Exposure to environmental contaminants and the impact on reproductive health

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Abstract

Reports are illustrating increasing evidence of perturbed reproductive health in a variety of species. Given the rate of change and the widespread occurrence amongst a variety of species, such observations allude to an environmental drive opposed to a natural genetic change. Extensive use and dissemination of plastics that contain anthropogenic organic chemicals is suggested as a plausible etiology of such adverse fertility trends given the drastic increase in global plastic production. Direct industrial emission and chemical migration from plastic product matrices, in which such chemicals originate, leads to environmental deposition. Once ubiquitous within the environment, such environmental chemicals, otherwise known as xenobiotics that are known to modulate endocrine signalling, are consistently available for uptake by humans and animals on a global scale. A variety of species are proposed as sentinel models to further explore the impact of a polluted ecosystem on reproductive health. Pregnant animal exposure to xenobiotics, during the key developmental programming window, is of great concern due to the potential for epigenetic modifications on the developing fetus. This review aims to discuss such concepts and routes of exposure, to highlight areas for further research within the field.

Keywords: Xenobiotics, reproduction, dog, fertility, environment

Introduction

Global plastic production reportedly stands at 320 x 10^6 tons per annum. Around 40% of such plastics are single use and contain a variety of anthropogengic organic chemicals. Direct industrial emission and migration from product matrices, in which such chemicals originate, leads to environmental deposition.[1] Exposure to environmental chemicals (ECs), often endocrine disruptive in nature, have been suggested in the etiology of adverse fertility trends.[2] Common anthropogenic organic chemical classes include; bisphenols, dioxins, phthalate ethers, parabens, polycyclic aromatic hydrocarbons, and perfluorinated compounds (PFCs), with existing overlap between some chemical congeners.[3,4] Such chemicals are typically produced through or utilized in a range of industrial and agricultural processes.[5] These include uses as plasticisers, flame-retardants, solvents, preservatives, additives, coatings, pesticides, herbicides, fungicides, and fertilizers. Given the ability of ECs to leach from products into nearby surroundings, chemicals remain ubiquitous within the environment; present in air, water, soil, and sediment.[6,7] Here, ECs are consistently available for uptake by humans and animals on a global scale, through a variety of means.[8]

With global increases reported in the occurrence of obesity, metabolic syndrome, and associated diseases (e.g., PCOS and diabetes) it has been postulated that this is also linked to exposure to chemicals, giving rise to the term 'metabolism disrupting chemicals'.[9] In humans, reports suggest that the etiology and pathophysiology of metabolic diseases could be due to environmental chemical exposure.[10] Many chemicals that appear to impact on metabolic function, also have endocrine disrupting activity and thus may adversely affect both reproductive function and metabolic disease.[11]

Exposure routes of environmental chemicals

One of the main deposition pathways for EC contamination is via a carnivorous diet. Consumption of contaminated meat, particularly when fat content is high, is considered as a main exposure route in carnivorous and omnivorous species, given the lipophilic nature of many ECs. This leads to biomagnification across trophic levels, with apex predators incurring the highest bodily burdens. Considering 3 aquatic species with high blubber content but differential dietary sources, the herbivorous dugongs has polybrominated diethyl ester biological burdens of 120 ng/g lipid weight.[12] This level, whilst high, is still 8 times less than the apex predator species, the killer whale.[13,14] Similar biomagnification of ECs has been reported in terrestrial ecosystems and agricultural food chains,[15] as part of human consumption.[16]

Dog is a close companion to man and shares the same habitat. For this reason, dog is exposed to the same environmental conditions, including environmental chemicals present in home. For this reason, dog is considered, an ideal sentinel model to investigate human exposure to environmental pol-

lutants.[14] Canine diet (commercially available pet foods) has common environmental chemicals.[17] Since similar chemical types were detected in dog semen and testes (collected from routine neuters), effects of environmental/gonadal contaminants on sperm quality parameters were tested. Deleterious chemical effects were reported on the quality of DNA and sperm motility in dog and human.[18] Food assessed for environmental chemicals contained meat sources from grazing animals (consumed by man who have a meat-based diet).[17]

Pasture contamination through the routine use of sewage sludge fertilizers (biosolids) is therefore of primary concern.[19] Such fertilizers promote the deposition of a broad range of toxic chemicals onto agricultural land, in addition to complex mixtures of microplastics.[20] The ability of chemicals originating from their polymer matrices, to leach into the surrounding environment or digestive tract, if ingested, provides an initial exposure route to biota. Fetuses from pregnant ewes grazed on such pastures, as well as their offspring, exhibit perturbations in in both female and male reproductive development.[17,21,22]

Highly chlorinated congeners, including polychlorinated biphenyls (PCBs), bond strongly to soil organic matter, reducing the uptake into plants through root structures, and instead are readily absorbed from the surrounding air.[8] However, when pasture concentrations are lower, direct consumption of contaminated soil may result in an additional exposure route to grazing species. Around 21,000 tonnes of surface soil are reported to contain PCBs[23] that are known reproductive toxicants.[17,18] Although banned in the 1970's, these ECs are reported to be present within UK soil at an average concentration of 2.52 mg/kg, with the highest concentrations reaching 80.6 mg/kg.[24] As alluded to above, biomagnification within the human can then ensue from a meat-based diet, by consuming animal species that have grazed on such treated lands. Theoretically, this would mean that a plant-based diet would result in lower biomagnification of contaminants.

Unpublished, preliminary data in the horse actually showcases concentrations of certain contaminants to be higher than sentinel models (e.g., dog) fed on meat-based diets (Harris, unpublished data). This is potentially indicative of alternative environmental exposure (e.g., water or plant-based feedstuffs). A common approach to breaking down contaminated materials in soil is a process called bioremediation. Using fungi or bacteria alongside plant material, ECs can be drawn from the soil into plant matter. Reports illustrate the ability of plant 'alfalfa' to remove PCBs from contaminated soil by uptake into roots and leaves.[25] Although this is a beneficial aspect for clearing contaminated soil, due to the digestible energy content of alfalfa, this feedstuff is suggested for pregnant herbivores and breeding stallions.[26] Giving rise to a potential exposure route for herbivorous diets.

A further risk factor is xenobiotic run off into water systems. Although a range of xenobiotics are reported present in water,[27,28] di-ethyl hexyl phthalate (DEHP) is a common plasticizer and a reported carcinogen,[29] known to perturb reproductive health at lower exposure concentrations.[17] This phthalate is present in tap water, bottled water and barrelled water supplies. Concentrations of DEHP were initially greater in tap water, but increased concentrations of DEHP were observed in plastic bottled water that was heated to 60°C, a finding expected due to the ability of chemicals to leach from the product matrices.[30] This increase in chemical pollutants is not restricted to plastic products. It is estimated that with every 1°C increase in environmental temperature, the volatility of polychlorinated bi-phenyls would rise by around 10 - 15%, increasing pollutant mobility and promoting further uptake within the ecosystem.[31] Such change could be an addition to the concept of biomagnification (Figure 1).

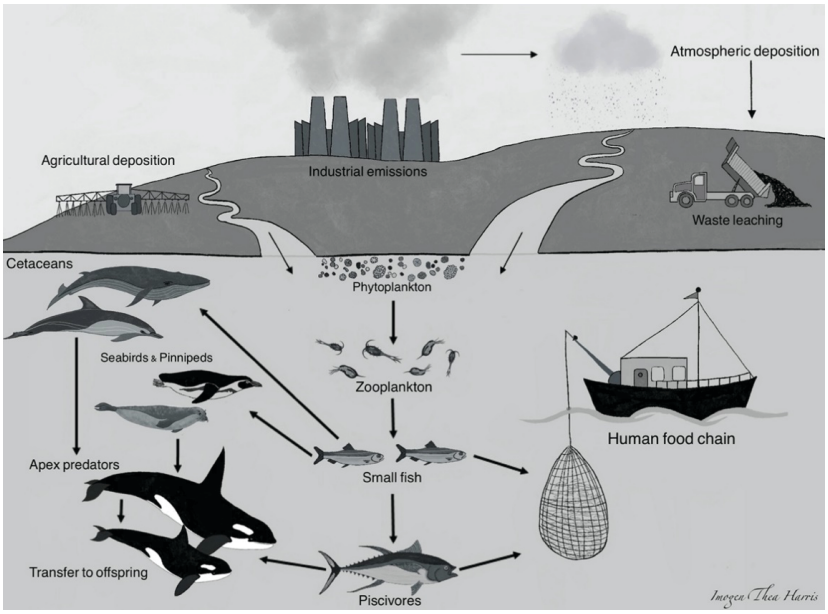

Figure 1. Biomagnification of xenobiotics within the aquatic ecosystem. Industrial processes, agricultural deposition and waste leaching gives rise to biomagnification within the ecosystem, starting from consumption of phytoplankton, magnifying within endangered apex predators. A similar process occurs on land. Figure not to scale. Figure is authors own (I.T.H).

Fetal environment

Environmental chemical exposure is likely to occur throughout life, beginning in utero, continuing postpartum, throughout adolescence, adulthood and gametogenesis. Placenta is a dynamic endocrine organ and has incorporating roles (e.g., homeostasis, fetal growth and sustaining pregnancy).[32] Perturbed placental function can impact fetal development and growth, contributing to chronic health issues in adult life.[33] Mono (2-ethylhexyl) phthalate, the primary metabolite of DEHP, is reported to perturb trophoblast differentiation, thereby acting as an endocrine disruptor to the very early developing placenta.[34] Intrauterine environment is where the fetus is most susceptible to the exposure of external ECs due to endocrine mediated developmental period.[35] It is thus concerning that ECs (e.g., phthalates[36] and bisphenols [BP][37]) have the ability to cross the placental barrier, exposing the developing fetus to a range of toxic chemicals. In an in vivo murine model, BP-A and BP-S altered 13 sets of identical placental genes, causing morphological defects within the midpregnancy placenta that persisted until parturition.[38] Such exposure may lead to the indirect disruption of essential developmental processes of the gonads and reproductive system, leading to chronic reproductive perturbations in an adult life.

Fetal transfer is complex, although placenta works to protect the fetus against exposure to xenobiotics there is the common consensus that ECs have an accumulatory nature towards fetal compartment due to cross talk of signalling pathways.[39] Analogue lipophilicity, polarity and hydrogen-bonding are reported to impact placental transfer efficiency.[37,40] Relatively lower concentrations of bisphenol congeners are actively transported to the fetal compartment.[41] Aryl hydrocarbon receptor is highly expressed within the placenta and a key receptor that works to protect the maternal-fetal interface and placental barrier from xenobiotic exposure. It is hypothesized that pollutants (e.g., bisphenol-A), interfere with the activity of the aryl hydrocarbon receptor, reducing the typical endocrinological function and metabolic activities of the placenta.[42] Due to the physiochemical specificities of chemical metabolites and their inability to be removed from the fetal compartment in their glucurono-conjugated forms, a back-metabolism cycle is initiated by which the bioactive forms are resynthesized. This cycle increases fetal exposure substantially.[43] For BP-S, although the placental transfer in the materno-fetal direction was only 0.4%, this back-metabolism increases fetal exposure to the bioactive form by 87%.[41] Additionally, it has been suggested that amniotic fluid acts as a reservoir by which the fetus is reexposed to BPS through swallowing and dermal adsorption, raising further concern over fetal exposure.[37,41]

ECs are also reported to have reprotoxic effects through endocrinological interactions.[44,45] Depending on specific chemical composition, steroidogenic perturbations are a result of affinities for different receptors. This is an area that certainly needs to be furthered within an appropriate sentinel model. Preliminary data assessing the addition of PCB-153 (2,2′,4,4′,5,5′-hexachlorobiphenyl) and DEHP on LH-induced testosterone secretion in the canine sentinel, to determine the impact of toxicants on endocrine function, did not appear to inhibit endocrine function.[17]

Male reproductive health

There is an increasing body of published evidence indicating that human male fertility and reproductive health has declined over the last 40 - 60 years. Geographically dependant temporal declines in human semen quality are becoming an increasing concern, with meta-analytical studies suggesting an approximate 50% decline in sperm concentration over the past 70 years.[46,47] Results from these meta-analytical studies remain heavily scrutinized, a result of developments in semen analysis methodologies, heterogeneity and the inclusion of historical data sets. Such limitations were suggested to be substantial factors influencing the adverse trends reported, thus questioning the true declines in semen quality.[48] Following the application of stricter inclusion criteria, in addition to completion in accordance with standardized protocols (meta-analysis of observational studies in epidemiology)[49] more recent meta-analyses continue to suggest a decline in fertility.[50] This rate of decline appears to have no plateau, raising substantial concerns for future male fertility.

Temporal declines are specific to the Western world, including Europe, North America, Australia and New Zealand, with trends failing to prevail in South Africa, Asia and South America.[50] Such trends, with distinct geographical variation would suggest the influence of environmental factors, although socio-economic contexts could pose as additional contributors. Parallel trends in sperm quality parameters to that in humans have been reported in a dog sentinel model, with an overall decline of 30% in progressive sperm motility.[17] Geographical variation is also evident within exposure to environmental chemicals. Dog sentinel model showcases how testicular chemical profiles vary regionally, alongside varying testicular pathological profiles. Testis collected from Finland had reduced pathologies compared to testis collected from Denmark and the UK. Such findings provide additional support to the concept that the environment likely influences reproductive function.[51] Many socio-economic interactions do not retain relevance in such species. Additionally, data were collated from a single lab with consistent analytical methods, thereby adding to the weight of evidence exhibited in the human and limiting the criticism over semen quality assessments. Decline in semen quality is proposed to be associated with bioaccumulation from meat-based diets, supported by the fact that a meta-analysis of temporal trends in the herbivorous stallion sperm quality does not appear to be overly altered. [52] However, collecting sperm quality data from a singular lab, preliminary data do actually have similar patterns of declining sperm quality within the herbivorous breeding stallion (Harris, unpublished).

As mentioned, dog, given the association to human lifestyle, is proposed as a useful sentinel model to assess the deleterious impact of environmental chemicals on human reproductive health. Undertaking an updated analysis of temporal trends in canine sperm quality, originally assessing sperm quality over a 26-year time frame,[17] continued to have a decline in sperm motility over time, yet not to as a substantial degree (Figure 2).

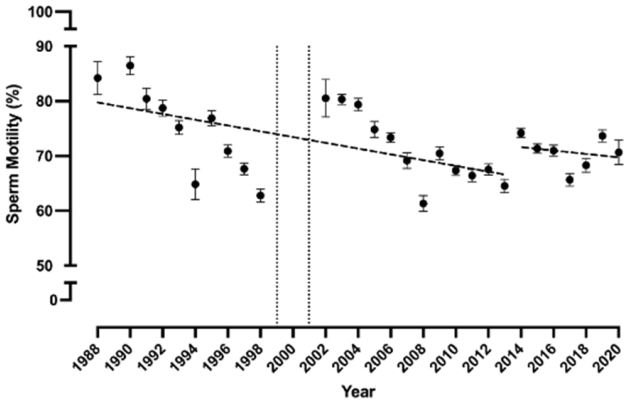

Figure 2. Updated analysis of temporal trends in canine sperm quality over a 32-year timeframe. Percent normal sperm motility. Data expanded utilising part of published data.[17] Scientific report articles are published under a CC BY license allowing for maximum dissemination where users are free to adapt data. Error bars represent ± 1 SEM. Vertical dotted lines represent time point within the programme where dogs with poor semen quality were removed from the programme. The cause of this is unknown. Diagonal lines showing declining sperm quality are plotted for graphical purposes only.

Temporal trends in sperm quality are also linked with global increases in reproductive perturbations; including testicular cancer (TCa) and genitourinary abnormalities, such as cryptorchidism and hypospadias.[2,53] This is evident within both the primate and dog. The reproductive trends present today are collectively termed testicular dysgenesis syndrome.[2] Globally, TCa incidences in humans have increased 2-fold over the past few decades, with most cases apparent in younger generations. Although a variety of factors could give rise to such changes, these trends are suggested to be a result of toxicant exposure at vital periods of sexual development.[54,55] Over a similar time frame, increases in cryptorchidism have been reported in male pups from the same population of stud dogs that exhibited a decrease in sperm motility.[17] Preliminary evidence within the canine additionally suggests an increased incidence of testicular tumours over a 40-year period.[56] Geographical variation in human reproductive perturbations are also heavily reported, with higher incidence rates in industrialised and agricultural areas, indicative of interactions between chemicals utilized within these industries.[19,57]

Female reproductive health

A further sensitive window of exposure is during the complex process of follicle development from the primordial follicle pool to mature preovulatory Graafian follicles. Ovarian somatic cells are specialised, multidisciplinary cells that are paramount for optimum reproductive function and follicle differentiation; with roles in germ cell support steroidogenesis and growth.[58] In canine sentinel model studies, certain environmental chemicals were present at higher concentrations in the dog ovary then in the testis (Van der Mescht, unpublished data). In addition, when coculturing murine ovarian tissue with the chemicals present within the canine ovary, there is an enhanced sensitivity to of the earlier follicle types (primordial and primary follicles). With the follicular population formed during development determining the reproductive lifespan of an individual, ensuring a balance between apoptosis, proliferation and differentiation is crucial. Primordial follicles are those that define the ovarian reserve, therefore modifications to such follicles could be detriment to fertility. Understanding how environmental chemicals affect the molecular and biochemical signalling of the granulosa cell, to support the oocyte, is an area that requires further study.

Key programming window and transgenerational impact of ECs

It has been suggested that EC exposure may manifest long after initial exposure due to epigenetic modifications originating from exposure at the critical window of genitourinary development in the foetus and new-born.[59,60] Modifications include DNA methylation, microRNA or histone alterations.[59] As discussed above, placenta is a dynamic organ that supports fetal development. Studies are beginning to indicate how exposure to phthalates during pregnancy is associated with genome wide modifications of placental DNA methylation, impacting fetal development.[61] Focussing on reproductive development, the bipotential gonad during early pregnancy incorporates many signalling pathways and molecules that instigate, and control, crucial embryological developmental pathways. Utilization of mouse knockout models provides insight into such genetic determinants of the bipotential gonad development. GATA binding protein 4 (Gata4) remains as 1 of the earliest markers crucial for formation and development of the gonadal ridge.[62] Loss of Gata4 gene expression is reported to inhibit formation of gonadal ridge.[62] Further development and maintenance of the gonadal ridge is determined by genes (e.g., Wilms' tumour suppressor 1 gene and steroidogenic factor 1) essential for early gonadal development.[63] Under the influence of WNT signalling, the binary fate decision of gonadal formation is chosen. A recent review discusses how the observed human male reproductive disorders might have a fetal origin, due to an androgen dependant programming window during early gestation.[64] One of the major signalling pathways throughout ovarian differentiation is that of WNT4/RSPO1 signalling.[65] In the absence of the male sex determinant gene, the cascade of genetic pathways to promote female development sees WNT mediated stimulation, like that of the ligand WNT4 induce the expression of downstream effectors,[66] such as follistatin and β-catenin.[67] β-catenin is a pro-ovarian signalling molecule which induces expression of the pivotal female development transcriptional target, FoxL2.[68-70] Should a toxicant impair the expression of male sex determining genes in an XY embryo, then it is plausible for WNT mediated stimulation to ensue, to follow the feminisation pathway.[71]

Epigenetic mechanisms, with adverse effects on reproductive potential, are considered to be transgenerational.[72,73] In killer whale populations, EC concentrations in calves are higher than in their lactating mothers.[74] Comparably, in suckling polar bear cubs, PCB concentrations surpassed that of maternal contamination.[75] Such research demonstrates the accumulatory nature of toxic ECs within the developing neonate. The subsequent exposure of such chemicals to the suckling offspring is likely to perturb reproductive development and future fertility, as reported in other species,[76] having future transgenerational impact. The transgenerational impact of contaminants

has been shown to induce delayed pubertal onset, impaired gametogenesis and impaired steroidogenic gene expression. Furthermore, maternal behaviours have also been shown to be impaired following transgenerational studies of toxicant mixtures, raising concern of not only reproductive health, but also wellbeing of future generations.[77] Within the sheep model, exposure to environmental contaminants has been shown to induce testis transcriptome modifications, which authors report, if not corrected by or during puberty, would likely have adverse outcomes for future generations adult life.[22] Reports discuss how epigenetic mechanisms are the means by which xenobiotics mediate such transgenerational effects.[78]

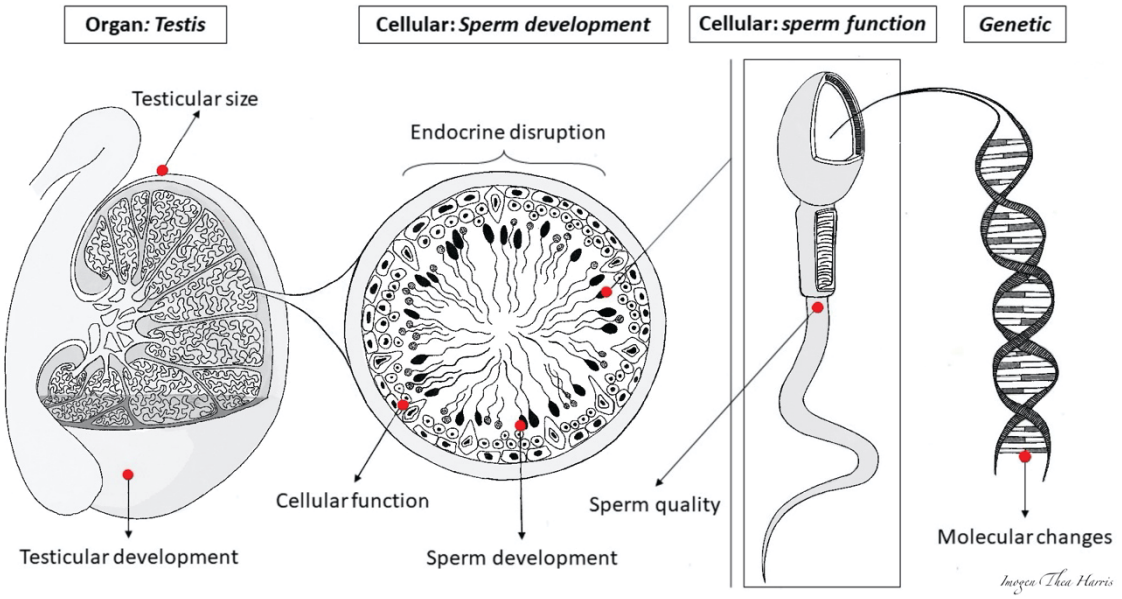

Figure 3. Avenues for xenobiotic perturbations within testicular tissue. As the housing unit of sperm development, testicular health has a significant impact on sperm quality and reproductive success, from pathological perturbations to epigenetic modifications. Figure is authors own (I.T.H).

Conclusion

The sentinel has historically been exploited as a model for biomonitoring environmental conditions in addition to health responses to toxic and infectious agents in other populations of species, including humans.[79] Common biomonitor species include ants,[1] birds,[80,81] sheep,[82] dogs,[14] and aquatic species (oysters, fish, and killer whales).[83-85] Many socio-economic interactions do not retain relevance in such species, although exception is given to species undergoing artificial reproductive techniques, which may still influence trends in reproductive health. Canids and felines share close environmental conditions with that of their owners, thus representing important models for bio-monitoring human health.[14] Although the precise drivers behind these reproductive temporal trends remain uncertain, there is increasing evidence that anthropogenic environmental change may be a key factor.[86] There is a distinct need to increase public understanding in order to promote a sustainable future. Although microorganisms are reported to be able to degrade toxic environmental compounds,[87] the use of the dog as a sentinel model, or additional sentinel models, would add substantial information in order to further or dispute previous research, and add to the weight of evidence that showcases how xenobiotics are perturbing the environment and future health of individuals. Only with further research can we work to drive change within a polluted environment.

Conflict of interest

There are no conflicts of interest to disclose.

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