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Review

Toxicant effects on mammalian oocyte mitochondria†

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Abstract

Oocyte mitochondria are unique organelles that establish a founder population in primordial germ cells (PGCs). As the oocyte matures in the postnatal mammalian ovary during folliculogenesis it increases exponentially in volume, and the oocyte mitochondria population proliferates to about 100 000 mitochondria per healthy, mature murine oocyte. The health of the mature oocyte and subsequent embryo is highly dependent on the oocyte mitochondria. Mitochondria are especially sensitive to toxic insults, as they are a major source of reactive oxygen species (ROS), they contain their own DNA (mtDNA) that is unprotected by histone proteins, they contain the electron transport chain that uses electron donors, including oxygen, to generate ATP, and they are important sensors for overall cellular stress. Here we review the effects that toxic insults including chemotherapeutics, toxic metals, plasticizers, pesticides, polycyclic aromatic hydrocarbons (PAHs), and ionizing radiation can have on oocyte mitochondria. This is very clearly a burgeoning field, as our understanding of oocyte mitochondria and metabolism is still relatively new, and we contend much more research is needed to understand the detrimental impacts of exposure to toxicants on oocyte mitochondria. Developing this field further can benefit our understanding of assisted reproductive technologies and the developmental origins of health and disease (DOHaD).

Summary sentence Oocyte mitochondria are unique organelles, which are sensitive targets to various toxicants.

Key words: oocyte, mitochondria, pesticides, metals, plasticizers, PAHs, ionizing radiation, chemotherapy.

Introduction

Folliculogenesis and Oocyte Maturation

Mammalian oocytes begin developing in the female fetus as primordial germ cells (PGCs). In the mouse embryo, PGCs arise on embryonic day 6.5 (E6.5), begin to proliferate rapidly and migrate to the developing gonadal ridge, arriving around E10.5. Now oogonia, they continue to proliferate, forming germ cell nests due to incomplete cytokinesis [1], and progressively enter meiosis starting on E13.5, at which point they become oocytes. Eventually, primary oocytes arrest in the diplotene stage of prophase I of meiosis I [2–4]. In the mouse, germ cell nests start reorganizing into primordial follicles prior to birth, beginning around E17.5 [5]. In the postnatal ovary, follicles are the functional unit containing the maturing oocyte and its supportive cells. During human fetal development, PGCs begin migrating by weeks 4 and 5, by the end of week 25 approximately 7 million oogonia populate the fetal ovary [6], and follicle assembly

begins during midgestation and continues into the third trimester [7]. Follicles are characterized by maturational stage with primordial follicles constituting the ovarian reserve, established before or around the time of birth in the majority of mammals [3, 4].

As follicles grow, the oocyte increases in volume by more than 100-fold, stockpiling maternal mRNAs, proteins, metabolic substrates, and organelles to support fertilization and preimplantation development [8]. This is referred to as cytoplasmic maturation, which in turn is required to support nuclear maturation. The mid-cycle luteinizing hormone (LH) and follicle-stimulating hormone (FSH) surges initiate ovulation and meiosis resumption in preovulatory follicles. The germinal vesicle (oocyte nucleus) is broken down, chromosomes align, guided by the meiotic spindle, and the first meiotic division occurs, extruding the first polar body; this successful cellular division constitutes nuclear maturation [2, 8]. All of these events outlined above from onset, resumption, and completion of meiosis, through preimplantation development require energy, which is derived from supportive granulosa and cumulus cells, or directly from the oocyte mitochondria [2, 9, 10].

Oocyte Mitochondria

Mitochondria are integral, highly specialized organelles responsible for energy production, cell signaling, cellular homeostasis, gene regulation, induction of cellular arrest, and apoptosis. Mitochondria contain the electron transport chain, located on the inner mitochondrial membrane, which generates an electrochemical gradient, referred to as mitochondrial membrane potential ($\Delta\Psi m$), that is used to drive ATP synthesis [11–15].

Mitochondria are the most abundant organelle in the mature oocyte. PGCs contain a founder population of only 10 mitochondria; as the oocyte matures this population expands to roughly 100 000 mitochondria per healthy mouse oocyte [2, 15–17] and 300 000–400 000 mitochondria per healthy human oocyte [2] at the time of ovulation. During folliculogenesis the oocyte depends on supportive granulosa and cumulus cells. Once ovulated, the gap junctions between the oocyte and the cumulus cells break down, leaving the oocyte dependent on its own metabolic reserves to sustain and support preimplantation development [2, 8, 18, 19]. Consequently, any deficiencies in mitochondria or their function more readily affect the oocyte after ovulation and are thought to be a major cause of female infertility and chromosomally abnormal conception [16].

Possessing few cristae and relatively quiescent in nature, oocyte mitochondria differ from somatic cell mitochondria [18, 20]. However, mitochondria are still the primary source of energy in the oocyte, with oxidation of pyruvate being the most utilized method of ATP generation during folliculogenesis and fatty acid β-oxidation being important after ovulation [14, 18, 21–25]. During folliculogenesis oocyte mitochondria are homogenously distributed. Upon germinal vesicle breakdown mitochondria localize around the meiotic spindle, presumably providing energy for extrusion of the first polar body. Once meiosis I is successfully completed, mitochondria reorganize with highly polarized mitochondria in the subcortical regions and a cloud of less polarized mitochondria around the meiotic spindle [18, 26, 27]. However, the latter conclusion that highly polarized mitochondria are localized in the subcortical region within the MII oocyte has recently been called into question as due to experimental artifacts [28].

It is widely accepted that the health and quality of an oocyte are highly dependent on the health, number, and quality of oocyte mitochondria [18, 29–32]. Mitochondrial health or quality can be defined through a myriad of parameters including, but not limited to, mitochondrial membrane potential ($\Delta\Psi m$), reactive oxygen species (ROS) production, mtDNA copy number, as well as ATP/ADP ratio [20, 23, 33, 34]. ROS are created by addition of electrons to molecular oxygen, yielding superoxide anion radical (SO), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH\bullet$). Consequently, mitochondria are the major producers of ROS in the cell via oxidative phosphorylation. It is important to note that ROS at low physiological levels are necessary cellular signaling molecules. Under conditions of oxidative stress, when ROS increase, cellular redox circuits are disrupted, perturbing cellular homeostasis and damaging cellular macromolecules [35–37].

Mitochondria in somatic cells have long been seen as sensitive targets of environmental toxicants with evidence of synthetic and natural compounds exerting their toxicity by affecting mtDNA integrity, inhibiting proteins along the electron transport chain, modifying $\Delta\Psi m$, and activating pro-apoptosis pathways [38–40]. More recently, oocyte mitochondria have been garnering attention as targets for reproductive toxicants. This review aims to summarize the current knowledge of how toxic insults specifically damage oocyte mitochondria, thus impacting oocyte quality, which could ultimately have broader health implications.

General Toxicant Metabolism and Cellular Responses

Most toxicants undergo metabolism upon uptake into a eukaryotic cell; a plurality of toxicants must undergo metabolism to undergo excretion, and in these cases, it is often the metabolite which exerts the negative, toxic effects on the cell [41–43]. In general, most toxicants undergo an oxidizing reaction (phase I metabolism) with the assistance of a myriad of oxidizing enzymes such as cytochrome P450s or catalase. Following oxidation, the now reactive metabolite must be made more water soluble to be successfully excreted; this is accomplished by phase II metabolism reactions, which include conjugation by glucuronidation, glutathione conjugation, acetylation, sulfonylation, and acylation [41–43].

Antioxidants play important roles in combating oxidative stress. GSH is a critically important antioxidant to reproductive health [36, 44–48]. It is an endogenous tri-peptide thiol that acts to clear ROS and reactive metabolites through several mechanisms—direct reduction of ROS-like hydroxyl radical, reduction with the help of glutathione peroxidases [36, 49] or glutathione transferase mediated GSH conjugation with reactive metabolites [42]. Other important endogenous antioxidants include melatonin [50, 51] and coenzyme Q10 [52]. Melatonin possesses an electron-rich aromatic indole ring making it a potent electron donor capable of chelating transition metals; further, it can activate melatonin receptors, upregulating antioxidant defense systems such as glutathione peroxidases or superoxide dismutases [50, 51]. Coenzyme Q10 is found in the plasma membrane and plays an important role in the electron transport chain acting as an electron carrier [52]. The reduced form of coenzyme Q10 is a likely antioxidant [52–56], although whether exogenous or endogenous coenzyme Q10 is a more effective antioxidant is up for debate [52].

When cellular stress response mechanisms are overloaded by toxicant exposure and irreparably damaged organelles, the cell will often begin to undergo mitochondria-mediated apoptosis [57]. Under conditions of cytotoxic stress, cytosolic Bax localizes to the mitochondrial outer membrane and becomes activated by interaction with BH3-only proteins [58], eventually creating pores through which cytochrome C is released into the cytosol [59]. Cytochrome C release

triggers the oligomerization of Apaf-1 [13], which then recruits and activates procaspase-9 to cleaved caspase-9 eventually leading to downstream activation of procaspase-3 to cleaved caspase-3, the effector caspase, at which point, the cell's fate is sealed [13, 60]. However, before a cell reaches that point, it may utilize autophagy [61, 62], a crucial process for the degradation of dysfunctional organelles, proteins, or other macromolecules, which has been shown to be an important player in primordial follicle assembly [3, 63], follicular response to toxicants [64], and germ cell death [65, 66].

Toxicants

Chemotherapeutics

Increased risk of premature ovarian failure for females of reproductive age and younger is a well-documented phenomenon and devastating tradeoff resulting from cancer treatment [67–72]. Cyclophosphamide (CPA) is a common chemotherapeutic drug of the alkylating agent class that undergoes metabolic activation by CYP2B1 and CYP3A4 to 4-hydroxycyclophosphamide and subsequently is converted to aldophosphamide, which is metabolized to phosphoramide mustard and acrolein [43]. Ovarian follicles are a primary target of CPA toxicity [73, 74]. Interestingly, intraperitoneal injection of rats with 300 mg/kg of CPA led to the induction of mitochondrial apoptosis in granulosa cells of secondary and antral follicles [36] and a significant decrease in ovarian GSH levels [75]. Apoptotic destruction of primordial follicle oocytes by CPA in mice was recently shown to require the BH3-only BCL-2 family protein PUMA [74]. These data suggest a crucial role for mitochondria in CPA-induced premature ovarian failure. This further begs the question, what are the effects of chemotherapeutics on oocyte mitochondria.

This question is only recently gaining interest from a few groups. Acrolein, a derivative of CPA and another chemotherapeutic, tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine 2-oxide (CTX), is a major source of ROS generation. Two studies by Jeelani et al. [76] explored the sensitivity of mature oocytes to the chemotherapeutic drug CTX, or its derivative, acrolein. In one study, denuded MII oocytes and cumulus-oocyte-complexes (COCs) were exposed to CTX and acrolein separately, at both 10 and 25 μM for 45 min. Both high concentrations of CTX and acrolein significantly decreased $\Delta\Psi m$ of exposed oocytes compared with controls, and acrolein caused activation of caspase 3/7 (CPA was not tested for caspase activation). In the other study, they observed an increase in ROS in acrolein exposed oocytes, but not CPA-exposed oocytes, compared with controls [77], suggesting that acrolein induces oxidative stress in the oocyte, by way of the mitochondria, ultimately inducing oocyte apoptosis. This demonstrates that the oocyte toxicity is caused by reactive metabolites of these chemotherapeutics, a well-characterized phenomenon for many toxicants.

Even more compelling evidence for mitochondrial toxicity of anticancer drugs comes from studies of nitrogen mustard, a chemical warfare agent from which the nitrogen mustard class of chemotherapeutic drugs was derived. After exposure for 48 h of adult mice to 0.1–1.6 mg/kg nitrogen mustard by intraperitoneal (i.p.) injection, oocytes had increased mitochondrial SO production and decreased mitochondrial volume. According to the authors, mitochondrial volume was calculated from the mitochondrial circumference measured using 3-D electron microscopy, although the exact calculation was not defined. Additionally, the remaining mitochondria were observed to possess fewer cristae and were surrounded by more "cellular debris" when visualized by electron microscopy [78]. Doxorubicin, another common chemotherapeutic agent, appears to largely act by poisoning the mitochondria via redox cycling resulting in ROS generation, ultimately causing mitochondria-mediated apoptosis [72]. While not exactly surprising, these data together suggest a major blind spot in the treatment and care for cancer patients moving forward, as chemotherapeutics have often been shown to exhibit "off-target" mitochondrial effects in somatic cells. Given the vulnerable nature of oocyte mitochondria more exploration needs to be pursued into understanding how chemotherapeutics alter oocyte mitochondria and metabolism.

Toxic Metals

Humans are exposed to toxic metals through various routes from drinking water to particulate matter in air pollution. In drinking water alone, humans are exposed to lead, chromium, arsenic, and cadmium [79]. Metal toxicity is of great concern regarding public health; however, very little attention has been paid to metal toxicity and female reproductive biology, let alone the effects on oocytes directly.

Arsenic is one of the most common metals and can form both organic and inorganic compounds in the environment and the human body. Although this is highly dependent on location, the most common sources of arsenic exposure for humans are drinking water and foods [80]. Arsenate (As(V)) and arsenite (As(III)) are the predominant oxidation states. These inorganic arsenics can be either methylated or demethylated, and those that contain arsenite are more toxic than those containing arsenate [81]. Arsenic-mediated generation of ROS includes the generation of SO, singlet oxygen, the peroxyl radical, nitric oxide, hydrogen peroxide, and others. Under physiological conditions, arsenite is oxidized to arsenate in the presence of water and oxygen to yield hydrogen peroxide via a spontaneous reaction [81].

Cultured CD-1 mouse zygotes exposed to 0–8 μg/ml arsenite for 2–96 h displayed compromised cleavage rates in a concentration and time-dependent manner [82]. The same group further demonstrated that exposure of zygotes to 8 μg/ml significantly decreased $\Delta\Psi m$ at 1 h of exposure, and ROS significantly increased compared to controls at 2 h of exposure. These effects were rescued by cotreatment with the antioxidant and glutathione precursor N-acetylcysteine [82]. These data provide strong support that arsenite toxicity in zygotes is caused by oxidative stress, mediated through mitochondrial dysfunction. The same group exposed CD-1 females to 8 mg/kg of arsenite, the accepted maternal NOAEL, or 16 mg/kg via i.p. injections every other day for 14 days [83]. MII oocytes harvested from treated mice displayed a 25% and 62.5% incidence, respectively, of meiotic spindle abnormalities, compared with controls, which had none. Further, zygotes derived from arsenite-exposed animals exhibited dose-dependent and significantly lower cleavage and development rates in vitro compared with controls [83]. While not directly related to mitochondria, the formation of the meiotic spindle and zygotic cleavage rates are energy intensive processes requiring large amounts of ATP [10, 84]. These data suggest that metals, and specifically arsenite, have detrimental effects on oocyte mitochondria. This is not surprising, considering cationic metals have been shown to preferentially accumulate in the mitochondria [39], and arsenite specifically is known to uncouple mitochondria, therefore reducing ATP production and increasing ROS production [85].

Cadmium is a heavy metal, and each year roughly 13 000 tons of cadmium are produced for nickel-cadmium batteries, pigments, chemical stabilizers, metal coatings and alloys [81]. The main route of exposure for the general population is through ingestion, as cadmium readily accumulates in plants from the soil. Inhalation is a primary route of exposure in occupational settings and is a secondary route of exposure for the general population. Cadmium alone is unable to generate free radicals, but it is hypothesized that cadmium can replace iron and copper within cytoplasmic or membrane-bound proteins, thus increasing the amount of unbound copper and iron ions in the cell that can generate OH• via Fenton reactions [81].

Zhu et al. [86] demonstrated that MII oocytes of females treated with 32 mg/L of cadmium in drinking water for 35 days had decreased ATP content and aberrant mitochondrial distribution compared with controls. In a more recent study, cadmium exposure was observed to disrupt meiotic spindle assembly and chromosome alignment, two ATP-dependent processes [87]. Further, exposure increased ROS and led to abnormal mitochondrial distribution within the oocytes. Additionally, oocytes were observed to have decreased levels of the epigenetic modifications 5-methylcytosine, Histone 3, lysine 9 trimethylation (H3K9me3), and Histone 3, lysine 9 acetylation (H3K9ac) following acute cadmium exposure, processes that are considered to be, at least in part, dependent on mitochondria [87]. These findings were later corroborated by Dong et al. [88], who exposed female mice to 0.5 mg/kg/day of cadmium chloride for 60 days and observed that oocytes from exposed females had increased incidence of disrupted spindle assembly and chromosome misalignment, as well as increased ROS. These limited data aggregated provide convincing evidence that arsenic and cadmium toxicity can impact oocytes and the mitochondria. However, there are limited data to understand the full effects of various toxic metals on the oocytes, let alone the offspring.

Pesticides

Many pesticidal compounds have been understood for decades to be reproductive toxicants. Methoxychlor (MXC) is an effective organochlorine insecticide that was commonly used on agricultural crops and livestock, in animal feed, barns, and grain storage prior to 2003 [89]. In vivo treatment of mice with greater than 32 mg/kg of MXC for 20 days was shown to induce follicular atresia selectively in antral follicles only [90], and MXC-induced atresia in cultured antral follicles, which was rescued by the glutathione precursor and antioxidant *N*-acetyl cysteine [91]. Even more interesting, mitochondria were isolated from whole CD-1 mouse ovaries and exposed in vitro to MXC or vehicle control and their respiration was measured directly. ADP-stimulated State 3 respiration, also known as ADP-stimulated respiration, was decreased by MXC exposure, and resting State 4 respiration, driven primarily by proton leak, was increased, resulting in decreased respiratory control ratio (RCR, ratio of State 3 to State 4) [91]. Additionally, adult, cycling, CD-1 female mice were either dosed with 20 mg/kg/day of MXC or vehicle control for 20 days. Following exposure, ovarian mitochondria were isolated, and their respiration was measured. Similar to the in vitro results, State 3 respiration and RCR were decreased, and State 4 respiration was increased [91, 92] (Please refer to Brand and Nicholls [93] for background on mitochondrial respiration). In vivo MXC treatment also increased hydrogen peroxide production by mitochondria isolated from treated ovaries and increased oxidative DNA and protein damage in whole ovaries [91].

Another common insecticide and piscicide, rotenone, has been shown to have detrimental effects on female reproduction. Exposure of female E11.5 gonads to 0.1 μM of rotenone in vitro resulted in a decreased proportion of germ cells initiating meiosis [94]. Additionally, rotenone treatment increased ROS generation and decreased ATP levels in exposed gonads [94]. Interestingly these effects were partially rescued with the administration of Coenzyme Q10, suggesting that the electron transport chain is imperative for meiotic initiation of developing oocytes [94]. In summary, the pesticides MXC and rotenone induce ovarian oxidative damage [91, 94], Coenzyme Q10 administration can rescue these effects of rotenone [94], and N-acetylcysteine can rescue the effects of MXC [91]. Together, these data suggest that the mitochondria could be a primary target of the pesticides.

Despite the many indications of mitochondrial sensitivity to pesticides, little has been done to explore the effect of pesticide exposure on oocyte mitochondria. As discussed above, whole ovary exposure observations of decreased mitochondrial function and increased oxidative stress [90, 91, 94] suggest that oocyte mitochondria may also be an important target. In one recent study, oocytes treated in vitro with 50 and 100 μM of MXC showed increased ROS formation, lipid peroxidation, increased incidence of aberrant mitochondrial distribution, and reduced $\Delta\Psi m$ compared with controls [95]. Methyl parathion is an organophosphate insecticide. Nair et al. [96] orally dosed female Swiss albino mice with a single dose of 5, 10, or 20 mg/kg methyl parathion; females were superovulated 1 week later. Exposed oocytes were observed to have increased meiotic spindle abnormalities, as well as decreased glutathione levels compared with controls [96]. These results indicate a role for mitochondria in the oocyte cellular response to pesticide exposure. ATP is necessary for proper spindle alignment, and glutathione is an important antioxidant found at very high concentrations in oocytes. This same group later found that methyl parathion led to aberrant mitochondria distribution patterns and increased indicators of oxidative and endoplasmic reticulum stress, effects that were rescued by cotreatment with the free radical scavenger, epigallocatechin-3-gallate [97].

A common herbicide, glyphosate, has also been shown to have detrimental effects on oocyte mitochondria [98]. Germinal vesicle stage oocytes were exposed in vitro to 500 μM glyphosate. After 14 h of exposure oocytes displayed increased ROS, abnormal spindle morphology, aggregated mitochondrial distribution, decreased $\Delta\Psi m$, and decreased expression of autophagy proteins [98]. While this exposure level is far higher than relevant human exposure levels to glyphosate [99], these data together suggest detrimental effects on mitochondria and that glyphosate hinders the ability of the oocyte to effectively clear and recycle damaged organelles. Altogether, there is a growing body of evidence that presents a compelling argument for further exploration of the effects of pesticides on oocyte metabolism, not only in the directly exposed organism, but also in subsequent generations.

Plasticizers

Plasticizers are relatively nonvolatile organic compounds which when incorporated into a material increase a polymer's flexibility, toughness, and ductility. The most common class of plasticizers humans are exposed to are phthalate esters, which are used in the manufacturing of products made from polyvinyl chlorides such as upholstery, flooring, food containers, and water bottles [100]. Phthalate esters are endocrine disruptors exhibiting estrogenic effects

on females, and the detrimental reproductive effects of phthalate exposure on females have been well documented [101–106]. In a compelling study, maternal di-(2-ethylhexyl) phthalate (DEHP) exposure (0, 0.05, 5 mg/kg/day) led to multigenerational and transgenerational reproductive abnormalities in females [101]. F1, F2, and F3 female offspring of exposed dams had reduced primordial follicle numbers, increased antral follicle numbers, and decreased blastocyst cleavage rates at the 0.05 mg/kg/day dose compared with controls [101].

More recently DEHP effects on oocyte mitochondria have been shown to have impacts far beyond the ovary. Oocytes derived from female mice treated with 40 µg/kg DEHP in corn oil for 14 days demonstrated increased ROS levels, decreased ATP levels, and decreased mitochondrial content, ultimately leading to meiotic defects in exposed oocytes compared with oocytes derived from females fed corn oil alone [107]. Interestingly, the detrimental effects of phthalate exposure seem to persist in embryos derived from the oocytes. Seven-day blastocysts derived from COCs exposed to MEHP (20–1000 nM) and estradiol (0–2000 ng/ml) in vitro displayed impaired transcription of three genes belonging to the electron transport chain, *Cyc1, Mt-co1,* and *Atp5b* (Cytochrome C1, Mitochondrially Encoded Cytochrome C Oxidase 1, and ATP Synthase F1 Subunit beta). MEHP treatment alone also led to mild decreases in *Cyc1* and *Atp5b* transcript levels in 7-day blastocysts [108].

Other plasticizers besides phthalates have been shown to be detrimental to oocyte mitochondria. Bisphenol A (BPA) treatment of MII oocytes (75 µm) for 22 h in vitro resulted in a significant increase in intracellular ROS generation, including mitochondrial SO production [109]. Further, this study demonstrated that treatment of MII oocytes decreased $\Delta\Psi m$ and increased BAX expression, suggesting activation of the mitochondrial apoptosis pathway [109]. Similar studies have yielded corroborating evidence. GV oocytes exposed for 12 h in vitro to 100 µM of the BPA substitute bisphenol AF had significantly increased vSO generation and increased SOD2 expression compared with controls [110]. Treatment of MI oocytes in vitro with 50–150 µM of the BPA substitute fluorene-9-bisphenol for 12 h resulted in a dose-dependent depletion of ATP, with a statistically significant depletion following exposure to 100 µM for 2 and 12 h and virtually no detection of ATP in oocytes treated with 150 µM after 12 h [111]. Additionally, exposure to 100 µM of fluorene-9-bisphenol led to an increase in cellular ROS and reduced mtDNA levels, often considered a proxy for mitochondrial load, in exposed oocytes [111]. Together these data suggest that exposures to phthalate and bisphenol plasticizers not only deplete the ovary of the precious ovarian reserve, but also damage the oocytes that survive exposure, and this damage is largely at the mitochondrial level. However, much more exploration of this topic into all commercially used plasticizers is necessary.

Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are the products of incomplete combustion of organic materials and are found in air pollution, cigarette, and cannabis smoke, as well as burnt and barbequed foods. Many PAHs are known carcinogens and reproductive toxicants [112]. PAHs require metabolic activation, mostly undergoing oxidation by prostaglandin endoperoxide synthase, epoxide hydrolase, or microsomal cytochrome P450s to produce metabolites that interact with cellular macromolecules which, in most cases, are thus oxidized [41, 113]. It has been well established that PAHs destroy ovarian follicles of all stages and corpora lutea, thus leading to premature ovarian failure in the females exposed [114].

Female mice exposed to a single dose of 80 mg/kg of the PAHs, benzo (a) pyrene (BaP), 7,12-dimethylbenz (a) anthracene (DMBA), or 3-methylcholanthrene (3MC) via i.p. injection, 40 h prior to sacrifice, displayed a 50% decrease in primordial follicles compared with controls, as early as 1 day after the injection of DMBA. Half the primordial oocytes were destroyed in 3MC and BaP exposed mice by 2 to 3 days after exposure [115]. In a later study, Mattison [116] demonstrated that BaP and 3MC acutely induced pyknotic degeneration of primordial follicle oocytes without apparent effects on later stages of follicle development, while DMBA induced pyknotic degeneration of oocytes and granulosa cells in growing follicles in addition to primordial oocytes. We have demonstrated that cultured preovulatory rat follicles exposed to DMBA underwent concentration and time-dependent granulosa and theca cell apoptosis, preceded by an increase in ROS following exposure [117]. Interestingly, preantral mouse follicles cultured in increasing concentrations of 1.5–45 ng/ml of BaP in vitro for 13 days displayed delayed antral follicle development and decreased follicular survival at the highest concentration compared with unexposed controls [118]. Further, more recently we observed that adult female mice exposed via oral dosing to their mothers with 2 or 10 mg/kg/day of BaP for 10 days in utero had decreased follicular counts at all stages, and mice genetically deficient in the antioxidant glutathione were more sensitive to these detrimental effects of prenatal exposure to BaP [119]. All these data indicate that PAH exposure is detrimental to the ovary. The evidence of increased ROS and the protective effects of glutathione, an antioxidant, suggest these deleterious effects may be, at least in part, due to mitochondrial dysfunction.

There is accumulating evidence that exposure to PAHs could result in persistent damage to the oocyte, and more specifically the oocyte mitochondria, thus inhibiting preimplantation development [120–122]. The high lipid content of mitochondria facilitates the accumulation of lipophilic compounds such as PAHs [123]. One of the primary metabolism pathways for PAHs is oxidative metabolism through cytochrome P450s, of which some isoforms are located on the inner mitochondrial membrane, yielding reactive metabolites which then react with other macromolecules generating ROS [35, 36, 41, 124–126]. This was demonstrated by Sobinoff et al. [120], who administered 0, 1.5, or 3 mg/kg/day of BaP for seven consecutive days by i.p. injection of PND4 female Swiss neonatal mice. At 6 weeks of age, oocytes were observed to have a BaP dose-dependent increase in mitochondrial SO production and increased lipid peroxidation levels, compared with controls. These results are consistent with persistent oxidized environment, initiated by the BaP exposure. In a later study, 4–6-week-old female ICR mice were orally dosed with 0, 10, 20, or 40 mg/kg/day of BaP in corn oil for 10 days. Oocytes derived from mice exposed to 40 mg/kg/day had increased levels of ROS compared with controls [121].

Moreover, mouse zygotes exposed in vitro to 5 and 50 nM BaP, for up to 96 h, demonstrated a concentration-dependent increase in ROS production and a significant reduction in blastocyst formation [122]. These findings support a recent study from Sui et al. [127], which observed that maternal exposure of 40 mg/kg/day BaP for 10 days preconception decreased polar body extrusion and increased incidence of aberrant meiotic spindle assembly in F1 oocytes. In this same study, oocytes from maternally exposed F1 females had decreased mitochondria content, ATP production, and decreased ROS generation although it is not clear whether the unit of statistical analysis was the F0 mother, F1 female, or the number of

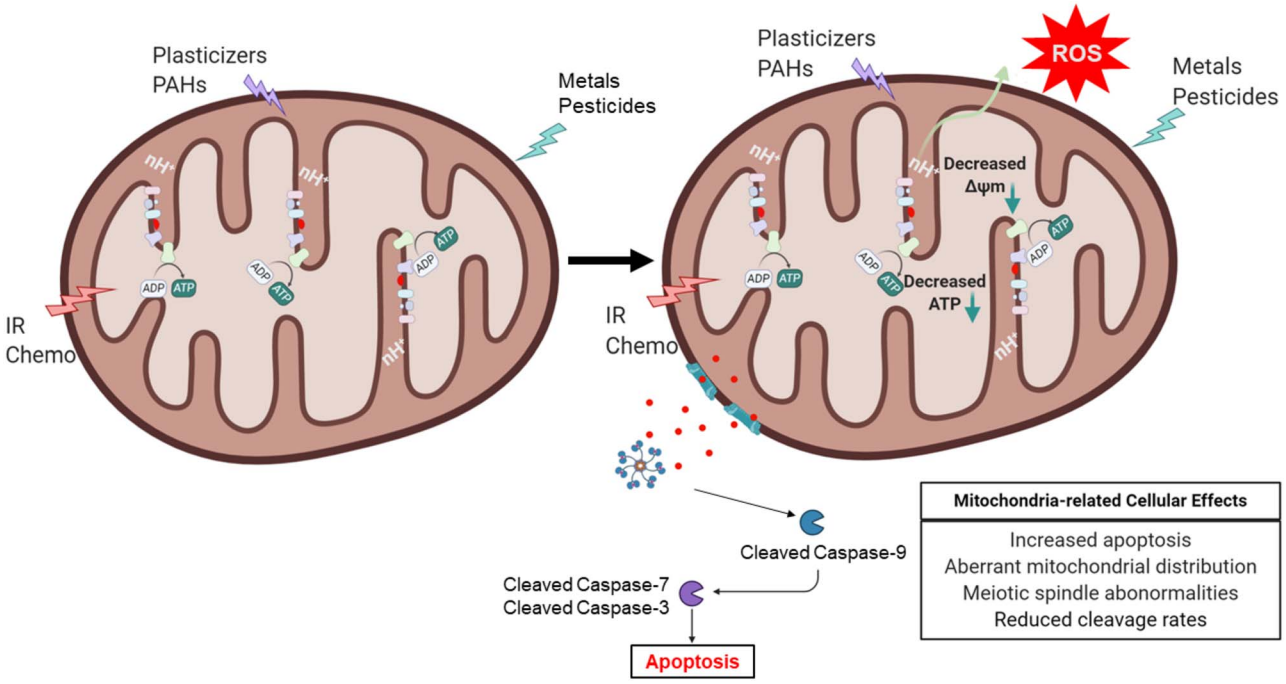

Figure 1. Overview of effects of chemotherapeutics, plasticizers, IR, PAHs, metals, and pesticides on oocyte mitochondria and the observed cellular effects related to mitochondria dysfunction. Electron transport is depicted on cristae, Bax/Bak pores depicted as blue channels, cytochrome C depicted as red circles.

oocytes [127]. These data together suggest that BaP, a model PAH, is metabolized in the oocyte making it more oxidized by increasing lipid peroxidation and disrupting mitochondrial function. However, much more work is needed to understand how various PAHs impact the mitochondria in the oocyte.

Ionizing Radiation

Ionizing radiation is energy emitted from a source strong enough to strip an electron from an atom, causing that atom to become charged, or ionized [128]. Ionizing radiation (IR) exists as electromagnetic or particulate energy. Electromagnetic radiation includes X- and gamma-rays, while particulate radiation is made up of energetic protons, neutrons, alpha particles, and heavy charged particles [35]. IR exposure of cells can directly disrupt the atomic structures of cells or damage cellular structures through radiolysis of water producing reactive oxygen and nitrogen species resulting in damage to proteins, nucleic acids, lipids, and other macromolecules in the cell [35].

Our work [129, 130] and that of others [131, 132] has cumulatively demonstrated that IR increases cellular stress in the ovary and oocyte through the increased production of ROS. We have shown that these increased ROS levels lead to more heavily oxidized and apoptotic cells in ovaries and overall fewer follicles [129, 130]. We observed that ovaries of female mice exposed to 0–50 cGy charged oxygen particles in vivo had dose-dependent increased incidence of DNA double-strand breaks, lipid peroxidation, and pro-apoptotic protein PUMA expression in both the oocytes and the granulosa cells of follicles [129]. These deleterious effects resulted in dose-dependent decreases in primordial, primary, and secondary follicles of exposed females. We observed similar results in females exposed to 0–50 cGy charged iron particles [130]. Females exposed to 30 or 50 cGy charged iron particles had statistically significant decreases in follicles at all stages of development at 1 and 8 weeks after IR exposure. Further, these decreases preceded by increased DNA double strand breaks, oxidative lipid damage, and protein nitration in the oocytes of primordial, primary, and secondary follicles [130]. Interestingly, the early effects of IR exposure in this study were mitigated by a chow diet supplemented with 150 mg/kg of alpha-lipoic acid, but ultimately this antioxidant was not effective in abrogating the persistent oxidative stress caused by IR 8 weeks after irradiation [130]. Together these data suggest at least the initial deleterious effects of IR exposure in the ovary are mediated, at least in part, through the mitochondria, however much more exploration is needed.

Interestingly, treatment with 100 µg of melatonin, an antioxidant, was significantly effective in protecting against gamma-radiation-induced primordial follicle loss, suggesting that radiation-induced depletion of primordial follicles could be attributed to ROS excess [133]. This supports that the deleterious reproductive effects of IR are mediated, in large part, through oxidative stress. Given the mitochondria consume 90% of the body's oxygen and can take up to 25% of the mass of the cell, these organelles are a likely target for radiation. In fact, radiation exposure initiates increased electron leakage from the basal level of the electron transport chain, with additional effects observed including perturbations in $\Delta\Psi m$, mitochondrial protein import, and oxidative damage to mitochondrial proteins [35].

In recent years there has been a growing interest in IR effects on oocytes directly and their mitochondria, though the exploration remains shallow at best. In a recent study, oocytes collected from prepubertal females exposed to 0.1 cGy of gamma radiation were analyzed for radiation-induced mitochondrial damage at 3, 6, and 24 h postirradiation. No differences were observed between control and irradiated oocytes when characterizing mitochondrial number and distribution patterns. However, $\Delta\Psi m$ was lost in oocytes at 3 and 6 h postirradation, but, interestingly, no differences were observed at 24 h between the two treatment groups, and ATP levels

were found to be the same across treatments [134]. More exploration of this topic needs to be pursued to determine if any discernable effects from IR persist in the mature oocyte following treatment, as the oocytes nearest to ovulation seem to be the most sensitive to radiation-induced cellular stress [135].

Implications for Fertility and Human Health

It is well established that oocyte mitochondria have a crucial impact on oocyte quality [2, 14, 16, 20, 136]. Proximally, oocyte mitochondria are of great interest in assisted reproductive technologies as their dysfunction has been implicated in reduced female fertility with aging and obesity [31, 137–139], characterized by decreased ATP production [140], compromised mtDNA integrity [26], $\Delta\Psi m$ [141], mitochondrial load [142], and redox imbalance [14]. Further, oocyte mitochondria have been suggested to be important mediators of the developmental origins of disease, as all the mitochondria in the oocyte and subsequent embryo are derived from a bottleneck of merely 10 maternal mitochondria beginning at the onset of PGC development [2, 31].

Of interest to this review is exposure to environmental toxicants and the subsequent impact on oocyte mitochondria. We have observed that many of the same mitochondrial dysfunctions documented in aging and obesity, which reduce female fertility, were documented following exposure to chemotherapeutics [76, 78], toxic metals [83, 88], pesticides [97, 98], plasticizers [108, 111], PAHs [121, 122], and ionizing radiation [134] (Summarized in Figure 1). When extrapolated further, maternal exposure to environmental toxicants and other stressors over the course of her life could irreparably damage or alter oocyte mitochondria which are then directly inherited by her offspring. In fact, a recent study has demonstrated that maternal preconception exposure to BaP significantly altered the quality of the oocyte mitochondria, and consequently, the oocyte [127]. While it is currently unknown if this mitochondrial dysfunction would persist once the oocyte is fertilized and beyond, this demonstrates the urgent need for more exploration. Persistent mitochondrial dysfunction could have wide-ranging effects as mitochondria are important organelles for many other cellular functions outside of metabolism, such as cell signaling and participating in regulation of the preimplantation epigenome [143].

Mitochondria play an indispensable role in the oocyte prior to and during development. Perhaps even more important is the role that they play in determining an individual's health later in life, as well as the health of their offspring. While this is still a relatively young field with more exploration needed on all fronts, we contend that more care and attention need to be paid to address environmental exposures, their proximal effects on oocyte mitochondria, their ultimate multigenerational and transgenerational effects on the developmental origins of health and disease, and reproduction for generations to come.

Conflicts of interest

The authors declare that they have no conflicts of interest to disclose.

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