Toxocologic Pathology
of the
Female Reproductive System

Sarah Beck
sbeck8@jhmi.edu
Overview

- Anatomy and physiology review
- Evaluation of the female reproductive tract
- Pathology
- Mechanisms of toxicity
- Examples of toxins
Objectives

• Be able to name some of the challenges to evaluating toxicologic pathology of the female reproductive tract

• Compare and contrast basic anatomy and physiology between rodents and humans

• Understand basic female reproductive cycle physiology (i.e., estrous cycle, hormone production/feedback)

• Know the three toxin classes and their effects on reproductive tissues

• Be able to name a few examples of specific toxins and discuss their effects
Challenges to Studying Female Reproductive Tract Toxicology

• Non-continuous cyclical process
  – Need a large number of animals
  – Need a longer test period (esp with non-rodent studies)
• Small gonads
• Normal variability in gonad size
  – Formation and regression of follicles/CL
• Delayed atrophy following insult compared to testes
• Few detailed descriptions of normal cyclical changes in the female hinders study of toxicologic effects
• Pregnancy effects
• Age effects
  – Most toxicologic studies are done with young, sexually immature animals
Development and anatomy

- Fallopian tube
- Ovary
- Uterus
- Endometrium
- Myometrium
- Serosa
- Cervix
- Vagina
Development - Internal

Muellerian Duct

Indifferent Gonad

Wolffian Duct
XX Female

Indifferent Gonads
- TDF
= Ovaries

Muellerian Duct
- Mullerian Inhibiting Factor
= Fallopian Tubes
Uterus

Wolffian Duct
- Testosterone
= Duct degeneration
Comparative Reproductive Anatomy

Rodent
- Ovary
- Oviduct
- Uterus
- Bladder
- Cervix
- Vagina
- Urethral opening

Primate
- Fallopian tube
- Uterus
- Ovary
- Cervix
- Labium minus
- External os
- Internal os
- Vagina
Comparative Reproductive Anatomy

Rodent

Primate
Mouse Ovary

- follicle
- corpus luteum
- medulla
- oocyte
- oviduct
- surface epithelium and tunica albuginea
Mouse Ovary

Primary and secondary follicles

- Theca
- Surface Epithelium
- zona granulosa
- oocyte
- primary follicle
- Secondary Follicle
- zona pellucida
Mouse Ovary
Tertiary follicle

Theca externa
Theca interna
Granulosa Cells
1° Oocyte
Zona Pellucida
Atretic Follicle
Antral Cavity

http://it.stlawu.edu/~mtem/devbiol/36c89bf2.jpg
Normal Anatomy

Many CLs
Human ovary

http://embryology.med.unsw.edu.au/Notes/images/week1/ovary/ovary.gif
Normal Anatomy: one CL

Atretic Follicles

Cortex

Medulla

Corpus Luteum

granulosa lutein cells

theca lutein cells

http://embryology.med.unsw.edu.au/Notes/week2_9.htm
Mouse, Uterus 40x
Mouse, Cervix 40x

- stroma
- stratified squamous epithelium
Mammary Gland Anatomy

Terminal duct lobular unit (TDLU) → functional unit of the breast
Human Terminal Duct Lobular Unit (TDLU) Whole Mount: functional unit

duct
acini
extralobular terminal ducts
lobules

Cardiff website
Human Mammary Gland Anatomy

- Alveoli
- Duct
- Lobules

www.bu.edu/histology
Mouse Mammary Glands

Why is this significant??

http://eulep.pdn.cam.ac.uk/
Mouse, Mammary Tissue 10x

fat pad

ducts

Lactating
# Functional Anatomy

<table>
<thead>
<tr>
<th>Organ</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovaries</td>
<td>Gamete Production, Hormone Production</td>
</tr>
<tr>
<td>Fallopian Tubes (Oviducts)</td>
<td>Gamete Transport, Fertilization</td>
</tr>
<tr>
<td>Uterus</td>
<td>Fetus Development</td>
</tr>
<tr>
<td>Vagina</td>
<td>Sperm Reception</td>
</tr>
<tr>
<td>Mammary Gland</td>
<td>Nourish offspring</td>
</tr>
</tbody>
</table>
Physiology
Steroidogenesis

**Theca interna cells:**
- cholesterol → androstenedione

**Granulosa cells:**
- androstenedione → estrogen
Mouse Ovary

Tertiary follicle

http://it.stlawu.edu/~mtem/devbiol/36c89bf2.jpg
# Estrogen Receptors

<table>
<thead>
<tr>
<th></th>
<th>Function</th>
<th>Ligand Affinity</th>
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<tbody>
<tr>
<td>$\alpha$ER</td>
<td>Increases transcription</td>
<td>Estrogen: 100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tamoxifen: 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DES (synthetic estrogen): 468</td>
</tr>
<tr>
<td>$\beta$ER</td>
<td>Decreases transcription</td>
<td>Estrogen: 100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tamoxifen: 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DES (synthetic estrogen): 295</td>
</tr>
</tbody>
</table>

http://www.bio.cmu.edu/Courses/BiochemMols/ER/ERIntro.html
A Rat’s Life

Birth

Puberty

“Menopause”

Days

Months

Conception

Germ cells migrate to ovary

Uterus Formation

Oocyte formation

Estrogen Level

Days

Birth

Months

5  10  15  20

6  12  18  24  30
The short cycle length of rodents (4-5 days) is attributed to a lack of a luteal phase (days post ovulation) with the lack of a functional progesterone-secreting corpora lutea (diesterus I = metestrus).

Staley, Nature 2005
Primate Menstrual Cycle

- **FSH**
- **LH**
- **Ovary**
- **Estradiol**
- **Progesterone**
- **Basal Body Temperature**

**Day 1**
- Menstruation Phase

**Day 7**
- Proliferative Phase

**Day 14**
- Ovulation
- Basal Body Temperature: 0.5°C

**Day 21**
- Secretory Phase
## Female Hormone Effects

<table>
<thead>
<tr>
<th></th>
<th>Ovary</th>
<th>Uterus</th>
<th>Vagina</th>
<th>Mammary Gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen</td>
<td>Atrophy</td>
<td>Endometrial hyperplasia</td>
<td>Proliferation and cornification</td>
<td>Fat deposition</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Myometrial hypertrophy</td>
<td></td>
<td>Duct development</td>
</tr>
<tr>
<td>Progesterone</td>
<td>Atrophy</td>
<td>Secretion</td>
<td>Secretion</td>
<td>Alveoli development</td>
</tr>
</tbody>
</table>

## Stages of Rat Estrus Cycle

<table>
<thead>
<tr>
<th></th>
<th>Proestrus</th>
<th>Estrus</th>
<th>Metestrus</th>
<th>Diestrus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ovary</strong></td>
<td>Primary and secondary follicles</td>
<td>Tertiary follicles and ovulation</td>
<td>Developing corpus luteum</td>
<td>Functional corpus luteum</td>
</tr>
</tbody>
</table>
| **Uterus**     | Medium sized epithelial cells  
Spindle shaped stromal cells | Tall epithelial cells 
Spindle shaped stromal cells | Tall epithelial cells 
Spindle shaped stromal cells | Small epithelial cells  
Round stromal cells  |
| **Vagina**     | Non-cornified epithelial cells | Cornified epithelial cells    | Cornified epithelial cells and neutrophils | Cornified and non-cornified vacuolated epithelial cells and neutrophils |
Cyclic uterine changes are less pronounced in rodents than in primates, but vaginal epithelium responds to hormonal changes (these changes are normal).
<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proestrus</td>
<td>Parabasal and basal cells, few intermediate and superficial squamous cells, occasional neutrophils</td>
</tr>
<tr>
<td>Estrus</td>
<td>Predominantly superficial squamous cells</td>
</tr>
<tr>
<td>Metestrus</td>
<td>Superficial and intermediate squamous cells with neutrophils</td>
</tr>
<tr>
<td>Diestrus</td>
<td>Parabasal cells, basal cells, vacuolated cells, with high numbers of neutrophils</td>
</tr>
</tbody>
</table>

Challenges

• Histopathological and cytological changes in vaginal mucosa may vary
  – Between animals
  – Within same animal (regional differences)

• Better characterized in some species than others

• More pronounced cyclic changes in some species than others
  – Cynomolgus macaques: slight and inconsistent changes
Stages of Mouse Mammary Development: Whole mounts

- Virgin
- Pregnancy
- Lactation
- Involution

www.mammary.nih.gov
Evaluation of the Female Reproductive System

• Control and experimental animals matched
  – Age
  – Strain
  – Stage of estrus cycle (Whitten effect)

• Pre-mortem
  – Hormone levels
  – Fertility/Fecundity

• Post-mortem
  – Organ weights
  – Histology +/- Follicle counts
  – IHC (ER/PR)
Pathology
Type I Toxicity:
Cause *inactivity* in ovary, uterus, and vagina

1. Decreased gonadotrophin secretion:
   - Stress
   - Reduced energy intake
   - Morphine
   - Cocaine
   - Marijuana
   - Gasoline by-products
   - Heavy Metals

2. Impairment of follicular growth:
   - Cyclophosphamide
   - Dibromochloropropane
   - Polycyclic aromatic amines
   - Radiation

3. Inhibitors of steroidogenesis:
   - Aminoglutethimide
   - Delta-1-testolactone
   - 4-hydroxyandrostenedione

Atrophy
Type II Toxicity: sex steroid hormones (natural or synthetic)

- Estrogen
  - Diethylstilbesterol
  - Tamoxifen
  - Clomiphene

- Progesterone
  - Ethynerone
  - Norethynodrel
  - Medroxyprogesterone
  - High dose androgens

Hypothalamus

GnRH

FSH

LH

Pituitary

Ovary

Causes inactivity in ovary and hyperactivity in uterus/vagina

Hypertrophy

Atrophy
Type III Toxicity: LH, FSH, or prolactin (or compounds that act like them)

Cause hyperactivity in ovary, uterus, vagina

Hypertrophy
## Types of Toxicity

<table>
<thead>
<tr>
<th>Type</th>
<th>Ovary</th>
<th>Uterus and Vagina</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>Inactivity</td>
<td>Inactivity</td>
<td>↓ FSH/LH&lt;br&gt;↓ Follicular development&lt;br&gt;↓ Hormone production</td>
</tr>
<tr>
<td>Type II</td>
<td>Inactivity</td>
<td>Hyperactivity</td>
<td>↑ Exogenous steroid hormones</td>
</tr>
<tr>
<td>Type III</td>
<td>Hyperactivity</td>
<td>Hyperactivity</td>
<td>↑ FSH/LH/GnRH</td>
</tr>
</tbody>
</table>
General Carcinogens

• Ionizing radiation (produces free radicals)
• Base analogs (point mutations)
  – 5-bromouracil
• Deaminating agents
  – Nitrous acid
• Intercalating agents
  – Ethidium bromide
• Alkylating agents (adds alkyl group)
  – Bromouracil
• Many of these are chemotherapeutic agents
Female Reproductive System Carcinogenesis

• Ovary
  – Lose negative feedback of estrogen and progesterone on the pituitary → unregulated increased stimulation by gonadotropins → constant ovary stim → hyperplasia/neoplasia of ovaries

• Uterus
  – Estrogen stimulation induces endometrial proliferation
  – Chronic inflammation/irritation

• Cervix/Vagina
  – Viral infection (HPV) → Chronic irritation/inflammation

• Mammary gland
  – Estrogen stimulation → mammary gland hyperplasia/neoplasia
Ovarian Tumors

Origin of three ovarian cancer types:

- Stromal cells: 5-10%
- Germ cells: 10-15%
- Epithelium (surface cells): 80%

http://ovariancancer.jhmi.edu/typesca.cfm
# Ovarian Tumors

<table>
<thead>
<tr>
<th></th>
<th>Cell of Origin</th>
<th>Gross</th>
<th>Histo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epithelial Tumors</strong></td>
<td>Surface epithelium, SES, rete ovarii</td>
<td>Cystic/papillary multinodular enlargement</td>
<td>Epithelial-lined arboriform papillae</td>
</tr>
<tr>
<td>(~60% ovarian tumors)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sex Cord-Stromal Tumors</strong></td>
<td>Endocrine apparatus (Granulosa cell, theca cell, corpus luteum)</td>
<td>Solid or cystic Uni- or bilateral Nodular or symmetric enlargement</td>
<td>Granulosa Cell -follicular/variable Luteoma -polygonal, lipid-laden cells Thecoma -spindle cells</td>
</tr>
<tr>
<td>(~10% ovarian tumors)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dysgerminoma</strong></td>
<td>Germ cell</td>
<td>Often large Grey-white Firm Homogenous</td>
<td>Sheets of large cells Prominent nuclei Little cytoplasm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Teratoma</strong></td>
<td>Germ cell</td>
<td>Solid to cystic +/- Hair, bone, teeth, cartilage</td>
<td>At least two germinal layers</td>
</tr>
<tr>
<td></td>
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</tr>
</tbody>
</table>
Ovarian Cystadenoma, Rat
Epithelial origin tumor
Granulosa Cell Tumor, Mare
Sex-cord stromal origin
Granulosa Cell Tumor Histology:
can form follicular structures
Call Exner Bodies
(characteristic of sex cord-stromal tumors)
Thecoma, Gerbil
Sex cord-stromal tumor
Thecal Cell Tumor (cow) Histology: predominantly spindle cells
# Ovarian Carcinogens

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Carcinogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulosa/thecal cell tumor</td>
<td>Benzo[a]pyrene, Iodine-131, Methylthiouracil, Progestins, Radiation, 7,12-Dimethylbenz[a]anthracene, 7,8,12-Trimethylbenz[a]anthracene</td>
</tr>
<tr>
<td>Tubular adenoma</td>
<td>Radiation</td>
</tr>
<tr>
<td>Fibroma</td>
<td>Mibolerone (androgen)</td>
</tr>
<tr>
<td>Leiomyoma (mesovarium)</td>
<td>Soterenol and Mesuprine (smooth muscle relaxants)</td>
</tr>
</tbody>
</table>
Uterine Tumors

- **Epithelial**
  - Endometrial adenoma/adenocarcinoma
- **Mesenchymal**
  - Myometrial leiomyoma/sarcoma
  - Fibroma/sarcoma
  - Fibroleiomyoma/sarcoma
- **Lymphosarcoma**
- **Placental**
Uterine Adenocarcinoma, Rabbit (epithelial origin)
Uterus, Leiomyoma, Guinea Pig
(mesenchymal origin)
Leiomyoma, histology
(again, spindle cells)

http://www.jeffersonhospital.org/obgyn/fibroid/leiomyoma2.jpg
<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Carcinogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma/adenoacanthoma</td>
<td>Diaminoxide, Estrogens, Intrauterine devices, Methylcholanthrene, N,N-Dimethyl-N-nitrosothiol, 3-Amino-9-ethylcarbazole, 4-Methyl-N’-nitro-N-nitrosoguanidine, 4-Nitroguaninoline-1-oxide, 4,4-Thiodianiline</td>
</tr>
<tr>
<td>Deciduoma</td>
<td>Estrogen + progesterone combination</td>
</tr>
<tr>
<td>Fibrosarcoma</td>
<td>2-Acetylaminofluorene, 3-Methylcholanthrene</td>
</tr>
<tr>
<td>Leiomyoma/leiomyosarcoma</td>
<td>Medroxalol (antihypertensive)</td>
</tr>
<tr>
<td>Papillary mesothelioma</td>
<td>Estrogens</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>Intrauterine devices, 7,12-Dimethylbenz[a]anthracene</td>
</tr>
</tbody>
</table>
# Mammary tumors (rat)

<table>
<thead>
<tr>
<th>Type</th>
<th>Russo et al. 1989</th>
<th>van Zwieten et al. 1994</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial</td>
<td>Papilloma</td>
<td>Adenoma</td>
</tr>
<tr>
<td></td>
<td>Cystadenoma/Adenoma</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td></td>
<td>Carcinoma</td>
<td></td>
</tr>
<tr>
<td>Epithelial-stromal</td>
<td>Fibroadenoma***</td>
<td>Fibroadenoma***</td>
</tr>
<tr>
<td></td>
<td>Carcinosarcoma</td>
<td>Carcinosarcoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adenolipoma</td>
</tr>
<tr>
<td>Stromal</td>
<td>Fibroma</td>
<td>Fibroma</td>
</tr>
<tr>
<td></td>
<td>Fibrosarcoma</td>
<td></td>
</tr>
</tbody>
</table>

*** Most common tumor in female Sprague-Dawley rats
Mouse Mammary Tumor
Mammary Gland Carcinogens

- 216 chemicals associated with mammary tumors
- 29 are produced at >1 million lb/yr
- 35 are air pollutants
- 25 have exposures to >5000 women
- 73 have been present in consumer products or as contaminants of food

Mammary Gland Carcinogens

- Urethane (beer)
- CCl4 (paint remover)
- Isoprene (car exhaust)
- Dichlorvos (flea collars)
- 3,3’=Dimethylbenzidine (pool test kits)
- Ochratoxin A (nuts)
- Doxorubicin (chemotherapeutic agent)

Examples!

http://publius.mu.nu/archives/rats-30nov04-girls.jpg
1. Diethylstilbestrol (DES)

- Synthetic, non-steroidal estrogen
- First synthesized in 1938 from coal tar
- Not patented, inexpensive
- FDA approved for a variety of conditions including:
  - Gonorrheal vaginitis
  - Atrophic vaginitis
  - Postpartum lactation suppression
  - Prostate cancer
  - Prevention of miscarriage
  - Breast cancer
- 5-10 million people exposed 1941-1971
1. Diethylstilbestrol (DES)

• 1971 NEJM Report:
  – Vaginal adenocarcinoma association found in “DES daughters”
    • Mothers started on DES during first trimester
    • Daughters 14-22 diagnosed with vaginal adenocarcinoma

• Further studies
  – 1\textsuperscript{st} generation:
    • ↑ Breast cancer
  – 2\textsuperscript{nd} generation:
    • ↑ Structural abnormalities
    • ↓ Fertility (elevated risk of ectopic pregnancy)
    • ↑ Adenosis
    • ↑ Clear cell adenocarcinoma of vagina/cervix
  – 3\textsuperscript{rd} generation: TBD
Vaginal Adenosis: Mullerian (uterine/vaginal epithelium precursor) type cells found within vaginal wall

Incidence of vaginal adenosis is nearly 100% if the drug was begun during or before the 8th week of pregnancy

http://pathology.catholic.ac.kr/pathology/specimen/female/fg4.jpg
Vaginal clear cell adenocarcinoma

Tumor of vacuolated, glycogen containing (Müllerian-type) cells developing in >0.14% of DES exposed females, with a low spontaneous background incidence.
Vaginal Adenosis and Adenocarcinoma in Mice Exposed Prenatally or Neonatally to Diethylstilbestrol

Retha R. Newbold and John A. McLachlan

Transplacental Toxicology Section, Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina 27709

Clinical findings supported by mouse tox path studies
2. Endocrine Disruptors

- Natural or synthetic chemicals
- Alter normal endocrine signaling by:
  - Binding steroid hormone receptors
  - Altering hormone synthesis/degradation
  - Modifying receptor production/functioning
- First reported in 1946
  - Virgin ewes in Australia
  - Mammary development, cystic endometrial hyperplasia
  - Grazing on red clover containing phytoestrogens
Cystic Endometrial Hyperplasia
Potential endocrine disruptors: A lot of them!!

- **Food products (include phytoestrogens)**
  - Soybeans
  - Legumes
  - Flax
  - Yams

- **Household products**
  - Nonylphenol
  - Octylphenol

- **Pesticides**
  - o,p'-DDT
  - Endosulfan
  - Atrazine
  - Nitrofen
  - Tributyl tin

- **Plastics**
  - Bisphenol A
  - Phthalates

- **Pharmaceuticals**
  - Birth control pills
  - DES
  - Cimetidine

- **Industrial chemicals**
  - Polychlorinated biphenyls (PCBs)
  - Dioxin
  - Benzo(a)pyrene

- **Metals**
  - Cadmium
  - Lead
  - Mercury

- **Byproducts of incineration, paper production, and fuel combustion**
Endocrine Disruptors: Potential Health Effects

- Reproductive Effects/Birth Defects
- Cancer
- Dysfunction
- Heart Disease
- Cognitive Disorders
- Sex Reversal
- Premature puberty
- Altered immune function
Phytoestrogens

• Three chemical classes:
  – Isoflavonoids
    • soybeans and soy products
  – Lignans
    • cereal brans
    • beans
    • flaxseeds
  – Coumestans
    • split peas
    • beans
    • lima beans
    • alfalfa and clover

• “The relevant research is complicated, inconsistent, and inconclusive”

Pro-Phytogen Plus
A natural alternative

Pro-Phytogen Plus is an all natural phyto-estrogen alternative, ideal for women who wish to affect hormone balancing naturally. Pro-Phytogen contains phyto-hormones that are identical to the human estrogens estradiol, estriol, and estrone, containing the co-factors necessary to allow this phyto-complex to be utilized by the human body. It has a stabilizing effect on the entire endocrine system, calming hot flashes and night sweats, and restoring normal sleep patterns for most women.

Pro-Phytogen Plus Capsules are an all-natural, 100% herbal product containing a proprietary combination blend of herbs traditionally used for natural female hormonal balancing and health enhancing qualities. The herbs used are traditional botanicals which are specific to female reproductive functions and overall health and balance that together have been known to balance female hormones and promote a natural sense of well being.

The herbs which create our Pro-Phytogen Plus are Saw Palmetto Extract (berry), Wild Yam Extract (Tuber), Hops (flower), Damiana (leaf), Dong Quai (root), Blessed Thistle (leaf and flower), Kava Kava (root), Dandelion (root), Motherwort (leaf), and Oat Fiber (acts as a natural filler).

Pro-Phytogen Plus Capsules may be used indefinitely as needed for hormone balance and to promote a sense of well being. Pro-Phytogen Plus Capsules may be used on their own or in conjunction with our Pro-Phytogen Cream.

Suggestions for Use
Take one capsule in the morning and one capsule again in the evening with a cool drink of water.

Ingredient Descriptions
3. PhIP

- 2-Amino-3-methylimidazo[4,5-b]pyridine (PhIP)
- Heterocyclic amine
- Potent mutagen/carcinogen
  - Induces DNA adducts and DNA mutations
- Reported to bind ER directly in vitro (Bennion et al, 2005)
- Produced during cooking of meats
- Associated with mammary, colonic, and prostate cancer in rats
### KFC Grilled Chicken Products Contain Carcinogenic Chemicals

*A Report from the Physicians Committee for Responsible Medicine*

**May 2009**

<table>
<thead>
<tr>
<th>KFC LOCATION</th>
<th>PART OF CHICKEN</th>
<th>PhIP</th>
<th>IQ</th>
<th>MeIQ</th>
<th>MeIQx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telegraph Ave, Oakland</td>
<td>Breast</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Telegraph Ave, Oakland</td>
<td>Wing</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Castro Valley Blvd, Castro Valley</td>
<td>Breast</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Castro Valley Blvd, Castro Valley</td>
<td>Wing</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>Hesperian Blvd, San Leandro</td>
<td>Drumstick</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
</tbody>
</table>
Rat Mammary Carcinoma

CANCER Supplement. 1994. 74(3):1063-1069

neoplasia.nci.nih.gov/ccs/Finished/results.html
PhIP and Mammary Cancer

• Effect of dietary fat (Snyderwine et al, 1998)
  – Low fat diet: benign tumors
  – High fat diet: 80% malignant tumors

• Effect of beer marinade (Nozawa et al, 2008)
  – Decreased tumor incidence (38.5% vs 73.3%)
  – Increased activity of GST (detoxification)
  – Decreased activity of CYP1A2 (activation)

....Red wine consumption may also counteract PhIP effects on prostate (Rybicki et al, 2011)
4. Bisphenol A (BPA)

- Organic compound used in plastics and resins
  - Line food cans
  - Plastic containers
  - Dental fillings and sealants
  - Water storage tanks and supply pipes
  - Etc...
- Degrades into monomeric form that leaches into foods and liquids
- Considered a weak environmental estrogen with low affinity for estrogen receptors
  - Evidence strongly binds estrogen-related receptor γ
  - But, can disrupt proper function of ER in variety of tissues
4. Bisphenol A (BPA)

• Developing mammary gland targeted by prenatal BPA exposure
  – Ductal hyperplasia developed in adult rats following prenatal exposure (Murray et al, 2007)
  – Alterations in mammary architecture (increased undifferentiated ducts) demonstrated in adult rats following continuous in utero exposure (Moral et al, 2008)
  – Key proteins involved in signal pathways including cellular proliferation are regulated by BPA (Betancourt et al 2010)
4. Bisphenol A (BPA)

- Other possible effects (many in animal studies)
  - Obesity
  - Thyroid hormone disruption
  - Increased susceptibility to prostate cancer (mice)
  - Promoting growth of neuroblastoma cells
  - Abnormal behavior (interference with memory, learning, and mood)
  - Suppresses DNA methylation (epigenetic effect)
  - Disruption of dopaminergic system
QUESTIONS??
Questions

1. Challenges to studying the female reproductive system toxicologic pathology
   - Cyclic
   - Variability between and within animals
   - Variation in gonad size
   - Delayed gonad atrophy
   - Pregnancy changes
   - Some animals better characterized than others
Questions

• 2. List several differences between female mouse and human anatomy and physiology.
  – Short vs long Fallopian tubes
  – Simplex uterus vs. bicornuate uterus
  – Many ovarian CLs simultaneously (mouse) vs. one (human)
  – Two main mammary glands vs. numerous mammary glands from neck to pelvis
  – Human cyclical epithelial changes occur mostly in uterine epithelium, mouse cyclical epithelial changes mostly in vaginal epithelium
Female Reproductive System

Primate

- Fallopian Tubes (Oviducts)
- Ovary
- Uterus:
  - Body
  - Horns
- Cervix
- Vagina

Rodent

- Ovary
- Uterus:
  - Body
  - Horns
Questions

3. What are the stages of the estrous cycle in animals?
   - Proestrus: ovarian follicles start to grow, endometrium develops
   - Estrus: sexually receptive stage
   - Metestrus: CL starts to form, estrogen influence dies
   - Diestrus: active CL producing progesterone
Questions

4. Which part of the female body is responsible for sex hormone (estrogen, androgen, progesterone) production? Specifically, which cells play the biggest role?

- The **ovary** produces sex hormones
- **Theca interna cells** (cholesterol $\rightarrow$ androstenedione) and **Granulosa cells** (androstenedione $\rightarrow$ estrogen)
Questions

• 5. Name the three toxin types and their general effects on reproductive tissues.

<table>
<thead>
<tr>
<th>Type I (ex., things that decrease GnRH and gonadotropins)</th>
<th>Ovary</th>
<th>Uterus and Vagina</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactivity</td>
<td>Inactivity</td>
<td>↓ FSH/LH</td>
<td>↓ Follicular development</td>
</tr>
<tr>
<td>Type II (sex steroid hormones)</td>
<td>Inactivity</td>
<td>Hyperactivity</td>
<td>↑ Exogenous steroid hormones</td>
</tr>
<tr>
<td>Type III (LH, FSH, or prolactin)</td>
<td>Hyperactivity</td>
<td>Hyperactivity</td>
<td>↑ FSH/LH/GnRH</td>
</tr>
</tbody>
</table>
Questions

6. What are some specific examples of reproductive toxic compounds and the pathology they cause?

- DES: Vaginal clear cell carcinoma
- Phytoestrogens: cystic endometrial hyperplasia (ewes eating red clover)
- PhIP: mammary carcinoma
- BPA: mammary tumors, poorly understood