Research in Reproductive Toxicology: changing the way we care for patients

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Disclosures

I have no disclosures.
Objectives

• Introduce the field of Reproductive Toxicology
• Review data related to gonadotoxic agents and multidrug resistance transporters in the ovary
• Discuss the integration of gene editing in reproductive toxicology
• Briefly mention controversy in gene editing of human embryos
### Figure 3. Leading Sites of New Cancer Cases and Deaths – 2017 Estimates

<table>
<thead>
<tr>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prostate</strong></td>
<td>161,360</td>
</tr>
<tr>
<td><strong>Lung &amp; bronchus</strong></td>
<td>116,990</td>
</tr>
<tr>
<td><strong>Colon &amp; rectum</strong></td>
<td>71,420</td>
</tr>
<tr>
<td><strong>Urinary bladder</strong></td>
<td>60,490</td>
</tr>
<tr>
<td><strong>Melanoma of the skin</strong></td>
<td>52,170</td>
</tr>
<tr>
<td><strong>Kidney &amp; renal pelvis</strong></td>
<td>40,610</td>
</tr>
<tr>
<td><strong>Non-Hodgkin lymphoma</strong></td>
<td>40,080</td>
</tr>
<tr>
<td><strong>Leukemia</strong></td>
<td>36,290</td>
</tr>
<tr>
<td><strong>Oral cavity &amp; pharynx</strong></td>
<td>35,720</td>
</tr>
<tr>
<td><strong>Liver &amp; intrahepatic bile duct</strong></td>
<td>29,200</td>
</tr>
<tr>
<td><strong>All sites</strong></td>
<td>836,150</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lung &amp; bronchus</strong></td>
<td>84,590</td>
</tr>
<tr>
<td><strong>Colon &amp; rectum</strong></td>
<td>27,150</td>
</tr>
<tr>
<td><strong>Prostate</strong></td>
<td>26,730</td>
</tr>
<tr>
<td><strong>Pancreas</strong></td>
<td>22,300</td>
</tr>
<tr>
<td><strong>Liver &amp; intrahepatic bile duct</strong></td>
<td>19,610</td>
</tr>
<tr>
<td><strong>Leukemia</strong></td>
<td>14,300</td>
</tr>
<tr>
<td><strong>Esophagus</strong></td>
<td>12,720</td>
</tr>
<tr>
<td><strong>Urinary bladder</strong></td>
<td>12,240</td>
</tr>
<tr>
<td><strong>Non-Hodgkin lymphoma</strong></td>
<td>11,450</td>
</tr>
<tr>
<td><strong>Brain &amp; other nervous system</strong></td>
<td>9,620</td>
</tr>
<tr>
<td><strong>All sites</strong></td>
<td>318,420</td>
</tr>
</tbody>
</table>

Estimates are rounded to the nearest 10, and cases exclude basal cell and squamous cell skin cancers and in situ carcinoma except urinary bladder.

©2017, American Cancer Society, Inc., Surveillance Research
Fertility Preservation for Patients With Cancer:  
American Society of Clinical Oncology Clinical Practice Guideline Update

Alison W. Loren, Pamela B. Mangu, Lindsay Nohr Beck, Lawrence Brennan, Anthony J. Magdalinski,  
Ann H. Partridge, Gwendolyn Quinn, W. Hamish Wallace, and Kutluk Oktay

**Adult Females**
- Present both embryo and oocyte cryopreservation as established fertility preservation methods
- Discuss the option of ovarian transposition (oophoropexy) when pelvic radiation therapy is performed as cancer treatment
- Inform patients of conservative gynecologic surgery and radiation therapy options
- Inform patients that there is insufficient evidence regarding the effectiveness of ovarian suppression (gonadotropin-releasing hormone analogs) as a fertility preservation method, and these agents should not be relied on to preserve fertility
- Inform patients that other methods (e.g., ovarian tissue cryopreservation, which does not require sexual maturity, for the purpose of future transplantation) are still experimental
Alkylating chemotherapeutics can lead to ovarian dysfunction

- Sexual Dysfunction
- Vasomotor Symptoms
- Increased cardiovascular risk
- Bone loss
- Infertility
Reproductive toxicology is the subject area dealing with the causes, mechanisms, effects and prevention of disturbances throughout the entire reproductive cycle, including fertility induced by chemicals.

*Drugs During Pregnancy and Lactation (Second Edition), 2007*
Reproductive Toxicology Fields

https://www.niehs.nih.gov/research/atniehs/labs/rdbl/index.cfm
What is Transgenerational Inheritance?

- Exposure occurs in the $F_0$ generation
- Exposure stops – not continuous, not across generations
- Health effect is evaluated in generation(s) not directly exposed.

ISIS used mustard gas against Kurds in Iraq, diplomat says

A man lies wounded after mustard gas attacks in Syria in 2015. A diplomat has confirmed that ISIS used mustard gas against Kurdish forces in Iraq last year. (AAP/LIU AHMAD/GETTY IMAGES)

North Korea’s Chemical Warfare Capabilities

By: Joseph S. Bermudez Jr.
October 18, 2013 | WMD

“...the South Korean government suggests that Pyongyang has 2,500 to 5,000 metric tons of some 20 chemical warfare agents.”
Nitrogen Mustard (NM) poses a risk for female soldiers

- Chemical warfare agent
- DNA mutagen implicated in NAD depletion
  - NAD: electron carrier important for oxidative phosphorylation
- Has been shown to result in mitochondrial destruction
  - Mitochondria are maternally inherited
- Presents an occupational hazard to female soldiers and their children
Mitochondria are abundant in the oocyte and maternally inherited

- Produce ATP in aerobic respiration
- 100,000 in a mature oocyte at fertilization
- Maternally Inherited
  - All mitochondria in the body come from the oocyte, inherited from mother

Mitochondria structural features, Molecular Expressions, Florida State University
https://micro.magnet.fsu.edu/cells/mitochondria/mitochondria.html
Nitrogen mustard exposed oocytes exhibit differences in aerobic metabolism

- Oxygen Consumption Rate (indicative of aerobic respiration)
- Oocytes incubated in 50nM NM for 2hr
- Future experiments – inject different doses of NM intraperitoneally
Low doses of NM oocytes cause more ROS compared to higher doses and controls.

MitoSOX treated oocytes

Correct Total Cell Fluorescence = 
integrated density x (area of cell - mean background fluorescence)
NM treated oocyte mitochondria display abnormal morphology

Nitrogen Mustard mitochondria are potentially more oblong/elongated compared to controls
Potential mitophagy in NM treated Oocytes

Ultrastructure of human oocytes after in vitro maturation
Mol Hum Reprod | © The Author 2015. Published by Oxford University Press on behalf of the European Society of Human Reproduction and Embryology.
Specific Aim:
To determine mitochondrial profiles and reproductive outcomes in a mother and offspring generations

• Inject mother with nitrogen mustard
• Mate F1 generation females with WT males
• Record reproductive outcomes
  • how fast they breed, how many in a litter, how many survive, how many reach sexual maturation
• Observe molecular outcomes in mitochondria of mother, F1, and F2 females
  • Send testes to andrology researchers to observe differences
Nitrogen Mustard Toxicity—can inducing Multidrug Resistance Transporters help?
Active Transporters

- Ligand
- ADP
- $P_i$
ABC Transport Families

- 49 different ABC transporters in the human body within 7 different families:
  - ABCA = ABCA 1-10, 12, 13
  - ABCB = ABCB 1-11
  - ABCC = ABCC 1-13
  - ABCD = ABCD 1-4
  - ABCE = ABCE1
  - ABCF = ABCF 1-3
  - ABCG = ABCG 1, 2, 4, 5, 8

ABCB1 = MDR-1 = P-glycoprotein
MDR-1 is expressed in the mammalian oocyte

Brayboy et al 2013, Fertility and Sterility
MDR-1 is expressed in the entire ovary.

MDR-1 is expressed throughout the mouse ovary, and triple knock out MDR ovaries exhibit significant cell death compared to WT controls after chemotherapeutic exposure.

Brayboy et al 2017 Reproductive Toxicology
Knock out of mdr1a/mdr1b/bcrp causes changes in ovarian follicle dynamics

Brayboy et al 2017, Reproductive Toxicology
P-glycoprotein mutant ovaries (mdr1a-/-) have over accumulation of metabolites

Brayboy et al
Unpublished
13 metabolites showed overabundance in P-gp mutant ovaries compared to CF-1 WT controls

(3) N- N-acetylphenylalanine

(12) *pyruvate*

(16) adenine

(146) argininosuccinate

(70) *acetyl-CoA*

(145) GMP

(69) dTMP

(149) *NADP*

(83) AMP

(115) *succinate*

(119) IMP

(127) *malate*

(132) CMP

Brayboy et al unpublished
Spontaneous P-gp mutants (*mdr1a-/-*) also display vulnerability to alkylating agents.
P-gp (mdr1a-/-) mutants have increased ROS in immature oocytes

Brayboy et al. unpublished
Loss of MDR-1 leads to changes in the ovarian transcriptome

Brayboy et al unpublished
Differentially expressed genes in \textit{mdr1a-/-} map to mitochondrial diseases

Brayboy et al. unpublished
Ideas for future research to inform clinical practice

• A knock in edit to increase MDR expression in the ovary during toxic exposure

• Use gene editing tools to remove endogenous MDR-1 and insert human SNPs of ABCB1 associated with susceptibility and cancer development

• Tissue specific knock-out of MDR-1 in the ovary to test toxicity
Tissue specific knock out of MDR-1 in the ovary to test toxicity

Ablain et al 2015 Developmental Cell
Overview of CRISPR in Human Reproduction

• Presents an bioethical dilemma
• Alternative to PGD, but unnecessary
• Question of mosaicism
• Off-target effects
• Eggs and sperm are easily manipulated
I am a FIRbee
FIR.mbl.edu

PRIMO

NIH
Eunice Kennedy Shriver
National Institute of
Child Health and
Human Development

ABO+G
The American Board of
Obstetrics & Gynecology
First in Womens Health

Marine Biological Laboratory
Woods Hole, MA
Frontiers
IN Reproduction

April 28 – June 10, 2018 | Application deadline January 18, 2018

COURSE DIRECTOR:
Rafael Furelow, University of Massachusetts, Amherst

This is an intensive, mentored training program in animal and human reproductive biology designed to transmit the latest conceptual advances in the field and provide hands-on experience with techniques used in research labs and in clinics. The ultimate goal is to train the future leaders in the field to improve reproductive outcomes in humans and animals. Topics covered include the hypofunctional pituitary-gonadal axis; ovarian cells, gametogenesis and fertilization; assisted reproductive technologies; placentaion and implantation strategies across species.

Substantial financial assistance is available.

"FIR allowed me to step out of my comfort zone and enabled me to do research I would have otherwise not had the opportunity to do." —2016 Alum

Marine Biological Laboratory | mbl.edu/courses