

Environmental influences on fertility: can we learn lessons from studies of wildlife?

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Wildlife are good models for understanding the impacts of environmental contamination on fertility because [1] exposure doses are representative of actual contaminant mixtures and concentrations in the environment, [2] wildlife populations are more genetically diverse than laboratory animal populations, and [3] both individual and population level effects of fertility compromise can be observed. (Fertil Steril® 2008;89:e21–4. ©2008 by American Society for Reproductive Medicine.)

Since the publication of Rachel Carson's legendary book "Silent Spring" in 1962 (1), wildlife have been seen as important sentinels of ecosystem health. The perception by many, however, is that the major endpoint worth examining is mortality, and thus, declining population numbers. A close reading of Carson's book and many scientific studies that have followed illustrates a very different series of endpoints. By no means is mortality excluded, but altered population size also can be attributed to alterations in fertility and fecundity of the individuals that comprise the population under study.

In the early 1990s, a growing series of studies began to associate environmental contamination with altered reproductive performance in wild populations of fish, amphibians, reptiles, and birds (2). This literature documented the endocrine disruptive effects of a wide array of environmental pollutants, including pesticides, contaminants in sewage such as surfactants (e.g., octylphenol and nonylphenol) and pharmaceutical agents (e.g., ethynylestradiol from birth control pills), plasticizers (e.g., phthalates), flame retardants (PCBs, PBDEs), and industrial pollutants (e.g., heavy metals, dioxin, PAHs) [for reviews, see (3–7)]. Many of these compounds alter estrogen, androgen, or thyroid signaling, which are essential for normal embryonic development and reproductive activity in all vertebrates studied to date (4, 8, 9). As more studies are published, it is becoming clear that multiple endocrine signaling pathways are targeted by these chemicals, as well as neural and immune signaling pathways (10–12). Moreover, the mechanisms of action of these various signal-disrupting compounds are not limited to receptor interactions; studies have shown these compounds can alter hepatic biotransformation and clearance of hormones as well as alterations in hormone synthesis and storage on plasma proteins (5). Furthermore, disrupted regulation of gene expression (e.g., DNA methylation, RNA stability, protein degradation)

has been observed in studies of chemically exposed humans and animals [reviewed by (13)]. In some cases, altered DNA methylation patterns have been shown to be heritable (14, 15).

Determination and differentiation of the gonad in vertebrates, other than mammals, can involve either specific genes (genetic sex determination—GSD) or environmental factors such as temperature (environmental sex determination—ESD) (16). Those with ESD have been hypothesized to be more sensitive to disruption by environmental factors, because in many of the species studied, estrogens play a central role in the differentiation of the ovary. Exposure of reptilian embryos to endogenous (estradiol-17 β), pharmaceutical (ethynylestradiol, diethylstilbestrol), or contaminant (DDT, DDE, bisphenol-A, trans-nonachlor) estrogens, during a critical window of development, induces sex reversal at male incubation temperatures leading to highly skewed female sex ratios (17–20). Interestingly, embryonic exposure to concentrations of contaminants too low to cause sex reversal, alter steroidogenesis of the ovary or testis in neonates and juveniles (21). Altered steroidogenesis, and consequently altered gametogenesis, is a common feature observed after embryonic or neonatal exposure to contaminants [e.g., see (22–24)]. In fish and amphibians with GSD, sex reversal does not occur following exposure to endocrine active compounds; rather, one observes aberrant gonadal morphology. For example, in male rats and fish, the presence of oocytes in the testis or altered Leydig or Sertoli cell morphology or abundance has been reported following contaminant exposure early in life (23, 25).

CONTAMINANTS, WILDLIFE, AND FECUNDITY

A number of studies have examined fertility and fecundity in wildlife, and some of the best examples come from fish and alligators. Below we briefly review these studies relative to implications for human health.

Freshwater Fish

Fertility in fish is best measured by egg fertilization rates and offspring survival. The most widely studied chemical from a fish fertility perspective is ethynylestradiol (EE2). EE2 is

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the synthetic estrogen found in birth control pills, and is, consequently, an increasingly important contaminant in treated sewage effluent.

In a well-done series of studies, Jobling and colleagues (25, 26) have described the high incidence of intersex (feminized male phenotype) among wild roach (*Rutilus rutilus*) from rivers that receive treated sewage effluent. Compared with normal males, intersex fish exhibit malformations of the reproductive ducts, including duct occlusion, concomitant with lower released milt volumes and decreased sperm density and motility. Furthermore, intersex fish are less likely to successfully fertilize eggs and produce viable offspring. Fertilization success was 75% lower among severely feminized fish compared with unaffected males (26).

Intersex has also been observed in pearl dace (*Margariscus margarita*) taken from a lake that was experimentally treated with EE2 over a 3-year period (27). Mean concentrations of EE2 ranged from 4.5–8.1 ng/L during the 3 years. This range is considered environmentally relevant. In addition to intersex, Palace et al. (27) observed ovarian edema and poor testicular development among exposed fish, compared with fish captured from a reference lake. Young-of-the-year size classes were also less abundant in the EE2-treated lake, suggesting reduced reproductive success at the population level. Interestingly, lake trout (*Salvelinus namaycush*) taken from the EE2-treated lake did not exhibit significant reproductive abnormalities, indicating differential sensitivity to EE2 among species (28).

Experimental studies with low, environmentally relevant concentrations of EE2 have yielded results similar to the field studies described above. Male fathead minnows (*Pimephales promelas*) with lifetime exposure to 0.32 and 0.96 ng/L EE2 exhibited reduced ability to fertilize eggs (29). Similarly, sand gobies (*Pomatoschistus minutus*), exposed for 7 months to 6 ng/L EE2 or 0.3% or 0.03% v/v sewage effluent, exhibited impaired male maturation, decreased female fecundity, and low egg fertility. As a result, fertile egg production by the EE2-exposed population was reduced by 90% compared with controls (30). In a third study, exposure of breeding zebrafish and their offspring to 5 ng/L EE2 resulted in a 56% decline in fecundity and no fertilization success among the F1 generation. The male infertility resulted from developmental abnormalities in the gonads (31).

Other aquatic contaminants associated with reduced fertility in fish include tributyltin (antifouling agent used on boats), bisphenol A (plasticizer), tetrabromobisphenol A (TBBPA) (widely used flame retardant), and nitrate. Haubruge et al. (32) observed that adult male guppies (*Poecilia reticulata*) exposed for 21 days to 11.2–22.3 ng/L tributyltin or 274–549 µg/L bisphenol A in experimental aquaria had significant declines (40%–75%) in total sperm counts compared with controls. Kuiper et al. (33) observed significantly decreased egg production among female zebrafish (*Danio rerio*) exposed for 30 days to 0.047–1.5 µM TBBPA. This exposure level resulted in accumulated body concentrations

of 3.9 µg/g lipid weight and up. This is higher than concentrations measured in wild fish (63–583 ng/g lipid weight). However, testing lower concentrations of TBBPA is limited by detection capabilities, leaving open the possibility that lower exposures can also reduce zebrafish fertility. Hatching of TBBPA-exposed larvae was decreased at doses as low as 0.023 µM, and posthatching mortality was 81% among juveniles exposed to 1.5 µM TBBPA. Females were more likely to survive than males.

Finally, Edwards et al. (34) observed that female mosquitofish (*Gambusia holbrooki*) caught from two springs in north-central Florida with nitrate concentrations of 4–5 mg/L NO₃-N were significantly less likely to be pregnant during the reproductive season, and those that were pregnant had smaller offspring on average, compared with fish captured from six springs with lower nitrate concentrations (0.2–1.7 mg/L NO₃-N). The US EPA drinking water standard for nitrate is 10 mg/L NO₃-N.

Alligators

The American alligator is now common to many inland waters of the southeastern United States. Previous studies from our laboratory over the last decade and a half have shown that alligators living in central Florida (USA) lakes polluted with agricultural and storm water runoff exhibit a number of alterations of the reproductive and endocrine systems. Many of these modifications appear to be developmental defects that are detectable at hatching and persist throughout juvenile life stages. Lake Apopka is a large, eutrophic, and polluted lake northwest of Orlando, Florida. In 1980, it was the site of a spill of the pesticide dicofol, and has been the recipient of extensive agricultural pesticide and nutrient runoff during the last 40 years. In the 5 years following the pesticide spill, juvenile recruitment plummeted on Lake Apopka because of depressed clutch viability and juvenile mortality (35). The juvenile population remained depressed until the early 1990s [see (35, 36)]. Recruitment increased the juvenile population during the 1990s, although pre-1980 population levels have not been observed.

The rise in juvenile recruitment reflects the rise in clutch viability. Clutch viability, the number of eggs that hatch versus the number laid, remained near or below 20% from 1983–1991 at Lake Apopka (35). Other lakes in Florida averaged approximately 50% (37). This is still significantly lower than the 85% clutch viability rate observed at Lake Woodruff National Wildlife Refuge (Guillette et al., unpublished data). The Lake Woodruff National Wildlife Refuge receives little to no agricultural runoff. Thus, alligators from Lake Apopka, as well as many other freshwater lake systems in Florida, exhibit reduced clutch viability when compared with reference sites in the same broad watershed.

Although clutch viability rose to approximately 40% and juvenile recruitment increased during the 1990s on Lake Apopka, viability of clutches and neonates was still depressed compared with nonpolluted populations. In addition, a number

of sublethal problems continued to be reported (38). Examinations of the reproductive and endocrine systems of hatchling and juvenile alligators from Lake Apopka have demonstrated alterations in plasma estrogen, androgen, and thyroid hormone concentrations as well as morphologic abnormalities of the testis and ovary (described below) (39–41). The alterations in plasma hormone concentrations have persisted and have been observed repeatedly [for review, see (7)].

Alterations in plasma testosterone concentrations throughout early life would lead to altered anatomic structures dependent on this hormone, and we have reported that neonatal and juvenile male alligators had reduced phallus size (42, 43). Recent work on a group of boys from mother's exhibiting normal pregnancies reported that a reduction (feminization) of anogenital distance in these boys was related to elevated maternal prenatal urinary concentrations of four phthalate metabolites (monoethyl phthalate, mono-*n*-butyl phthalate, monobenzyl phthalate, and monoisobutyl phthalate) (44). Swan et al. (42) also reported that reduced anogenital distance was correlated with reduced penis volume and elevated incidence of cryptorchidism. Previous studies of phthalate- or pesticide-exposed rodents support the above observations of a syndrome of incomplete virilization in males. It is now apparent that numerous environmental contaminants have the potential to act as antiandrogenic compounds, altering androgen signaling during development in various vertebrate species, including humans (8).

In addition to the genital abnormalities described above, we have also observed at least one major organizational defect of the ovary that could explain the continued reduction in clutch viability. Exposure of the developing ovary to endocrine active compounds, especially those with estrogenic activity, alters folliculogenesis leading to a pathologic condition termed the multiocytic follicle [see (45)]. We observed such a condition in alligator neonates obtained from eggs collected from Lake Apopka (39). The same ovarian pathology was first reported in neonatal mice exposed to diethylstilbestrol developmentally (46–48). Experimental studies exposing neonatal mice to phytoestrogen genistein, have described similarly altered folliculogenesis (49, 50), as have studies in which the inhibin α subunit was overexpressed in the developing ovary (51). Multiocytic follicles are associated with infertility and early embryonic loss in diethylstilbestrol-treated mice (52), and we have hypothesized that this condition could be the underlying basis for reduced clutch viability in contaminant-exposed alligator populations. Current research is testing this hypothesis.

What induces the endocrine alterations and altered fertility described above? We have hypothesized that embryonic exposure to contaminants acting as endocrine disruptors, causes permanent organizational changes in the developing alligator, both male and female (53). If this hypothesis is to have a factual basis, embryos and subsequent neonatal and juvenile alligators must be exposed to contaminants that disrupt endocrine signaling at physiologically relevant concentrations. This prerequisite appears to be fulfilled, as we have

reported in a number of studies that many of the contaminants found in the embryonic or juvenile environment exhibit competitive binding to receptors and/or disrupt hormone synthesis or hepatic clearance at concentrations found in the environment (54–57).

In conclusion, as studies of the underlying mechanisms of infertility expand over the next decade, it is essential that environmental factors be considered as major players in this phenomenon. A growing literature has established a basis for concern, as various ubiquitous contaminants have been shown to depress, but not necessarily preclude, fertility in a wide array of vertebrate species. The concentrations of chemicals needed to induce such effects are well within the exposure levels experienced by some human populations. Moreover, exposure during embryonic and neonatal periods appears to be critical, as our understanding of the development of the reproductive system clearly demonstrates that potential adult fertility is affected by contaminant exposure events occurring early in life.

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