

Environmental contaminants

Is male reproductive health at risk?

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Contaminants such as cadmium, bisphenol A and lead pollute our environment and affect male reproductive function. There is evidence that toxicant exposure adversely affects fertility. Cadmium and bisphenol A exert their effects in the testis by perturbing blood-testis barrier function, which in turn affects germ cell adhesion in the seminiferous epithelium because of a disruption of the functional axis between these sites. In essence, cadmium mediates its adverse effects at the blood-testis barrier by disrupting cell adhesion protein complexes, illustrating that toxicants can dismantle cell junctions in the testis. Herein, we will discuss how environmental toxicants may affect reproductive function. We will also examine how these adverse effects on fertility may be mediated in part by adipose tissue and bone. Lastly, we will briefly discuss how toxicant-induced damage may be effectively managed so that fertility can be maintained. It is hoped that this information will offer a new paradigm for future studies.

Introduction

Spermatogenesis is a complex and step-wise cellular process that results in the production of ~25,000 sperm each minute in healthy adult males, and it takes place within seminiferous tubules in the mammalian testis under the strict regulation of testosterone, follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol 17 β .¹⁻⁵ Seminiferous tubules from one adult testis, if stretched out end to end, would add up to a mighty 20 m in length—a vast surface area given that

this organ produces millions of germ cells each day. For simplicity, these 20 m of tubules undergo continuous cyclic cellular changes known as the seminiferous epithelial cycle of spermatogenesis which can be divided into six organizational stages in the human;⁶⁻⁸ however, the number of stages is species-specific, and 12 and 14 stages have been described in the mouse and rat, respectively.^{9,10} This means that a typical cross-section through an adult mammalian testis shows several seminiferous tubules at different stages of the epithelial cycle, each of which is comprised of a unique arrangement of developing germ cells that depend on ‘nurse-like’ Sertoli cells for structural and functional support. For instance, one Sertoli cell can sustain between 30 to 50 developing germ cells,¹¹ illustrating an enormous responsibility on the part of Sertoli cells to maintain adhesion with individual germ cells. If adhesion was to be compromised by an environmental toxicant, for instance, germ cells would slough from the seminiferous epithelium, spermatogenesis would be arrested and subfertility or infertility may result.

To appreciate spermatogenesis, it is important to first understand the complex process of germ cell development, which occurs throughout the reproductive lifespan of a male. Morphological studies conducted several decades ago in rodents identified three different types of spermatogonia: type A, intermediate and type B that sit atop the basement membrane, a specialized type of extracellular matrix found in the testis. In the rodent testis, type A spermatogonia can be further subdivided into A_{single} (A_s), A_{paired} (A_{pr})

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and $A_{aligned}$ (A_{al}). In brief, an A_s spermatogonium divides by mitosis to produce A_s (which replenish the stem cell pool) or A_{pr} (which follow the differentiation path) cells. (Because of species differences in the way germ cells were originally defined, readers are asked to also refer to a description of germ cell development in the human.⁶⁻⁸ For instance, progenitor A_{dark} , progenitor A_{pale} and committed A_{pale} are the only type A spermatogonia present in the human testis.) In rodents, the latter cell type (i.e., A_{pr}) then divides by mitosis to generate a chain of A_{al} cells which is followed by a series of differentiation and mitotic steps to yield type B spermatogonia. These cells subsequently develop into primary spermatocytes (i.e., preleptotene spermatocytes) whose fate it is to cross the blood-testis barrier (BTB) and enter into the adluminal compartment of the seminiferous epithelium. Once in the adluminal compartment, spermatocytes enter and complete meiosis I and II to produce spermatids. Finally, spermatids undergo spermiogenesis via 19 steps, the last stage of spermatogenesis which produces mature spermatozoa that are released from the seminiferous epithelium at late stage VIII in the rat (i.e., spermiation). This entire process—beginning with a single type A spermatogonium and theoretically ending with 4,096 sperm—takes ~58 d to complete in the rat.^{9,10,12}

Throughout spermatogenesis, germ cells also traverse the seminiferous epithelium, with more developed germ cells inching toward the luminal edge in preparation for spermiation at late stage VIII of the seminiferous epithelial cycle.^{13,14} This suggests that an enormous amount of Sertoli-germ cell junction restructuring is taking place in the epithelium. Extensive restructuring also occurs at the blood-testis barrier (BTB), a Sertoli cell structure constituted by co-existing and mutually interacting junctions types [i.e., tight junctions (TJs), basal ectoplasmic specializations (basal ES), desmosomes and gap junctions (GJs)] that facilitate the entry of preleptotene spermatocytes, which are derived from type B spermatogonia, into the adluminal compartment for further development.^{15,16} As such the BTB is also crucial for spermatogenesis and fertility.

Environmental health is defined as “the branch of public health that protects against the effects of environmental hazards that can adversely affect health or the ecological balances essential to human health and environmental quality”.¹⁷ From this definition, the term endocrine disruptor was derived to describe a chemical having the ability to disrupt the endocrine system and/or endocrine organs of both humans and wildlife. As such, these definitions imply that environmental toxicants affect Sertoli-Sertoli and Sertoli-germ cell junctions, thereby leading to germ cell loss from the seminiferous epithelium in the testis and resulting in subfertility or infertility. In this review, we will discuss why environmental toxicants [e.g., cadmium (Cd) and bisphenol A (BPA)] have a predisposition to affect testis function. First, we discuss current data relating to two major environmental toxicants that are known to directly affect male reproductive function. Second, we focus on the effects of environmental toxicants on critical tissues and organs (e.g., adipose tissue, bone) within the mammalian body whose functions, if compromised, can indirectly contribute to male subfertility or infertility. Finally, we consider the ways in which toxicant-induced damage may be clinically managed so that male fertility can be protected. It is hoped that this review on environmental toxicants will ignite further interest in the field of reproductive medicine and that future studies will lead to an in-depth molecular dissection of the regulatory mechanism(s) that govern toxicant-induced testicular damage.

Effects of Environmental Toxicants on Male Reproductive Function

The following sub-sections provide an overview of two environmental toxicants, namely Cd and BPA. Emphasis is placed on findings that have made a significant contribution to our understanding of toxicant-induced male subfertility and infertility.

Cadmium. Cd is an environmental contaminant found most notably in tobacco smoke and industrial pollution, but also in water and the food chain where it has been causing increasing public

concern. Cd is bio-accumulative; it has a long biological half-life (i.e., >25 y), which is attributable to its slow and poor excretion; and depending on its dose, route and duration of exposure, it can adversely affect target organs such as the kidney, liver, gastrointestinal tract, lung, testis, bone and adipose tissue.¹⁸⁻²⁰ The primary route of exposure appears to be via inhalation, and average concentrations of Cd in non-smokers and smokers typically run at ~1 and ~5 µg/L serum, respectively. Another main route of exposure involves the gastrointestinal tract where Cd can enter epithelial cells via divalent metal ion transporter 1 (DMT1, an Fe transporter) and ZRT-, IRT-like protein (ZIP8, a Zn and Mn transporter) to cause havoc [e.g., increase production of reactive oxygen species (ROS)²¹] in the cytosol before being exported into the systemic circulation. In the liver, Cd can bind to metallothionein (a metal-binding protein that provides some protection against toxicity), followed by its endocytosis by kidney proximal tubule cells and subsequent degradation in lysosomal compartments.²² Not surprisingly, exposure to high levels of Cd can increase cancer risk; yet, Cd is not considered genotoxic, that is, it brings about carcinogenesis indirectly via aberrant gene expression, oxidative stress, inhibition of apoptosis or disruption of cell junctions, illustrating that its injurious effects are numerous and extensive. For instance, Cd was found to evoke spontaneous shedding of E-cadherin, which was triggered by γ -secretase (a multi-subunit protease complex) to produce a C-terminal fragment, thereby resulting in adherens junction disassembly and increased migration/invasion in several cancer cell lines.^{23,24} Still, the mechanisms behind Cd toxicity, as well as how this toxicity can be avoided or reduced, remain poorly understood.

Other *in vitro* and *in vivo* studies have shown Cd to be an endocrine disrupting chemical that can bind steroid receptors to mimic, enhance or inhibit the action of estrogens and androgens, thereby also potentially affecting any system in the body that is controlled by hormones (e.g., reproductive and skeletal systems).²⁵⁻³⁰ For instance, a significant decrease in serum and testicular testosterone levels was noted in mice exposed to acute doses

of CdCl₂ [i.e., 1 mg/kg daily from post-natal day (PND) 35 to PND 70],³¹ illustrating that this compound disrupts the hypothalamic-pituitary-testicular axis by creating a hormonal imbalance and inadvertently affecting male fertility.^{32,33} There were other irreversible effects as well; histological findings included interstitial edema and hemorrhage, testicular weight loss (resulting from the detachment of the germinal epithelium) and necrosis, and testicular atrophy and calcification, which occurred gradually over ensuing months, revealing that the testis is extremely vulnerable to Cd-induced damage (Fig. 1). At the molecular level, Cd is known to interact with extracellular calcium binding domains present within E-cadherin, which modifies its adhesive properties, obliterates its localization at the plasma membrane (via γ -secretase-mediated proteolysis and ectodomain shedding) and disrupts cell-cell junctions such as those found between Sertoli cells, as well as those between Sertoli and germ cells.³⁴⁻³⁶ However, it is still undetermined if Cd can trigger the shedding of E-cadherin in the testis; this is an interesting question that requires further experimentation. Translocation of β -catenin (CTNNB1, a cadherin binding protein) from the plasma membrane and into the nucleus, where it interacts with the T-cell factor/lymphoid enhancer factor (TCF/LEF) machinery to activate transcription of wingless-related MMTV integration (Wnt) target genes, has also been reported (albeit in kidney proximal tubule cells) after Cd exposure.³⁷ This observation is quite interesting because sustained activation of Wnt- β -catenin signaling in Sertoli cells of *Ctnnb1^{tm1Mmt/+};Amhr2^{tm3(cre)Bhr/+}* mice was shown to result in seminiferous tubule deterioration, increased apoptosis, germ cell loss, testis weight loss, progressive atrophy and infertility.^{38,39} Likewise, *Nkd1^{-/-}* mice were also reported as being infertile (Naked1 is negative regulator of Wnt- β -catenin signaling),⁴⁰ strongly suggesting that deregulated Wnt signaling might be behind Cd-induced infertility, as well as testicular carcinogenesis. Cd has also been reported in diverse biological contexts to trigger other related or parallel signaling cascades such p38 mitogen-activated protein kinase (MAPK) and c-Jun

N-terminal kinase (JNK) pathways,^{41,42} both of which are known to be activated by environmental stress (e.g., ROS) and linked to subfertility and infertility.⁴³ As an interesting case in point, p38 MAPK was activated via phosphorylation in the rat testis following acute exposure to CdCl₂ (i.e., 3 mg/kg, single dose), whose adverse effects on germ cell adhesion were partially blocked by SB202190,^{41,44} a highly selective inhibitor of p38 α and p38 β (but not p38 γ and p38 δ) isoforms. The harmful effects of CdCl₂ at the BTB were probably also partially blocked by SB202190 because CdCl₂ is known to disrupt barrier function by reducing the levels of TJ (e.g., occludin, zonula occludens-1) and basal ES (e.g., N-cadherin, β -catenin) proteins, which in and of itself can result in germ cell loss from the seminiferous epithelium.⁴⁴ Moreover, CdCl₂ was found to disrupt interactions between residual proteins remaining at the BTB [e.g., occludin/zonula occludens-1 (ZO-1)/focal adhesion kinase (FAK) and N-cadherin/ β -catenin],^{45,46} which further destabilized barrier function. Wnt has also been described as an important regulator of p38 MAPK signaling in normal cells. Taken collectively, these findings show that Cd targets, at least in part, crucial proteins within major signaling modules to bring about subfertility or infertility. Precisely which additional proteins and signaling pathways are involved is a matter of further investigation.

Bisphenol A. BPA (4,4' isopropylidenediphenol) is a xenoestrogen with a short half-life (i.e., <6 h) and one of the world's most produced chemicals via industrial activities. It is used in the manufacturing of numerous products (e.g., polycarbonate plastics, epoxy resins that line food and beverage cans, dental sealants and composites, flame retardants and coating of water pipe walls, as well as other common consumer items such as compact discs and paper receipts received from supermarkets, bank teller machines and gas stations). Unfortunately, BPA can leach from the linings of food and beverage containers (including those that contain infant formulas and pet foods), baby bottles and pipe walls to contaminate food and water,

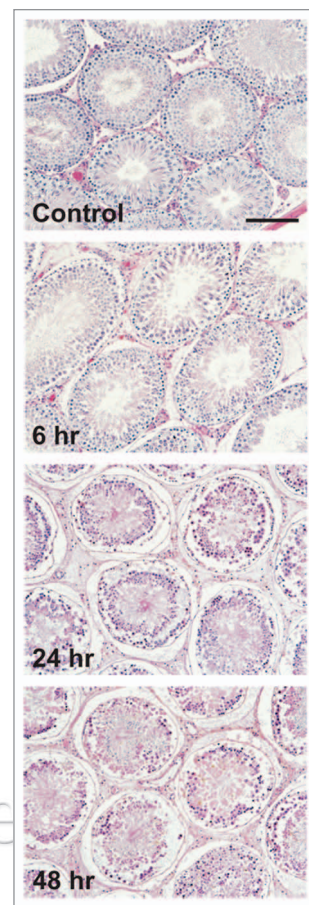


Figure 1. Adverse effects of Cd on the testis as assessed by histological analysis. Rats (~300 g b.w.) were treated with a single dose of CdCl₂ (suspended in 0.9% saline) at 3 mg/kg b.w. via intraperitoneal injection (i.p.). Thereafter, rats in groups of n = 3 were terminated at 6, 24 and 48 h. Paraffin sections were stained with hematoxylin and eosin for histological analysis. It is noted that by 24 h post treatment, germ cells were found to detach from the basement membrane in the tunica propria; and by 48 h, virtually all elongating/elongated and round spermatids, as well as spermatocytes were found in the tubule lumen in >98% of tubules. Bar = 100 μ m in top panel, which applies to all other micrographs.

thereby potentially posing a serious risk to human health as this compound has been implicated in several developmental and reproductive diseases.^{47,48} BPA is known to bind classical nuclear estrogen receptors (i.e., ER α and ER β), albeit at a significantly lower affinity than endogenous estradiol 17 β , to activate or repress estrogen responsive genes.⁴⁹ In subsequent studies, BPA was found to also bind G protein-coupled receptor 30 (GPR30, an

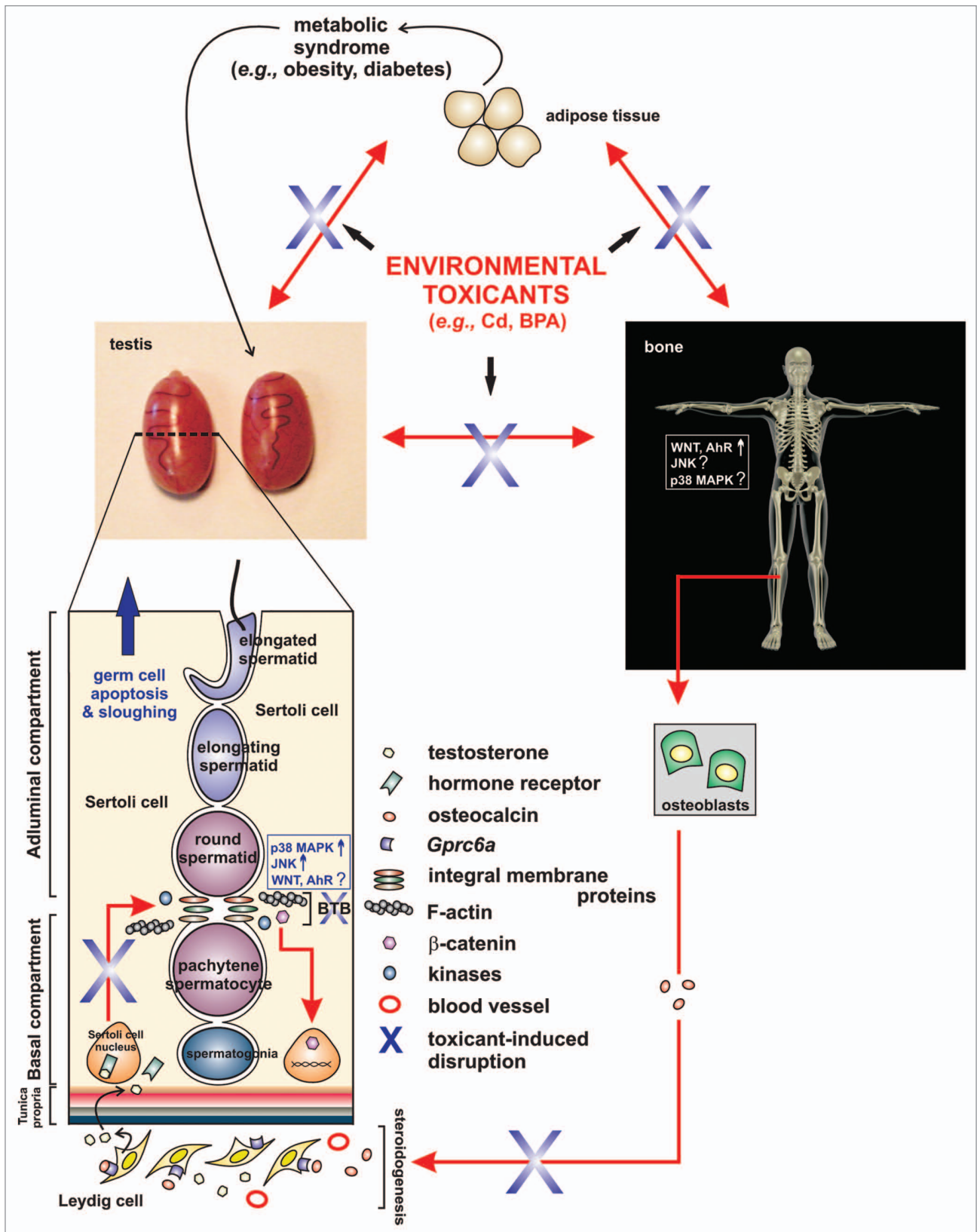


Figure 2. A schematic drawing illustrating crosstalk between adipose tissue, the skeleton and the testis that together maintain male reproductive function. As described in the text, osteocalcin released from osteoblasts in bones can regulate steroidogenesis, which can be impeded by environmental toxicants. Environmental toxicants can exert their effects on crosstalk between the skeleton-testis, adipose tissue-testis, adipose-skeleton and bone-Leydig cells. One of the net results of these destructive effects involves a disruption of BTB function, germ cell adhesion and steroidogenesis via changes in mitogen-activated protein (MAP) kinase, oxidative stress and others.

orphan seven-transmembrane spanning receptor that is structurally different from ER^{50,51}) with high affinity, resulting in the production of cAMP and in the activation of alternative estrogen signaling cascades [e.g., extracellular-regulated kinases 1 and 2 (ERK1/2) and phosphoinositide-3-kinase (PI3K)], that is, via non-genomic pathways that are outside of the classical ER pathway.⁵¹⁻⁵⁶ Inappropriate MAPK activation such as that initiated by the binding of BPA to GPR30 may also lead to a variety of human cancers⁵⁷ because estrogens and xenoestrogens can increase cell proliferation directly or indirectly via growth factors, and this can favor tumorigenesis.^{58,59} In addition, BPA binds estrogen-related receptor γ (ERR γ , an orphan nuclear receptor that does not directly bind estradiol 17 β)^{60,61} and aryl hydrocarbon receptor (AhR, a transcription factor that plays an important role in xenobiotic metabolism),⁶² illustrating that BPA has many cellular targets. In the latter case, in utero administration of BPA significantly increased AhR expression in testes from embryos.^{63,64} Crosstalk between AhR and MAPK signaling pathways has been reported in normal cells,⁶⁵ but it remains to be determined if environmental toxicants can alter crosstalk between these signaling cascades. BPA may also have anti-androgenic activity.⁶⁶⁻⁶⁸ For example, BPA at a wide range of concentrations [i.e., 2 μ g–400 mg/kg daily from gestational day (GD) 11 to GD20] was found to significantly downregulate steroidogenic acute regulatory protein (StAR, a transport protein critical for steroid biosynthesis in steroid-producing organs such as the testis, ovary and adrenal gland⁶⁹) in fetal rat testes.^{70,71} A decrease in cytochrome P450 (P450arom, an enzyme that catalyzes the irreversible conversion of testosterone to estrogen^{72,73}) was also observed in fetal testes following exposure to BPA at high doses (i.e., 400 mg/kg from GD11 to GD20),⁷⁰ revealing that BPA can bring about a hormonal imbalance. These findings are compelling because oral dosing of rats with BPA (i.e., 50 mg/kg daily from PND20 to PND25) was found to disrupt the BTB,⁷⁴ a structure long-known to be hormone-dependent,^{75,76} and prolonged disruption of the BTB by environmental toxicants can lead to infertility

or subfertility.^{77,78} This is because the BTB creates a unique environment (i.e., adluminal compartment) essential for germ cell development; without a functioning BTB, germ cells cannot develop into mature sperm. In yet another related study, perinatal exposure of rats to BPA (i.e., 1.2 and 2.4 μ g/kg daily from GD12 to GD21) reduced sperm counts, increased germ cell sloughing from the seminiferous epithelium and resulted in subfertility in F₁, F₂ and F₃ offspring,⁷⁹ clearly illustrating that in utero exposure to BPA can produce long-lasting adverse epigenetic effects that extend into future generations. More specifically, BPA appears to affect epigenetic mechanisms (e.g., DNA methylation) within cells, which implies that we are directly affected by the lives of our ancestors—by the food they ate, by the air they breathed, by their occupations, by their personal experiences, so on and so forth.^{80,81} Thus, the consequences of BPA are incalculable.

Environmental Toxicants, Metabolic Diseases and Male Reproductive Function

Globally, 50–80 million people are infertile, an estimate that is likely to increase drastically in the future. Several factors are known to underlie male infertility, and as discussed previously, exposure to environmental toxicants is one of these factors. While it is not yet completely understood why the testis is so vulnerable to damage by environmental toxicants, it is clear that toxicants affect many, if not all, mammalian organs in some adverse way. Presently, there is increasing evidence from both laboratory animal and human studies to support the contention that Cd and BPA can directly contribute to the onset of metabolic diseases (e.g., obesity and diabetes mellitus) and that this may indirectly impede male fertility. For example, administration of BPA (i.e., 10 μ g/kg daily for 2 d) stimulated insulin production by pancreatic β -cells in adult mice. At more than ten times this dose of BPA, mice developed insulin resistance and hyperinsulinemia,⁸² which can mature into diabetes if clinically untreated in humans. Conversely, adults with the highest levels of circulating BPA were more than twice

as likely to develop diabetes than those with lower levels of this toxicant.⁸³⁻⁸⁵ Cd exposure also associates with the development of type II diabetes;⁸⁶ a significant increase in fasting blood glucose levels was observed in subjects with high serum and/or urinary Cd levels. Not unexpectedly, diabetic rats were found to display decreases in testosterone, sperm counts and motility and testicular weight,^{87,88} suggestive of abnormal spermatogenesis and subfertility or infertility. Likewise, many diabetic men are known to have hypothalamic-pituitary-testicular axis disturbances (e.g., a reduction in testosterone) and to be infertile.^{89,90} Furthermore, it comes as little surprise that obesity, which in and of itself is a risk factor for diabetes, cancer, infertility and a host of other morbidities, has also been linked to environmental toxicant exposure. Today, obesity is a serious public health concern as more than 30% of Americans are obese; and while over-eating, inactivity and genetic predisposition are major contributors of the obesity crisis, environmental toxicant exposure is probably also a contributing factor. In the case of BPA, for example, an increase in adipose tissue mass was noted in pubertal mice that were fed a low-fat diet but exposed to BPA (i.e., 1 μ g/ml or 10 μ g/ml water from GD10 to PND30) when compared with control mice.⁹¹ BPA is lipophilic, and it can accumulate in fat stores to increase the number and/or size of adipocytes, thereby resulting in weight gain. Adipocytes also express ERs to which BPA bind.⁹² Other in vitro and in vivo studies performed in both laboratory animals and humans showed BPA at environmentally relevant doses to reduce the secretion of adiponectin,⁹³ a hormone produced by adipose tissue that can protect an individual from diabetes, by inhibiting the activation of protein kinase B (PKB, also known as AKT, a downstream regulator of the PI3K signaling pathway).⁵⁶ PKB β /Akt2 null mice also displayed insulin resistance with animals developing β -cell failure and diabetes,⁹⁴⁻⁹⁶ illustrating that BPA targets an important regulator of human physiology. It remains to be determined if BPA also interferes with the production of leptin, a protein that controls body weight, metabolism and reproductive function.⁹⁷ Taken collectively, these

findings illustrate that there is a crucial connection between environmental toxicant exposure and metabolic diseases.

Environmental Toxicants, Bone and Male Reproductive Function

Environmental toxicants are also known to accumulate in and to directly affect mature bone by disturbing the balance between osteoclastic bone resorption and osteoblastic bone formation, and this may indirectly compromise male fertility. For example, exposure to Cd—even at low doses—has been found to disrupt bone metabolism, leading to a decrease in bone mineral density (i.e., osteoporosis) and an increase in fracture incidence.⁹⁸ Interestingly, the injurious effects of Cd on the skeletal system were shown to be relatively rapid, analogous to what occurs in the testis after Cd exposure. In mice, bone demineralization was observed within 24 h of an oral dose of Cd (i.e., 200 mg/kg, single dose), and an *in vitro* study on the effects of Cd on bone organ and cell culture models reported similar findings.⁹⁹ It is possible that Cd may be affecting osteoblasts' ability to produce collagen type I, a structural protein whose role it is to assemble a scaffold for bone mineralization. In the testis, Cd was found to disrupt the organization of collagen fibrils residing within the tunica propria (it is not known if type I and/or type IV collagen was affected),¹⁰⁰ indicating that Cd targets a critical understructure on which all cells of the seminiferous epithelium reside. Other studies relating to the skeleton have reported the harmful effects of environmental toxicants to be also mediated by AhR.¹⁰¹ These results are in line with the effects of BPA on AhR expression in the testis, as discussed above, suggesting that these effects may be coordinately regulated across different organs in the mammalian body. In a more recent study, Oury et al. reported on an intriguing connection between the skeleton and the testis;¹⁰² these authors showed that osteocalcin directly affects the production of testosterone by Leydig cells in the testis which is needed for many aspects of reproductive function, including BTB maintenance and germ cell survival. These effects were mediated by Gprc6a

(a G-protein coupled receptor present on the Leydig cell surface) binding and cAMP response element-binding (CREB, a transcription factor) activation, which resulted in the regulation of steroid producing enzymes (e.g., StAR, P450arom and 3 β -hydroxysteroid dehydrogenase).¹⁰² When mice deficient for osteocalcin were studied, decreases in testis weight, sperm count and serum testosterone were noted, as well as a decrease in the number of offspring born, revealing that osteocalcin is yet another important regulator of male fertility, in addition to insulin^{89,103} and leptin.^{104,105} These findings raise many important questions that should be addressed in future studies.

Concluding Remarks and Future Perspectives

Herein, we have commented on the effects of environmental toxicants on male reproductive function. As briefly discussed, Cd and BPA are known to compromise testis function directly by affecting Sertoli-Sertoli and Sertoli-germ cell junctions. However, they are known to affect other tissues and organs (e.g., adipose tissue, bone) as well, which may compromise testis function indirectly, thereby leading to subfertility or infertility. At this point in our trend toward global industrialization, it would be foolish to contend that human exposure to environmental toxicants can be completely eliminated. While some of our exposure to environmental toxicants can be controlled by personal choices made in our adult lives (e.g., deciding not to smoke cigarettes/cigars, choosing glassware over polycarbonate plasticware), at this point it is impossible to conclude that these choices can protect an individual from the harmful effects of environmental contaminants. Inhibiting signaling pathways in the testis that are activated by environmental toxicants, which has been proposed by some investigators in the field, may be an interesting approach to avoid subfertility or infertility. However, there are several caveats associated with this theoretical approach. First, any inhibitor developed would have to target the testis specifically, and it would have to inhibit the adverse effects of a broad spectrum

of environmental toxicants, not just Cd and BPA. Second, environmental toxicants target key signaling cascades (e.g., PI3K, PKB) that regulate important cellular functions, and inhibiting these signaling cascades may inadvertently result in other more severe effects. Finally and most importantly, it is rather difficult to prove cause-and-effect. In other words, it is difficult to prove that exposure to environmental toxicants at environmentally relevant doses over a long period of time can cause subfertility or infertility, even though there is ample scientific evidence to suggest that this may be the case. Additional mechanistic studies are desperately needed to understand the molecular mechanism(s) underlying toxicant-induced male reproductive dysfunction. These may open up new ways in which environmental toxicant-induced testicular damage may be therapeutically managed in the future.

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