

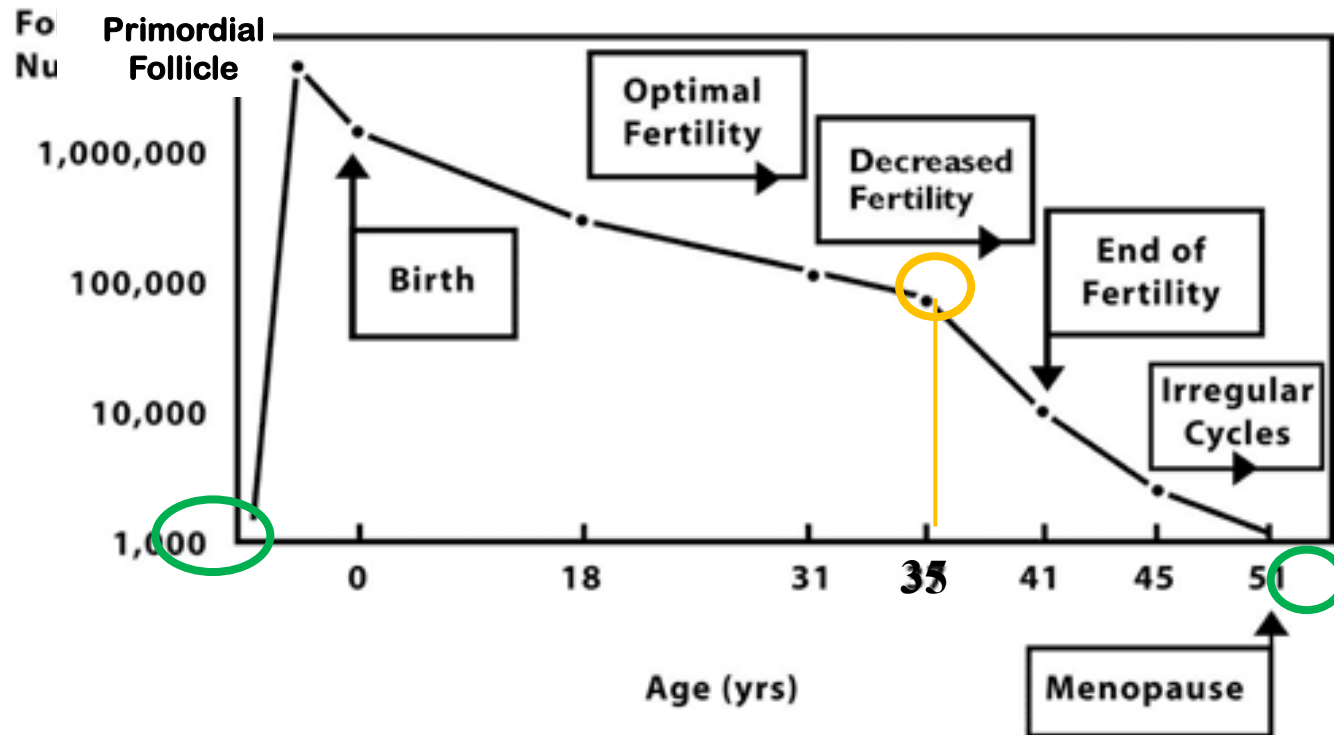
# Fertility Preservation in Children at Risk for Gonadal Dysfunction

**Veronica Gomez-Lobo, MD**

**Director of Pediatric and Adolescent Gynecology Program  
*Eunice Kennedy Shriver* National Institute of Child Health and  
Human Development**

September 2021 NACHHD Council Meeting

# Oocytes Decline Over Time



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





## Project Details



Description >



Details



Sub-Projects

### Natl Phys Coop to Preserve Fertility for Female Cancer Patients

Project Number  
1PL1CA133835-01

Contact PI/Project Leader  
CHANG, R JEFFREY

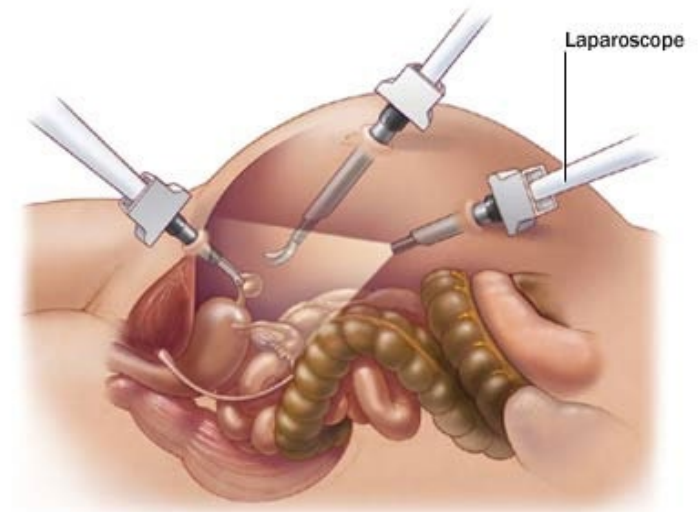
# Ovarian Tissue Cryopreservation (OTC)

- 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

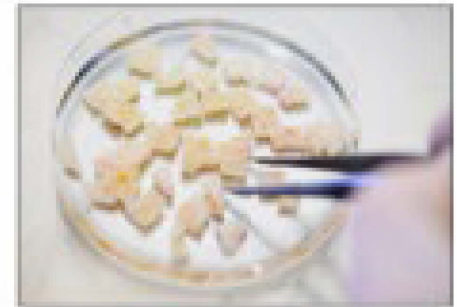




Retrival of one ovary



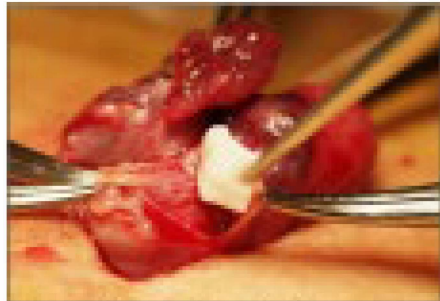
Preparation of cortical tissue



Freezing and storage



Thawing



Transplantation

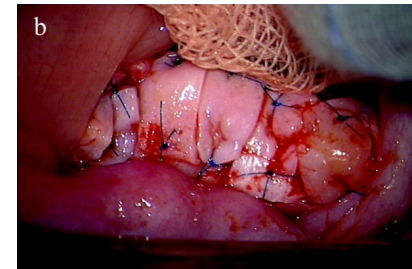
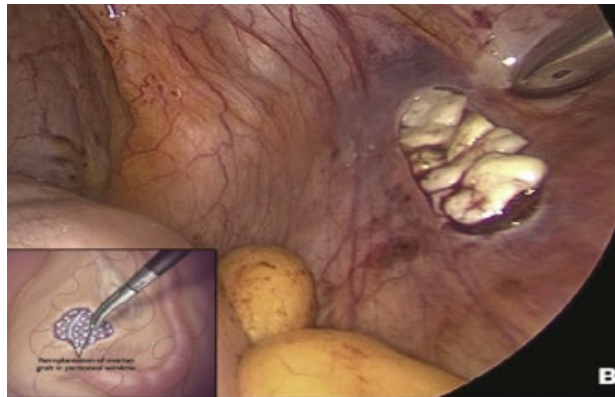
# Ovarian Tissue Transplantation

Orthotopic:

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

Limitations:

- Loss of  $\sim 2/3$  of primordial follicles





# Function after Ovarian Tissue Transplantation

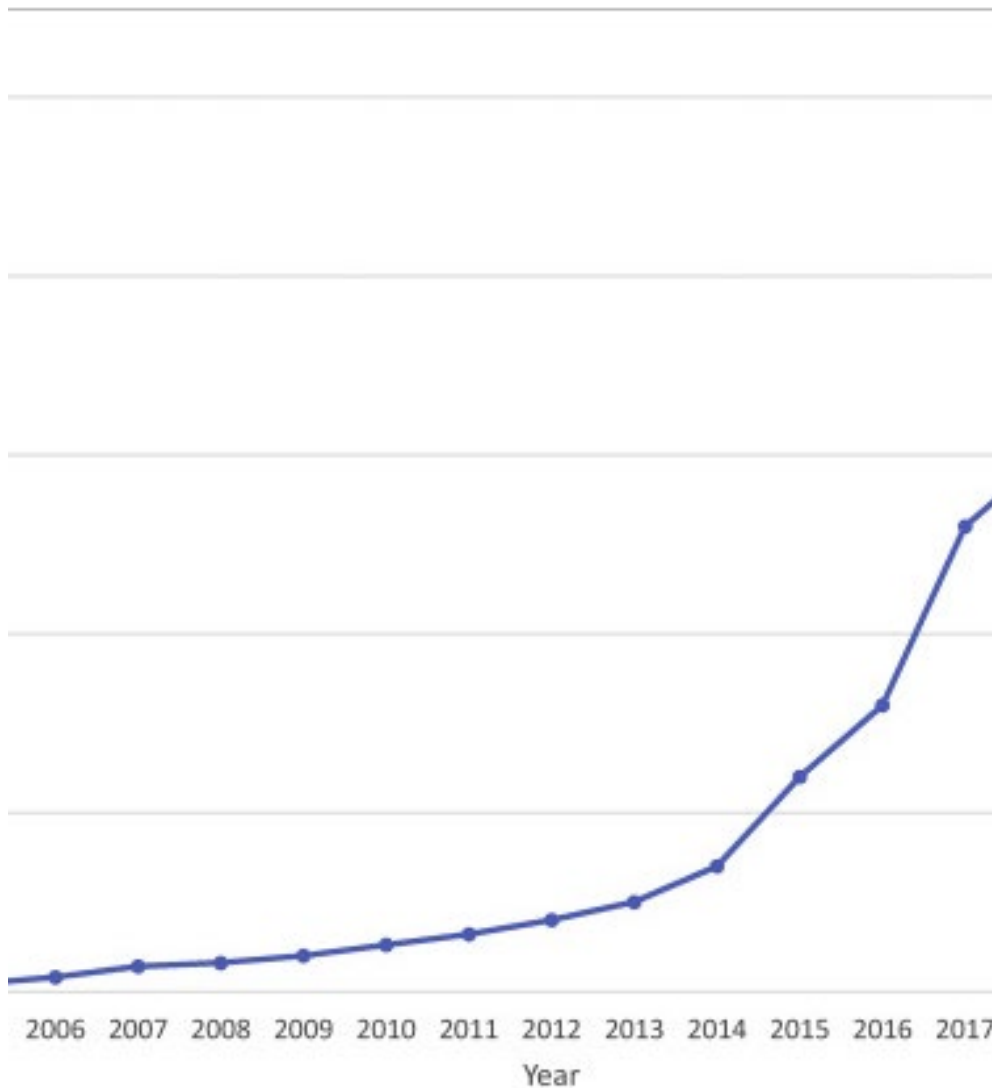
- Tissue function up to 10 years after transplant
- Function after 14 years of storage
- Multiple pregnancies 2-3 in same patient reported

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

# Fertility preservation in patients undergoing gonadotoxic therapy or gonadectomy: a committee opinion

Practice Committee of the American Society for Reproductive Medicine  
American Society for Reproductive Medicine, Birmingham, Alabama

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**

ASRM December 2019

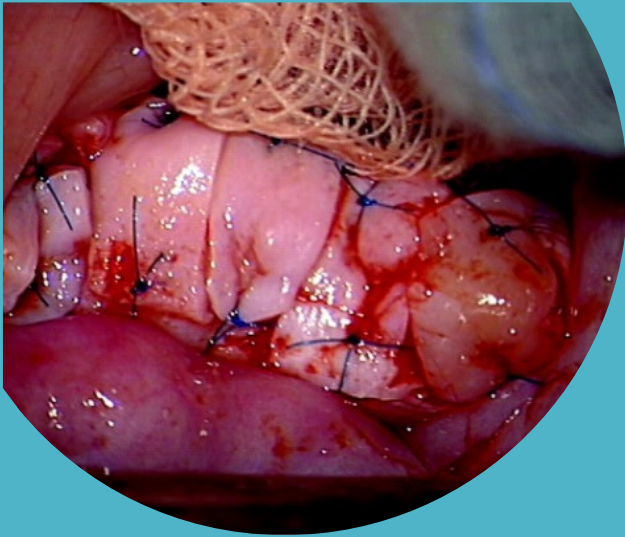


## Ovarian tissue cryopreservation as standard of care: what does this mean for pediatric populations?




Leena Nahata<sup>1,2</sup> · Teresa K. Woodruff<sup>3</sup> · Gwendolyn P. Quinn<sup>4</sup> · Lillian R. Meacham<sup>5,6</sup> · Diane Chen<sup>7</sup> · Leslie C. Appiah<sup>6,7</sup> · Courtney Finlayson<sup>3,8</sup> · Kyle E. Orwig<sup>9</sup> · Monica M. Laronda<sup>10</sup> · Erin E. Rowell<sup>3,11</sup> · Antoinette Anazodo<sup>12,13</sup> · Olivia Frias<sup>14</sup> · Julie Sroga Rios<sup>14,15</sup> · Stacy Whiteside<sup>16</sup> · Veronica Gomez-Lobo<sup>17</sup> · Maggie Dwiggin<sup>18</sup> · Krista J. Childress<sup>19,20</sup> · Holly R. Hoefgen<sup>21</sup> · Jennifer M. Levine<sup>22</sup> · Yasmin Jayasinghe<sup>23</sup> · Molly Moravek<sup>24</sup>

- 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

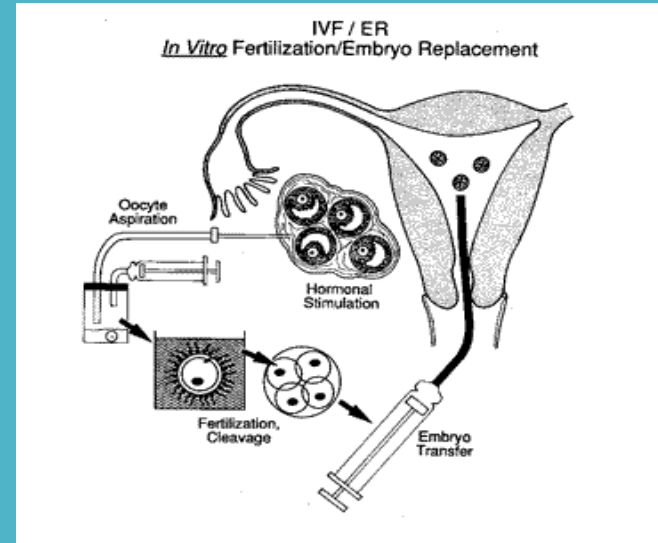
# Fertility Preservation in Special Populations



# Non-Oncologic Indications

	Adult women (≥18 y) (n = 1076)	Girls (1-17 y) (n = 178)	All patients (n = 1254)
<b>Benign indications</b>	<b>278 (25.8)</b>	<b>124 (69.7)</b>	<b>402 (32.1)</b>
Genetic predisposition to POI	17 (1.6)	76 (42.7)	93 (7.4)
 Turner's syndrome	16 (1.5)	74 (41.1)	90 (7.2)
 Galactosemia and other	1 (.1)	2 (1.1)	3 (.2)
Gynecologic benign	51 (4.7)	9 (5.1)	60 (4.8)
 Impending ovarian failure	16 (1.5)	8 (4.5)	24 (1.9)

# Fertility Preservation after Puberty



Oocyte  
Cryopreservation

Embryo Cryopreservation

# Conditions Associated with Accelerated or Early Follicle Loss

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

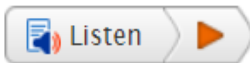
This disease is grouped under: [Numeric sex chromosome variations](#)



## GARD Information Navigator

Try our interactive tool for help finding information, services, experts, financial aid, and more!

## 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\*



Turner Syndrome Foundation

For additional information about Turner syndrome, please visit: [www.turnersyndrome.org](http://www.turnersyndrome.org)



AAACME  
AMERICAN ACADEMY OF CME, INC.



Scherer Clinical Communications

Developed by the Turner Syndrome Foundation, the American Academy of CME, Inc., and Scherer Clinical Communications. Funding for this material was provided through an educational grant from Novo Nordisk Inc.



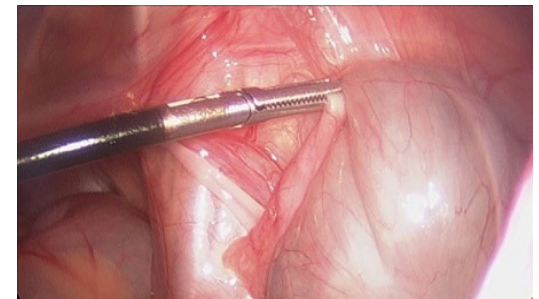
Novo Nordisk

# 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)

Mol Hum Reprod. 2003 Apr;9(4):219-25.



# Turner Ovarian Function:

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

J Clin Endocrinol Metab.1997 Jun;82(6):1810-3.

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

Human Reproduction Open, pp. 1-8, 2019  
doi:10.1093/hropen/hoz016

human  
reproduction  
open

OPINION

## Time to consider ovarian tissue cryopreservation for girls with Turner's syndrome: an opinion paper

Yadava Bapurao Jevu<sup>1,\*</sup>, Tarek Gelbaya<sup>2</sup>, and Muhammad Fatum<sup>3</sup>

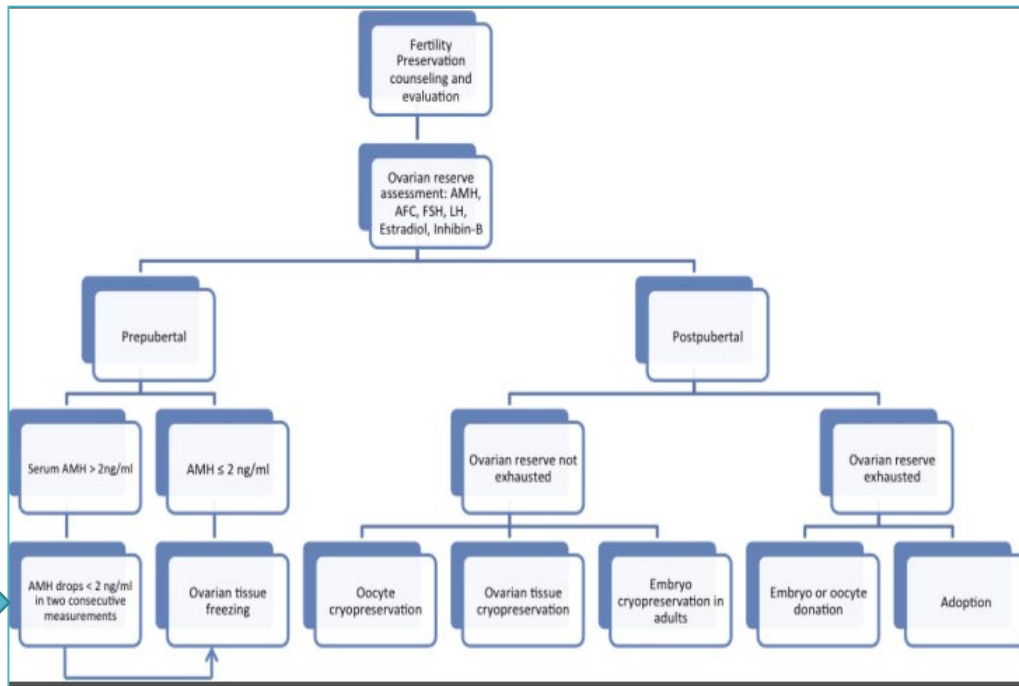
Human Reproduction, Vol.35, No.5, pp. 1061-1072, 2020  
Advance Access Publication on April 29, 2020 doi:10.1093/humrep/deaa007

human  
reproduction

ORIGINAL ARTICLE *Infertility*

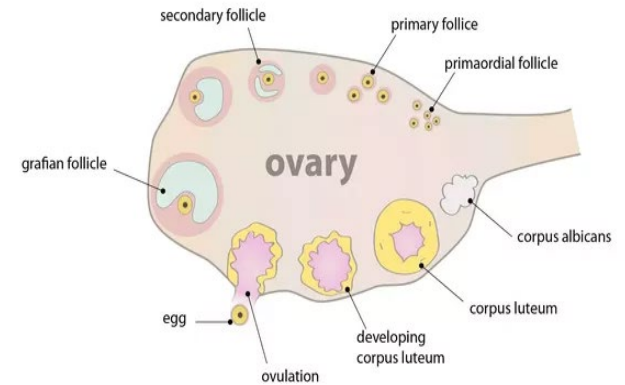
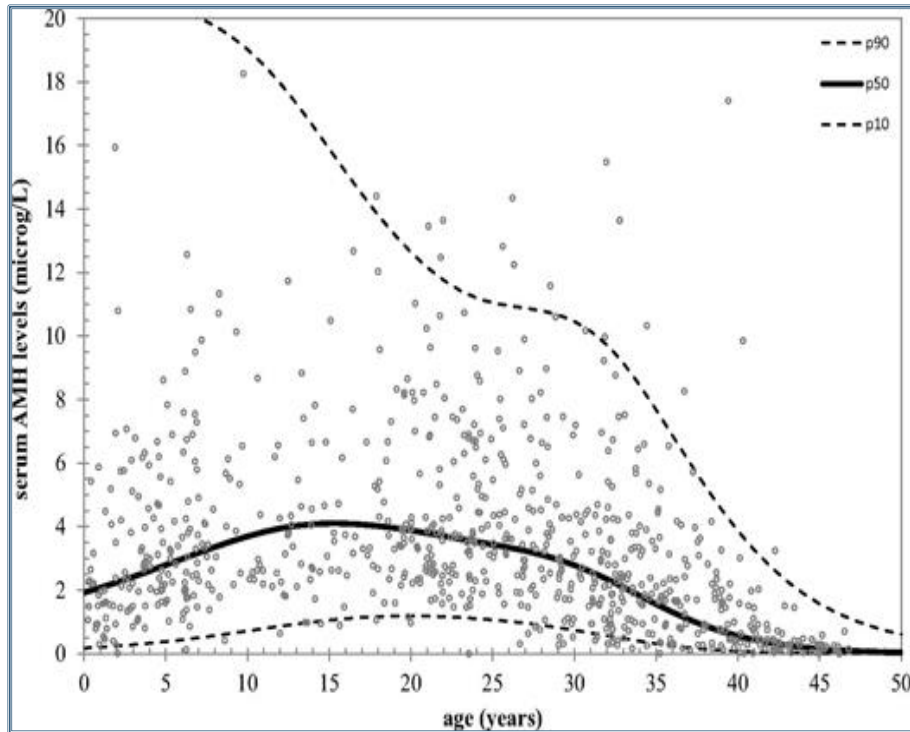
## International consensus: ovarian tissue cryopreservation in young Turner syndrome patients: outcomes of an ethical Delphi study including 55 experts from 16 different countries

M.J. Schleedoorn<sup>1,\*</sup>, B.H. Mulder<sup>1</sup>, D.D.M. Braat<sup>1</sup>, C.C.M. Beerendonk<sup>1</sup>, R. Peek<sup>1</sup>, W.L.D.M. Nelen<sup>1</sup>, E. Van Leeuwen<sup>2</sup>, A.A.E.M. Van der Velden<sup>3</sup>, and K. Fleischer<sup>1</sup>, on behalf of the Turner Fertility expert panel<sup>1</sup>









□ J Pediatr Adolesc Gynecol 2017;29(5):409-416

# Anti-Mullerian Hormone (AMH)



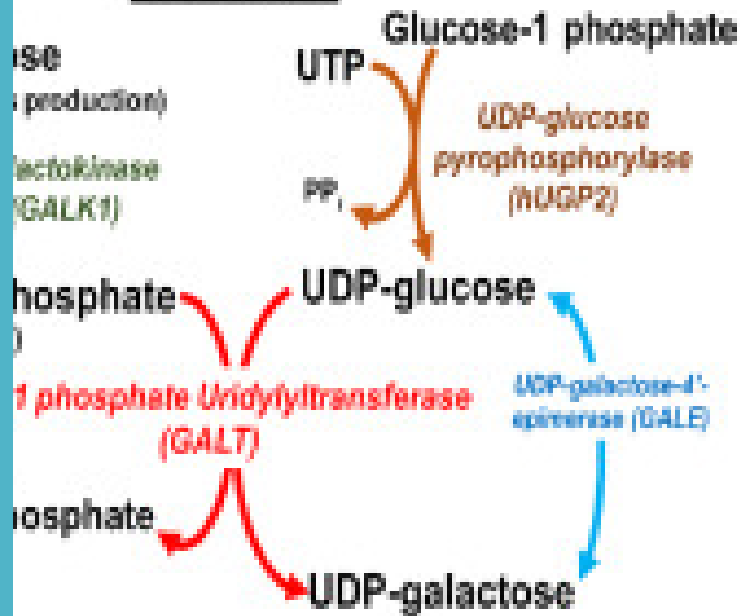


# OTC in Turners

Subject no.	Age at cryopreservation (y)	OTC center	Karyotype	Spontaneous menarche	AMH (ng/mL)	FSH (IU/L)	Nongrowing follicles per mm <sup>2</sup> in ovarian cortex
1	5.0	Edinburgh	45,X	NA	0.73	NA	106 
2	8.8	Copenhagen	45,X (161/200, 80%) 46,X,r(X) (39/200, 20%)	NA	<0.067	4.4	0 
3	13.5	Edinburgh	45,X 46,X,r(X)	Yes (11 y)	0.412	5.5	3
4	13.5	Copenhagen	45,X (7%) 46,XX (93%)	Yes (13 y)	NA	3.1	47 
5	14.4	Copenhagen	46,X, del(X) (p11) (10/10, 100%)	NA	<0.040	4.2	0
6	14.4	Copenhagen	46X i(Xq10) (40%) 46,XX (60%)	Yes (14 y)	1.618	4.5	20 
7	14.7	Melbourne	45,X (43%) 46 X,add (X) (q28) (56%)	No	<0.4	82.9	0
8	14.8	Melbourne	45,X (8%) 46,XX (92%)	Yes (13 y)	20.2	5.1	519 
9	15.4	Edinburgh	45,X 46,X,r(X)	Yes (14 y)	0.297	5.1	3
10	17	Copenhagen	45,X 46,X,i(Xq)	NA	NA	31	0
11	17.4	Melbourne	46X, deletion X(p11.23)	Yes (11 y)	3.2	12	0
12	17.8	Copenhagen	45,X (60%) 46,XX (40%)	NA	NA	NA	138 
13	20.7	Edinburgh	45,X 47,XXX	Yes (13 y)	0.365	0.4	1
14	22.3	Edinburgh	45,X 46,XX	Postpubertal	0.06	<0.1	3
15	22.4	Copenhagen	45,X	NA	NA	13	0

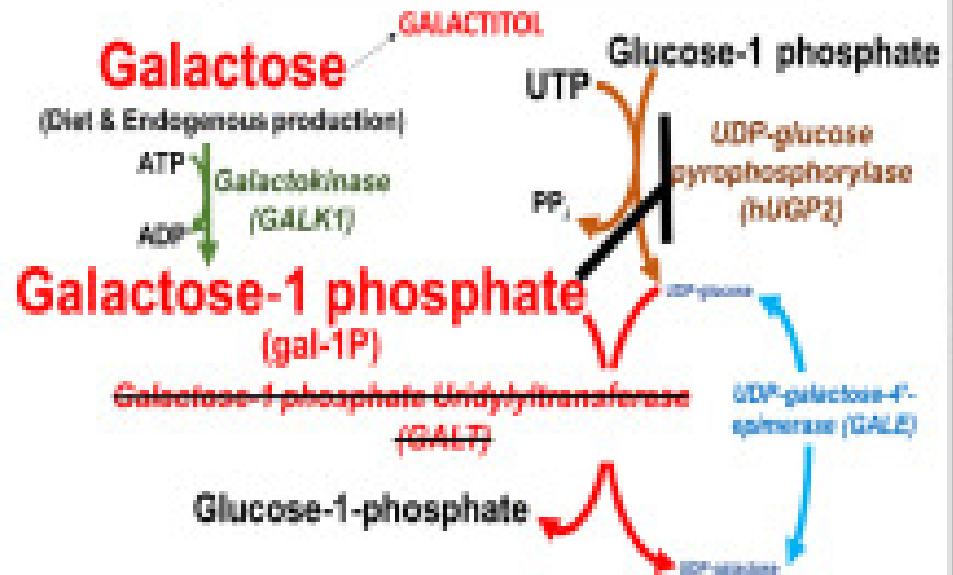
Fertil Steril March 2019

## Normal



B

## Classic Galactosemia



# 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

Fertil Steril. 2017 Jul;108(1):168-174.

# Ovarian Tissue in Classic Galactosemia

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

# 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



## Successful fertility preservation following ovarian tissue vitrification in patients with primary ovarian insufficiency

Nao Suzuki<sup>1</sup>, Nobuhito Yoshioka<sup>1</sup>, Seido Takae<sup>1</sup>, Yodo Sugishita<sup>1</sup>, Midori Tamura<sup>1</sup>, Shu Has<sup>1</sup>, Kazuhiro Kawamura<sup>1</sup>\*

## In Vitro Activation of Follicles and Fresh Tissue Auto-transplantation in Primary Ovarian Insufficiency Patients

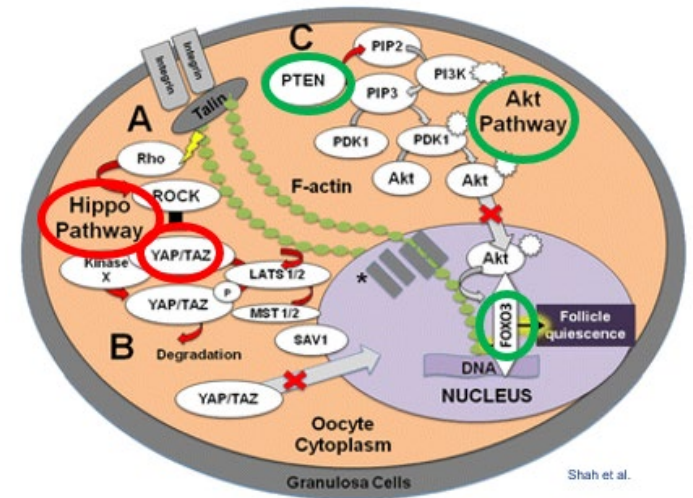
Jun Zhai,\* Guidong Yao,\* Fangli Dong,\* Zhiqin Bu,\* Yuan Cheng, Yorino Sato, Linli Hu, Yingying Zhang, Jingyuan Wang, Shanjun Dai, Jing Li, Jing Sun, Aaron J. Hsueh, Kazuhiro Kawamura, and Yingpu Sun

-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).

# In-Vitro Activation



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.



Jacqueline Yano Maher, MD

# Follicular Activation and Inhibition

- 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?

## NICHD Protocol # 000106: Gonadal Tissue Freezing for Fertility Preservation in Girls at risk for Ovarian Dysfunction and Primary Ovarian Insufficiency

- 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.



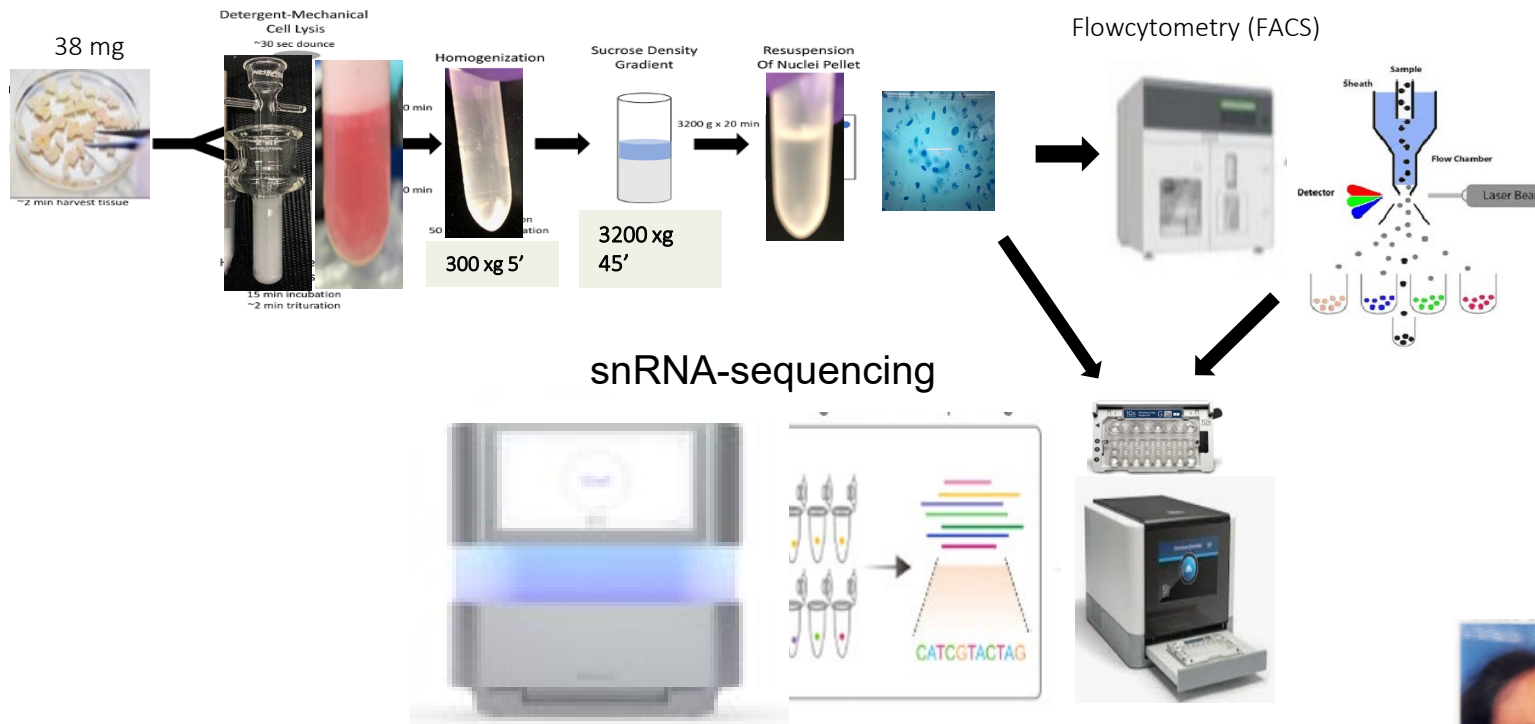
DIVISION OF  
INTRAMURAL  
RESEARCH

Molecular Genomics Core



Ryan Dale, PhD

# 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.

# Establishing the “Normal”

**“... 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



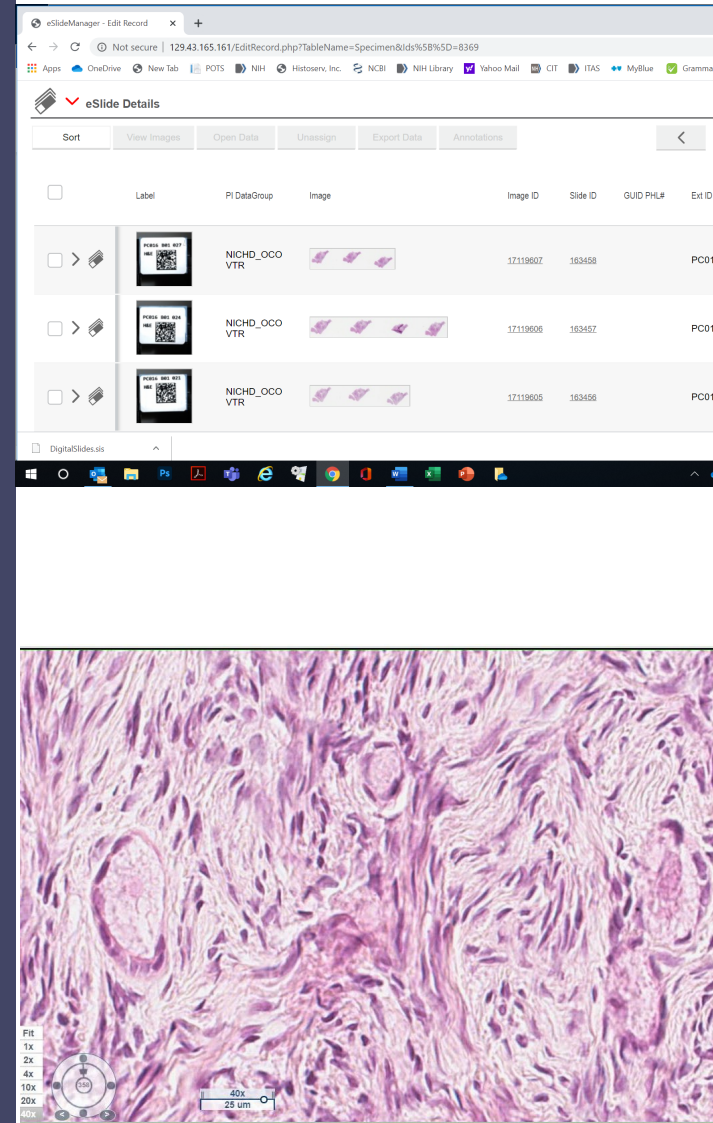
# NICHD/Oncofertility Ovarian Tissue Image Database

- 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.**



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

## **Ovarian Anatomy Nomenclature Workshop**

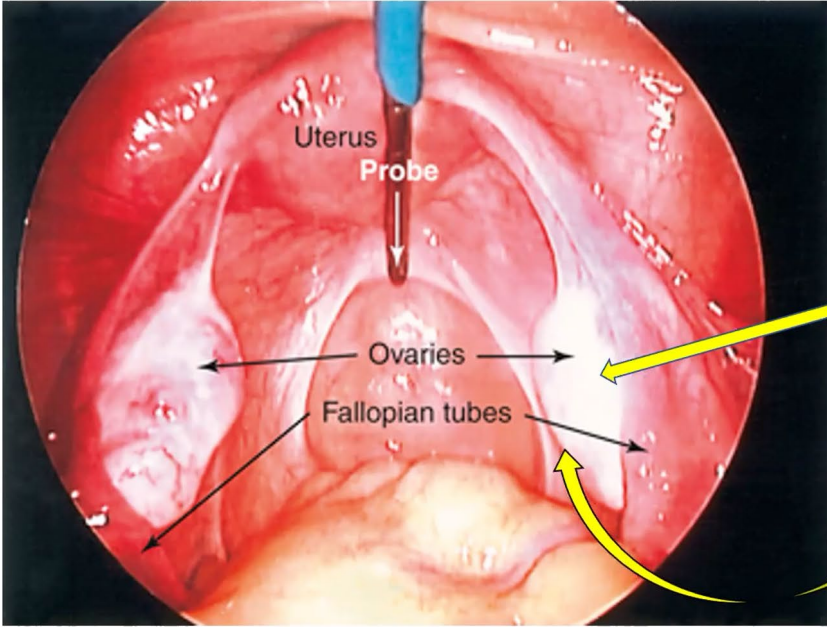
Pediatric and Adolescent Gynecology Program

Division of Intramural Research

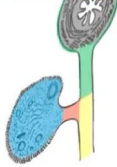
**Meetings on May 7, 25 and June 25**

# Gross Anatomy Orientation

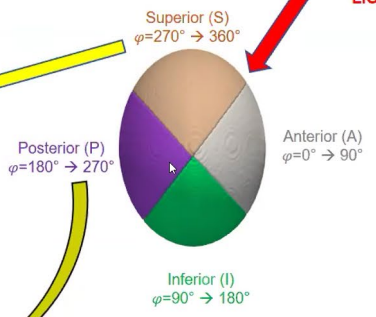
Use in Clinical Practice



Circumferential (CIRC)  
described by variable  $\varphi$



MESOVARIUM  
(ATTACHMENT OF  
OVARY TO BROAD  
LIGAMENT)  
 $\varphi$



# 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

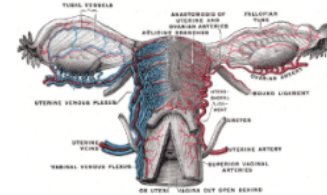
## latin term

ovarium [ <http://en.wikipedia.org/wiki/Ovary> ]

## definition

the gonad of a female organism which contains germ cells

## depicted by



## external definition

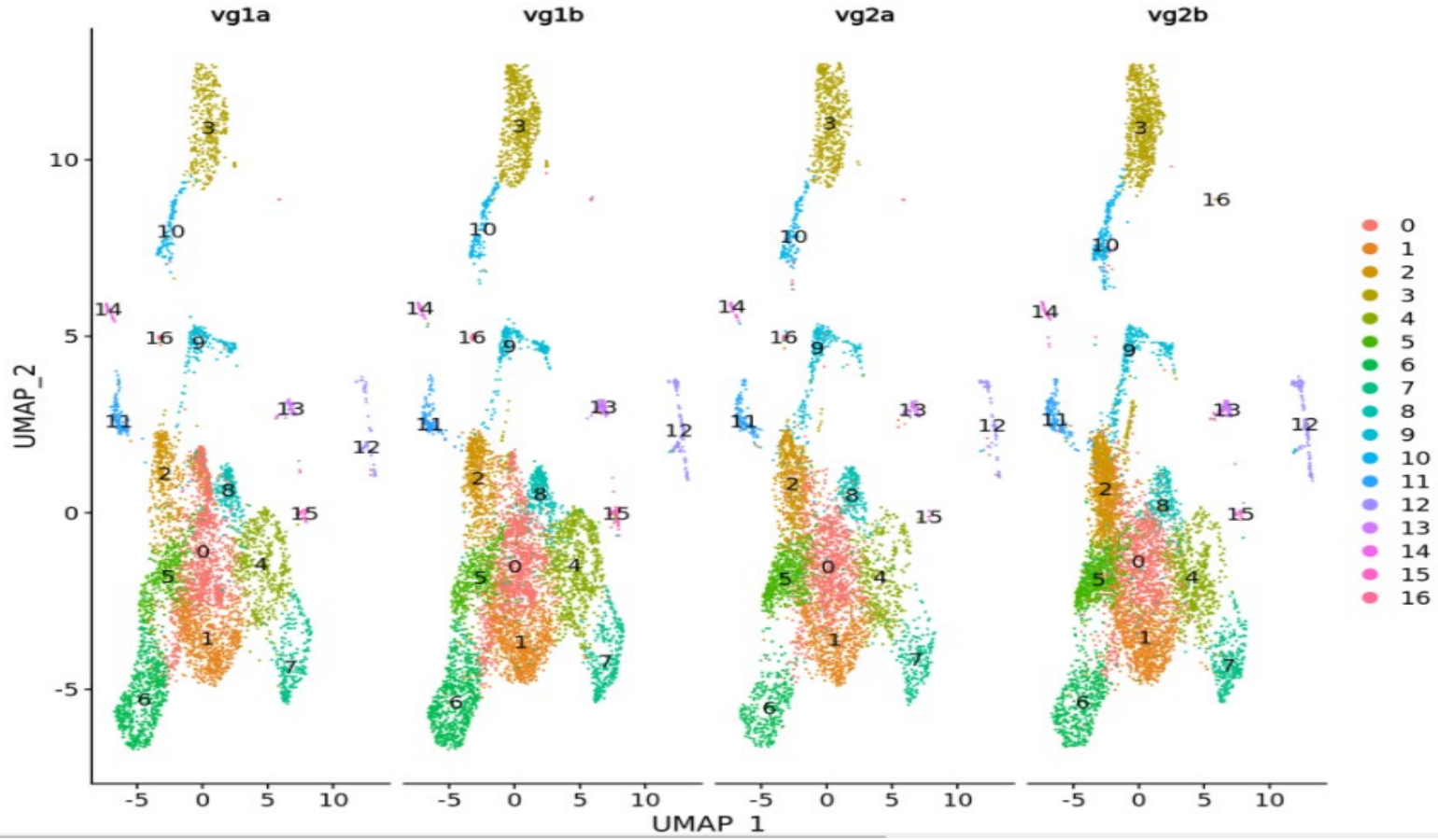
**NIH** Eunice Kennedy Shriver National Institute  
of Child Health and Human Development

**Ovarian Anatomy Nomenclature Workshop**

Pediatric and Adolescent Gynecology Program

Division of Intramural Research

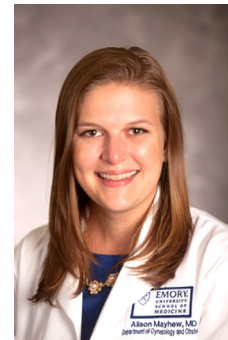
# Cell clustering



# 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



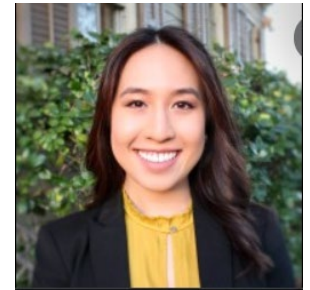
## Students:

Sarina Hanfling

*IRTA*

Victoria Huynh

*MRSP*



- **Bo-Hyon Yun, MD, PhD**  
Yonsei University College of  
Medicine (Research Volunteer)





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

*Healthy pregnancies. Healthy children. Healthy and optimal lives.*

Thank You!

Veronica.Gomez-lobo@nih.gov