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RESEARCH ARTICLE

Environmental Pollutants as Endocrine Disruptors and Their Effect on Male Fertility

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ABSTRACT

Endocrine disruptors interfere with the endocrine system and lead to a range of developmental, reproductive, immunological and metabolic diseases in human as well as in animals. Studies report that these endocrine disruptors are released from various man made sources such as industry production like plasticizers, day to day usable products like pesticides and life style factors such as smoking alcohol etc which have an adverse effect on male fertility. Impact may vary from affecting spermatogenesis to testicular cancers. With the increasing fertility problems around the need of the time is to look into the problem, identifying the mechanism responsible and developing methods for preventing infertility in males.

Key words: *Environmental Pollutants, Endocrine Disruptors, Male Fertility*

INTRODUCTION

The phrase 'endocrine disruption' has seemingly become inextricably linked with terms like 'environmental oestrogens' and 'falling sperm counts'. The term endocrine disruptor chemicals (EDCs) were firstly coined in 1991 at the Wingspread Conference Center in Wisconsin. The paper by Theo Colborn *et al.* (1993) was one of the earliest papers about this phenomenon. EDCs are referred to those exogenous substances that can interfere with the endocrine system and then lead to a range of developmental, reproductive, immune, neurological, or metabolic diseases in human and animals (Kavlock *et al.,* 1996). Many EDCs are man-made chemicals produced and are released into the environment by industry production such as plasticizers, organotins, pesticides, or alkylphenols. There is, however, very good evidence that lifestyle factors (e.g. smoking, alcohol consumption and/or use of cosmetics) can have an impact on fertility (Sharpe & Franks 2002; Sharpe & Irvine, 2004). Reports of declining sperm counts over the past 50 years and other disturbing trends alerted scientists to the possibility that exposure to chemicals in the environment may damage male reproductive health (Carlsen *et al.,* 1992). Testicular cancer, the most common malignancy in men 15-44 years of age, has increased markedly in incidence in this century in virtually all countries studied. The incidence of hypospadias (a developmental malformation of the male urethra), cryptorchidism (undescended testicle) and testicular cancer may have increased in some human populations and appears to be increasing in wildlife (Toppari *et al.,* 1996; Fisher, 2004, Sharpe, 2010). The causes of these trends have not been identified and relevant toxicological data about male reproductive effects of environmental toxicants are limited. Recent research efforts have focused on the possibility that exposures to hormonally active compounds, particularly during developmental and in utero are to blame for changes in semen quality, increasing rates of testicular cancer, and malformations of the male urogenital tract (Sharpe, 2010). The

ability to investigate environmental determinants of these indicators of male reproductive health is currently limited by available methodologies and data.

THE MALE REPRODUCTIVE SYSTEM: ENVIRONMENTAL INFLUENCE

Global changes in semen quality are suggested to be produced by the enhanced exposure to environmental chemicals contained in pesticides, food sources, cosmetics, plastics, electronics, and other synthetic materials (Carlsen *et al.,* 1992). The biological basis for this hypothesis is the action of certain chemical compounds, both naturally occurring and anthropogenic (man-made), on endogenous hormone receptors and hormone-dependent pathways. These chemicals are termed hormonally active agents, environmental estrogens, hormone mimics, and endocrine disrupters/disruptors (US. EPA, 1998a; 1998b). A wide range of mechanisms of action are described for endocrine disrupters, including agonists of the estrogen receptor (ER) genistein, diethylstilbestrol (DES; Roy *et al.,* 1997), and bisphenol A (BPA; Kuiper *et al.,* 1998); androgen receptor (AR) antagonists such as vinclozolin (Wong *et al.,* 1995), linuron, procymidone (Gray *et al.,* 1999), phthalates (Foster *et al.,* 2001), and p,p′-dichlorodiphenyl dichloroethylene (p,p′-DDE; Kelce *et al.,* 1995) and aryl hydrocarbon receptor (AhR) agonists, which include dioxins (Toyoshiba *et al.,* 2004), polychlorinated biphenyls (PCB), polycyclic aromatic hydrocarbons (PAH), and polychlorinated dibenzofurans (PCDF; Peterson *et al.,* 1993). Exposure to endocrine-disrupting chemicals may occur through environmental routes (air, soil, water, food) or via occupational exposures (Sharpe & Irvine, 2004).

THE TESTIS: MALE REPRODUCTIVE ORGAN

The testis is both an endocrine gland and a reproductive organ, responsible for the production of hormones and male gametes and an important target for endocrine disruption. The testis consists of two types of tissues: seminiferous tubules, supported by Sertoli cells, and the interstitial compartment, comprised of Leydig cells (Fisher, 2004; Akingbemi, 2005). Testicular functions (spermatogenesis steroidogenesis) are regulated by the hypothalamic-pituitary-testicular (HPT) axis which involves the pituitary gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH; Jana *et al.,* 2006). Testicular functions are proposed to be regulated by a number of hormones and growth factors in addition to FSH, LH, and androgens, including insulin-like growth factor, oxytocin, and transforming growth factor-α and estrogens (Pryor *et al.,* 2000).

SPERMATOGENESIS

Spermatogenesis is the formation of the male gamete or spermatozoa. Spermatogenesis is dependent on the integrity of the architecture of the seminiferous tubules and Sertoli cells and endocrine regulation and is regulated by testosterone and FSH. In response to LH, Leydig cells produce androgens, including testosterone, which along with FSH bind to their respective Sertoli cell receptors to regulate spermatogenesis. Spermatogenesis requires unique associations between Sertoli cells and developing male germ cells such that the seminiferous tubules are lined by Sertoli cells and joined by tight junctions forming the blood–testis barrier (BTB; Walker & Cheng, 2005). There are three major phases of spermatogenesis: (1) spermatogonial phase, (2) spermatocyte phase, and (3) spermatid phase. In the first phase the diploid spermatogonia undergo mitosis and create stem cells and diploid primary spermatocytes. During the second phase the primary spermatocytes

undergo two rounds of meiosis, producing haploid spermatids. Finally, the spermatids begin a differentiation phase, sometimes referred to as spermiogenesis, during which the immature gametes develop into mature spermatozoa (O'Donnell *et al.,* 2001). Spermatids continue their differentiation (spermiogenesis) while physically associated with the Sertoli cells. Spermiogenesis includes polarization of the spermatid, formation of the acrosome cap and flagellum, condensation, elongation of the nucleus, and cytoplasmic remodelling to produce the characteristic appearance of the mature spermatozoa. Spermatozoa are morphologically mature but immotile and are then released into the lumen of the seminiferous tubules (spermiation). At this stage these immotile testicular spermatozoa are not yet capable of fertilization (O'Donnell *et al.,* 2001). The BTB between Sertoli cells comprises a co-existing tight junction (TJ), desmosome, gap junction and a testis specific adherens junction (AJ) called the basal ectoplasmic specialization (ES). The basal ES is typified by the presence of actin filament bundles 'sandwiched' between the plasma membrane and the cisternae of endoplasmic reticulum in two neighbouring Sertoli cells. However, recent studies show that the unique structural aspects of the BTB, such as the presence of focal adhesion protein FAK, also render the testis highly susceptible to damage from environmental toxicants. Third, during spermiogenesis when round spermatids differentiate into elongated spermatids, genetic material in the spermatid head condense to form the tightly packed nucleus with the formation of an acrosome above the head region and elongation of the spermatid tail. During this time, spermatids migrate towards the adluminal compartment of the seminiferous tubule until elongated spermatids are released into the tubule lumen via the disassembly of another ES, the apical ES, at spermiation. The apical ES anchors developing spermatids in the seminiferous epithelium until they are fully developed. Thus, disruption of the apical ES (e.g. by environmental toxicants) causes the premature release of spermatids that are structurally defective (e.g. lack of acrosome and/or tail) and which are incapable of fertilizing the ovum (Wong & Cheng, 2011).

SPERM MATURATION

The immotile spermatozoa are transported from the lumen of the seminiferous tubules by peristaltic contractions of adjacent myoid cells. The spermatozoa are suspended in a fluid secreted by Sertoli cells and migrate through a series of ductules within the testis (rete testis), passing through the efferent ductules and eventually entering the epididymis. The efferent ductules concentrate the spermatozoa by reabsorbing fluid (O'Donnell *et al.,* 2001). There is evidence from transgenic mice that this fluid resorption is regulated by estrogen (Hess *et al.,* 1997). The segments of the epididymis, caput, corpus, and cauda secrete proteins, and endocytose secreted proteins from the epididymal lumen to contribute to the maturation of the spermatozoa (O'Donnell *et al.,* 2001). It is within the epididymis that the spermatozoa gain motility machinery. However, these spermatozoa remain immotile as they are pushed through the rest of the reproductive tissues via peristaltic contractions. It is during this final passage that seminal fluid is produced by the seminal vesicles, which contributes about 70% to the semen, and the prostate gland, which contributes another 10–30%. Seminal fluid is comprised of proteins, enzymes, fructose, mucus, vitamin-C, flavins, phosphorylcholine, and prostaglandins (Purvis *et al.,* 1986). Decreases in seminal fluid volume may therefore indicate diminished seminal vesicle or prostate functions.

THE ROLE OF ANDROGENS IN MALE REPRODUCTIVE TRACT DEVELOPMENT

Male reproductive tract development is a dynamic process requiring the interaction of many factors and hormones. One of the major factors essential for the development of the male internal and external male reproductive tract are the androgens, testosterone and dihydrotestosterone (DHT) (Phillips & Tanphaichitr, 2008). Androgens are produced by the testes during fetal and neonatal development and are essential for the maintenance of the Wolffian duct that differentiates into the epididymis, vas deferens and the seminal vesicles. The masculinization of these reproductive structures is mediated by testosterone. The masculinization of the external genitalia and prostate is largely mediated by DHT which is a more potent metabolite of testosterone and is produced by the action of the enzyme 5α reductase. The central role of androgens in driving these developmental processes illustrates why chemicals that can interfere with the synthesis or action of androgens can have deleterious consequences for the developing male genital tract. Administration of the antiandrogen, flutamide (an androgen receptor antagonist), during male reproductive tract development resulted in abnormalities in the formation of the external genitalia - hypospadias and cryptorchidism; internally, agenesis of the epididymis, vas deferens and prostate (Mylchreest *et al.,* 2000). Within the testis, degeneration of the seminiferous epithelium and Leydig cell hyperplasia was common (although this may be a consequence of the cryptorchidism rather than an anti-androgenic effect). The male pups also displayed retained thoracic nipples and a reduced anogenital distance (feminised) which are both indicative of reduced androgen action in fetal life (Mylchreest *et al.,* 2000). In summary, both testosterone- and DHT-mediated male reproductive tract developments are impaired by flutamide when administered over the period of reproductive tract differentiation.

PROBLEMS WITH MALE REPRODUCTIVE HEALTH

SEMEN QUALITY:

Reports suggesting that sperm counts have declined in certain areas of industrialized countries throughout the world have contributed to concern about a possible worldwide decline in human semen quality (Swan *et al.,* 1997). Potential confounders include increasing donor age, duration of abstinence, frequency of ejaculation, and even the season of sample collection, all of which influence sperm variables. Other suggested confounders include smoking, chemicals and radiation exposures, stress, ethnicity, and a variety of physical conditions including varicocele, infection, and genital abnormalities such as hypospadias and cryptorchidism. Theories explaining the apparent geographic disparities in sperm counts are currently only speculative, and include environmental, socioeconomic, racial, and methodologic differences (Swan *et al.,* 1997).

TESTICULAR CANCER:

Testicular cancer is often quoted as the commonest cancer of young men. The increase in testicular cancer has been linked to a birth cohort effect, suggesting that factors affecting in utero development may be important (Bergstrom *et al.,* 1996). Testicular germ cell cancer arises from cells which have similar characteristics to fetal germ-cells; these pre-malignant cells are termed carcinoma-in situ (CIS) cells (RajpertDe Meyts *et al.,* 2003). It is thought that the factors that promote normal germ cell division may also be important in promoting CIS proliferation. Abnormal intrauterine hormone levels i.e. decreased androgen

and/or increased oestrogen levels are believed to be important in the occurrence of testicular cancer (Sharpe & Skakkebaek 1993).

CONGENITAL ABNORMALITIES (CRYPTORCHIDISM AND HYPOSPADIAS):

Cryptorchidism and hypospadias are abnormalities normally detected at birth (congenital abnormalities). Cryptorchidism occurs when the testis does not descend into the scrotal sac; this is generally unilateral but can be bilateral. Hypospadias is a developmental abnormality of the penis in which the urethral opening is not located at the tip of the glans penis but can occur anywhere along the shaft decreased androgen and/or increased oestrogen levels have also been implicated in the occurrence of cryptorchidism, hypospadias and low sperm counts (Sharpe & Skakkebaek 1993). Cryptorchidism is the most common congenital abnormality of the newborn (2–4% incidence) and trends for hypospadias suggest a progressive increase; based on registry data, hypospadias is the second most common (0.3–0.7% at birth) congenital malformation (Sharpe, 2003). Prospective studies are underway, which employ standardised diagnostic criteria, to collect robust data about the current incidence of cryptorchidism and hypospadias.

LINK BETWEEN THESE MALE REPRODUCTIVE HEALTH ISSUES

The strongest evidence suggesting a link between these male reproductive tract disorders, aside from the (largely imperfect) data which suggests they are all increasing in incidence, is the fact that epidemiologically the occurrence of one disorder is a risk factor for the occurrence of another (Skakkebaek *et al.,* 2001, Sharpe, 2003). This has led to the proposal that low sperm counts, hypospadias, cryptorchidism and testicular germ cell cancer are interrelated disorders comprising a 'testicular dysgenesis syndrome' (TDS; Skakkebaek *et al.,* 2001, Sharpe, 2003, 2010). The disorders that comprise TDS all have their roots in fetal development, suggesting that a possible causal link lies in abnormal hormone synthesis or action during reproductive tract development. In male rodents, neonatal administration of DES induces a reduction in the number of Sertoli cells (the major somatic cell type which supports spermatogenesis) (Sharpe *et al.,* 2003). There is also data suggesting DES administration to humans induces an increase in the incidence of cryptorchidism, although it is less certain whether hypospadias and testicular cancer show any significant increase (Stillman, 1982). DES only induces male reproductive tract abnormalities after administration at very high doses, which are probably not relevant to environmental considerations. However, what is of more concern is that, when administered at high doses, DES and other potent oestrogens are capable of reducing androgen levels and expression of the androgen receptor protein relative to control rats (McKinnell *et al.,* 2001, Rivas *et al.,* 2002). This raises the important question of whether some of the genital tract abnormalities that arise from in utero administration of potent oestrogens are caused by lowered androgen levels and/or action.

ANTI-ANDROGENIC COMPOUNDS IN THE ENVIRONMENT

There are a number of commonly used environmental chemicals that have been identified as having anti-androgenic properties. These chemicals have been administered to pregnant rodents during the period of reproductive tract development. When the male pups were examined, they displayed many of the abnormalities associated with flutamide administration. Some chemicals (vinclozolin, procymidone, linuron, p,p′DDE

(1,1,1dichloro-2,2-bis(p-chlorophenyl)ethane) act as androgen receptor antagonists, others (phthalate esters) reduce androgen synthesis, but it is likely that other modes of action are also involved in the toxicity induced by these compounds (Gray *et al.*,2001). There are several anti-androgenic compounds (i.e. vinclozolin, linuron, p,p′ DDE and phthalates etc.) and common environmental toxins reported to be involved in male reproductive toxicity.

Polychlorinated Biphenyls (PCB):

PCB make up a group of synthetic organic chemicals containing about 200 individual compounds. PCB produces reproductive toxicity through the production of free radicals. Rats exposed to mixtures of PCB demonstrated decreased superoxide dismutase and catalase activity in the testes following exposure (Peltola *et al.,* 1994). Some PCB bind to ERs and consequently PCB may exert their toxic effects through estrogenic activity (McKinney & Waller, 1998). Alternatively, some PCB congeners have different mechanisms of action and therefore different effects on biological systems. Prenatal and lactational exposure to PCB may exert adverse effects on male reproduction during subsequent adulthood. The effects of PCB exposure on semen quality in men from the general population appear to affect differentiation of spermatids (spermiogenesis) and post testicular development (sperm maturation), which would manifest as decreased sperm morphology and motility, respectively (Guo *et al.,* 2000). A wide range of study designs and locations, measurement methods, and PCB exposure levels, reports of inverse associations between PCBs and sperm motility have been consistent which may suggest a lack of exposure threshold for a PCB-related effect on sperm motility. Several studies have also reported inverse associations between PCBs and circulating testosterone levels in men, though the specific form of testosterone (i.e. total, bound, or free testosterone) associated with exposure has not been fully consistent between studies. (Meeker *et al.* 2010)

DDT and p,p ′-DDE:

The persistent pesticide, DDT, is broken down in the environment, and one of its metabolites is p,p′-DDE, which has been shown to act as an androgen receptor antagonist both in vivo and in vitro (Kelce *et al.*,1995). Studies in which p,p′-DDE was administered to rats during development (gestational day (GD)14–18; 100 mg/kg/day) affected androgen dependent aspects of male development such that it reduced anogenital distance, caused nipple retention and, depending on the rat strain, induced hypospadias (You *et al.,* 1998). Another DDT derivative, methoxychlor and its metabolites, have been shown to interact with both oestrogen receptors and the androgen receptor (AR). The methoxychlor metabolite, 1,1-Trichloro-2,2-bis (4 hydroxyphenyl) ethane, is an oestrogen receptor (ER)-αagonist, an ER- β antagonist and an androgen receptor (AR) antagonist (Gray *et al.,* 2001). This illustrates that these chemicals may act by more than one mechanism to induce effects on the exposed population. Significantly higher seminal concentrations of p,p′-DDE were also reported in infertile patients compared to a fertile control group in India. Seminal fluid levels of fructose, γ-glutamyl transpeptidase, and acid phosphatase were positively correlated with p,p′-DDE concentrations in infertile men. The high concentration of fructose, a marker for seminal vesicle function and an important energy source for sperm, may indicate non-utilization of fructose by sperm. DDT exposure may be associated with abnormal metabolism in sperm, including decreased fuel utilization, in infertile men (Pant *et al.,* 2004). Both semen volume and total sperm number were inversely correlated to p,p'-

DDE levels. Low semen Quality observed in DDT exposed workers (Salazar –Gracia *et al.*,2004) Thus, androgen levels and semen quality are adversely affected by high p,p′-DDE body burden. The studies examining DDT exposure and semen quality report consistent effects on sperm motility and sperm morphology, similar to the PCB studies. The increased SHBG concentrations associated with serum p,p′-DDE described by Ayotte *et al.*,(2001) provides a possible mechanism for observed reductions in plasma testosterone and sperm number. By inducing SHBG synthesis, p,p′-DDE may exert its antiandrogenic effects by reducing the amount of bioavailable testosterone, thereby impairing spermatogenesis. Exposure of DDT during embryonic stage in domestic rooster lead to cloacal deformities in adulthood, causing abnormal semen flow, deformation in testes, reduction in seminiferrous tubular area. (Blomquist *et al.,* 2006) Inhibin level was decreased and FSH, E2 levels was increased due to DDT exposure (Dalvie *et al.*, 2004; Dalvie and Myers 2006). Detailed Sperm Study conducted by Aneck Hahn *et al.* (2007) decrease semen volume, sperm motility, increased in the percentage of sperm with cytoplasmic droplets and incident of teratozoospermia and oligozoospermia. Insufficent sperm chromatin condensation present was positively correlated with the concentration with pp DDT also adverse effect on testicular function and reproductive hormone (De Jager *et al.*, 2006) Borman *et al.* (2010) examined 3310 newborn boys in malarial area and found nearly 11% had urogenital birth defects but Tren *et al.* (2010) disagreed to this entire study.

Dioxins:

Tetrachlodibenzo-p-dioxin (TCDD) is a carcinogen, demonstrated to target the endocrine system in experimental animals. Humans are exposed to dioxins through pulp and paper industry emissions, use of contaminated herbicides (now reduced in industrialized countries), and waste incineration emissions. Dioxins are lipophilic, slowly metabolized, and thus are not easily eliminated leading to bioaccumulation. Secondary dioxin exposures include dietary uptake via contaminated breast milk, meat, fish, and other dairy. Dioxins along with polycyclic aromatic hydrocarbons (PAH) and polyhalogenated biphenyls bind to the aryl hydrocarbon receptor (AhR). AhR ligands induce cell proliferation, differentiation, and apoptosis, although the mechanisms of these stimulations are not fully understood. It is known that human sperm possess AhR and may therefore be directly susceptible to dioxin (Khorram *et al.,* 2004). A range of endocrine effects are reported in experimental animals following dioxin exposure. These include disruption of the HPT axis feedback mechanisms leading to alterations in serum levels of testosterone, dihydrotestosterone (DHT), E2, and LH, as well as modifications of the metabolism processes/events of estrogens and androgens (Birnbaum & Tuomisto, 2000). Increases in serum dioxin-like activity were associated with decreased seminal volume resulting in elevated sperm concentrations. Total testosterone levels were significantly reduced in men with high serum dioxin-like activity. However, there was no significant association with LH, inhibin B, FSH, total sperm numbers, or sperm morphology (Dhooge *et al.,* 2006). A possible mechanism for infertility may be mediated by dioxin interacting with AhR on human sperm with implications for capacitation, acrosome reaction, sperm–egg binding, and fertilization.

Phthalates esters:

Phthalate esters are abundant industrial chemicals used in the production of plastics and are present in many personal care products including cosmetics. Phthalates are a family of compounds and only a few induce male reproductive tract abnormalities. Gray *et al.,*

(2000) compared the ability of six phthalate esters (diethylhexyl phthalate, DEHP; benzylbutyl phthalate, BBP; diisononyl phthalate, DINP; dimethyl phthalate, DMP; diethyl phthalate, DEP; dioctyl terephthalate, DOTP; all administered at 750 mg/kg body weight from GD14 to postnatal day (PND) 3) to induce malformations of the reproductive tract. This study assessed changes in many androgenic endpoints and found that only DEHP, BBP and to a lesser degree DINP induced alterations in all aspects of androgen-regulated male reproductive endpoints. Exposure to diethyl hexyl and dibutyl phthalates is associated with adverse effects on sperm motility (Fredricsson *et al.,* 1993). Animal studies consistently demonstrated that phthalate esters are male reproductive toxicants (Park *et al.,* 2002), with exposure associated with testicular atrophy, spermatogenetic cell loss, and damage to the Sertoli cell population. Phthalate monoesters target Sertoli cell functions in supporting the spermatogenesis process. This may be due to the effect of phthalates in reducing the ability of Sertoli cells to respond to FSH (Hauser *et al.,* 2005). Initial reports on the effects of phthalates on male reproductive tract development focussed on the gross changes such as reduced anogenital distance, hypospadias, malformed epididymis and, in later studies, nipple retention (Mylchreest *et al.,* 2000). Only a few studies give a more detailed account of the histological changes observed in the testis after in utero phthalate exposure and demonstrate that the fetal testis is directly affected by phthalates during fetal and neonatal testis differentiation (Parks *et al.,* 2000; Fisher *et al.,* 2003). Some of these alterations are permanent and affect the function of the testis in adult life and are similar to the histological changes which are now being shown in patients with testicur dysgenesis syndrome (TDS; Skakkebaek *et al.,* 2003). It has been shown that DBP and DEHP are both capable of inhibiting the production of testosterone by the fetal testis (Parks *et al.,* 2000; Fisher *et al.,* 2003). Testosterone synthesis by the fetal testis is first detectable by GD15, reaches a peak at around GD18/19, and remains high until birth. However, phthalate treatment induces a 60–85% reduction in testosterone synthesis during this critical developmental window, reducing testosterone levels to a similar level to those found in females (Parks *et al.,* 2000). This reduction in testosterone is a factor in the occurrence of hypospadias and cryptorchidism observed after phthalate treatment. This is not to suggest that phthalate exposure causes TDS in humans, merely that the administration of very high doses of DBP to pregnant rats induces a TDS-like syndrome in the male offspring that shows many analogous features to human TDS. In both the human syndrome and the rodent model, abnormal testicular development or dysgenesis is evident by the abnormal organisation of these tissues. In humans, histological evidence of testicular dysgenesis (immature seminiferous tubules with undifferentiated Sertoli cells, microcalcifications and Sertoli cell only (SCO) tubules, Leydig cell hyperplasia, morphologically distorted tubules and the presence of carcinoma in situ (CIS) cells) have been found in biopsies of the contralateral testes of testicular germ cell cancer patients and in biopsies from patients with infertility, hypospadias and cryptorchidism (Skakkebaek *et al.,* 2003). These studies support the hypothesis that all of these disorders (low sperm counts, cryptorchidism, hypospadias and testicular cancer) are associated with TDS. The in utero administration of DBP to rodents during the sensitive period of tissue morphogenesis permanently alters the testis and produces foci of testicular dysgenesis (immature seminiferous tubules with undifferentiated Sertoli cells, SCO tubules, Leydig cell hyperplasia, morphologically distorted tubules and the presence of abnormal germ cells) which persist in the adult animal (Fisher *et al.,* 2003). The downstream consequences of altered Sertoli cell (and

subsequently Leydig cell) function may be a key cause of many of the observed changes in both human TDS and the rat TDS-like model due to the central role of this cell type in driving testis morphogenesis in both rodents and humans. Several population studies evaluated phthalate ester exposure and semen quality. A randomized controlled study of men with unexplained infertility reported a negative correlation between seminal plasma phthalate ester concentration and sperm morphology (Rozati *et al.,* 2002). Environmental phthalate levels measured by urinary metabolite, were reported to be associated with increased DNA damage in sperm (Duty *et al.,* 2003). The studies measuring phthalate levels and semen quality seem to suggest an effect on sperm morphology and motility, rather than on total sperm numbers. Hauser *et al.*, (2005) suggest a mechanism by which PCB exposure may extend the bioavailability of phthalate metabolites, which in turn adversely affect semen quality. As human exposure consists of phthalate mixtures, along with xenobiotics, studies designed to test or measure single phthalate esters fail to appropriately characterize risks associated with these chemicals. Phthalate esters are reproductive toxicants in laboratory rats, particularly when exposure occurs in the womb. (Howdeshell, 2008)

Phytoestrogens:

Phytoestrogens are nonsteroidal plant-derived compounds with potent estrogenic activity. There are four main groups of phytoestrogens: isoflavonoids, flavonoids, coumestans, and lignans. Phytoestrogens exert their action via multiple mechanisms. Phytoestrogens interact with both ERα and ERβ, thereby inducing weak estrogenic and antiestrogenic actions (Kuiper *et al.,* 1998). Coumestrol and genistein, two phytoestrogens, exhibit a higher affinity for ERß than for ER-α (Whitten & Naftolin, 1998). Some phytoestrogens exert an inhibitory action on steroidogenic enzymes (Strauss *et al.,* 1998). For example, isoflavonoids and lignans inhibit 5αductase activity, thereby reducing the conversion of testosterone to the active form DHT. A number of phytoestrogens, including lignans, isoflavonoids daidzein and equol, enterolactone, and genistein, were found to induce SHBG production in the liver (Adlercreutz *et al.,* 1987). There are few studies measuring the effects of phytoestrogens on semen parameters in men. Testicular volume was not influenced by phytoestrogen supplementation; nor did serum E2, testosterone, FSH, or LH differ between the supplement-taking group and the control group who did not take supplements. Finally, phytoestrogen supplementation did not produce changes in seminal volume, sperm concentration, sperm count, and sperm motility (Mitchell *et al.,* 2001).

Studies examining the actual dose of the chemical that entered circulation revealed that there was very minimal exposure to the neonates through lactation so the male rats were exposed primarily prenatally and in adulthood but not during the neonatal period (Doerge *et al.*,2006). There was a large multi-generational study conducted in rats exposed to genistein through the diet throughout the lifetime of the animal (Delclos and Newbold, 2007). The dose range finding portion of this study exposed rats to genistein in the diet starting on gestational day 7 and through lactation until weaning and then in the diet until postnatal day 50 (adulthood). The doses used in that study were approximately 0.3, 1.7, 6.4, 16, 38, and 72 mg/kg to the dam and 0.6, 3, 11, 29, 69, and 166 mg/kg to the pups after weaning. In addition to females developing ductal/alveolar hyperplasia in the mammary gland, males in this study developed mammary gland hypertrophy at doses at or above 11 mg/kg and mammary gland hyperplasia at doses at or above 29 mg/kg. Males in this study

had reduced prostate weight following the highest dose. There were very few additional effects on the male reproductive tract in this study. Males on the soy diet had lower serum testosterone concentrations and higher numbers of Leydig cells at the discontinuation of soy formula use. As adults, the soy fed marmoset had larger testes and lower serum testosterone levels than its twin demonstrating that the impacts were persistent (Sharpe *et al.,* 2002; Tan *et al.,* 2006). There is a surprising paucity of data on the developmental effects of phytoestrogens in males (reviewed in 2009) (Cederroth *et al.*, 2009).Despite their limitations, information gathered from the published human studies combined with the large number of animal studies already available clearly demonstrates that phytoestrogens have the ability to permanently reprogram adult tissue responses after a developmental exposure, and that these altered tissue responses are important for reproductive health. These findings should be taken into account when recommendations are made regarding dietary or therapeutic phytoestrogen intake, while keeping in mind developmentally sensitive time points. (Jefferson *et al.,* 2012)

Pesticides, Fungicides and Herbicides:

The U.S Environmental Protection Agency (EPA) defines a pesticide as "any substance or mixture of substances intended for preventing, destroying, repelling, or lessening the damage of any pest," which may include plants, weeds, animals, insects, and fungus. Urinary levels of metabolites of eight currently used pesticides were measured and correlated with semen quality. Men with elevated metabolite levels of alachlor and atrazine (herbicides) and diazinon (2-isopropoxy-4-methyl-pyrimidinol insecticide) were significantly more likely to have poor semen quality than controls (Swan, 2006). Sperm concentration, motility, and, to a lesser extent, morphology were reduced in men with elevated exposure to carbaryl/naphthalene (as measured by urinary levels of the metabolite 1-naphthol) and to chlorpyrifos (as measured by urinary levels of the metabolite 3,5,6-trichloro-2-pyridinol [TCPY]) (Meeker *et al.,* 2004). The mechanism of action of carbaryl may be related to the production of reactive oxygen species (ROS) rather than endocrine disruption. Carbaryl produced lipid peroxidation at low concentrations, which in turn induced the sperm plasma membrane to lose its fluidity and integrity, thereby impairing sperm motility (Meeker *et al.,* 2004). Generally, the studies reviewed here demonstrated a relationship between pesticide exposure and reduced semen quality. However, toxicology studies using animal models are essential to understand the biological mechanisms underlying the adverse reproductive affects caused by pesticide exposure in the male.

Vinclozolin:

Vinclozolin is a dicarboximide fungicide that has two active metabolites, M1 and M2, which have anti-androgenic properties. In vivo and in vitro experiments demonstrate that these compounds act as potent androgen receptor antagonists, and administration to pregnant rats results in abnormalities of androgen-regulated sexual differentiation similar to those induced by flutamide, e.g. reduced anogenital distance, nipple retention, hypospadias, undescended testes and small or absent accessory glands (Gray *et al.,* 2001). Studies have tried to define the 'sensitive window' for exposure to vinclozolin, and have determined that administration to pregnant rats during gestational day (GD) 14–19 induced reproductive tract malformations, with treatment over GD16–17 causing the most severe malformations (Wolf *et al.,* 2000). This illustrates that the whole period of male reproductive tract

differentiation is sensitive to the effects of anti-androgens. In the present study, guppies (Poecilia reticulata) were fed with the anti-androgenic fungicide vinclozolin at concentrations ranging from 1.8 to 180 mg/kg from 8–14 wk of age. Male sperm count and the intensity of his sexual display behavior were significantly reduced in treatment groups, which was in line with the results of previous studies. Here, we show further that these impairments translate into reduced fertility, measured as the size of the female's first clutch. Also, this reduced fertility was correlated to the male sperm count, but not to the intensity of the male sexual display. Finally, by crossing exposed with unexposed animals, we show that the adverse effect of vinclozolin on reproduction is mediated through the male alone. (Bayley *et al.*, 2003)

Linuron:

Linuron is a urea-based herbicide which acts as a weak androgen receptor antagonist in vitro and in vivo, and disrupts androgen-dependent male reproductive tract development after gestational exposure (Gray *et al.,* 2001). When administered to pregnant rats (GD 14–18; 100 mg/ kg/day) the male pups displayed a reduced anogenital distance and retention of areolas (Gray *et al.,* 1999). Linuron failed to induce either hypospadias or undescended testes, suggesting that linuron affects testosterone-but not DHT-mediated development, though how this occurs is not known (McIntyre *et al.*, 2002). When administered in utero, linuron disturbs the timing of the sequences that must properly take place during fetal development. Linuron interrupts the signals at key times, leading to the development of a variety of birth defects that only affect the male, since it is only the male that requires androgen for their normal reproductive development. (American Association For Clinical Chemistry, 2003).

Tobacco Smoke:

An important study by Robbins *et al.,* (1997) did investigate the interactions of caffeine, alcohol, and cigarette smoking on sperm aneuploidy, determining that incidence of sperm abnormalities decreased after controlling for age and other lifestyle factors. PAH (polycyclic aromatic hydrocarbons), the major carcinogenic components of cigarette smoke (Vine, 1996), were found to activate aryl hydrocarbon receptor (AhR), suggesting that tobacco smoke may represent a chemical mixture with endocrine disrupting activity. There is extensive evidence demonstrating that exposure to tobacco smoke is associated with reduced semen quality (Vine, 1996). Testicular biopsies from boys exposed in utero/neonatal to tobacco smoke demonstrated a decreased number of spermatogonia and gonocytes per tubule cross section (Thorup *et al.,* 2006). Serum LH and testosterone were positively correlated with smoking (Ramlau-Hansen *et al.,* 2007) Tobacco smokers are 60% more likely to be infertile than non-smokers (Regulated Family Services, 2009) Most of the reports showed that smoking reduces sperm production, sperm motility, sperm normal forms and sperm fertilising capacity through increased seminal oxidative stress and DNA damage. Few papers reported non significant differences in semen parameters between smokers or non-smokers. It is concluded that although some smokers may not experience reduced fertility, men with marginal semen quality can benefit from quitting smoking (Mostafa,2010) Male rats were given nicotine 0.5 mg/kg (low dose) and 1.0 mg/kg (high dose) per body weight for 30 days. Sperm motility and count significantly decreased while the percentage of abnormality significantly increased in treatment groups. However, there was an insignificant decrease in the viability and semen volume of the treated groups. The

present study showed that nicotine has a dose-dependent deleterious effect on the sperm characteristics and that fertility is ameliorated by nicotine cessation in male rats (Oyeyipo *et al.,* 2011)

MEDICATIONS AND MALE REPRODUCTIVE TOXICITY

There are a variety of prescription medications that can lead to male infertility, often temporary but sometimes permanent. Arthritis medication, depression drugs, high blood pressure medication, drugs for digestive problems as well as antibiotics and cancer drugs are just a few of the medications that can lead to interferences with sperm production, sexual function and ejaculation (Nudell *et al.,* 2002). Here is a look at some of the common medications and drugs that can cause a man to experience fertility problems.

Antihypertensive:

Although most men who are treated for hypertension are older, the recent focus on the importance of blood pressure control has led to greater numbers of younger patients on antihypertensives. Many of these medications are commonly associated with erectile dysfunction but most do not directly affect fertility. One exception is spironolactone, which acts as an anti-androgen and has been associated with impaired semen quality. Calcium channel blockers (e.g. nifedipine) have been reported to cause reversible functional defects in sperm, impairing their ability to fertilize eggs without affecting sperm production or standard semen analysis parameters; however, not all investigators report these types of effects. Diuretics can affect function by decreasing penile blood flow, and beta-blockers may affect libido and erectile function (Benoff *et al.,* 1994).

Hormones:

Diethylstilbestrol (DES) was given to pregnant women in the 1950s, and reports of epididymal cysts and cryptorchidism (undescended testes) in males with prenatal DES exposure have raised concerns about fertility; however, follow-up studies on adult men with prenatal DES exposure have revealed no adverse effects on fertility (Wilcox *et al.,* 1995). Exogenous androgens are well known to induce hypogonadotropic hypogonadism. This may be induced directly by testosterone supplementation or by use of synthetic anabolic steroids, leading to azoospermia. This hypogonadism is usually reversible but may take 3 to 6 months, and some patients do not recover pituitary function. It is important to remember that testosterone replacement therapy in younger men may lead to infertility. Dehydroepiandrosterone (DHEA) is a natural steroid prohormone precursor of androsterone, testosterone, and estrogen. DHEA is commonly taken and easily available over the counter. Antiandrogens and estrogens can adversely affect fertility by altering the HPG axis or decreasing libido or erectile function, while progesterones act by decreasing libido or erectile function (Nudell *et al.,* 2002). The original medical applications of anabolic steroids were for breast cancer, aplastic anemia, treatment of an gioneurotic edema, growth failure in young males, stimulating sexual development in hypogonadal males, and possibly for the treatment of osteoporosis, however, these uses are limited (Robert, 2012).

Antiandrogens:

Finasteride and dutasteride are antiandrogens that act by inhibiting 5-alpha-reductase. Finasteride has also been used to treat male-pattern baldness. These drugs increase the risk of low ejaculate volumes and libido, as well as cause erectile and ejaculatory dysfunction; however, men taking low doses of finasteride for hair loss have shown no

changes in semen parameters (Overstreet *et al.,* 1999). Anti androgens supplementation can also cause cancer in testis. (Fisher, 2004)

Antibiotics:

Many antibiotics have been reported to exert adverse effects on male fertility; however, there are few human data on the majority of these medications. Sulfasalazine, used in the treatment of ulcerative colitis, is well known to cause defects in human sperm concentration and motility. Aminoglycosides, type of antibiotics is generally used for serious bacterial infections, like TB, and are administered under medical supervision. Aminoglycosides can negatively impact sperm production while neomycin has been shown to reduce both sperm count and motility. Macrolides, in addition to being used to treat chlamydia and Legionnaires disease, macrolides are similar to penicillin and can be used in place of it in people with a penicillin allergy. Macrolides research has mainly focused on animals, where it has been found that the antibiotic can decrease sperm motility as well as kill off sperm. It is believed that the antibiotic produces similar results in humans (Schlegel *et al.,* 1991).High doses of nitrofurantoin have been reported to cause early maturation arrest at the primary spermatocyte stage but the more common short-term low-dose therapy is not likely detrimental. While in vitro data on erythromycin, tetracycline, and gentamycin suggest the potential for adverse effects on fertility, documentation of an in vivo effect in humans is lacking (Hargreaves *et al.,* 1998). A recent study conducted by researchers at the University of Nevada revealed that tetracycline– an antibiotic used to treat infections like pneumonia and urinary tract infections, among a number of other conditions– may have an effect not just on a male's sperm viability, but his offspring's as well. (Jeanne Zeh, 2012) another drug Perfloxacin produced toxic effect on testicular function in animals (Adikwu and Brambaifa, 2012)

Psychotherapeutic Agents:

Many psychotherapeutic agents affect male fertility by suppressing the HPG axis and decreasing erectile function and libido. Indeed, one of the most significant side effects of the antidepressants is elevation of serum prolactin, leading to significant but reversible suppression of spermatogenesis (Nudell *et al.,* 2002). Psychotherapeutic agents include antipsychotics, tricyclic and selective serotonin reuptake inhibitor (SSRI) or selective norepinephrine reuptake inhibitor (SNRI) antidepressants, monoamine oxidase inhibitors (MAOIs), phenothiazines, and lithium. There are now large numbers of patients taking SSRI or SNRI medications, many of which have significant fertility effects. Retrospective trial has demonstrated an association between SSRI use and decreased sperm motility (Relwani *et al.,* 2011). Evidence also shows that SSRIs have a spermicidal effect in vitro (Kumar *et al.,* 2006)

Anticancer drugs:

Doxorubicin hydrochloride, Goserelin acetate, methotrexate, or fluorouracil all are the drugs used to treat various types of cancer. However, these drugs have significant side effects on sexual behaviour, altered fertility and sperm count (Nudell *et al.,* 2002).

Chemotherapeutic agents:

Chemotherapy for the treatment of cancer can have devastating effects on male fertility through the impairment of spermatogenesis; indeed, alkylating agents, antimetabolites, and the vinca alkaloids are all gonadotoxins. The alkylating agent, cyclophosphamide alters

male fertility; treatment with 1-2 mg/kg for more than 4 months increases the incidence of azospermia and oligospermia in adult male patients (Qureshi *et al.,* 1972). In patients with testicular cancer, the cumulative dose of cisplatin determines whether spermatogenesis is impaired irreversibly. Most patients will become azoospermic, with the majority recovering spermatogenesis within 4 years. The majority of Hodgkin's disease or leukemia patients become azoospermic after chemotherapy; this may or may not lead to permanent sterility. After treatment with mitoxantrone, vincristine, vinblastine, and prednisone combination therapy plus abdominal radiotherapy for Hodgkin's disease, sperm counts and motility were restored to pre-treatment levels in most patients (Magelseen *et al.,* 2006). 1.5.8.7 Miscellaneous medications Cimetidine has been reported to have antiandrogenic effects that induce gynecomastia and decreases in sperm count. Immune modulators are commonly used but, unfortunately, clear human data regarding male fertility for interferon or the immunosuppressant mycophenolate mofetil are lacking. Although cyclosporine has been found to induce impaired fertility in rats, there are no human data available (Nudell *et al.,* 2002). Epilepsy has been associated with decreased testosterone levels and increased estrogen levels leading to reductions in libido and to erectile dysfunction. Medications used to treat epilepsy (eg, valproate, oxcarbazepine, and carbamazepine) may worsen hormonal abnormalities and have been associated with some sperm morphologic defects (Isojarvi *et al.,* 2004).

Despite these design limitations, the literature does suggest that certain medications may, in fact, prove detrimental to male reproductive potential. Specifically, there is good data that SSRIs, CCBs (calcium channel blockers), certain AABs (Alpha-adrenergic blockers), and HAART (Highly active antiretroviral therapy) medications certainly could contribute to MFI (male factor infertility). However, many men still father healthy children even while taking medications. Therefore, as the detrimental effects of these medications were never universally observed, counseling men taking these medications should include discussing the risk that the medications may, but also may not, pose to their reproductive health. In many instances, the detrimental aspects of these drugs on semen parameters is likely outweighed by the significant medical benefit conferred to one taking the medications, especially in life-extending treatments such as HAART. (Brezina *et al.*, 2012)

RECREATIONAL AND ILLICIT SUBSTANCES AND MALE REPRODUCTIVE TOXICITY

Heavy marijuana use has been associated with gynecomastia, decreased serum testosterone levels, decreased sperm concentration, and pyospermia (white cells in the semen indicating possible infection) (Close *et al.,* 1990). Patients experience variable sensitivity to marijuana, and it may take 2 to 3 months for symptoms to improve. Oligospermia (abnormally low sperm concentration in the ejaculate) and defects in sperm morphology and motility have been reported in users of cocaine. Opiates have also been shown to decrease libido and erectile function through induction of hypogonadotropic hypogonadism. This also is important to note when prescribing opioids for pain. Chronic opioid use whether, oral or intrathecal, may lead to sexual dysfunction (Bracken *et al.,* 1990). Cumulative evidence suggests that cigarette smoking may have a deleterious effect on male fertility by reducing sperm production, motility, and morphology. Cigarette smoking may also lead to development of pyospermia, decreased sperm penetration, and hormonal alterations (Nudell *et al.,* 2002; Close *et al.,* 1990). Long-term abuse of alcohol has detrimental effects in the HPG axis. Alcoholics exhibit significant decreases in semen

volume, sperm count, motility, and number of morphologically normal sperm. They also show signs of pyospermia. Alcohol in excess can thus exert profound deleterious effects on all aspects of the male reproductive system. However, there is no evidence that moderate alcohol intake impairs male fertility (Nudell *et al.,* 2002; Close *et al.,* 1990).

The environmental Toxicants chemicals which are reported to disrupt the sex hormones and/or damage the male in animal studies are Heavy Metals (Mainly cadmium, Lead and arsenic) ,Volatile organic compounds (Toluene, benzene and xylene) ,Phthalates DBP = di(n)butylphthalate DiBP =di(iso)butylphthalate, Paraben, Triclosan, Triclocarban, BPA (Bisphenol A). Penta-BDE (Penta-brominated diphenyl ether), PCBs, Diesel fuel Exhaust, Alkylphenols Nonylphenol Octylphenol, Linuron Diuron, Vinclozolin Procymidone Iprodione Prochloroz Fenarimol, Fenarimol Fenitrothion Chlorpyrifos-methyl, Ketoconazole, Pyrethroid pesticides Permethrin Beta-cyfluthrin Cypermethrin Certain sun-screens 4-MBC 3-BC, Heat, Ionizing radiation, Non-ionizing radiation, microwaves, electromagnetic fields, Chemotherapeutic drugs (Cisplatin, cyclophosphamide, procarbazine, and doxirubicine, and vincristine etc.) (Woodruff *et al.,* 2008)

CONCLUSION

In view of the fact that the preliminary concerns arose about environmental chemicals or toxins and declining sperm counts, there has been an explosion of research in this area. The initial 'environmental oestrogen' hypothesis has been superseded by a more refined definition of EDCs. It is now accepted that there are a plethora of ways in which the environmental chemicals can potentially act on the endocrine as well as male reproductive systems. Though supportive data must need to determine whether human male reproductive health is declining or not. However; the hypothesis of a 'testicular dysgenesis syndrome' is an important advancement and may aid our understanding of the underlying aetiology of these disorders. Within the reproductive tract, the male is exquisitely vulnerable to the effects of anti-androgens during development due the dependence on the synthesis and action of androgens for the masculinization of the male reproductive tract. The ability of phthalates to suppress androgen synthesis during development and to induce testicular dysgenesis together with cryptorchidism and hypospadias has close parallels with human TDS. However, the crucial question regarding whether the level of environmental chemicals is sufficient to impact on human male reproductive health remains unanswered, although advances will be made from studying the effects of multi-component EDC mixtures in both in vitro and in vivo test systems. Moreover, it has been observed that in wildlife, there is an increasing rate of testicular cancer, to the debate regarding trends in sperm counts, there has been increasing concern that hazardous substances in the environment adversely affect male reproductive health.

By better defining the problems, learning about the mechanisms responsible for adverse effects, and developing panels of relevant biomarkers, we will make progress toward preventing future adverse effects on male reproductive health.

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