Fertility Preservation in Children at Risk for Gonadal Dysfunction

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Eunice Kennedy Shriver National Institute of Child Health and Human Development

September 2021 NACHHD Council Meeting
Oocytes Decline Over Time

1,000,000
100,000
10,000
1,000

Primordial Follicle

Birth
Optimal Fertility
Decreased Fertility
End of Fertility
Irregular Cycles
Menopause

Age (yrs)

E.R. TE VELDE ET AL., 1998
Current Standard of Care for Preservation of Gametes in Females

Oocyte Cryopreservation (2013)

Since December 2019: Ovarian tissue Cryopreservation
<table>
<thead>
<tr>
<th>Description</th>
<th>Project Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natl Phys Coop to Preserve Fertility for Female Cancer Patients</td>
<td></td>
</tr>
<tr>
<td>Project Number</td>
<td>Contact PI/Project Leader</td>
</tr>
<tr>
<td>1PL1CA133835-01</td>
<td>CHANG, R JEFFREY</td>
</tr>
</tbody>
</table>
Ovarian Tissue Cryopreservation (OTC)

- Only option for prepubertal children
- No delay in cancer treatment
Ovarian Tissue Removal

- Ovarian tissue is removed laparoscopically
- To date most remove an entire ovary
Ovarian Tissue Transplantation

Orthotopic:
- remaining ovary
- ovarian fossa
- broad ligament, peritoneal pocket

Limitations:
- Loss of ~2/3 of primordial follicles
Function after Ovarian Tissue Transplantation

- Tissue function up to 10 years after transplant
- Function after 14 years of storage
- Multiple pregnancies 2-3 in same patient reported

Table 3
Factors affecting the longevity of ovarian tissue graft

1. Age at the time of cryopreservation
2. Baseline ovarian reserve
3. History of cancer treatment
4. Techniques of ovarian tissue preparation
5. Freezing-thawing protocols
6. Number of cortical sections grafted
7. Transplantation techniques and graft sites
8. Degree of ischemia after transplantation
9. Number of follicles survived in ovarian grafts
Pregnancy After OTC

- Worldwide ~200 live births
  - 1 prepubertal
  - 1 premenstrual
- 23%-41% Live birth rate (51% Live birth after natural conception)

- All pregnancies occurred after transplantation back into the individuals
Ovarian tissue banking is an acceptable fertility-preservation technique and is no longer considered experimental. Ovarian tissue banking is the only method to preserve fertility for prepubertal girls since ovarian stimulation and IVF are not options.
• Only one pregnancy in tissue obtained in a pre-pubertal child
• Research is still needed
• The benefit of this technique in special populations has not been studied
Fertility Preservation in Special Populations
## Non-Oncologic Indications

<table>
<thead>
<tr>
<th>Benign indications</th>
<th>Adult women (≥18 y) (n = 1076)</th>
<th>Girls (1-17 y) (n = 178)</th>
<th>All patients (n = 1254)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic predisposition to POI</td>
<td>17 (1.6)</td>
<td>76 (42.7)</td>
<td>93 (7.4)</td>
</tr>
<tr>
<td>Turner’s syndrome</td>
<td>16 (1.5)</td>
<td>74 (41.1)</td>
<td>90 (7.2)</td>
</tr>
<tr>
<td>Galactosemia and other</td>
<td>1 (.1)</td>
<td>2 (1.1)</td>
<td>3 (.2)</td>
</tr>
<tr>
<td>Gynecologic benign</td>
<td>51 (4.7)</td>
<td>9 (5.1)</td>
<td>60 (4.8)</td>
</tr>
<tr>
<td>Impending ovarian failure</td>
<td>16 (1.5)</td>
<td>8 (4.5)</td>
<td>24 (1.9)</td>
</tr>
</tbody>
</table>
Fertility Preservation after Puberty

Oocyte Cryopreservation

Embryo Cryopreservation
Can cryopreservation allow the girls to “stop the clock” on follicle loss and allow them to thaw the functioning tissue when they are ready to have children?
NICHD Protocol # 000106: Gonadal Tissue Freezing for Fertility Preservation in Girls at risk for Ovarian Dysfunction and Primary Ovarian Insufficiency

Will offer ovarian tissue cryopreservation to:
1. pre-pubertal children with Turner syndrome and classic galactosemia
2. adolescents with recent premature ovarian insufficiency

Modeled after the Oncofertility Consortium Protocol:
80% stored for patient
20% for research
Turner Syndrome
Turners: 1/2500 Girls

Summary

*Turner syndrome* is a chromosomal disorder that affects development in females. It results when a female's cells have one normal X chromosome and the other sex chromosome is either missing or structurally altered.
Frequent Clinical Manifestations of Turner Syndrome

- Short stature
- Webbed neck
- Retrognathic face, micrognathic chin, high-arched palate
- Delayed pubertal development
- Increased carrying angle of forearm
- Short 4th metacarpal, large hands
- Droopy eyelids, strabismus
- Prominent, low-set ears
- Broad chest, widely spaced nipples
- Knob knees
- Nail dysplasia
- Peripheral lymphedema

Other Possible Features:
- Congenital heart defects
- Frequent otitis media
- Hearing loss (usually conductive)
- Hypothyroidism
- Myopia
- Hypertension and dyslipidemia
- Urinary tract infections
- Malocclusion, abnormal tooth development
- Difficulty with mathematics
- Problems with social interaction
- Verbally gifted

*In addition to short stature, one or more, but rarely all, of these features may be present.

For additional information about Turner syndrome, please visit: www.turnersyndromefoundation.org
Turner Syndrome and Premature Ovarian Insufficiency (POI)

Accelerated follicular atresia

- Mid-gestation evaluation of apoptosis by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) analysis in human fetal ovaries
  - 46 XX approximately 3-7% of oocytes were apoptotic (N=16)
  - Turners ovaries: 50-70% of the oocytes were TUNEL positive (N=4)

Turner Ovarian Function:

- Spontaneous Puberty: 36-50%
- Spontaneous Menarche: 14-20%
- Spontaneous pregnancy: ~5%

References:

J Pediatr Endocrinol Metab. 2014 Sep;27(9-10):845-9;
J Pediatr Endocrinol Metab. 2014 Sep;27(9-10):845-9
Horm Res Paediatr 2018;89:90-97
Hum Reprod. 2016 Apr;31(4):782-8
Fertility and Sterility, 2011-06-30, Volume 95, Issue 8, Pages 2507-2510
Turner Syndrome and OTC

Anti-Mullerian Hormone (AMH)
# OTC in Turners

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Age at cryopreservation (y)</th>
<th>OTC center</th>
<th>Karyotype</th>
<th>Spontaneous menarche</th>
<th>AMH (ng/mL)</th>
<th>FSH (IU/L)</th>
<th>Nongrowing follicles per mm² in ovarian cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.0</td>
<td>Edinburgh</td>
<td>45,X</td>
<td>NA</td>
<td>0.73</td>
<td>NA</td>
<td>106</td>
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<tr>
<td>2</td>
<td>8.8</td>
<td>Copenhagen</td>
<td>45,X(161/200, 80%) 46,XX,r(X) (39/200, 20%)</td>
<td>NA</td>
<td>&lt;0.067</td>
<td>4.4</td>
<td>0</td>
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<tr>
<td>3</td>
<td>13.5</td>
<td>Edinburgh</td>
<td>45,X</td>
<td>Yes (11 y)</td>
<td>0.412</td>
<td>5.5</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>13.5</td>
<td>Copenhagen</td>
<td>45,X (7%) 46,XX (93%)</td>
<td>Yes (13 y)</td>
<td>NA</td>
<td>3.1</td>
<td>47</td>
</tr>
<tr>
<td>5</td>
<td>14.4</td>
<td>Copenhagen</td>
<td>46.X, del(X) (p11) (10/10, 100%)</td>
<td>NA</td>
<td>&lt;0.040</td>
<td>4.2</td>
<td>0</td>
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<tr>
<td>6</td>
<td>14.4</td>
<td>Copenhagen</td>
<td>46X (Xq10) (40%) 46,XX (60%)</td>
<td>Yes (14 y)</td>
<td>1.618</td>
<td>4.5</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>14.7</td>
<td>Melbourne</td>
<td>45,X (43%) 46,X,add (X) (q28) (56%)</td>
<td>No</td>
<td>&lt;0.4</td>
<td>82.9</td>
<td>0</td>
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<tr>
<td>8</td>
<td>14.8</td>
<td>Melbourne</td>
<td>45,X (8%) 46,XX (92%)</td>
<td>Yes (13 y)</td>
<td>20.2</td>
<td>5.1</td>
<td>519</td>
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<tr>
<td>9</td>
<td>15.4</td>
<td>Edinburgh</td>
<td>45,X</td>
<td>Yes (14 y)</td>
<td>0.297</td>
<td>5.1</td>
<td>3</td>
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<tr>
<td>10</td>
<td>17</td>
<td>Copenhagen</td>
<td>45,X</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>31</td>
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<tr>
<td>11</td>
<td>17.4</td>
<td>Melbourne</td>
<td>46X, deletion X(p11.21)</td>
<td>Yes (11 y)</td>
<td>3.2</td>
<td>12</td>
<td>0</td>
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<tr>
<td>12</td>
<td>17.8</td>
<td>Copenhagen</td>
<td>45,X (60%) 46,XX (40%)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>13</td>
<td>20.7</td>
<td>Edinburgh</td>
<td>45,X</td>
<td>Yes (13 y)</td>
<td>0.365</td>
<td>0.4</td>
<td>1</td>
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<tr>
<td>14</td>
<td>22.3</td>
<td>Edinburgh</td>
<td>45,X</td>
<td>Postpubertal</td>
<td>0.06</td>
<td>&lt;0.1</td>
<td>3</td>
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<tr>
<td>15</td>
<td>22.4</td>
<td>Copenhagen</td>
<td>45,X</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>13</td>
</tr>
</tbody>
</table>
Classic Galactosemia
Classic Galactosemia

Rare inborn error of galactose metabolism with a birth prevalence of about 1/30 000-60 000. Detected through newborn screening (NBS)

With early diagnosis and rigorous dietary restriction of galactose, most infants survive and grow.

Long term sequelae
- Neurodevelopmental impairment
- Primary ovarian insufficiency which affects >80% of women many of whom present with primary or secondary amenorrhea
Premature Ovarian Insufficiency in Classic Galactosemia

Mechanism of follicle depletion not understood, possible explanations:

- **Direct toxicity of galactose and metabolites on ovarian tissue,**
- **Glycosylation abnormalities causing abnormal function of FSH and FSH receptor,**
- **Direct effect on ovarian function from GALT**
- **Epigenetic changes**
Menstruation and Pregnancy in Classic Galactosemia

- Spontaneous menarche occurred in 25/56 (45%)
- 5 females who sought to conceive, 4 had pregnancies

  Puberty and fertility in classic galactosemia. Endocr Connect. 2021 Jan

- 85 women with POI and classic galactosemia
- 9/21 conceived spontaneously
- 27 mo- 61.3% of couples had conceived

# Ovarian Tissue in Classic Galactosemia

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>genotype</th>
<th>Follicle density</th>
<th>Total tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3&lt;sup&gt;1&lt;/sup&gt;</td>
<td>p.Q188R</td>
<td>2521 Follicles/mm&lt;sup&gt;3&lt;/sup&gt;</td>
<td>48 mm&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.9&lt;sup&gt;1&lt;/sup&gt;</td>
<td>p.Q188R</td>
<td>1444</td>
<td>156 mm&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.7&lt;sup&gt;1&lt;/sup&gt;</td>
<td>p.S236I</td>
<td>1041</td>
<td>100 mm&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>3.9</td>
<td>p.Q188R</td>
<td>631</td>
<td>28 mm&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>4.5&lt;sup&gt;1&lt;/sup&gt;</td>
<td>p.Q188R and p.R333Q</td>
<td>17</td>
<td>36 mm&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>11.7&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Clinical classic</td>
<td>0</td>
<td>12 mm&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>?&lt;sup&gt;2&lt;/sup&gt;</td>
<td>?</td>
<td>0</td>
<td>?</td>
</tr>
<tr>
<td>5 day&lt;sup&gt;3&lt;/sup&gt;</td>
<td>?</td>
<td>“Abundant and normal folliculogenesis”</td>
<td>none</td>
</tr>
<tr>
<td>17&lt;sup&gt;4&lt;/sup&gt;</td>
<td>?</td>
<td>“fibrous stroma almost devoid of follicles”</td>
<td>none</td>
</tr>
<tr>
<td>17&lt;sup&gt;5&lt;/sup&gt;</td>
<td>?</td>
<td>“ovarian stroma, small group of hilar cells and no follicles”</td>
<td>none</td>
</tr>
<tr>
<td>21&lt;sup&gt;6&lt;/sup&gt;</td>
<td>?</td>
<td>“increase in fibrous tissue and that a few hyalinized atretic follicles were present with no intermediate or evolving Graafian follicles”</td>
<td>none</td>
</tr>
</tbody>
</table>
Premature Ovarian Insufficiency in Adolescents
Premature Ovarian Insufficiency (POI)

- POI has been previously referred to as “premature ovarian failure” or “early menopause”
  - 1/10,000 of women under the age of 20 have POI not related to cancer therapy

- In many cases, ovarian function is still present, but in an intermittent and unpredictable manner that can persist for decades
  - Approximately 5-10% of women with POI conceive spontaneously after diagnosis

- The mechanism of POI can be follicular dysfunction or follicle depletion
IVA in ovarian tissue from women with POI:
fragmented the thawed tissue to disrupt the Hippo pathway,
then treated the tissue with PI3 kinase stimulators and PTEN inhibitors.

- 51 patients, 15 had follicular development, 3 had live births and 1 had a miscarriage
(K. Kawamura et al., 2013; Suzuki et al., 2015; Zhai et al., 2016).
IVA has developed based by altering 2 of the pathways:

1. the Hippo signaling pathway via ovarian fragmentation
2. Akt pathway via PTEN inhibitors and PI3 kinase stimulators.
The mechanism for how some primordial follicles are selected and activated to develop while others are able to stay dormant into adulthood remains unclear.

- Manipulation of inhibition and activation of follicles could assist in
  - or assisting women with few remaining follicles to achieve pregnancy
  - prolonging fertility in women
Classic Galactosemia, Turner Syndrome and early POI: Knowledge Gaps

- Can OTC arrest follicle loss?
- What is the quality of follicles and stroma present in the ovarian cortical tissue?
- What is the mechanism of ovarian dysfunction and follicle loss?
  - *Options for possible prevention?*
- What is the optimal age to perform OTC?
- What if the AMH is undetectable?
- Does Laparoscopically removing an ovary further decrease the ovarian follicle pool?
- Is the follicle loss that occurs after transplantation increased in these conditions?
The first aim for project one is to determine if children with Turner syndrome, classic galactosemia and adolescents with recent premature ovarian insufficiency, have ovaries containing viable follicles.

- Evaluate if these correlate with currently known ovarian reserve markers

The second aim will be to elucidate of mechanisms of follicle loss in these conditions.

- Will compare to age matched cadaveric donors
- Will identify crucial signaling pathways regulating follicle activation and loss through collaborations with NICHD Core laboratories using methods including RNA seq and single cell analytics.
Single Nucleus RNA Sequencing

- Single-cell RNA sequencing (sc-RNA seq) techniques have emerged as powerful tools to identify and characterize different cell types in heterogeneous tissues.

- Single-nucleus RNA (sn-RNA seq) sequencing provides an alternative way to obtain transcriptome profiles and can be performed on frozen tissue.

- Using this technology, mechanisms of follicle loss or dysfunction may be elucidated.
Ovarian Nuclear Isolation and snRNA Sequencing

To date:
• Optimized nucleus isolation in bovine and human tissue
• Successful sn-RNA sequencing of human ovarian tissue

Hong Lou, M.D.
“...general knowledge of ovarian tissue biology in this young population remains limited because such tissue is not readily available for investigation”

Francesca Duncan, Ph D
NICHD/Oncofertility Ovarian Tissue Image Database

- Over 2000 images of ovarian tissue collected during OTC
- Working with NCI Artificial Intelligence Core to
  - Develop the ability to have a computer count and classify follicles
  - develop machine learning to evaluate differences in tissues

G. Thomas Brown
National Institutes of Health | NIH · Laboratory of Pathology
MD, PhD
A refined definition of ovarian anatomy will be critical not only for accurately detailing the heterogeneity of cellular composition and function throughout this tissue, but also for standardizing tissue collection and allowing comparisons for both clinical and research purposes.

Meetings on May 7, 25 and June 25
Gross Anatomy Orientation

Use in Clinical Practice

Circumferential (CIRC) described by variable $\phi$

Superior (S) $\phi=270^\circ \rightarrow 360^\circ$

Posterior (P) $\phi=180^\circ \rightarrow 270^\circ$

Inferior (I) $\phi=90^\circ \rightarrow 180^\circ$

Anterior (A) $\phi=0^\circ \rightarrow 90^\circ$

MESOVARIIUM (ATTACHMENT OF OVARY TO BROAD LIGAMENT) $\phi$
Ontology of the Ovary

- reproductive organ
  - female reproductive organ
    - ovary
      - capsule of ovary
      - corpus luteum
      - epithelium of female gonad
    - left ovary
      - median ovary
      - mesenchyme of ovary
      - mesovarium
      - ooblast
      - ovarian cortex
      - ovarian fibroblast
      - ovarian follicle
      - ovarian medulla
      - ovarian surface epithelial cell
      - ovary septum
      - ovary sex cord

- latin term

- definition
  - the gonad of a female organism which contains germ cells

- depicted by

- external definition

Ovarian Anatomy Nomenclature Workshop
Pediatric and Adolescent Gynecology Program
Division of Intramural Research
Cell clustering

[Graph showing cell clustering with UMAP axes and color-coded clusters for vg1a, vg1b, vg2a, and vg2b.]
Pediatric and Adolescent Gynecology

Faculty

- Lauren Damle, MD
- Tazim Dowlut-McElroy, MD
- Jacqueline Maher, MD
- Allison Mayhew

Fellows

- Ariel Cohen, MD
- Swetha Naroji, MD
- Jessica Long, MD
Research Team

Sofia Getachew, MSPM
   Fellowship Program Analyst

Harveen Kaur, MPH
   Clinical Research Coordinator

Hong Lou, MD
   Lab Manager

Students:
   Sarina Hanfling
      IRTA
   Victoria Huynh
      MRSP

• Bo-Hyon Yun, MD, PhD
   Yonsei University College of Medicine (Research Volunteer)
Thank You!

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