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Prenatal toxicant exposure and ovarian aging and cancer

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Overview

- Understand the critical events during ovarian development and their timing.
- Review the experimental and epidemiological evidence that prenatal exposure to environmental toxicants can lead to accelerated ovarian aging and ovarian tumors later in life.
- Understand what is known about mechanisms by which prenatal exposures to toxicants cause ovarian failure and ovarian tumors.
Critical Windows of Ovarian Development

GW: gestational week. Adapted from Pepling, 2006, Genesis 44:622-632; Pepling et al, Reproduction, 2010
Ovarian follicular development

- Primordial
- Primary
- Secondary
- Antral
- Mature/Preovulatory

Ovarian surface epithelium

Corpora Lutea

Ovulation

Ovulated Oocyte
Age-Related Decline in Human Follicles

Germ Cell/Follicle Number

Age (yrs)

Birth
Optimal Fertility
Decreased Fertility
Premature Ovarian Failure
Irregular Cycles

E.R. te Velde et al, 1998
Age-related decline in oocyte quality

- Aneuploidy
- Telomere shortening
- Decreased mitochondria fraction
- Increased ER and Golgi fractions
- Decreased pregnancy rates, increased pre- and post-implantation death

Ottolenghi et al, 2004; Broekmans et al, 2007; Tarin et al, 2001; Keefe and Liu, 2009
Reactive Oxygen Species Generation and Detoxification

\[
\begin{align*}
O_2 + e^- & \rightarrow O_2^- \rightarrow O_2^- \\
2H^+ O_2^- & \rightarrow SOD \rightarrow H_2O_2 + O_2 \\
Fe^{++} + O_2^- & \rightarrow CAT \rightarrow O_2 + OH^- + OH^- \\
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\end{align*}
\]

Oxidative damage to DNA, protein, lipids
Evidence for roles of oxidative stress in ovarian aging

- Increased ROS, decreased antioxidant activity with age in oocytes, cumulus cells, and follicular fluid of women undergoing IVF (Tatone et al, 2006; Matos et al, 2009; Wiener-Megnazi et al, 2004; Das et al, 2006)

- Increasing oxidative protein, lipid, and DNA damage and decreased antioxidant expression in ovaries with age in mice (Lim and Luderer, 2011)

- Decreased GSH and expression of GSH-related genes in oocytes of aged compared to young mice (Tarin, 1996; Hamatani et al, 2004; Pan et al, 2008)
Glutathione Synthesis

1: glutamate cysteine ligase (γ-glutamyl-cysteine synthetase) heterodimer composed of Gclc and Gclm

2: glutathione synthetase

γ-glutamyl-cysteinyl glycine (GSH)
Gclm-/- mice have decreased ovarian GSH

Lim et al, Endocrinology, 2015
Ovarian GSH redox state is oxidized in *Gclm*-/ mice

![](image)

Lim et al, Endocrinology, 2015
Accelerated age-related decline in ovarian follicles in \textit{Gclm-/-} mice

Lim et al, Endocrinology, 2015
Decreased fertility in *Gclm*-/- mice

Nakamura et al, Endocrinology, 2011
What causes small litter size in Gclm-/- mice?

<table>
<thead>
<tr>
<th></th>
<th>Gclm+/+ (mean±SEM)</th>
<th>Gclm-/- (mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oocytes ovulated</td>
<td>8.8±0.7</td>
<td>8.3±1.1</td>
</tr>
<tr>
<td>natural estrous cycle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oocytes ovulated,</td>
<td>44.6±8.0</td>
<td>52.5±5.9</td>
</tr>
<tr>
<td>superovulation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live fetuses</td>
<td>6.8±0.9</td>
<td>2.4±0.8*</td>
</tr>
<tr>
<td>Dead fetuses</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>Resorptions</td>
<td>0.9±0.4</td>
<td>1.7±0.4</td>
</tr>
<tr>
<td>Total implantations</td>
<td>7.9±0.8</td>
<td>4.3±0.7*</td>
</tr>
</tbody>
</table>

Nakamura et al, Endocrinology, 2011
Impaired zygote development in $Gclm^{-/-}$ females

Nakamura et al, Endocrinology, 2011
GSH deficiency causes premature ovarian failure

- GSH deficiency causes chronic ovarian oxidative stress and premature ovarian failure with accelerated age-related decline of ovarian follicles and decreased oocyte quality.
- These findings support the hypothesis that chronic ovarian oxidative stress causes POF.
- Do environmental toxicants that cause ovarian oxidative stress also cause POF?
Chromium (Cr)

- Heavy metal used in industrial processes such as electroplating, leather tanning, steel production, and wood preservative manufacturing.
- Drinking water contamination with Cr is widespread.
- Cr exists in multiple oxidations states, of which CrVI and CrIII are the most common.
- CrVI anion (chromate) in biological systems readily crosses cell membranes; it is then reduced to CrIII by ascorbate, GSH, and cysteine.
- Process of reduction generates reactive species that form DNA adducts, leading to double strand breaks and genomic instability.

Prenatal CrVI depletes ovarian germ cells and inhibits germ cell nest breakdown.

Pregnant rats were administered 25 mg/L CrVI in drinking water from GD 9.5 to 14.5. Ovaries were analyzed at GD 15.5 or PND 1.

Prenatal CrVI accelerates the age-related decline in fertility

Potential mechanisms of CrVI-induced germ cell destruction

- Induction of apoptosis with increased proapoptotic proteins and decreased antiapoptotic proteins.
- Inhibition of cell cycle and proliferation-related proteins.
- Inhibition of phosphorylation of AKT, which is required for primordial follicle activation.
- Induction of X-prolyl aminopeptidase and resulting down-regulation of collagen synthesis in the developing ovary and opposite effects with up-regulation of collagen synthesis postnatally.

Culture of fetal ovaries with CrVI induces oxidative stress

Ovaries were collected from rat fetuses at 13.5 days post-coitum and cultured for 12 days, Cr was added to the media on culture day 2. Culture media were collected for measurement of antioxidant enzyme activities and LPO. LPO: Lipid hydroperoxides (products of lipid peroxidation) GPx: Glutathione peroxidase SOD: Superoxide dismutase

(Stanley et al, 2015, Toxicol Appl Pharmacol 289:58-69)
Can antioxidants protect against transplacental CrVI ovarian toxicity?

- Vitamin C (asorbate) and the free radical scavenger edavarone mitigate ovarian follicle depletion by lactational exposure to CrVI, but antioxidants have not been tested in the prenatal exposure model.

Summary of CrVI effects on the developing ovary

Germ cell apoptosis prenatally. Accelerated germ cell nest breakdown and follicle assembly and increased follicular atresia postnatally.
Ovarian cancer

- Leading cause of death from gynecological cancers.
- 5th leading cause of cancer-related death among women.
- 90% of malignant ovarian cancers in humans are epithelial.
- 85% occur post menopause.
- In mouse models, diverse stimuli that cause premature ovarian failure also cause ovarian tumors.

(SEER; Capen et al, 1995; Vanderhyden et al, 2003, 2005)
Pathophysiology of ovarian cancer

- Epithelial tumors thought to arise from ovarian or oviductal surface epithelium stem cells and are associated with mutations of BRCA1 and 2, Retinoblastoma (Rb1), KRAS, and p53.
- Granulosa cell tumors arise from granulosa cells.
- Oxidative stress is implicated in epithelial cancers, possibly by causing oxidative DNA damage in surface epithelium cells that leads to mutations.
- Germ cell depletion and high levels of gonadotropin hormones may play a promotional role.

Premature ovarian failure and arsenic (As)

- Inorganic As exposure occurs mainly via drinking water contamination; other uses include wood preservatives and pesticides.
- Administration of 0.4 ppm As in drinking water to adult rats depletes ovarian follicles, decreases ovarian steroidogenesis, and disrupts estrous cycling.
- However, no studies have examined the effects of prenatal As exposure on ovarian follicle numbers or fertility in the offspring.

As induces ovarian oxidative stress

- As in drinking water increases lipid peroxidation and decreases SOD activity in ovaries of adult rats.
- Co-administration of the antioxidants GSH or sodium selenite or high protein diet are protective against ovarian toxicity of As in adult rats.

Prenatal arsenic (As) causes ovarian tumors in mice

Pregnant C3H mice were administered As (sodium arsenite at 42.5 or 85 ppm) in drinking water from GD 8 to 18 (Waalkes et al, 2003, Toxicol Appl Pharmacol 186:7-17).

<table>
<thead>
<tr>
<th>Group</th>
<th>Incidence</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Benign</td>
<td>Malignant</td>
<td>Total tumors</td>
<td></td>
</tr>
<tr>
<td>Control (n = 25)</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>42.5 ppm (n = 23)</td>
<td>4</td>
<td>2</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>85 ppm (n = 24)</td>
<td>8*</td>
<td>1</td>
<td>9*</td>
<td></td>
</tr>
<tr>
<td>Trend p =</td>
<td>0.025*</td>
<td>0.456</td>
<td>0.015*</td>
<td></td>
</tr>
</tbody>
</table>
Prenatal arsenic (As) causes ovarian tumors in mice

- Pregnant mice were administered As (sodium arsenite at 42.5 or 85 ppm) in drinking water from GD 8 to 18.
- As dose-dependently increased epithelial and total ovarian tumors in offspring.
- Similar effects were observed with the inorganic As metabolite methylarsenous acid, suggesting that this may be the ultimate carcinogen.

Ionizing radiation (IR)

- IR directly damages DNA, but the majority of the cellular damage it causes is due to generation of ROS from indirect ionization of water.
- Ionizing radiation to the pelvis for cancer therapy causes amenorrhea and POF.
- Ionizing radiation exposure is associated with increased risk of ovarian cancer.

Ionizing radiation destroys germ cells in human fetal ovaries

6-10 wk human fetal ovaries (mitotic stage) were cultured for 14 d (A) or 0-2 d (B) after irradiation with the indicated doses of gamma radiation at time 0 of culture. (Guerquin et al, 2009, Hum Reprod 24:670)
Fetal mouse ovaries (GD 12.5, mitotic stage) were cultured for 24 or 48h after irradiation with the indicated doses of gamma radiation at time 0 of culture. (Guerquin et al, 2009, Hum Reprod 24:670)
What is the critical window for ovarian tumors induction by IR?

Mice irradiated with 3.8 Gy γ-rays at GD 17 or the indicated postnatal ages, then followed for tumor development until death.

However, when mice were irradiated with daily doses of x-rays GD11-13 compared to GD14-16, no ovarian tumors were induced over the 14% incidence in controls with the earlier irradiation, but a 20% incidence was observed with the later irradiation (Schmahl and Kriegel, 1980, J Can Res Clin Oncol 98:65).
Sources of PAHs
Polycyclic Aromatic Hydrocarbon (PAH) Metabolism

Xue and Warshawsky, 2005, TAAP
PAHs and ovarian toxicity

- Treatment of peripubertal mice with PAHs destroys primordial and primary ovarian follicles and causes premature ovarian failure (studies by Mattison and coworkers; Borman et al, 2000)
- In utero exposure to BaP decreased fertility in female mice (MacKenzie and Angevine, 1981)
- Women who smoke have earlier onset of menopause (Harlow, 2000)
- Daughters of women who smoked during pregnancy have decreased fecundity (Weinberg et al, 1989) and earlier age at menopause (Strohsnitter et al, 2008)
PAHs and ovarian cancer

- Smoking is associated with increased risk of ovarian cancer (Terry et al, 2003; Gates et al, 2010)
- Treatment of adult mice with PAHs causes ovarian tumors
- *In utero* treatment of mice with DMBA or Dibenz[def,p]chrysene causes ovarian tumors in adulthood (Görttler et al, 1981; Shorey et al, 2012)
  - Single 60 mg/kg dose of DMBA induced ovarian cancers on any of GD 6-20, but highest incidence was with GD 8-13 dosing
Hypothesis: BaP is a transplacental ovarian toxicant and tumorigen, and Gclm deletion increases sensitivity to these effects of BaP.
Dose-dependent depletion of oocytes by in utero exposure to BaP in F1 Gclm+/- females
Greater decrease in offspring production with prenatal BaP exposure in F1 $Gclm^{-/-}$ than $Gclm^{+/+}$ females

P<0.001, effects of genotype, BaP dose, BaP dose x genotype

Lim et al, 2013, Cancer Research
Gclm−/− females are more sensitive to germ cell depletion by *in utero* exposure to BaP

P<0.001, effects of BaP dose, genotype, BaP x genotype interaction

Lim et al, 2013, Cancer Research
### Effects of prenatal BaP and *Gclm* genotype on ovarian tumor prevalence at 7.5 months

<table>
<thead>
<tr>
<th>Genotype</th>
<th>% no tumors (N)</th>
<th>% unilateral tumor (N)</th>
<th>% bilateral tumor (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Gclm-</em>/*, 0mg/kg BaP</td>
<td>100 (12)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Gclm+/</em>, 0mg/kg BaP</td>
<td>100 (13)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Gclm-</em>/*, 2mg/kg BaP</td>
<td>89 (8)</td>
<td>11 (1)</td>
<td>0</td>
</tr>
<tr>
<td><em>Gclm+/</em>, 2mg/kg BaP</td>
<td>100 (8)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Gclm-</em>/*, 10mg/kg BaP</td>
<td>0</td>
<td>0</td>
<td>100 (2)</td>
</tr>
<tr>
<td><em>Gclm+/</em>, 10mg/kg BaP</td>
<td>17 (1)</td>
<td>33 (2)</td>
<td>50 (3)</td>
</tr>
</tbody>
</table>

P<0.001, effects of BaP, genotype, and genotype x dose interaction on tumor multiplicity

Lim et al, 2013, Cancer Research
Cytokeratin positive tumor in *Gclm*--/--, 10 mg/kg BaP-exposed female

Lim et al, 2013, Cancer Research
Conclusions

• *In utero* exposures to Cr, BaP, and IR deplete ovarian germ cells.

• *In utero* exposures to As, BaP, and IR induce ovarian tumors later in life.

• Oxidative stress and induction of germ cell apoptosis appear to be involved in germ cell depletion by all of these agents.

• Other agents that induce oxidative stress may be good candidates for investigation as transplacental ovarian toxicants and tumorigens.
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