfr-alpha mediates testis cord organization and fetal Leydig cell development in the XY gonad. *Genes Dev* 2003;17:800-10.
- Bryan TE, Gildersleeve RP, Wiard RP. Exposure of Japanese quail embryos to o,p'-DDT has long-term effects on reproductive behaviors, hematology, and feather morphology. *Teratology* 1989;39:525-35.
- Buehr M, McLaren A. Mesonephric contribution to testis differentiation in the fetal mouse. *Development* 1993;117:273-81.
- Byskov AG. Differentiation of mammalian embryonic gonad. *Physiol Rev* 1986;66:71-117.

- Canto P, Soderlund D, Reyes E, Mendez JP. Mutations in the desert hedgehog (DHH) gene in patients with 46, XY complete pure gonadal dysgenesis. *J Clin Endocrinol Metab* 2004;89:4480–3.
- Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Evidence for decreasing quality of semen during past 50 years. *BMJ* 1992;305:609–13.
- Chao HR, Wang SL, Lin LY, Lee WJ, Papke O. Placental transfer of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls in Taiwanese mothers in relation to menstrual cycle characteristics. *Food Chem Toxicol* 2007;45:259–65.
- Choi H, Perera F, Pac A, Wang L, Flak E, Mroz E et al. Estimating individual-level exposure to airborne polycyclic aromatic hydrocarbons throughout the gestational period based on personal, indoor, and outdoor monitoring. *Environ Health Perspect* 2008;116:1509-18.
- Clarke AM, Garland KK, Russell LD. Desert hedgehog (Dhh) gene is required in the mouse testis for formation of adult-type Leydig cells and normal development of peritubular cells and seminiferous tubules. *Biol Reprod* 2000;63:1825-38.
- Cobellis L, Latini G, De Felice C, Razzi S, Paris I, Ruggieri F et al. High plasma concentrations of di-(2-ethylhexyl)-phthalate in women with endometriosis. *Hum Reprod* 2003;18:1512–5.
- Cook JD, Davis BJ, Cai SI, Barrett JC, Conti CJ, Walker CL. Interaction between genetic susceptibility and early-life environmental exposure determines tumor-suppressor-gene penetrance. *Proc Natl Acad Sci USA* 2005;102:8644–9.
- Couse JF, Korach KS. Estrogen receptor-alpha mediates the detrimental effects of neonatal diethylstilbestrol (DES) exposure in the murine reproductive tract. *Toxicology* 2004;205:55–63.
- Cupp AS, Skinner MK. Expression, action, and regulation of transforming growth factor alpha and epidermal growth factor receptor during embryonic and perinatal rat testis development. *J Androl* 2001;22:1019-29.
- Davis BJ, Maronpot RR, Heindel JJ. Di-(2-ethylhexyl)phthalate suppresses estradiol and ovulation in cycling rats. *Toxicol Appl Pharmacol* 1994;128:216–23.
- De Felici M, Dolci S. Cellular interactions of mouse fetal germ cells in in-vitro systems. *Curr Top Dev Biol* 1987;23:147-62.
- Degen GH, Bolt HM. Endocrine disruptors: update on xenoestrogens. *Int Arch Occup Environ Health* 2000;73:433–41.
- Denham M, Schell LM, Deane G, Gallo MV, Ravenscroft J, DeCaprio AP. Relationship of lead, mercury, mirex, dichlorodiphenyldichloroethylene, hexachlorobenzene, and polychlorinated biphenyls to timing of menarche among Akwesasne Mohawk girls. *Pediatrics* 2005;115:e127–34.
- Duty SM, Calafat AM, Silva MJ, Ryan L, Hauser R. Phthalate exposure and reproductive hormones in adult men. *Hum Reprod* 2005;20:604–10.
- Ekelund R, Bergman A, Granmo A, Berggren M. Bioaccumulation of 4-nonylphenol in marine animals—a re-evaluation. *Environ Pollut* 1990;64:107–20.
- Emmen JM, McLuskey A, Adham IM, Engel W, Grootegoed JA, Brinkmann AO. Hormonal control of gubernaculum development during testis descent: gubernaculum outgrowth in vitro requires both insulin-like factor and androgen. *Endocrinology* 2000;141:4720-7.
- Enders GC, May JJ. Developmentally regulated expression of a mouse germ cell nuclear antigen examined from embryonic day 11 to adult in male and female mice. *Dev Biol* 1994;163:331–40.

- Farr SL, Cooper GS, Cai J, Savitz DA, Sandler DP. Pesticide use and menstrual cycle characteristics among premenopausal women in the Agricultural Health Study. *Am J Epidemiol* 2004;160:1194–204.
- Ferlin A, Zuccarello D, Zuccarello B, Chirico MR, Zanon GF, Foresta C. Genetic alterations associated with cryptorchidism. *JAMA* 2008;300:2271-6.
- Fisher JS, Macpherson S, Marchetti N, Sharpe RM. Human ‘testicular dysgenesis syndrome’: a possible model based on in-utero exposure of the rat to dibutyl phthalate. *Hum Reprod* 2003;18:1383–94.
- Ford CE, Evans EP, Burtenshaw MD, Clegg HM, Tuffrey M, Barnes RD. A functional ‘sex-reversed’ oocyte in the mouse. *Proc R Soc Lond B Biol Sci* 1975;190:187–97.
- Forman D, Moller H. Testicular cancer. *Cancer Surv* 1994;19-20:323-41.
- Fowler PA, Abramovich DR, Haites NE, Cash P, Groome NP, Al-Qahtani A et al. Human fetal testis Leydig cell disruption by exposure to the pesticide dieldrin at low concentrations. *Hum Reprod* 2007;22:2919–27.
- Fowler PA, Dorà NJ, McFerran H, Amezcaga MR, Miller DW, Lea RG et al. In-utero exposure to low doses of environmental pollutants disrupts fetal ovarian development in sheep. *Mol Hum Reprod* 2008;14:269-80.
- Fowler P, O’Shaughnessy P, Rhind S, Ayres J. PAH exposure letter. *Environ Health Perspect* 2009;117:A140.
- Fox GA, Lundberg R, Wejheden C, Lind L, Larsson S, Orberg J et al. Health of herring gulls (*Larus argentatus*) in relation to breeding location in the early 1990s. III. Effects on the bone tissue. *J Toxicol Environ Health A* 2008;71:1-9.
- George FW. Developmental pattern of 5 alpha-reductase activity in the rat gubernaculum. *Endocrinology* 1989;124:727-32.
- Ginsburg M, Snow MH, McLaren A. Primordial germ cells in the mouse embryo during gastrulation. *Development* 1990;110:521-8.
- Gondos B, Berndston WE. Postnatal and pubertal development. In: Russell LD, Griswold MD (eds): *The Sertoli cell* (pp 115-154). Clear Water, FL: Cache River Press, 1993.
- Gray LE, Ostby J, Furr J, Wolf CJ, Lambright C, Parks L et al. Effects of environmental antiandrogens on reproductive development in experimental animals. *Hum Reprod Update* 2001;7:248-64.
- Guillette Jr LJ, Gross TS, Masson GR, Matter JM, Percival HF, Woodward AR. Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. *Environ Health Perspect* 1994;102:680–8.
- Guillette Jr LJ, Woodward AR, Crain DA, Pickford DB, Rooney AA, Percival HF. Plasma steroid concentrations and male phallus size in juvenile alligators from seven Florida lakes. *Gen Comp Endocrinol* 1999;116:356–72.
- Ha M-H, Lee D-H, Jacobs DR. Association between serum concentrations of persistent organic pollutants and self-reported cardiovascular disease prevalence: results from the National Health and Nutrition Examination Survey, 1999-2002. *Environ Health Perspect* 2007;115:1204-9.
- Habert R, Lejeune H, Saez JM. Origin, differentiation and regulation of fetal and adult Leydig cells. *Mol Cell Endocrinol* 2001;179:47-74.

- Hakulinen T, Andersen A, Malker B, Pukala E, Schou G, Tulinius H. Trends in cancer incidence in the Nordic countries. A collaborative study of the five Nordic Cancer Registries. *Acta Pathol Microbiol Immunol Scand* 1986;288(Suppl):1–151.
- Hardy MP, Nonneman D, Ganjam VK, Zirkin BR. Hormonal control of Leydig cell differentiation and mature function. In: Whitcomb R, Zirkin BR (eds): *Understanding male fertility: basic and clinical approaches* (pp 125-142). New York: Raven Press, 1993.
- Harris LE, Steinberg AG. Abnormalities observed during the first six days of life in 8,716 live-born infants. *Pediatrics* 1954;14: 314–26.
- Hayes TB, Collins A, Lee M, Mendoza M, Noriega N, Stuart AA et al. Hermaphroditic, demasculinized frogs after exposure to the herbicide, atrazin, at low ecologically relevant doses. *Proc Natl Acad Sci U S A* 2002;9:5476-80.
- Herath CB, Jin W, Watanabe G, Arai K, Suzuki AK, Taya K. Adverse effects of environmental toxicants, octylphenol and bisphenol A, on male reproductive functions in pubertal rats. *Endocrine* 2004;25: 163–72.
- Hilscher B, Hilscher W, Delbrück G, Le-rouge-Bénard B. Autoradiographische Bestimmung der S-Phasen-Dauer der Gonozyten bei der Wirtsratte durch Einfach- und Doppelmarkierung. *Z Zellforsch Mikrosk Anat* 1972;125:229-51.
- Hochereau-de Reviers MT, Perreau C, Pisselet C, Locatelli A, Bosc M. Ontogenesis of somatic and germ cells in sheep fetal testis. *J Reprod Fertil* 1995;103:41-6.
- Hollander D. Environmental effects on reproductive health: the endocrine disruption hypothesis. *Fam Plann Perspect* 1997;29: 82-6.
- Holsberger DR, Jirawatnotai S, Kiyokawa H, Cooke PS. Thyroid hormone regulates the cell cycle inhibitor p27Kip1 in postnatal murine Sertoli cells. *Endocrinology* 2003; 144:3732-8.
- Hood E. Are EDCs blurring issues of gender? *Environ Health Perspect* 2005;113: A670-7.
- Howell WM, Black DA, Bortone SA. Abnormal expression of secondary sex characters in a population of mosquitofish, *Gambusia affinis holbrooki*: evidence of environmental-induced masculinisation. *Copeia* 1980;4:676-81.
- Huang PC, Kuo PL, Guo YL, Liao PC, Lee CC. Associations between urinary phthalate monoesters and thyroid hormones in pregnant women. *Hum Reprod* 2007;22:2715–22.
- Hughes CL Jr. Phytochemical mimicry of reproductive hormones and modulation of herbivore fertility by phytoestrogens. *Environ Health Perspect* 1988;78:171–4.
- Huhtaniemi I. Endocrine function and regulation of the fetal and neonatal testis. *Int J Dev Biol* 1989;33:117-23.
- Huhtaniemi IT, Nozu K, Warren DW, Dufau ML, Catt KJ. Acquisition of regulatory mechanisms for gonadotropin receptors and steroidogenesis in the maturing rat testis. *Endocrinology* 1982;111:1711-20.
- Hunt PA, Koehler KE, Susiarjo M, Hodges, CA, Ilagan A, Voigt RC et al. Bisphenol a exposure causes meiotic aneuploidy in the female mouse. *Curr Biol* 2003;13:546–53.
- Hutchison GR, Scott HM, Walker M, McKinnell C, Ferrara D, Mahood IK, et al. Sertoli cell development and function in an animal model of testicular dysgenesis syndrome. *Biol Reprod* 2008;78:352-60.

- Ikezuki Y, Tsutsumi O, Takai Y, Kamei Y, Taketani Y. Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure. *Hum Reprod* 2002;17:2839-41.
- Imajima T, Shono T, Zakaria O, Suita S. Prenatal phthalate cause cryptorchidism postnatally by inducing transabdominal ascent of the testis in fetal rats. *J Pediatr Surg* 1997;32:18-21.
- Johnston H, Baker PJ, Abel M, Charlton HM, Jackson G, Fleming L et al. Regulation of Sertoli cell number and activity by follicle-stimulating hormone and androgen during postnatal development in the mouse. *Endocrinology* 2004;145:318-29.
- Karl J, Capel B. Sertoli cells of the mouse testis originate from the coelomic epithelium. *Dev Biol* 1998;203:323-33.
- Kerr JB, Knell CM. The fate of fetal Leydig cells during the development of the fetal and postnatal rat testis. *Development* 1988;103:535-44.
- Kitamura K, Yanazawa M, Sugiyama N, Miura H, Iizuka-Kogo A, Kusaka M et al. Mutation of ARX causes abnormal development of forebrain and testes in mice and X-linked lissencephaly with abnormal genitalia in humans. *Nat Genet* 2002;32:359-69.
- Klonisch T, Fowler PA, Hombach-Klonisch S. Molecular and genetic regulation of testis descent and external genitalia development. *Dev Biol* 2004;270:1-18.
- Kocer A, Reichmann J, Best D, Adam IR. Germ cell sex determination in mammals. *Mol Hum Reprod* 2009;15:205-13.
- Koch HM, Calafat AM. Human body burdens of chemicals used in plastic manufacture. *Phil Trans R Soc B* 2009;364:2063-78.
- Kortenkap A. Breast cancer, oestrogens and environmental pollutants: a re-evolution from a mixture perspective. *Int J Androl* 2006;29:193-8.
- Kung T, Murphy KA, White LA. The aryl hydrocarbon receptor (AhR) pathway as a regulatory pathway for cell adhesion and matrix metabolism. *Biochem Pharmacol* 2009;77:536-46.
- Lambright CS, Wilson VS, Furr JR, Wolf CJ, Noriega N, Gray LE Jr. Effects of endocrine disrupting chemicals on fetal testes hormone production. *The Toxicologist* 2003;72:272.
- Langer P, Tajtakova M, Fodor G, Kocan A, Bohov P, Michalek J et al. Increased thyroid volume and prevalence of thyroid disorders in an area heavily polluted by polychlorinated biphenyls. *Eur J Endocrinol* 1998;139:402-9.
- Lau C, Butenhoff JL, Rogers JM. The developmental toxicity of perfluoroalkyl acids and their derivatives. *Toxicol Appl Pharmacol* 2004;198:231-41.
- Leto S, Frensilli FJ. Changing parameters of donor semen. *Fertil Steril* 1981;36:766-70.
- Li H, MacLean G, Cameron D, Clagett-Dame M, Petkovich M. Cyp26b1 expression in murine Sertoli cells is required to maintain male germ cells in an undifferentiated state during embryogenesis. *PLoS One* 2009;4(10):e7501.
- Luo X, Ikeda Y, Parker KL. A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. *Cell* 1994;77:481-90.
- Mackay S. Gonadal development in mammals at the cellular and molecular levels. *Int Rev Cytol* 2000;200:47-99.

- Markman S, Leitner S, Catchpole C, Barnsley S, Muller CT, Pascoe D et al. Pollutants increase song complexity and the volume of the brain area HVC in a songbird. *PLoS One* 2008;3:e1674.
- Martineau J, Nordqvist K, Tilmann C, Lovell-Badge R, Capel B. Male-specific cell migration into the developing gonad. *Curr Biol* 1997;7:958-68.
- Mayerhofer A, Lahr G, Seidl K, Eusterschulte B, Christoph A, Gratzl M. The neural cell adhesion molecule (NCAM) provides clues to the development of testicular Leydig cells. *J Androl* 1996;17:223-30.
- McLaren A. Meiosis and differentiation of mouse germ cells. In: Evans CW, Dickinson HG (eds): *Controlling events in meiosis*. 38th Symposium of the Society for Experimental Biology (pp 7-23). Cambridge, UK: Company of Biologists, 1984.
- McLaren A, Southee D. Entry of mouse embryonic germ cells into meiosis. *Dev Biol* 1997;187:107-13.
- Miller KP, Borgeest C, Greenfeld C, Tomic D, Flaws JA. In utero effects of chemicals on reproductive tissues in females. *Toxicol Appl Pharmacol* 2004;198:111-31.
- Milnes MR, Bryan TA, Katsu Y, Kohno S, Moore BC, Iguchi T et al. Increased post-hatching mortality and loss of sexually dimorphic gene expression in alligators (*Alligator mississippiensis*) from a contaminated environment. *Biol Reprod* 2008;78:932-8.
- Moral R, Wang R, Russo IH, Lamartiniere CA, Pereira J, Russo J. Effect of prenatal exposure to the endocrine disruptor bisphenol A on mammary gland morphology and gene expression signature. *J Endocrinol* 2008;196:101-12.
- Mylchreest E, Sar M, Cattley RC, Foster PM. Disruption of androgen-regulated male reproductive development by di(n-butyl) phthalate during late gestation in rats is different from flutamide. *Toxicol Appl Pharmacol* 1999;156:81-95.
- Newbold RR, Tyrey S, Haney AF, McLachlan JA. Developmentally arrested oviduct: a structural and functional defect in mice following prenatal exposure to diethylstilbestrol. *Teratology* 1983;27:417-26.
- Newbold RR, Hanson RB, Jefferson WN, Bullock BC, Haseman J, McLachlan JA. Proliferative lesions and reproductive tract tumors in male descendants of mice exposed developmentally to diethylstilbestrol. *Carcinogenesis* 2000;21:1355-63.
- Newbold RR, Padilla-Banks E, Snyder RJ, Phillips TM, Jefferson WN. Developmental exposure to endocrine disruptors and the obesity epidemic. *Reprod Toxicol* 2007;23:290-6.
- Nishino K, Yamanouchi K, Naito K, Tojo H. Characterization of mesonephric cells that migrate into the XY gonad during testis differentiation. *Exp Cell Res* 2001;267:225-32.
- Orth JM. Proliferation of Sertoli cells in fetal and postnatal rats: a quantitative autoradiographic study. *Anat Rec* 1982;203:485-92.
- Orth JM, Boehm R. Endorphin suppresses FSH-stimulated proliferation of isolated neonatal Sertoli cells by a pertussis toxin-sensitive mechanism. *Anat Rec* 1990;226:320-7.
- O'Shaughnessy PJ, Baker P, Sohnius U, Haavisto AM, Charlton HM, Huhtaniemi I. Fetal development of Leydig cell activity in the mouse is independent of pituitary gonadotroph function. *Endocrinology* 1998;139:1141-6.

- Palmer SJ, Burgoyne PS. In situ analysis of fetal, prepuberal and adult XX---XY chimaeric mouse testes: Sertoli cells are predominantly, but not exclusively, XY. *Development* 1991;112:265-8.
- Palombi F, Farini D, Salanova M, de Grossi S, Stefanini M. Development and cytodifferentiation of peritubular myoid cells in the rat testis. *Anat Rec* 1992;233:32-40.
- Park SY, Tong M, Jameson JL. Distinct roles for steroidogenic factor 1 and Desert hedgehog pathways in fetal and adult Leydig cell development. *Endocrinology* 2007;148:3704-10.
- Parker KL, Schimmer BP. Steroidogenic factor 1: a key determinant of endocrine development and function. *Endocr Rev* 1997;18:361-77.
- Parks L, Ostby J, Lambright C, Abbott B, Clinefelter GD, Barlow N et al. The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat. *Toxicol Sci* 2000;58:339-49.
- Payen E, Pailhoux E, Merhi RA, Gianquinto L, Kirszenbaum M, Locatelli A et al. Characterization of ovine SRY transcript and developmental expression of genes involved in sexual differentiation. *Int J Dev Biol* 1996;40:567-75.
- Pearce N, Sheppard RA, Howard JK, Fraser J, Lilley BM. Time trends and occupational differences in cancer of the testis in New Zealand. *Cancer* 1987;59:1677-82.
- Petersen C, Boitani C, Froysa B, Soder O. Transforming growth factor-alpha stimulates proliferation of rat Sertoli cells. *Mol Cell Endocrinol* 2001;181:221-7.
- Pike MC, Chilvers CE, Bobrow LG. Classification of testicular cancer in incidence and mortality statistics. *Br J Cancer* 1987;56:83-5.
- Pojana G, Gomiero A, Jonkers N, Marcomini A. Natural and synthetic endocrine disrupting compounds (EDCs) in water, sediment and biota of a coastal lagoon. *Environ Int* 2007;33:929-36.
- Puga A, Ma C, Marlowe JL. The aryl hydrocarbon receptor cross-talks with multiple signal transduction pathways. *Biochem Pharmacol* 2009;77:4:713-22.
- Rhind SM. Endocrine disrupting compounds and farm animals: their properties, actions and routes of exposure. *Domest Anim Endocrinol* 2002;23:179-87.
- Rhind SM. Are endocrine disrupting compounds a threat to farm animal health, welfare and productivity? *Reprod Domest Anim* 2005;40:282-90.
- Rhind SM. Anthropogenic pollutants – a threat to ecosystem sustainability? *Phil Trans R Soc B* 2009;364:3391-401.
- Rhind SM, Smith A, Kyle CE, Telfer G, Martin G, Duff E et al. Phthalate and alkyl phenol concentrations in soil following applications of inorganic fertiliser or sewage sludge to pasture and potential rates of ingestion by grazing ruminants. *J Environ Monit* 2002;4:142-8.
- Safe S, Wormke M, Samudio I. Mechanisms of inhibitory aryl hydrocarbon receptor-estrogen receptor crosstalk in human breast cancer cells. *J Mammary Gland Biol Neoplasia* 2000;5:295-306.
- Sharpe RM. Regulation of spermatogenesis. In: Knobil E, Neill JD (eds): *The physiology of reproduction* (pp 1363-1434). New York: Raven Press, 1994.
- Sharpe RM, Skakkebaek NE. Male reproductive disorders and the role of endocrine disruption: advances in understanding and identification of areas for future research. *Pure Appl Chem* 2003;75:2023-38.

- Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Hum Reprod* 2001;16:972-8.
- Skinner MK. Cell-cell interactions in the testis. *Endocr Rev* 1991;12:45-77.
- Spitz MR, Sider JG, Pollack ES, Lynch HK, Newell GR. Incidence and descriptive features of testicular cancer among United States whites, blacks, and Hispanics, 1973-1982. *Cancer* 1986;58:1785-90.
- Staub C, Rauch M, Ferriere F, Trepos M, Dorval-Coiffec I, Saunders PT et al. Expression of estrogen receptor ESR1 and its 46-kDa variant in the gubernaculum testis. *Biol Reprod* 2005;73:703-12.
- Stone JM, Cruickshank DG, Sandeman TF, Matthews JP. Trebling of the incidence of testicular cancer in victoria, Australia (1950-1985). *Cancer* 1991;68:211-9.
- Sweeney T, Brooks AN. Maternal exposure to oestrogenic toxicants suppress ovine fetal FSH secretion and decrease pituitary FSH responsiveness to a GnRH challenge. *J Reprod Fert* 1996;17:46.
- Tam PP, Snow MH. Proliferation and migration of primordial germ cells during compensatory growth in mouse embryos. *J Embryol Exp Morphol* 1981;64:133-47.
- Thundiyil JG, Solomon GM, Miller MD. Transgenerational exposures: persistent chemical pollutants in the environment and breast milk. *Pediatr Clin North Am* 2007;54:81-101.
- Toppari J, Larsen JC, Christianen P, Gierwerman A, Grandjean P, Guillette LJ Jr et al. Male reproductive health and environmental xenoestrogens. *Environ Health Perspect* 1996;104 (Suppl 4):741-803.
- Tsutsumi O. Assessment of human contamination of estrogenic endocrine-disrupting chemicals and their risk for human reproduction. *J Steroid Biochem Mol Biol* 2005;93:325-30.
- Tyler CR, Jobling S, Sumpter JP. Endocrine disruption in wildlife: a critical review of the evidence. *Crit Rev Toxicol* 1998;28:319-61.
- Van Haaster LH, De Jong FH, Docter R, De Rooij DG. The effect of hypothyroidism on Sertoli cell proliferation and differentiation and hormone levels during testicular development in the rat. *Endocrinology* 1992;131:1574-6.
- Van Haaster LH, de Jong FH, Docter R, de Rooij DG. High neonatal triiodothyronine levels reduce the period of Sertoli cell proliferation and accelerate tubular lumen formation in the rat testis, and increase serum inhibin levels. *Endocrinology* 1993;133:755-60.
- Venners SA, Korrick S, Xu X, Chen C, Guang W, Huang A et al. Preconception serum DDT and pregnancy loss: a prospective study using a biomarker of pregnancy. *Am J Epidemiol* 2005;162:709-16.
- Wilhelm D, Palmer S, Koopman P. Sex determination and gonadal development in mammals. *Physiol Rev* 2007;87:1-28.
- Wilkinson TJ, Colls BM, Schluter PJ. Increased incidence of germ cell testicular cancer in New Zealand Maoris. *Br J Cancer* 1992;65:769-71.
- Willingham E, Crews D. Sex reversal effects of environmentally relevant xenobiotic concentrations on the red-eared slider turtle, a species with temperature-dependent sex determination. *Gen Comp Endocrinol* 1999;113:429-35.

Yao HH, Whoriskey W, Capel B. Desert Hedgehog/Patched 1 signaling specifies fetal Leydig cell fate in testis organogenesis. *Genes Dev* 2002;16:1433-40.

You L, Sar M. Androgen receptor expression in the testes and epididymides of prenatal and postnatal Sprague-Dawley rats. *Endocrine* 1998;9:253-61.

Younglai EV, Foster WG, Hughes EG, Trim K, Jarrell JF. Levels of environmental contaminants in human follicular fluid, serum, and seminal plasma of couples undergoing in vitro fertilization. *Arch Environ Contam Toxicol* 2002;43:121-6.