# Fertility Preservation in Breast Cancer Patients: Issues of Timing

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# Cancer Treatment and Fertility

- Life preserving treatments

  Chemotherapy
  Radiation treatment
  Surgery

- Can threaten fertility

- Fertility preservation options
- Impact of fertility treatments
- Tamoxifen and survivorship
- Oncofertility in clinical practice

## ❖ Who is at Risk?

- More than 1.4 million new cancer patients are diagnosed in U.S. annually
- 10 million new cases diagnosed globally
- 10% are in their reproductive years (up to age 45)
- 11,630 children in 2013, 83% expected to survive
- Approximately 11% of breast cancer patients are diagnosed under the age of 45






# Oncofertility: State of the Problem



- Fertility preservation for men with cancer: option for decades
- Women with cancer have fewer options
- Three main gaps
  - Information gap
    - Lack of fundamental knowledge about impact of treatment for patients and providers
  - Data gap
    - Precise gonadotoxicity of cancer drugs unknown
  - Option gap
    - Communicating recent scientific and medical breakthroughs

# Effect of Infertility on Survivorship

- Infertility associated with diagnosis of depression 2X that of fertile population
- Adult survivors of childhood cancer report increased anxiety regarding finding a mate, not prepared for long-term side effects of treatment
- Overall, young men and women have equal concerns regarding fertility
- Young breast cancer survivors: 57% report substantial concern about fertility, 29% concerns influenced treatment decisions

Duffy C, Allen S, The Cancer Journal, 2009
Schover L, Medical and Pediatric Oncology, 1999
Syrjala K et al, JCO, 2007

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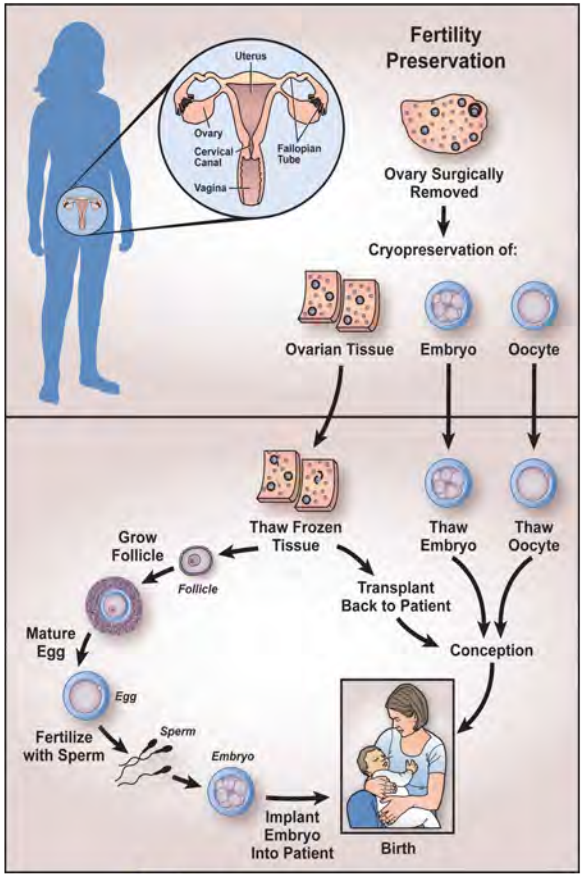
# Options for Women and Girls

- Natural Pregnancy
- Embryo/Egg Banking
- Ovarian Cryopreservation
- Ovarian Tissue Transplantation
- Donor Egg, Adoption
- Surrogacy

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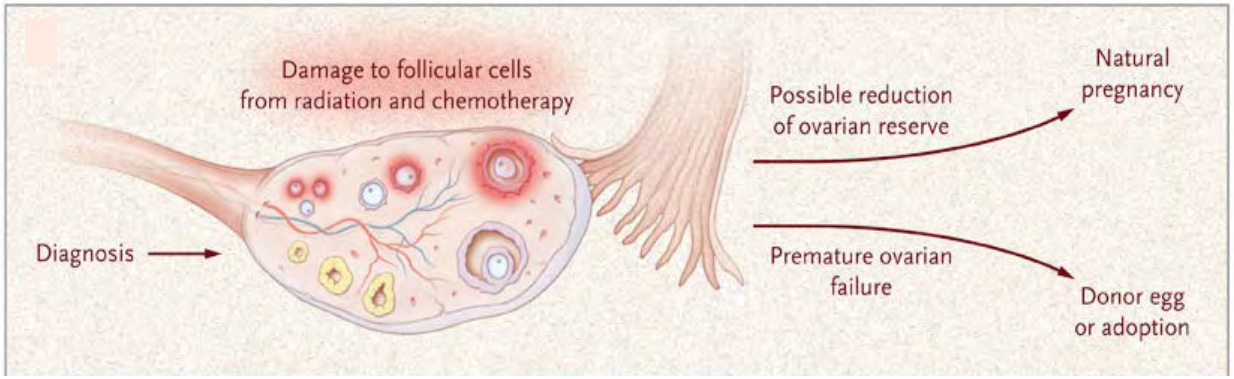
# Early Stage Breast Cancer Management

- Early stage patients with favorable biology: radiation and antiestrogen therapy (5 - 10 years)
- Fertility measures should not be taken during radiation therapy
- Indirect evidence supports delay in antiestrogen treatment to allow for pregnancy




*Jeruss and Woodruff, NEJM, 2009*

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# Chemotherapy Indicated: Impact on Fertility

- Determination ovarian reserve complex- basal levels of AMH, FSH, inhibin B, estrogen, antral follicle count
- Most regimens include alkylating agents- pose greatest risk for ovarian failure, OR 3.98 compared to unexposed patients
- 12M amenorrhea
  - AC (n=75) 44% ≤ age 40; 81% > age 40
  - AC+T (n=116) 61% ≤ age 40; 85% > age 40
- Amenorrhea permanent for AC and AC+T regimens
  - ≤ age 40 60% (n= 52)
  - > age 40 82% (n= 23)
- Trastuzumab: oligo/anhydramnios
- Effects of tamoxifen on the ovary thought to be reversible though some controversy

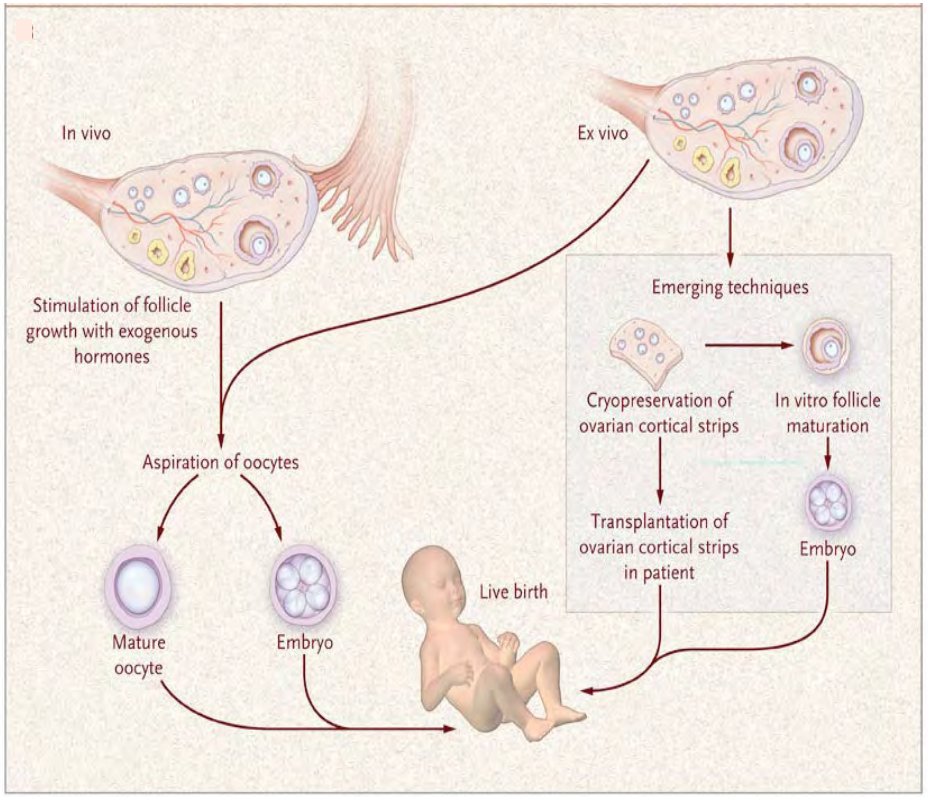
Tham et al. Am J Clinical Oncology, 30, 2007
Han et al. Breast Cancer Res Treat, 115, 2009
Swain et al. Breast Cancer Res Treat, 113, 2009
Pant et al. J Clin Oncol, 26, 2008.

# Fertility Preservations Options: Chemotherapy Indicated

## Hormonally Based Options

- Patient may elect to undergo hormone stimulation, chemotherapy may start 1 month from diagnosis

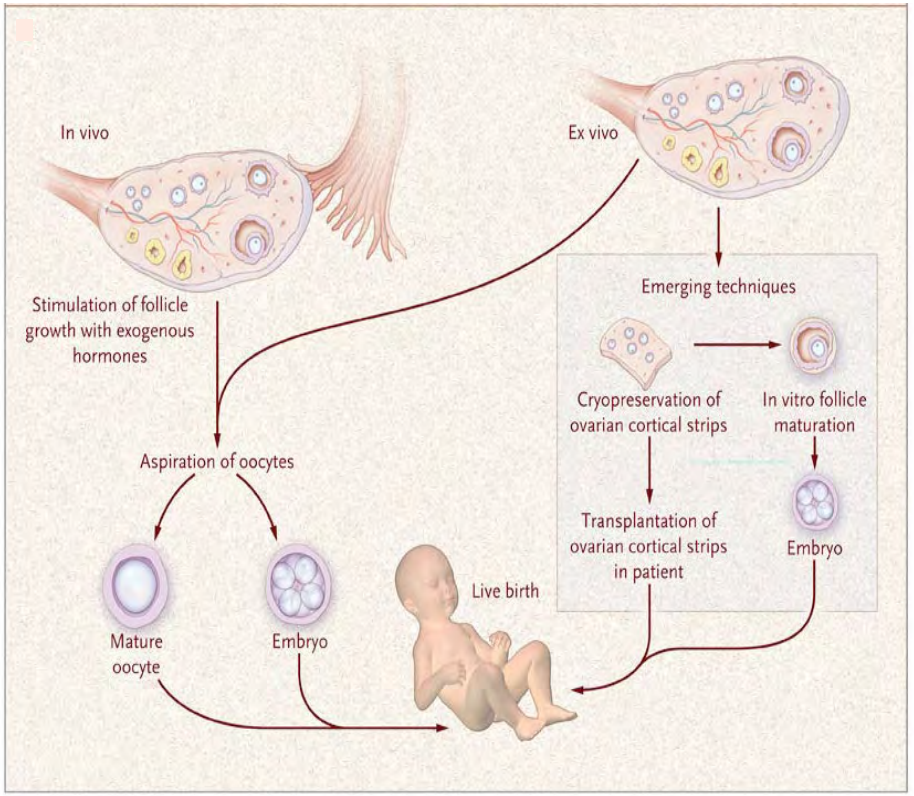
- Cryopreservation
  - Embryo
  - Mature oocyte



# Hormone Independent Options

## Hormone Independent

- Ovarian tissue retrieved at time of diagnosis
  - Cryopreserve cortical strips or aspirated oocytes
    - In vitro follicle maturation
    - Autologous transplantation of cortical strips
- Outcomes
  - Natural cycle IVF: success rate low
  - IVFM: live births in murine model, human studies progressing
  - Transplantation: + live births, risk for re-exposure of cancer cells, not for BRCA positive patients




- Donor egg, surrogacy
- Adoption

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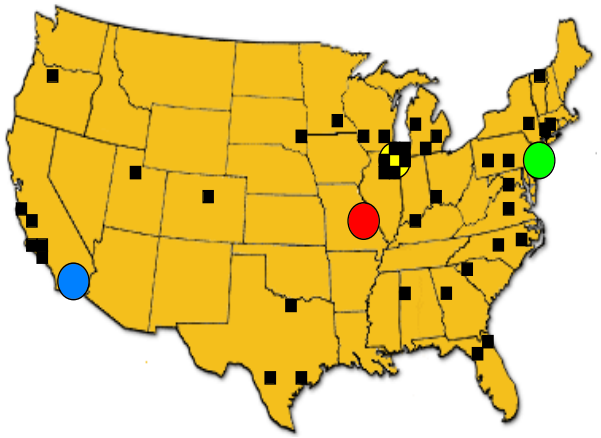
# Multidisciplinary Approach

- Group of clinicians, scientists, psychologists who meet regularly to discuss case management
- Evaluate patient options in context of treatment and diagnostic concerns (BRCA patients)
- Help establish ethical guidelines for fertility preservation
- Forum to discuss state of science and share established protocols and research initiatives

## National Physicians Cooperative



**NPC Core Centers**

- Northwestern University/Children's Memorial Hospital, Chicago, IL
- University of California - San Diego, CA
- University of Missouri
- University of Pennsylvania - Children's Hospital of Philadelphia, PA
- NPC Allied Centers

# Young Breast Cancer Patients

- 200,000 women diagnosed each year; 25,000 younger than 45
- Diagnosis often traumatic for younger patients- isolation
- Initial consultation focused on explaining diagnosis, treatment plan, and reassurance
- Can be difficult to also discuss fertility preservation
- Patients present in all phases of life from single to committed relationships

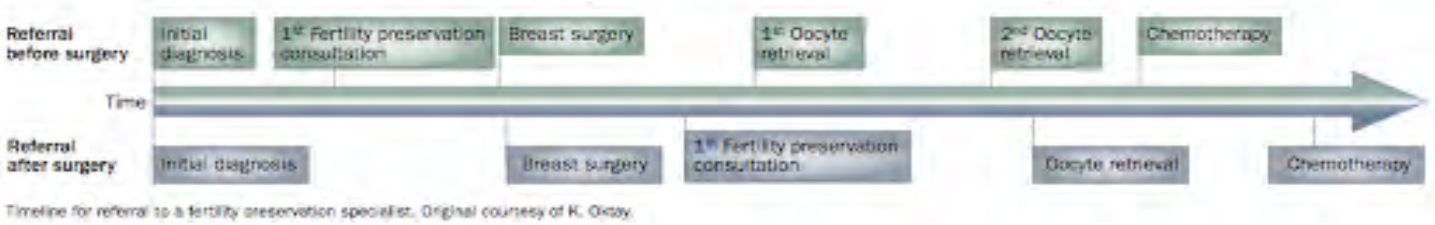
# The Initial Consultation

- Establish a connection with patient
- Take history and review medical record
- Physical exam
- Review workup and diagnosis
- Discuss treatment options
- Establish plan of care
- Address fertility concerns and discuss options for fertility preservation

# Critical Factors for Success

- Primary treating physician supportive of fertility preservation- timeliness is essential
- Patient navigator readily available to meet/call patient
  - Patients concerned about moving ahead with cancer care
- Reproductive specialist on call for fertility "emergencies"
- Entire multidisciplinary oncology team willing to modify plan to accommodate fertility preservation when possible
- Ideally, fertility preservation should be seamlessly integrated into care

# Timeline for Treatment

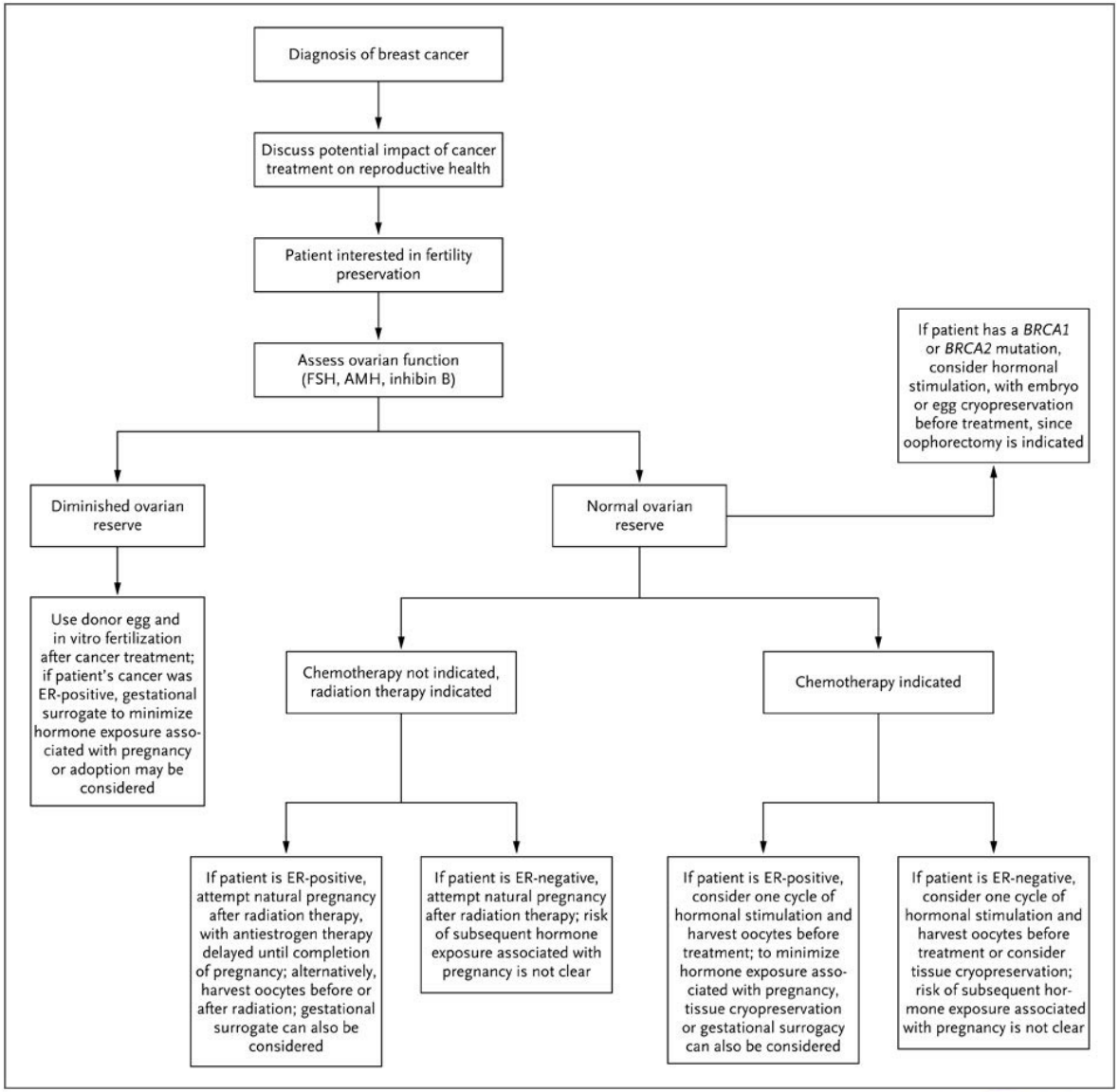



Timeline for referral to a fertility preservation specialist. Original courtesy of K. Oktay.

- Study of 93 patients: 35 referred for fertility preservation prior to surgery and 53 post surgery
- Mean age 35
- Higher percentage of patients who had referral prior to surgery underwent 2 retrieval cycles resulting in larger number of oocyte and embryos for these patients
- Controversial interval between surgery and chemotherapy (9 weeks)
- Recommendation: allow for one cycle prior to initiation of treatment

Lee S et al. JCO, 2010.

# Algorithm of care for breast cancer patients




Jeruss and Woodruff, NEJM, 2009

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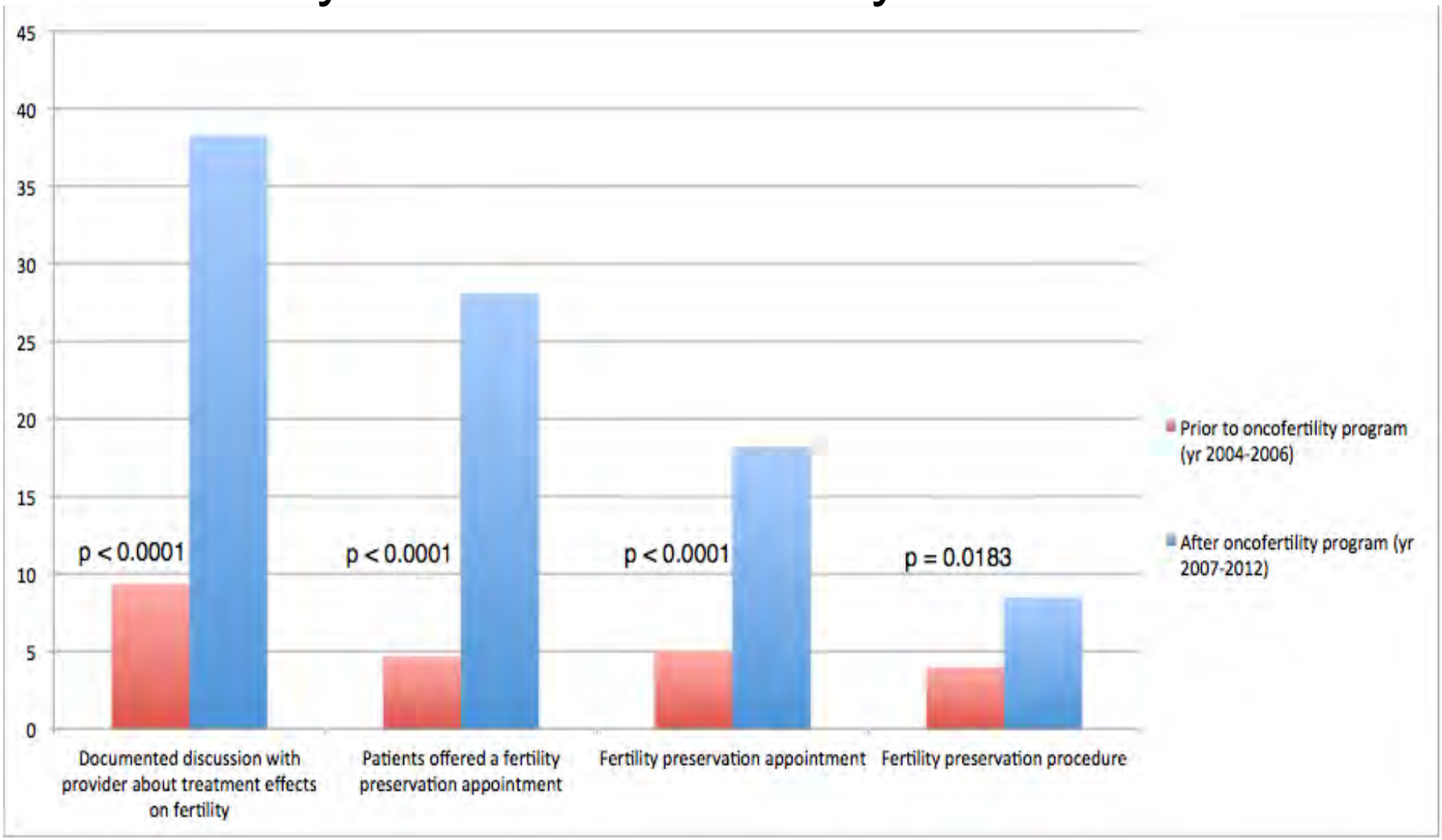
# Historic Comparison of Fertility Consultations Physician-Patient Fertility Discussion




Llarena N, Estevez S, et al. *J Surg Oncol*, 2017.

# Impact of Fertility Concerns on Tamoxifen Initiation and Persistence

# Processing Risk and Benefit

- Chemotherapy reduces recurrence risk by 25%, mortality 25-35%
- Oncotype Dx for node negative ER+ breast cancer patients
  - Low recurrence score: chemo of little benefit
  - Intermediate: benefit of chemo unclear
  - High: benefit likely greater than risk
- Chemo compliance 70-100%, despite substantial side effects
- Tamoxifen: Meta-analysis of 55 clinical trials
  - Significant decrease in annual recurrence risk, mortality, and contralateral breast cancer
- 20% patients fail to adhere during 1st year of treatment, at 5 years, up to 50% discontinued treatment
- Younger women have lower rates of adherence when compared to women ages of 50-70
- Recent study found women < 40 had highest risk of tamoxifen discontinuation
- WHY???

Mittal et al. *Patient Educ Couns.* 2005.
Hershman et al. *J Clin Oncol.* 2010.
The Oncologist 2011,16:742-751.

# Treatment Duration and Outcomes

**Table 1** Percentage of Recurrence-Free Patients

| Average Duration of Therapy (Years) | Nodal Status | Treatment (N) | Years Since Randomization | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| 1 | Negative | Tamoxifen (1079) | 97.4 | 93.5 | 89.4 | 85.6 | 82.7 | 79.6 | 77.8 | 76.7 | 75.9 |
| | | Control | 96.3 | 92.2 | 86.4 | 83.9 | 79.9 | 76.8 | 74.4 | 72.9 | 71.2 |
| 1 | Positive | Tamoxifen (2685) | 92.0 | 80.3 | 71.3 | 63.7 | 58.3 | 54.1 | 50.8 | 47.8 | 45.4 |
| | | Control | 88.6 | 74.3 | 63.7 | 56.1 | 50.2 | 46.1 | 43.0 | 40.4 | 37.9 |
| 2 | Negative | Tamoxifen (3131) | 98.3 | 95.0 | 92.1 | 89.48 | 87.1 | 85.3 | 83.0 | 81.5 | 79.7 |
| | | Control | 97.1 | 91.9 | 87.6 | 3.9 | 81.4 | 79.0 | 77.3 | 76.2 | 74.6 |
| 2 | Positive | Tamoxifen (4180) | 92.1 | 83.3 | 76.5 | 69.05 | 63.5 | 59.6 | 56.5 | 54.1 | 51.1 |
| | | Control | 88.0 | 73.3 | 62.6 | 6.1 | 51.4 | 47.4 | 45.3 | 42.7 | 40.7 |
| About 5 | Negative | Tamoxifen (2611) | 98.0 | 95.0 | 92.1 | 89.4 | 87.4 | 85.5 | 84.0 | 82.3 | 80.5 |
| | | Control | 95.2 | 87.9 | 82.2 | 78.2 | 74.9 | 71.9 | 69.3 | 67.4 | 65.6 |
| About 5 | Positive | Tamoxifen (1127) | 95.1 | 88.9 | 83.9 | 79.3 | 75.6 | 73.1 | 69.3 | 65.4 | 61.6 |
| | | Control | 90.4 | 76.8 | 69.4 | 63.1 | 58.3 | 54.9 | 52.6 | 49.2 | 46.6 |

Data from the EBCTCG showing patient outcomes for 1, 2, and 5 years of tamoxifen therapy. Percentage of recurrence-free patients by year since randomization. Patients with 5 years of therapy had better outcomes, particularly in the node-positive groups. From Gradishar and Hellmund, 2002.

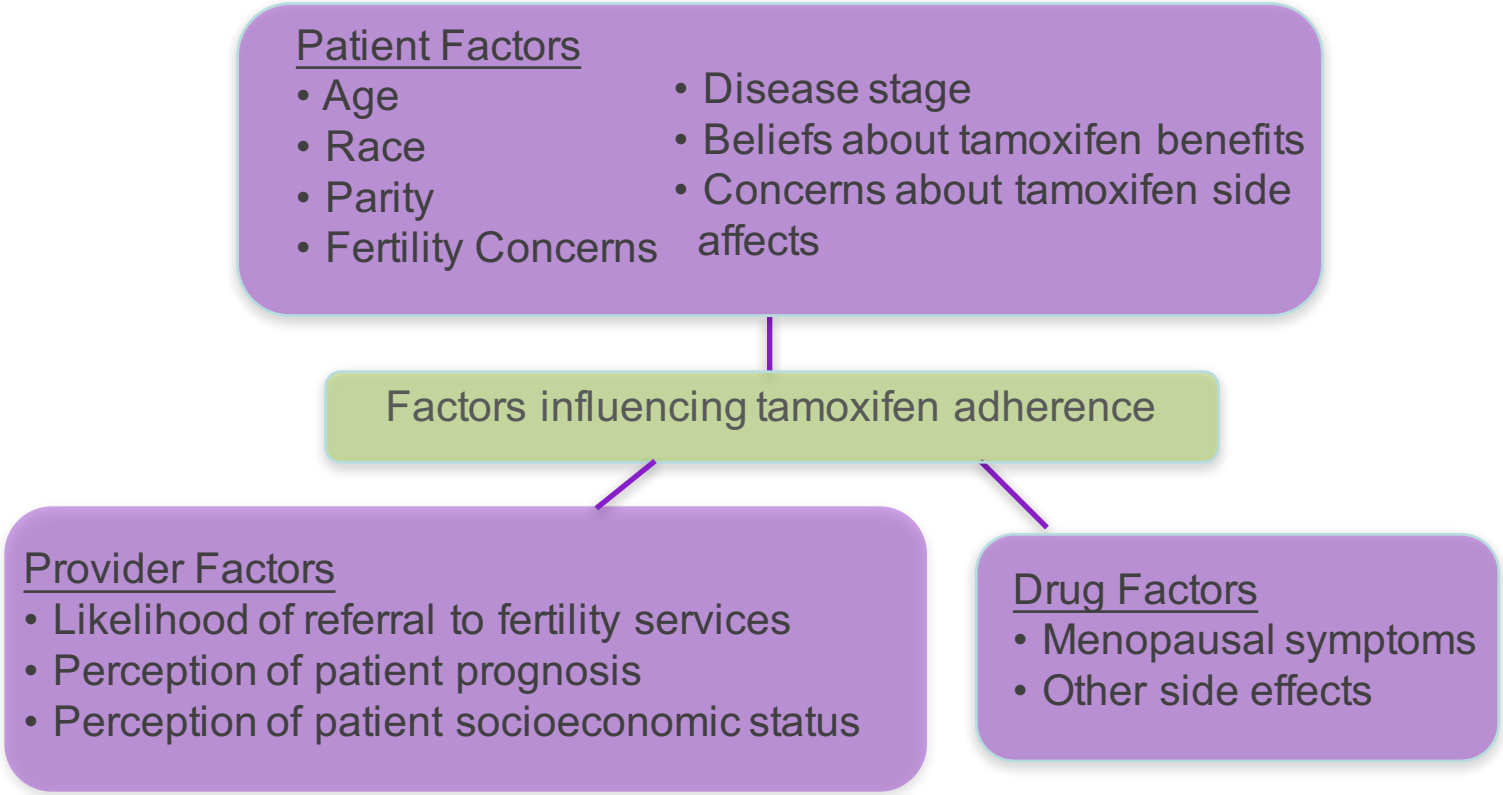
- In order for young breast cancer patients to realize full benefit of tamoxifen therapy, which may **significantly improve** outcomes, we must understand factors that impact adherence in this patient population.

# Tamoxifen and Fertility

- Tamoxifen is a teratogen and pregnancy should be avoided during the recommended 5 -10 year duration of therapy
  - As fertility begins to decline significantly after the age of 35, the considerable length of recommended therapy may be a critical deterrent
- Studies on adjuvant tamoxifen adherence have not examined fertility concerns as a potential reason for noncompliance

# Impact of Fertility Concerns on Tamoxifen Use

**Aim:** To evaluate patient- and provider-level factors that influence tamoxifen use among breast cancer patients age 45 and younger




**Hypothesis:** Fertility concerns may contribute to the poor tamoxifen use observed among young breast cancer patients.

# Factors Associated with Non-initiation of Tamoxifen

## Univariate Factors

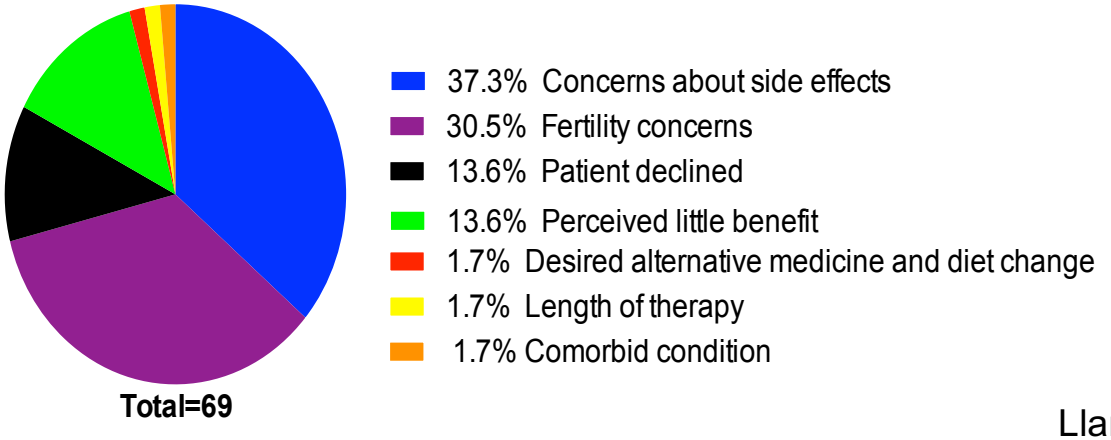
- Smoking history (p = 0.04)
- Parity (p = 0.038)
- Fertility concerns ( p = 0.009)
- Surgery type (p = 0.049)
- Chemotherapy (p = 0.012)
- Radiation (p = < 0.001)
- Stage (p = < 0.001)

**Multivariate Model of Non-initiation of Tamoxifen**

| Patient Characteristic | Odds Ratio | 95% Confidence Interval |
|---|---|---|
| Stage 0 cancer | 28.07 | 10.99 – 71.64 |
| Declined XRT | 7.97 | 3.15 – 20.15 |
| Desired fertility at diagnosis | 5.04 | 2.29 – 11.07 |
| No chemotherapy | 5.02 | 1.92 - 13.10 |

Multivariate logistic regression model of tamoxifen non-initiation and delayed initiation among premenopausal patients age ≤ 45 diagnosed with stage 0-III ER+ and/or PR+ breast cancer from 2007-2012 (n = 515).

Reasons for non-initiation or delayed initiation

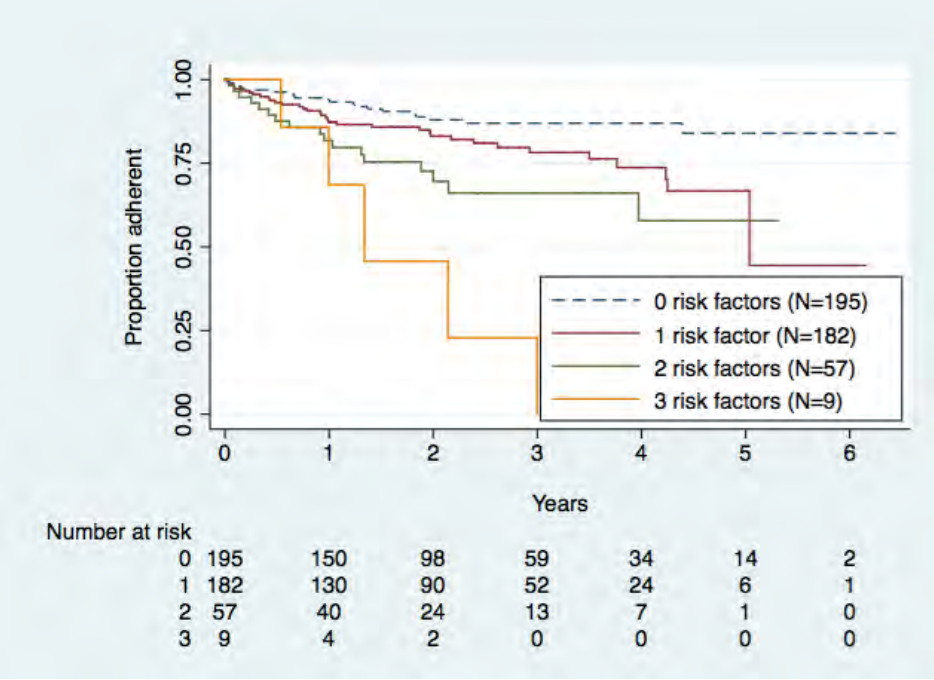



0 points: 2/198
1 point: 19/196
2 points: 30/94
3 points: 17/26
4 points: 1/1

Llarena N, Estevez S, et al. *JNCI*, 2015.

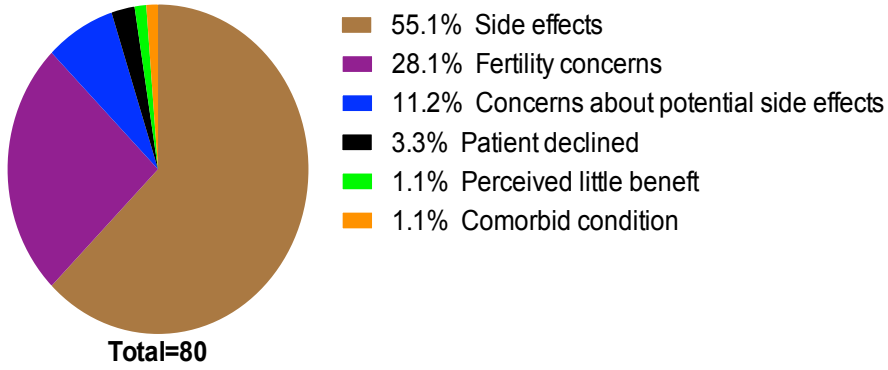
# Multivariate Model of Early Discontinuation of Tamoxifen

| Patient Characteristic | Hazard Ratio | 95% Confidence Interval |
|---|---|---|
| Interest in fertility at diagnosis | 1.78 | 1.09 – 3.38 |
| Radiation therapy not indicated | 2.10 | 1.30 – 3.38 |
| Current or former smoker | 1.73 | 1.09 – 2.75 |




Multivariate Cox model of tamoxifen early discontinuation among premenopausal patients age ≤ 45 diagnosed with stage 0-III ER+ and/or PR+ breast cancer from 2007-2012 (n = 515).

## Reasons for early discontinuation




Llarena N, Estevez S, et al. *JNCI,* 2015.

# Evidence for Tamoxifen Delay

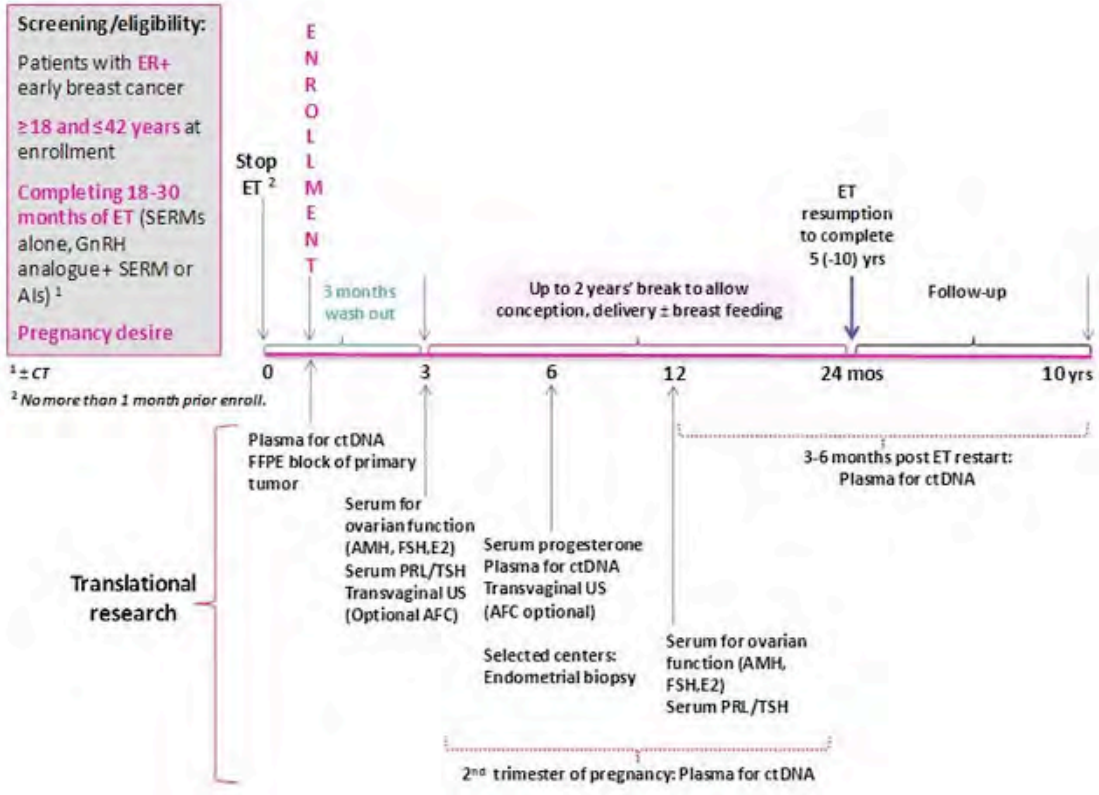
- Indirect evidence from Early Breast Cancer Trialists' Collaborative Group suggests tamoxifen can be delayed
- Multi-institutional French study: patients who delayed tamoxifen for 2 years and then completed a 5-year course showed significantly improved disease-free survival (35% recurrence reduction) compared to controls
- Wisconsin Tamoxifen Study, tamoxifen delayed 7-8 years, patients showed benefit in treatment versus controls
- These data support potential for tailored delay in tamoxifen therapy allowing time for pregnancy, with expectation for counseling to ultimately complete 5-10 years of therapy.

Hershman et al. *J Clin Oncol.* 2010;
Gradishar and Hellmund, *Clin Breast Cancer* 2002;
Delozier et al. Ann Oncol. 2000;
Love RR J Natl Cancer Inst. 1999.

# IBCSG 48-14 POSITIVE

**A study evaluating the pregnancy outcomes and safety of interrupting endocrine therapy for young women with endocrine responsive breast cancer who desire pregnancy.**

## DESIGN




**Study Chair**
Monica Ruggeri

**Statistician**
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**Trial Coordinators**
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**Date of Activation**
July 3, 2104

**Targeted Accrual**
500

- Protocol Documents
- Current Status
- Data Management Resources
- Former Protocol Documents
- Pathology

# Future Directions

- Young survivors are a unique patient population contending with distinct survivorship issues
- For ER+ breast cancer patients age 45 and under, fertility concerns are associated with tamoxifen non-initiation and non-persistence
- Efforts to improve tamoxifen initiation/adherence should address possible modifiable risk factors including fertility concerns, smoking cessation, treatable side effects, personal recurrence risk, and treatment impact
- Despite the importance of fertility to young cancer patients and ASCO recommendations on this topic, referral rates to fertility specialists can be improved upon
- High-risk data acquisition is moving forward
- Validation of primary findings

# Implementation

- Pregnancy not associated with increased risk for disease recurrence or adverse outcomes (9 retrospective studies)
- Regarding fertility treatments: epidemiological and basic research do not show negative effect
- ER/PR positive breast cancer hormonally driven- questions regarding safety of hormone stimulation (elevated estrogen effect) are being prospectively monitored
- Stimulation with letrozole
- Gestational surrogacy

# Young Patient Who Desired Treatment

- 34 year old mother of 2 in supportive marriage
- Diagnosed with high grade stage IIIA breast cancer
- Fertility preservation discussed postoperatively, patient very interested
- Successfully pursued embryo cryopreservation
- Often stated knowledge of fertility preservation helped her persevere through treatment
- Patient had a baby 3 years ago, doing well back on tamoxifen
- Can we reconcile prognosis known prior to fertility preservation?

# Young Patient Who Refused Treatment

- 36 year old high level executive, elite athlete, married
- Diagnosed with invasive, ER/PR/HER2 positive disease, at surgery found to be node positive
- Fertility preservation discussed at initial consultation and post-operatively
- Patient refused, focused on athletic goals
- 9 months into treatment patient and husband return for follow-up and state desire for children
- Patient amenorrheic, 6 months of trastuzumab therapy remaining
- At completion of trastuzumab therapy, pt remained amenorrheic and stated regret at not pursuing fertility preservation prior to therapy

# Practice Guidelines

- Cases illustrate significance of fertility preservation integration into plan of care
- Success depends on early/open communication with patients, flexibility in scheduling appointments/procedures, patient navigator
- Presence of multidisciplinary team to see patients and discuss cases on short notice
- Current ASRM/ASCO/NCCN guidelines advocate for education regarding fertility preservation options
- Why guidelines not followed: lack of knowledge re options, uncertainty about success of fertility measures, language/cultural barriers, coverage


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VIDEOS
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Learn More >
1/5
I just received a cancer diagnosis and haven't yet started treatment.
What are my options for preserving fertility? >
Survivor Stories
© 2008, Northwestern University | Disclaimer | Contact Us | Site Map
For questions about your fertility preservation options call 866-708-FERT

- Established a national referral line for patients and providers

  866-708-FERT (3378)

- Two websites launched to enhance patient referral to local programs and serve as a resource for patients and providers

  www.myoncofertility.org
  (Patients)

  www.oncofertility.northwestern.edu
  (Researchers and Physicians)

- Participation in multicenter research studies and access most up to date technologies and information

# Conclusions

- The connections between fertility and cancer are complex
- Obligation to consider how our practices affect the whole patient
- Work together as multidisciplinary team
- Counsel patients about risk and how to process this risk
- Intersection between clinical recommendations and patient choice

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UCSF
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# Fertility Preservation for Children, Adolescents, Transgender Youth, and Young Adults

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Director, Male Reproductive Health
Associate Professor, Department of Urology

# Disclosure

I have no disclosures and no conflicts of interest

# Outline

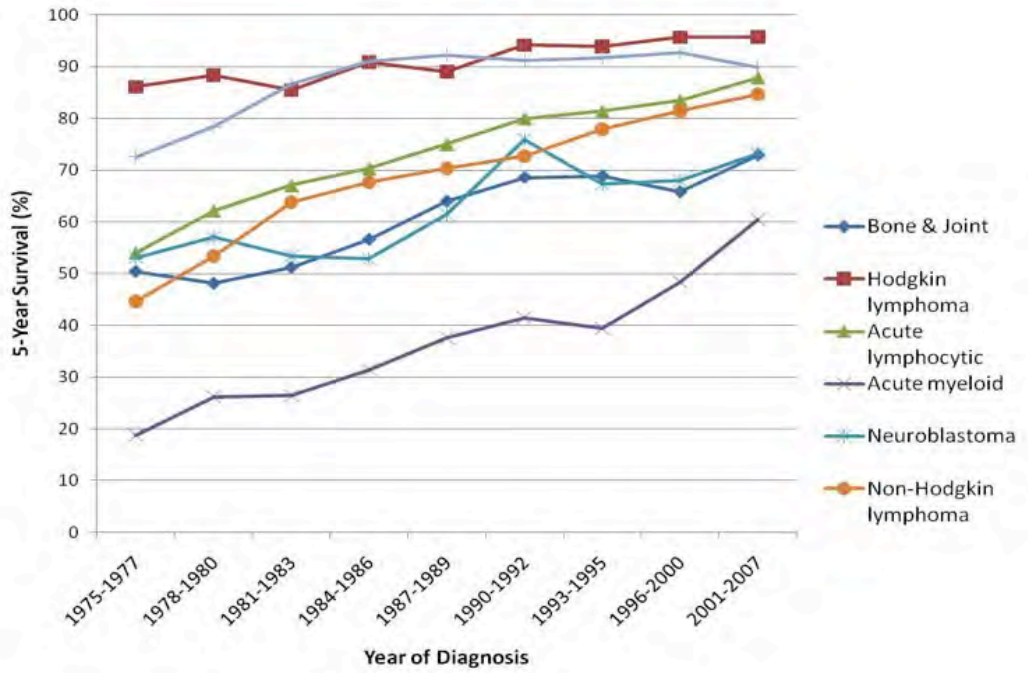
- Background
- Spermatogenesis overview
- Pre-pubertal fertility preservation (experimental)
- Post pubertal fertility preservation (Standard of care, complexities)
- Chemotherapy: reproductive risk and safety concerns

# Learning Objectives

- At the conclusion of this course, participants should be able to:
- Describe currently available options for **male fertility preservation** including recent advances in sperm and testicular tissue cryopreservation.
- **Formulate individualized treatment plans** for patients throughout the reproductive spectrum who are interested in undergoing fertility preservation through cross-discipline collaboration.
- **Appreciate the interdisciplinary approach** necessary to achieve effective fertility preservation and survivorship care.

# Men and Boys at Risk for Infertility

- More than 850,000 males diagnosed with cancer in the U.S. in 2014
- 7,000 boys annually
- 50+ BMT UCSF
- Reproduction one of top concerns after treatment
- Adult & adolescent fertility preservation




Howlader N, et al. SEER Cancer Statistics Review, National Cancer Institute. Bethesda, MD.

UCSF

# Classification of Male Infertility

- Pre-testicular
  - Disruption of the brain centers that regulate sperm production (hypothalamus and pituitary radiation/surgery)
- Testicular
  - Disruption of sperm production at the level of the testicle or abnormal sperm function
- Post-testicular
  - Obstruction, ejaculatory dysfunction, hypospadias

Brugh VM III *et al. Med Clin NA* 2004; 88:367-85
Nudell DM *et al.* In *Clinical Urology 3rd ed.* 2001. 643-74

# What to look for in the History?

| | Pre-testicular | Testicular/Sperm | Post-Testicular |
|---|---|---|---|
| **Medical** | B-thalassemia | Cancer (of any type) | Epididymitis |
| | Pituitary tumor | Chemo-, radiation therapy | Cystic Fibrosis |
| | Sickle cell anemia | Fevers, heat, exposures | CBAVD / UAVD |
| | Kallman's | Orchitis (mumps) | Cord injury/Spina Bifida |
| | | Klinefelter's, varicocele | Multiple sclerosis, DM |
| | | Kartagener's (ICS, bronchitis) | |
| **Surgical** | Pituitary | Torsion, detorsion | Vasectomy, Hernia, trauma |
| | | Orchiopexy | Hypospadias |
| | | | Turp |
| | | | Retroperitoneal, pelvic |

- Timing of intercourse: Sperm survives max 5-7 days; Oocytes 12-24 hours
- Length of time trying: 85% couples conceive 12 months trying
- Lubricants

# 29 y/o testicular cancer

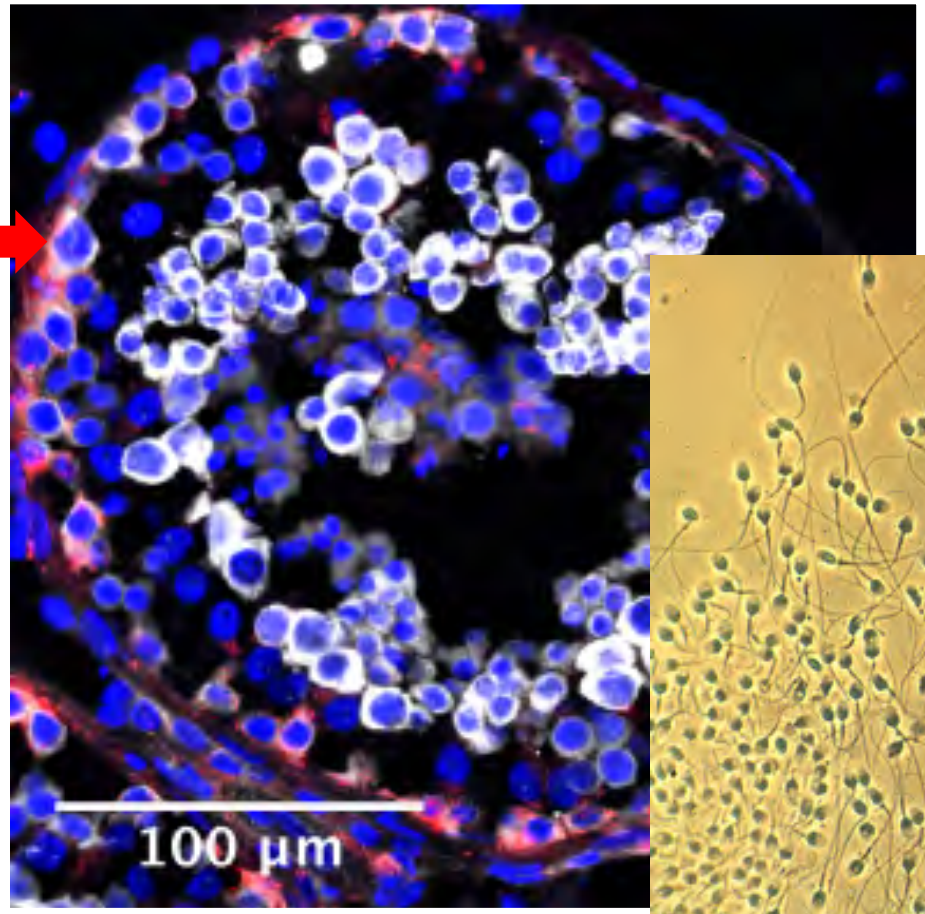
- HPI
  - 29 y/o with desire for fertility after testicular cancer treatment. Right orchiectomy 3 years ago. Did not bank sperm. BEP x3
  - Partner: 27 y/o reg cycles, G0
- PMH/PSH: right orchiectomy
- Meds: None currently
- Exposures: none
- Pex: well m nad
- Normal phallus, nl meatus; 16cc left; Empty right scrotum, nl epid, normal vas/epididymis

# 29 y/o testicular cancer

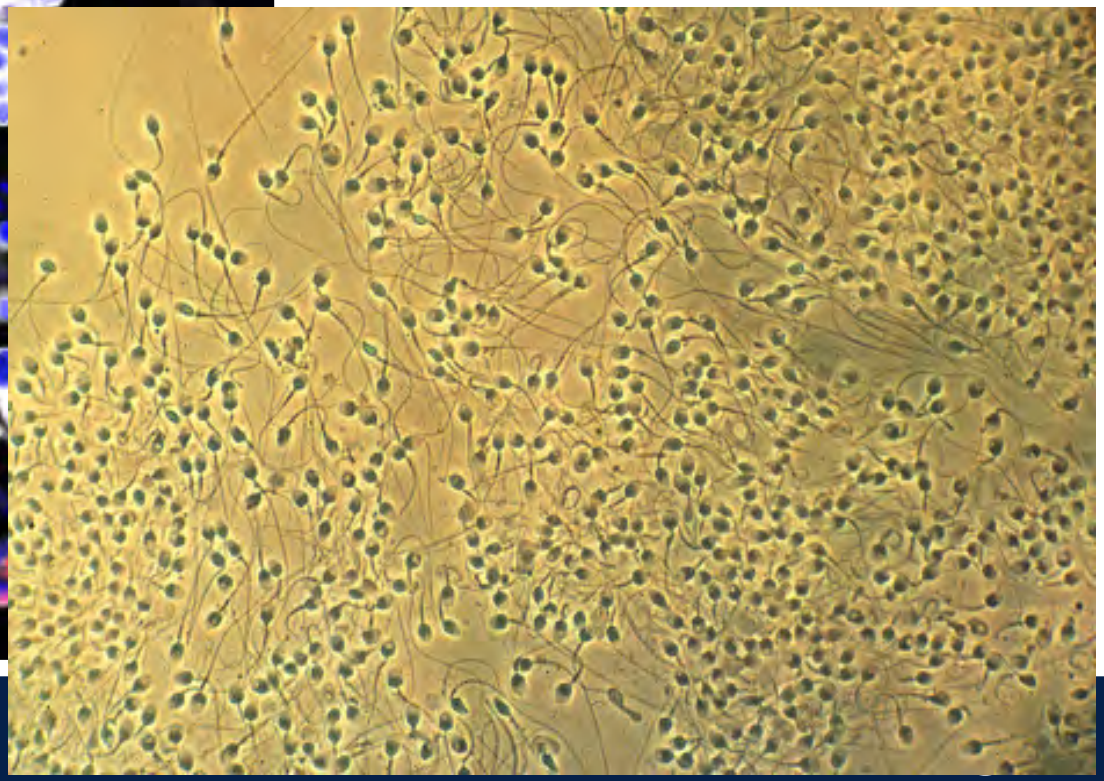
- Labs:
  - FSH 37, T 350, LH 15
  - SA x 2: azoospermia
- Diagnosis: OA vs. NOA?
- Discuss Options: NOA / Cancer patients
  - Timing of sex after chemo? How long should patients wait?
  - How toxic is semen after chemo exposure?
  - Micro-TESE with IVF/ICSI
  - Donor sperm with IUI
  - Adoption

**Spermatogenesis:** production of sperm from primordial germ cells

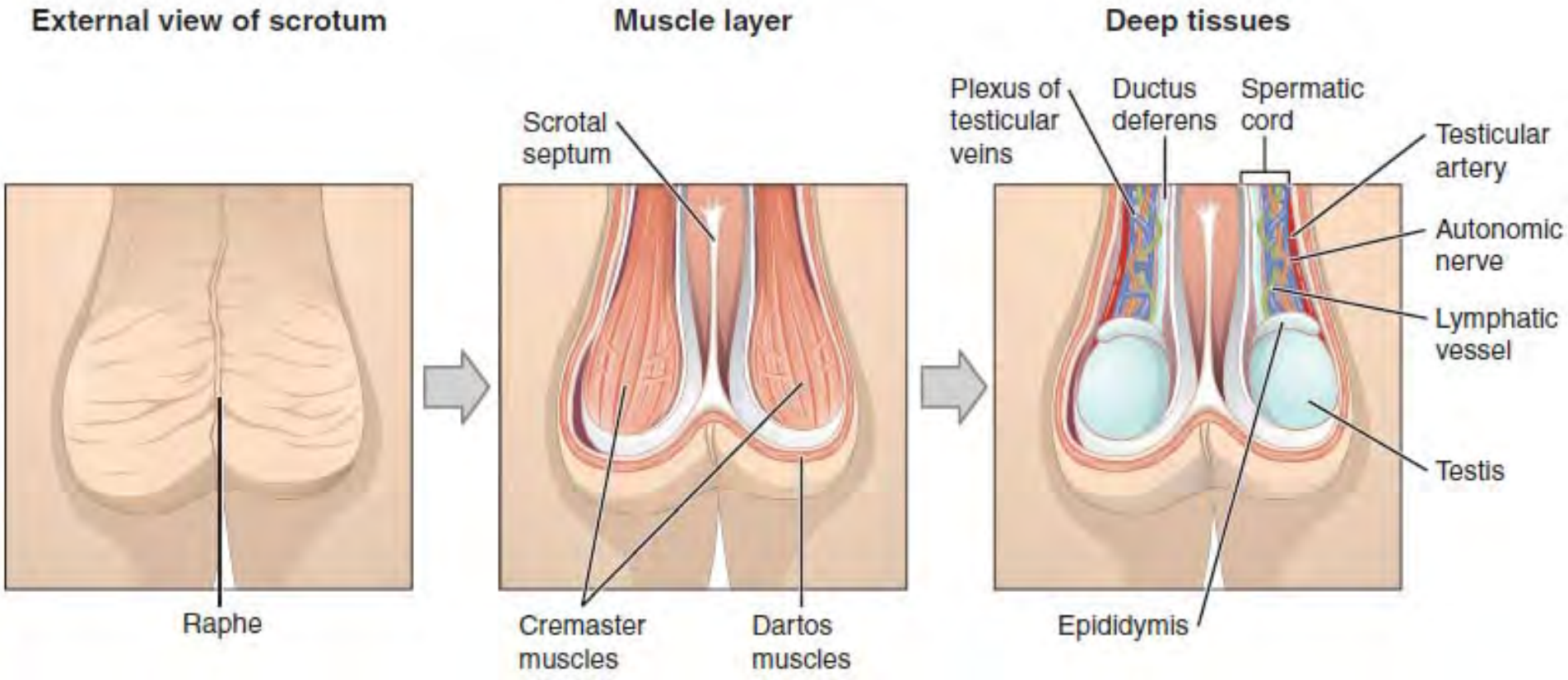
Spermatogonia




Spermatozoa

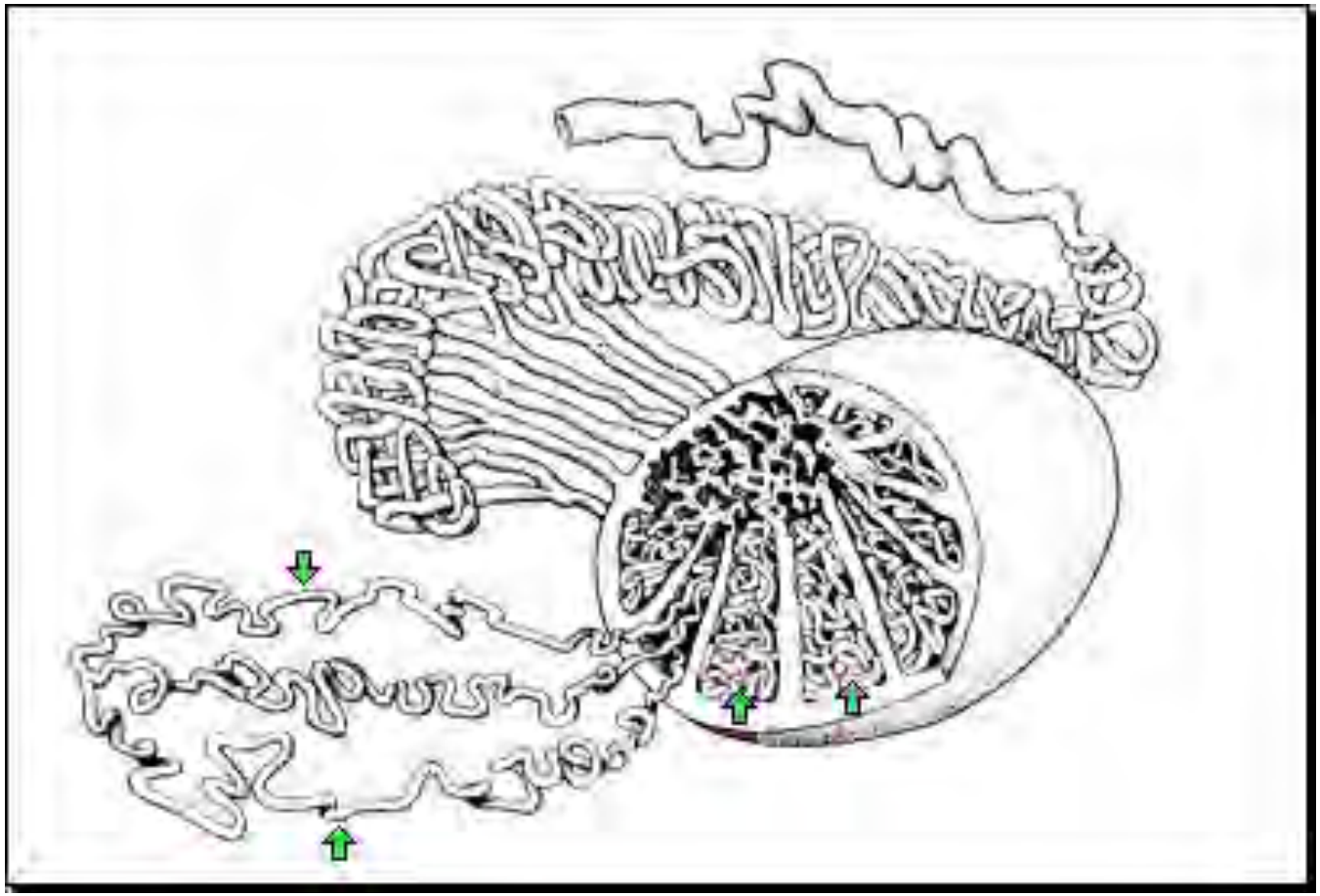


# GU Anatomy


External view of scrotum
Muscle layer
Deep tissues
Raphe
Scrotal septum
Cremaster muscles
Dartos muscles
Plexus of testicular veins
Ductus deferens
Spermatic cord
Testicular artery
Autonomic nerve
Lymphatic vessel
Testis
Epididymis

# Testicular framework

**Seminiferous tubules**



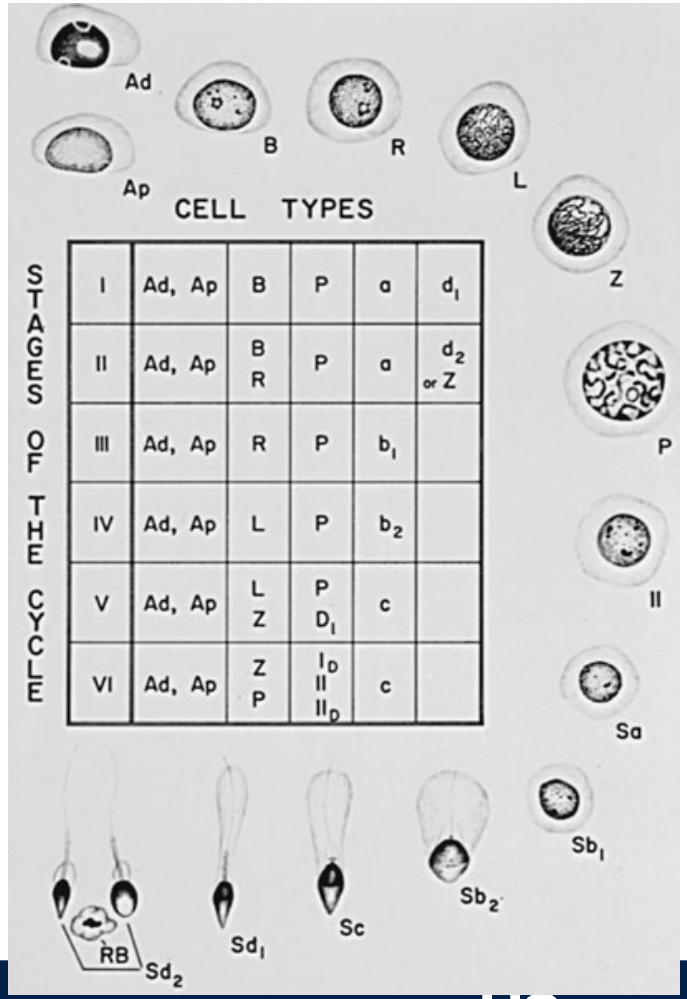
http://trc.ucdavis.edu/mjguinan/apc100/modules/Reproductive/mammal/images/testis02.jpg

# Spermatogenesis

1. Mitotic division
2. Meiotic division

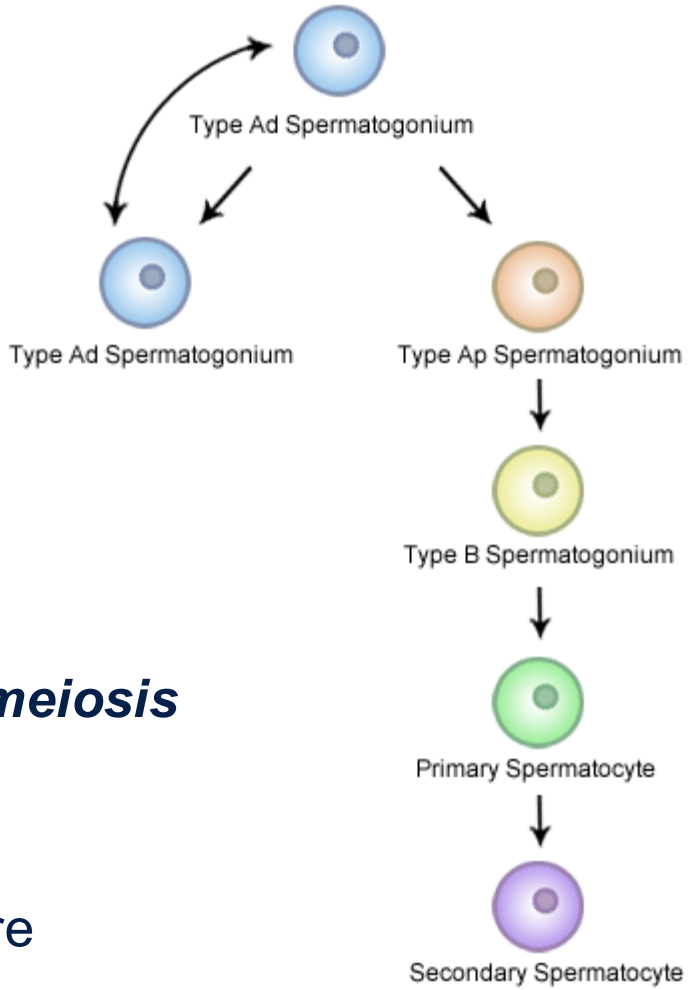
Spermatogenesis

3. Cellular remodeling- Spermiogenesis

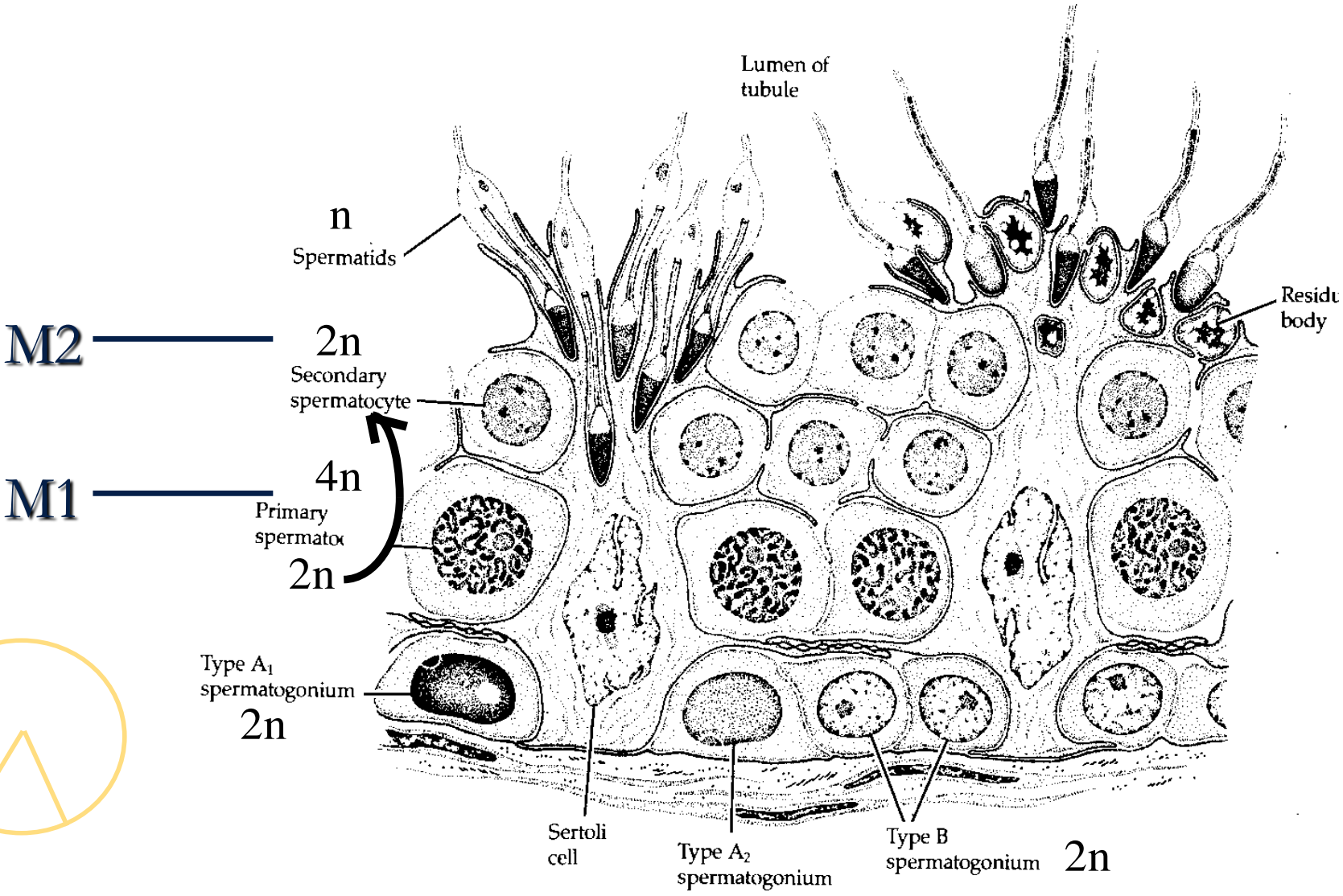


# Two Main Types of Spermatogonia

- **Stem cells**
  - Divide sporadically by ***mitosis***
  - Relatively quiescent, dormant cells
  - Do not develop into spermatozoa
  - Type Ad in primates
- **Differentiating**
  - Divide at fixed, regular intervals through ***meiosis***
  - Do develop into spermatozoa
  - Intermediate type (Ap) and B type cells are committed to next step of differentiation



# Spermatogenesis- Meiosis in the Testis




Mruk DD, Cheng CY. The Mammalian Blood-Testis Barrier: Its Biology and Regulation. Endocrine reviews. 2015;36(5):564-591. doi:10.1210/er.2014-1101.

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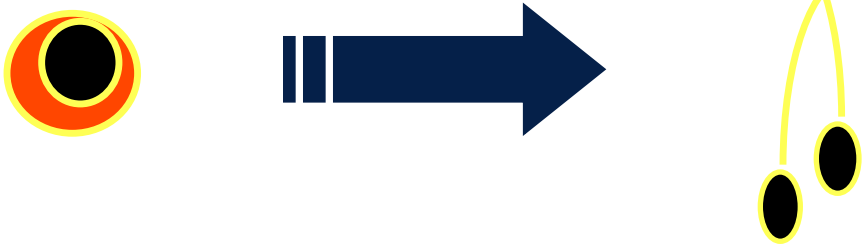
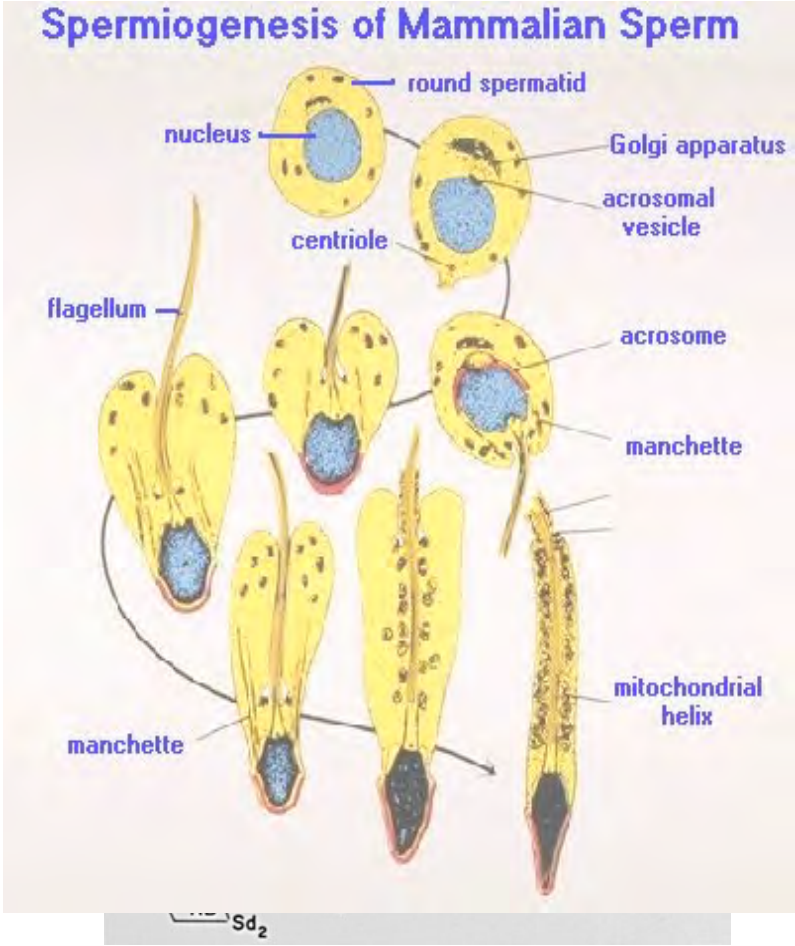
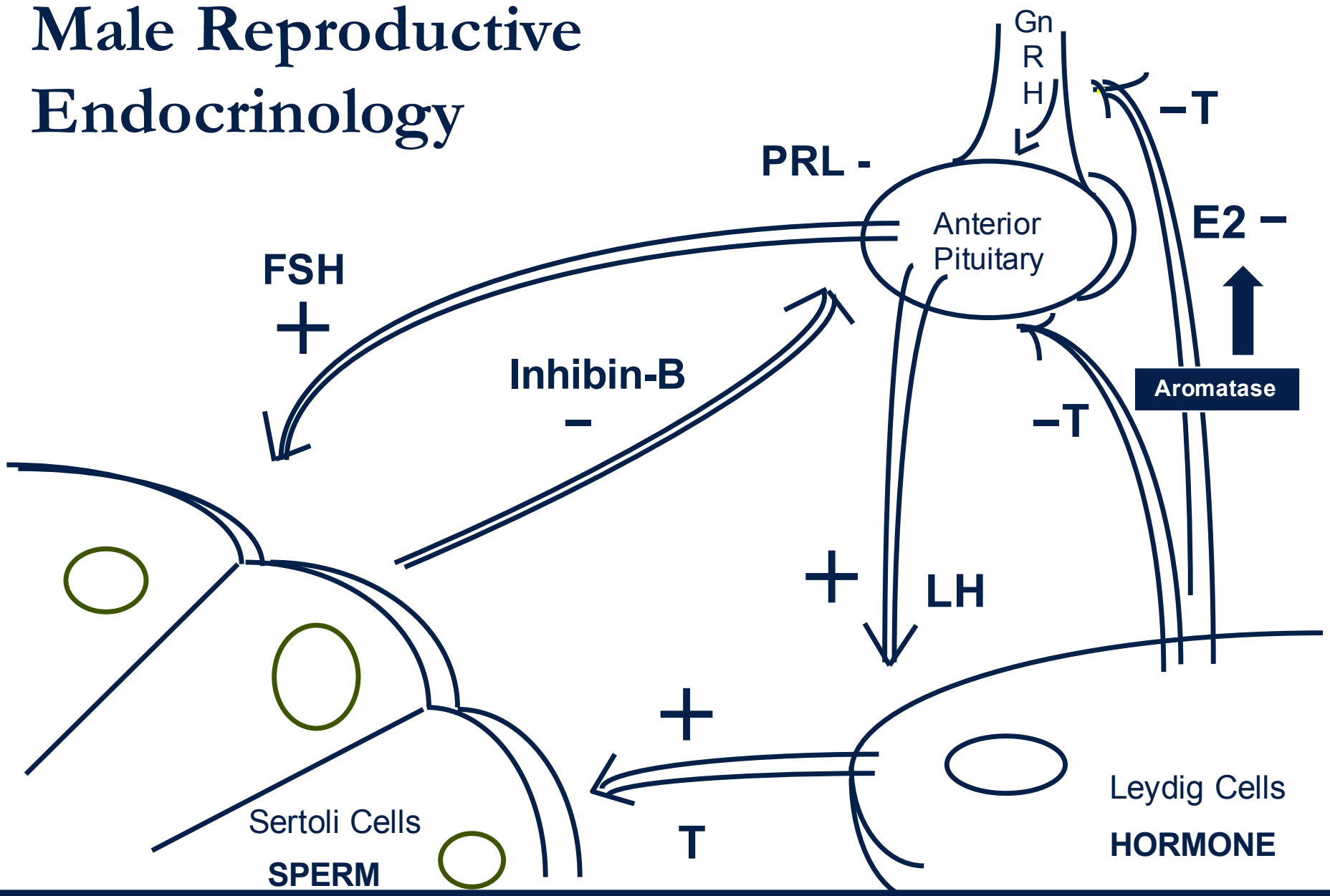
# Spermiogenesis

1. Acrosome forms
2. Flagellum constructed
3. Mitochondria organize near mid-piece
4. Nuclear compaction (10 fold)
5. Residual cytoplasm extruded

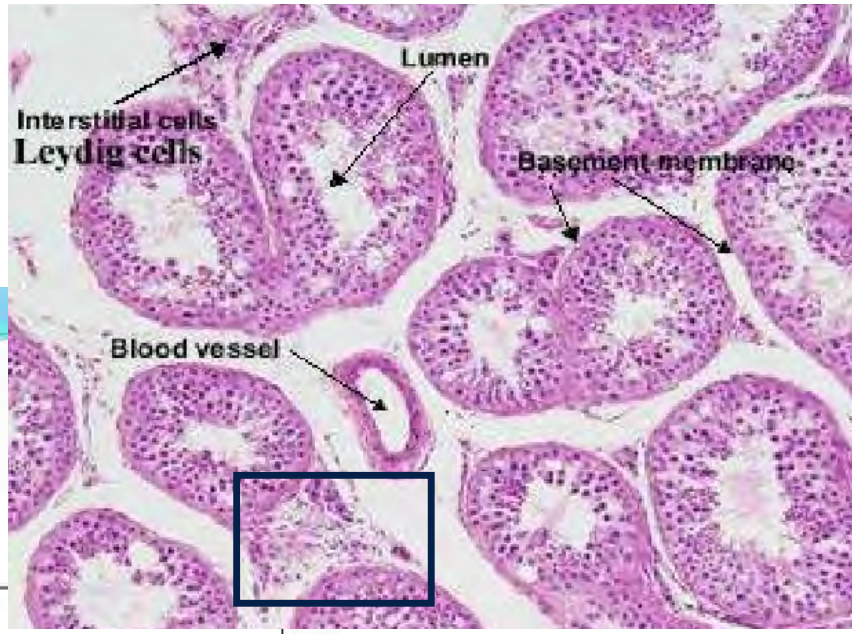


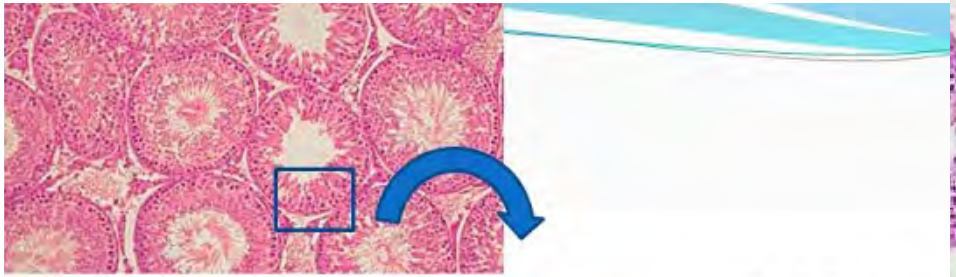


# Male Reproductive Endocrinology

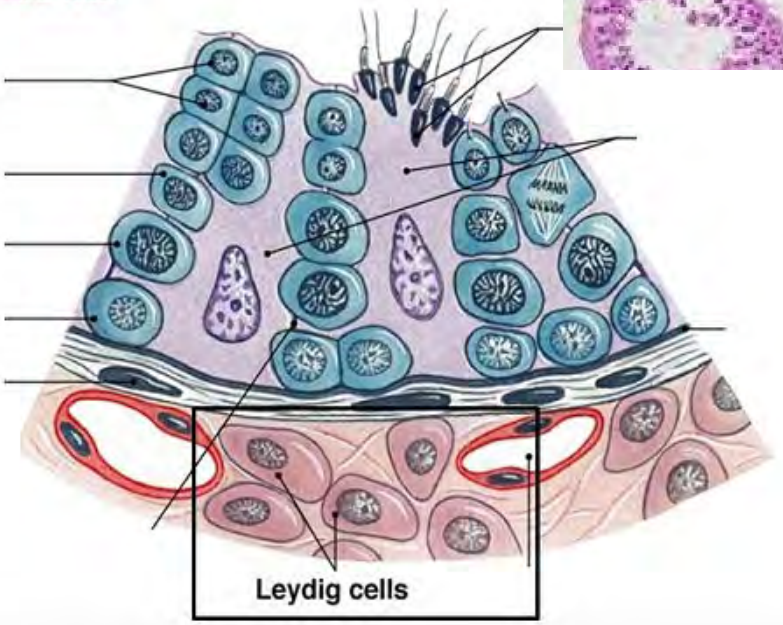


# Leydig cells

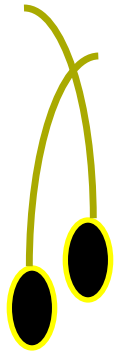


***Leydig cells***
produce
testosterone

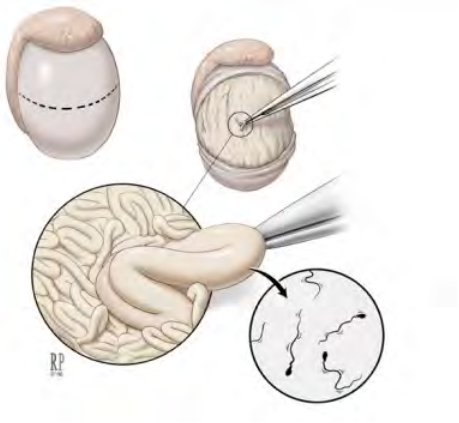


# Spermatogenesis Summary



- Mitosis duplicates diploid chromosomes
- Meiosis has genetic recombination/crossovers and leads to haploid chromosomes
- Spermatogenesis involves both replication processes
- Spermiogenesis involves elaborate differentiation of sperm cells
- Reproductive hormones essential for spermatogenesis

# 29 y/o NOA



- Micro-TESE
  - Done day of or day before oocyte retrieval or cryopreserved in advance
  - Local, spermatic cord block, and sedation
  - 2+ hours surgery time, 2-3 hours+ lab time (can be 16hrs+)
  - Probability success depends on histology

| Histology on Biopsy | Micro-TESE Sperm |
|---|---|
| Sertoli Cell only | 25-45%** |
| Maturation Arrest | 40-60% |
| Hypospermatogenesis | 80-90% |

# Micro-TESE: Outcomes

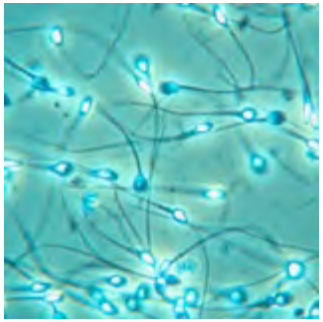
- Sperm retrieved in 37% of patients overall.
- Hypospermatogenesis positively associated with success.
- Cyclophosphamide exposure negatively associated with success.
- 50% clinical pregnancy rate
- 42% live birth rate

**Table 3.** Sperm Retrieval by Indication per Procedure (overall)

| Indication | No. | Total Patients (%) | Sperm Retrieved? Yes | Sperm Retrieved? No | Retrieval Rate (%) |
|---|---|---|---|---|---|
| Hodgkin's lymphoma | 30 | 35.7 | 9 | 21 | 30.0 |
| Leukemia | 13 | 15.5 | 7 | 6 | 53.8 |
| Non-Hodgkin's lymphoma | 12 | 14.3 | 5 | 7 | 41.7 |
| Testicular cancer | 13 | 15.5 | 11 | 2 | 84.6 |
| Sarcoma | 7 | 8.3 | 1 | 6 | 14.3 |
| Neuroblastoma | 4 | 4.8 | 2 | 2 | 50.0 |
| Other | 5 | 6.0 | 1 | 4 | 20.0 |

# What should our patient have done?

# Cancer and Fertility Preservation Pre-Chemo



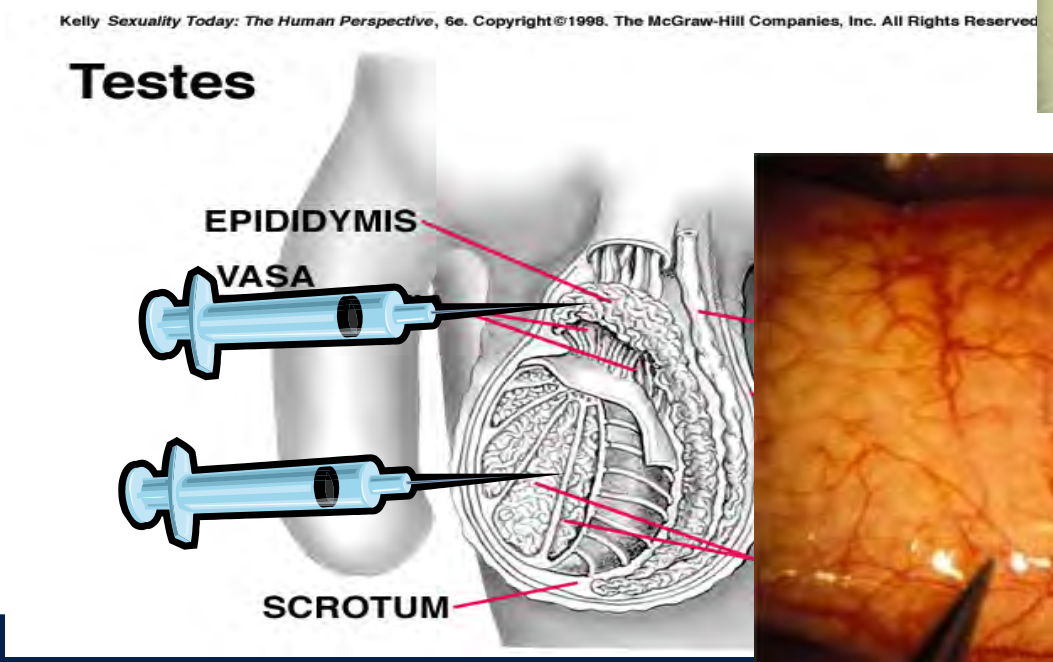
## Current approach

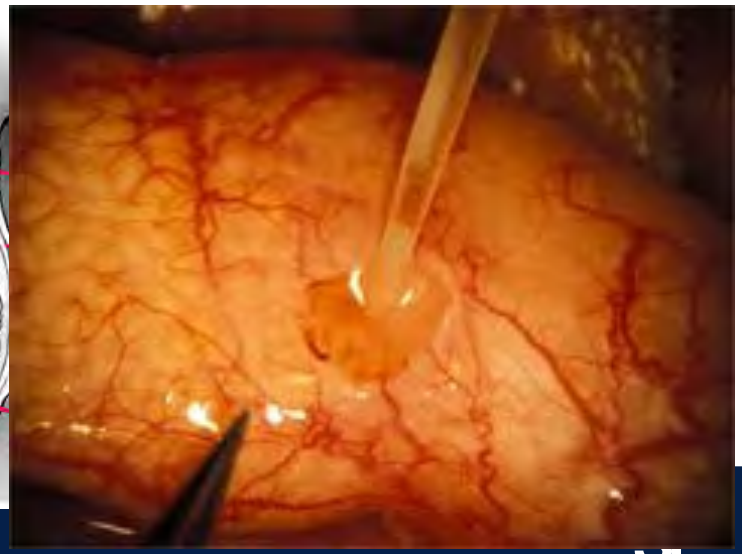
| |
|---|
| Referral to fertility specialist* **ASCO, AAP, ASRM guidelines** |
| Semen cryopreservation for appropriate candidates |
| Testicular sperm extraction |
| Approach requires IUI or IVF |

- How much to bank? How does it thaw?
- When should patient be referred?
- How about erectile dysfunction?
- What if he can't produce a sample?

# Sperm Aspiration or Extraction (OA)

- Percutaneous Epididymal Sperm Aspiration (PeSA)
- Testicular Sperm Aspiration (TeSA)
- Testicular Sperm Extraction (TeSE)
- Microsurgical Epididymal Sperm Aspiration (MESA)



# Electroejaculation (EEJ)

- Sensation, need for anesthesia

1. Cath, empty bladder, 50 cc sperm buffer
2. Rectal exam, proctoscope
3. Probe, apply voltage
4. Collect antegrade sample
5. Cath and collect bladder sample
6. Anoscope

- Can collect enough sperm for IUI or IVF / ICSI
- May need to be done repeatedly to achieve goals

# Pre-pubertal Case

**A 3 yo boy is brought to the emergency room in status epilepticus.** A MRI reveals hypervascular left peri-ventricular mass with extensive edema and midline shift. He undergoes a gross-total resection of the tumor. There was extensive blood loss, but he tolerates the procedure well without any complications. The final pathology was a choroid plexus carcinoma.

-His pediatric oncologist recommends treatment per clinical trial protocol ACNS 0334 arm B: vincristine, methotrexate, etoposide, cytoxan, cisplatin

- He is currently stable and plans are being made to initiate chemotherapy next week

# Pre-pubertal Case

He will be receiving a lumbar puncture and Broviac catheter next week

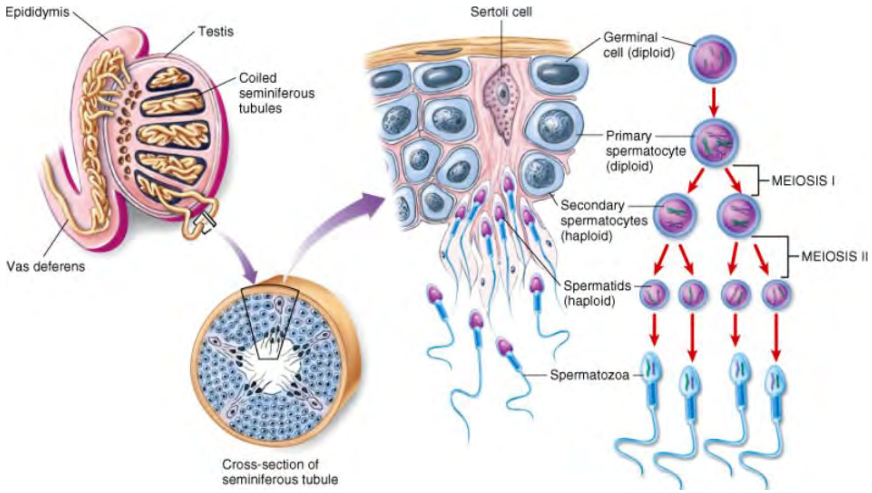
-His parents ask you about the risk of infertility associated with the planned therapy.

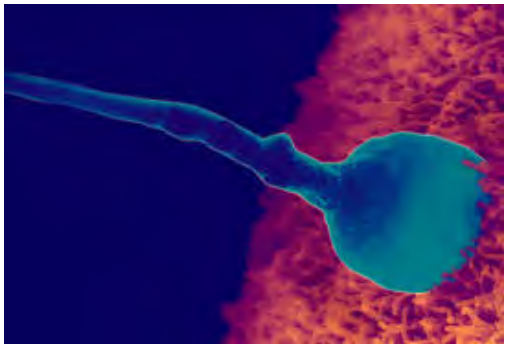
-His parents ask you about measures to preserve his fertility.

**-What do you tell them?**
**-What about cost and logistical challenges?**

# Treatment Effects on Fertility

- Azoospermia
- Oligospermia
- Poor quality sperm, functional infertility
- Inability to ejaculate (spine or pelvic surgery)
- Hypogonadism



# Lower Risk

- Vincristine, methotrexate, dactinomycin, mercaptopurine, mitoxantrone, vinblastine

- Toxicity:
  - NOVP (mitoxantrone, vincristine, vinblastine, prednisone): Azoospermia 38% & severe oligospermia in 62% after 1 month
  - Normospermia in 63% after 4.5 months
  - **There is no NO RISK CHEMOTHERAPY! Will not improve function!**

Sonmezer M, Oktay K. Fertility preservation in female patients. Hum Reprod Update 2004;10:251-66.
2-Meistrich, Wilson, Mathur , Lipshultz, et al. Rapid recovery of spermatogenesis after adult chemo. JCO 1997.

# Higher Risk

- Cisplatin, Carboplatin, Doxorubicin, BEP (Bleomycin, etoposide, cisplatin), ABVD (adriamycin, bleomycin, vinblastine, dacarbazine)

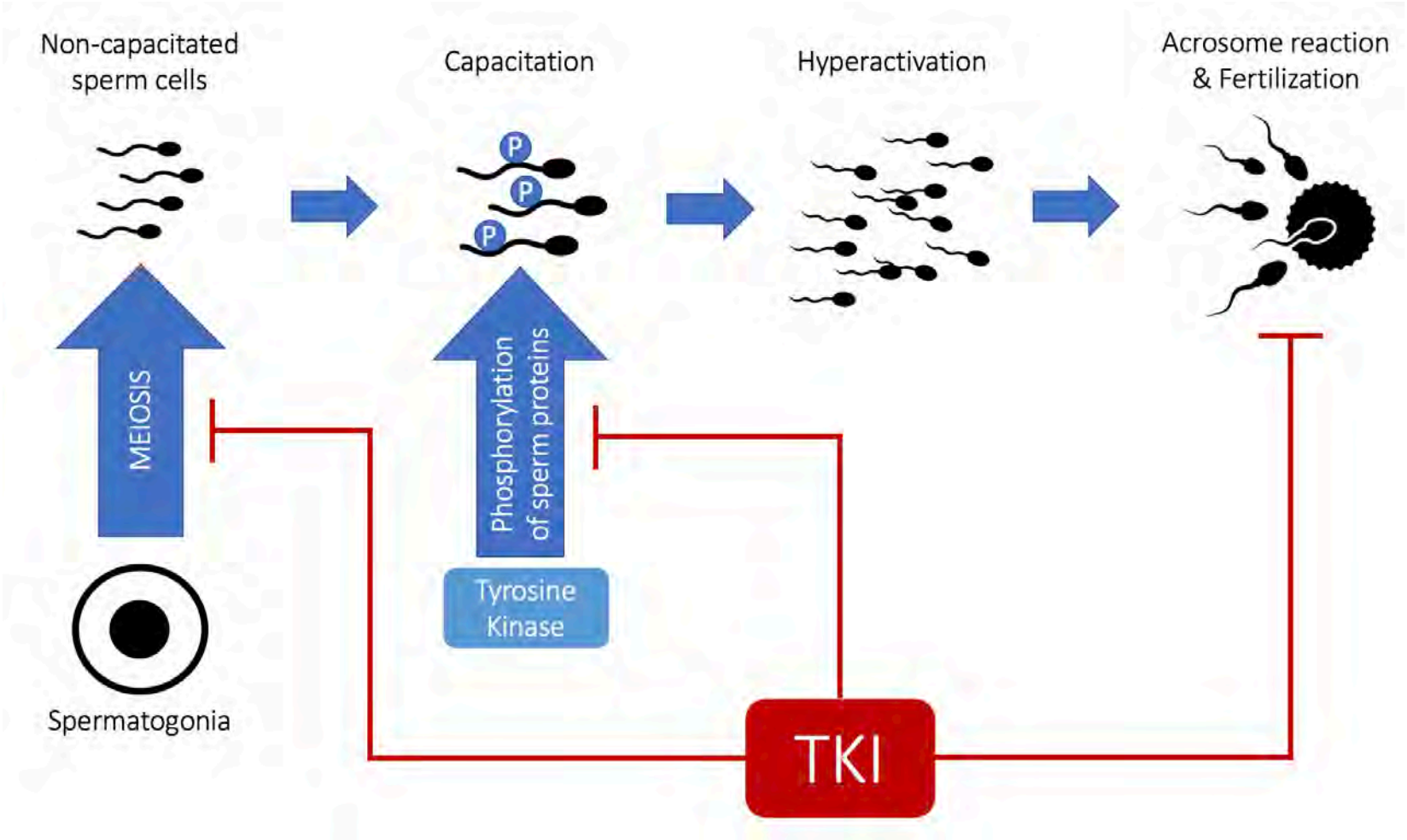
- Toxicity:
  - **Cisplatin-** Azoospermia 27% boys 20 yrs after tx
  - **ABVD -**40% azoospermia & 38% severe oligo after 4-8 cycles. 90% recovery after 1-5 years
  - **BEP-** 30% decline in counts. Recovery in 80% within 5-8 years

# Highest Risk

- Cyclophosphamide, busulfan, ifosfamide, thio-TEPA, melphalan, procarbazine, chlorambucil, MOPP, CHOP

- Toxicity:
  - 80%+ probability azoospermia for most chemo agents
  - Permanent for many
  - Germ cell failure for many agents

# Effect of Targeted Cancer Therapy on Male Reproductive Function




Smith JF, Syritsyna O, Tran N, Rosen M, Jalalian L, Lishko P. Effect of Tyrosine Kinase Inhibitors on Male Reproductive Function. American Society of Andrology, 2013.

UCSF

# Radiation, Hypogonadism, and Male Infertility

- Location (pelvis, gonads, whole body) of exposure
- Gonad dose
  - Azoospermia temporary if <3 Gy
  - Azoospermia permanent if >3 Gy
- Endocrine function:
  - Leydig cells preserved if < 12 Gy exposure (increased LH in some)
  - Hypogonadism if gonadal exposure > 20 Gy (pre-pubertal)
  - Hypogonadism if > 30 Gy (post-pubertal)

van Casteren NJ, van der Linden GH, Hakvoort-Cammel FG, Hahlen K, Dohle GR, van den Heuvel-Eibrink MM. Effect of childhood cancer treatment on fertility markers in adult male long-term survivors. Pediatr Blood Cancer 2009;52:108-12.

You perform an open testicular biopsy concurrent with his lumbar puncture and central line placement

**-What options are possible for this tissue in the future?**

# Transgender Case

**The mother of a 13 yo transgender female calls you about fertility preservation her daughter.** Since age 9, she's been taking Lupron and is planning to start estradiol and spironolactone. Tanner stage 2 with 4ml testicles bilaterally.

**-How do these medications affect male fertility?**

**-What fertility preservation options are possible for pre- and post-pubertal transgender patients receiving therapy?**

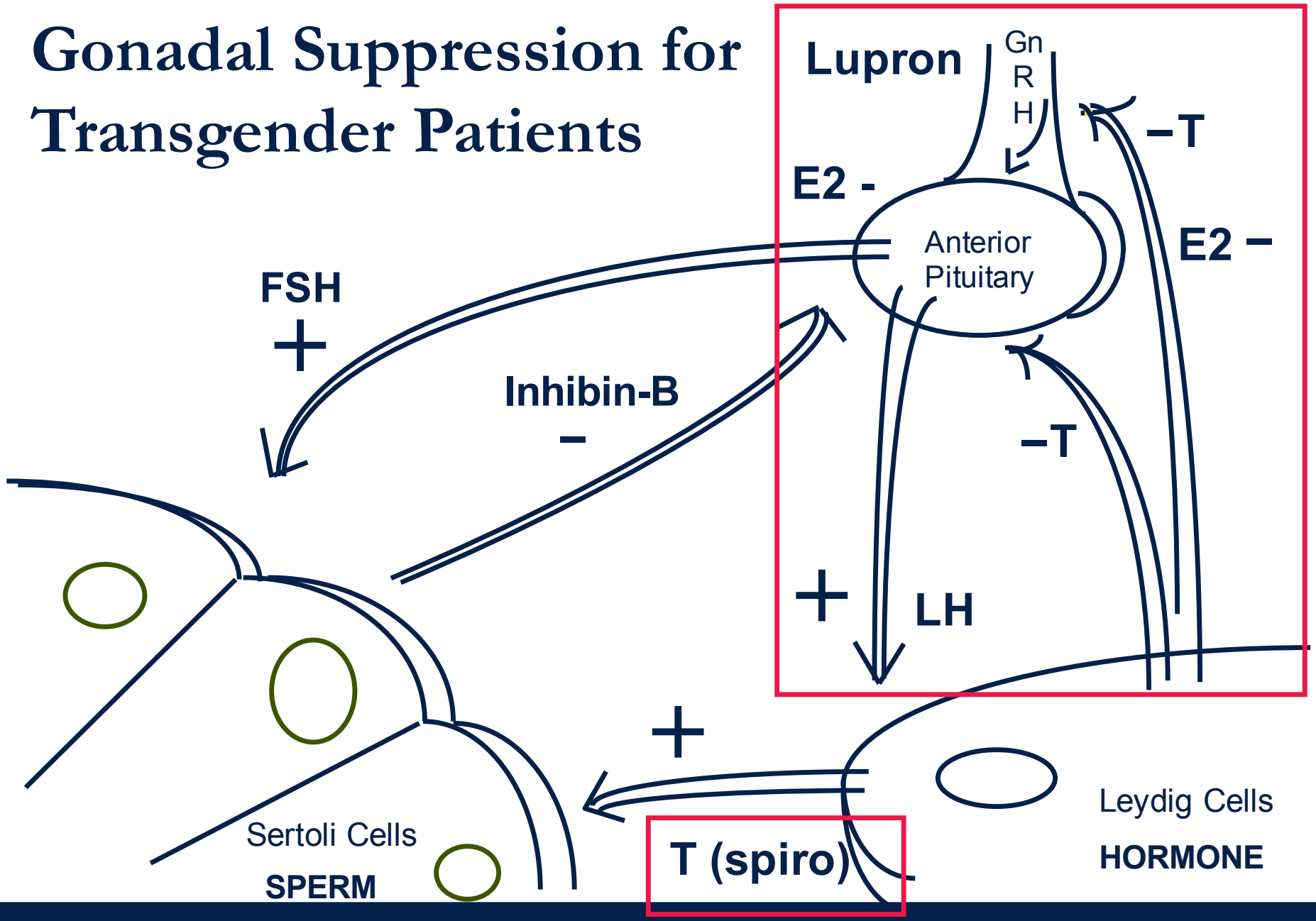
# What about FP options for transgender adolescents and young adults?

- Commonly use estradiol, spirinolactone to suppress gender dysphoria
- Suppression of peripubertal children with Lupron

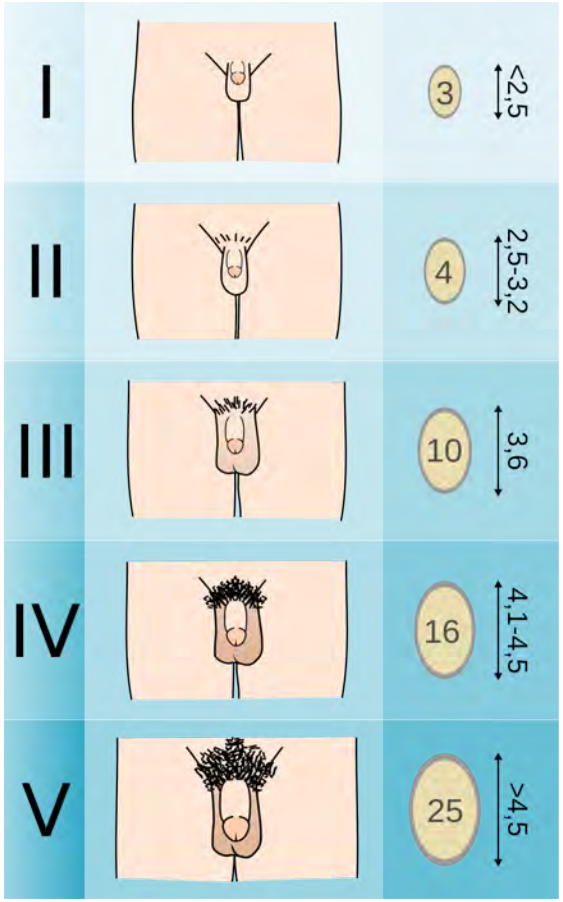
- Is it possible to bank sperm for post-pubertal patients on hormone suppression?
- What about peripubertal and prepubertal transgender youth?

Adeleye A, Reid G, Mok Lin E, Smith JF. FERTILITY PRESERVATION FOR TRANSGENDER FEMALES AFTER GENDER AFFIRMING TREATMENT IS EFFECTIVE. ASRM 2017.

# Gonadal Suppression for Transgender Patients



# Clinical Assessment and Development of Spermatogenesis



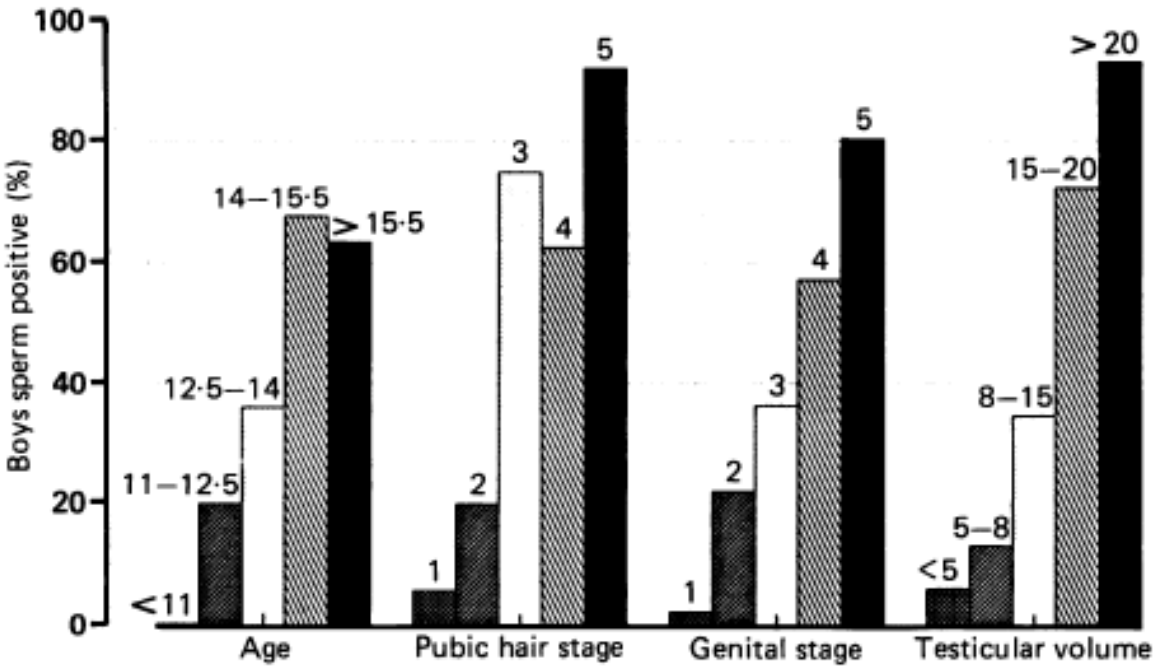




*Figure 2 Overall cumulative probability of spermaturia according to age, Tanner stages for pubic hair (1–5) and genitals (1–5), and testicular volume (ml) (n=129).*

Schaefer F, Marr J, Seidel C, Tilgen W, Schärer K. Assessment of gonadal maturation by evaluation of spermaturia. Arch Dis Child. 1990

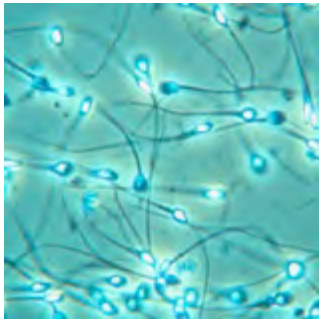
UCSF

# Sperm Cryopreservation for Transgender Females

- N=14 transgender females (natal males)
- 43 semen specimens, 10 collected after discontinuing therapy for at least 3 months
- On therapy vs. off therapy:
  - Semen volume dramatically lower (0.7ml vs. 2.7 ml)
  - Concentration 12 million/ml vs. 48 million/ml
  - Motility 17% vs. 43%
  - Total motile count 2.3 million vs. 56 million
  - Vials frozen 1.1 vs. 3.5
- FP is possible for these patients even on therapy (though less successful)

Adeleye A, Reid G, Mok Lin E, Smith JF. FERTILITY PRESERVATION FOR TRANSGENDER FEMALES AFTER GENDER AFFIRMING TREATMENT IS EFFECTIVE. ASRM 2017.

# Cancer and Fertility Preservation Pre-Chemo



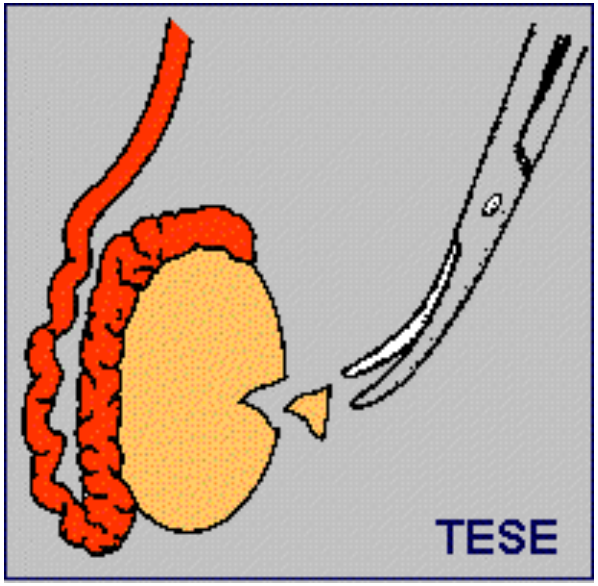
## Current approach

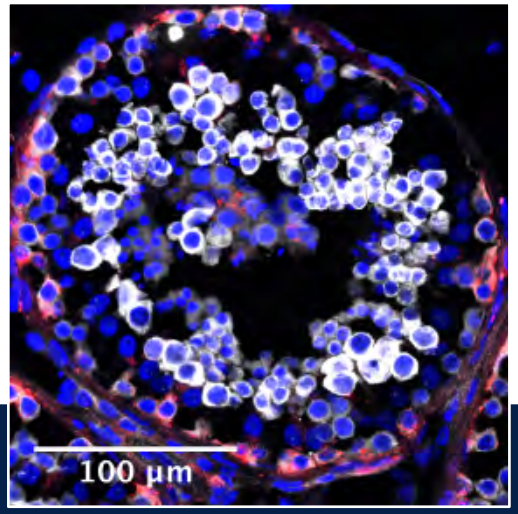
| |
|---|
| Referral to fertility specialist* **ASCO, AAP, ASRM guidelines** |
| Cryopreservation for appropriate candidates |
| Testicular sperm / Sperm stem cell extraction |
| Approach requires IUI or IVF |

## Future approach

| |
|---|
| Growth of sperm from stem cells *in vitro* then IVF / ICSI |
| Fertility **RESTORATION**: testicular biopsy followed by testicular cell transplant, conceive naturally |

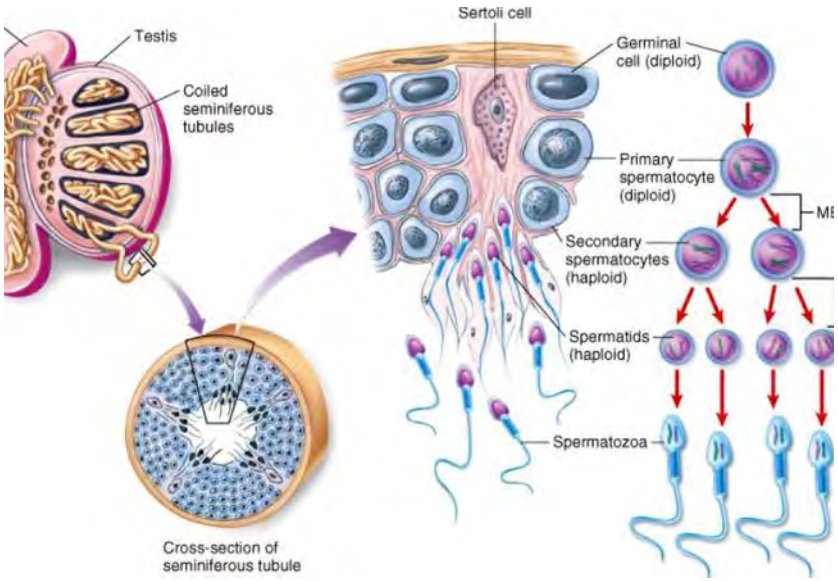
# Testicular Sperm Stem Cell Extraction (Pedi-TeSE)




- 30 minute surgical procedure, combined with other procedures
- Testicular tissue removed, cryopreserved. Size? Cryo technique?
- Sperm stem cells, no sperm in pre-pubertal males (& trans females)

# Fertility Preservation for Pre-Pubertal Males: Options and Challenges




1. **Autologous spermatogonial stem cell transplantation**
   - Successful many species including monkeys
   - Natural conception or IVF/ICSI
2. ***In vitro* maturation of sperm stem cells**
   - Start with small amount source material
   - Will require IVF/ICSI

# Primate Autologous Testicular Cell Transplant

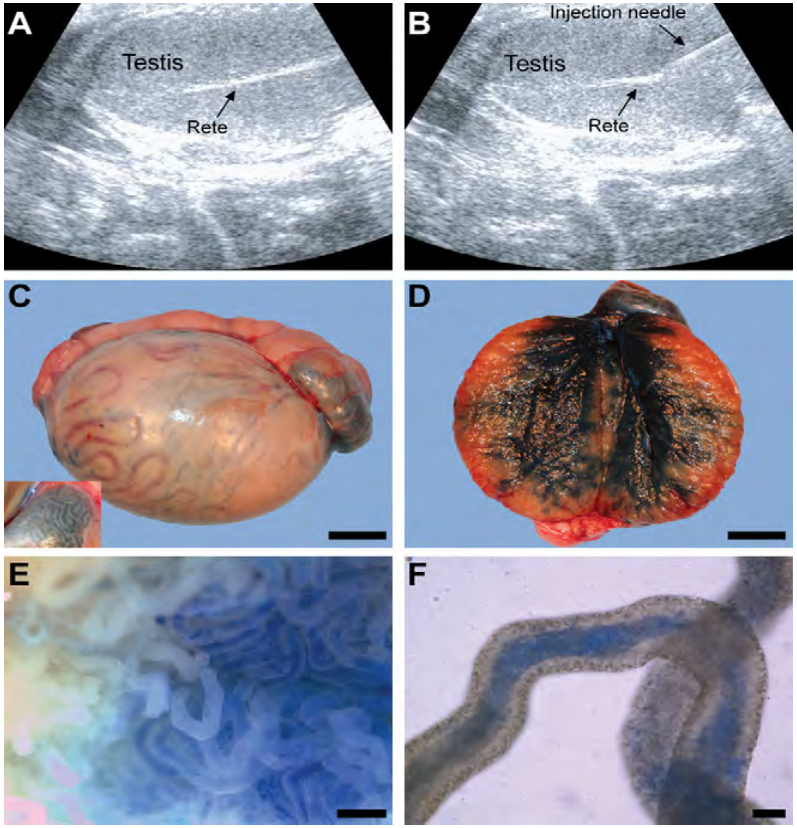



**Figure 1. Rhesus SSC Transplantation by Ultrasound-Guided Rete Testis Injection**

- Prepubertal (and postpubertal) Rhesus macaque monkeys underwent orchiectomy prior to busulfan (n=5)
- Tissue processed to single cells
- Bone marrow transplant after busulfan
- Biopsy revealed sperm production in all prepubertal monkeys

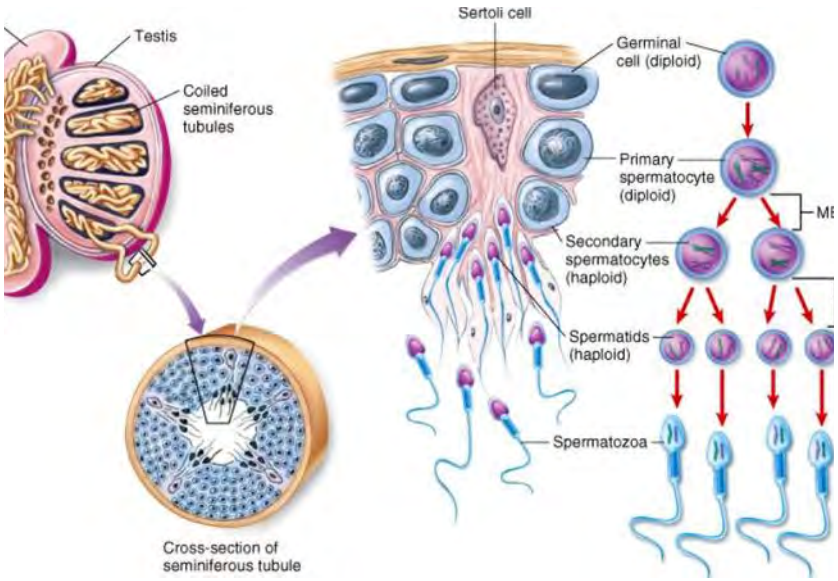
Hermann BP, Sukhwani M, Winkler F, et al. Spermatogonial stem cell transplantation into rhesus testes regenerates spermatogenesis producing functional sperm. Cell Stem Cell. 2012

UCSF

# Autologous Testicular Cell Transplantation: What do you inject?

- **Testicular tissue, processed to single cells:**
  - Success in many animal models from mice to monkeys
  - Risk of malignant cell transfer
- **Selected cell transplant**
  - Requires amplification of SSC and supporting cells (maybe?)
  - Minimize risk malignant cell transfer
  - Need in vitro culture system, understanding niche

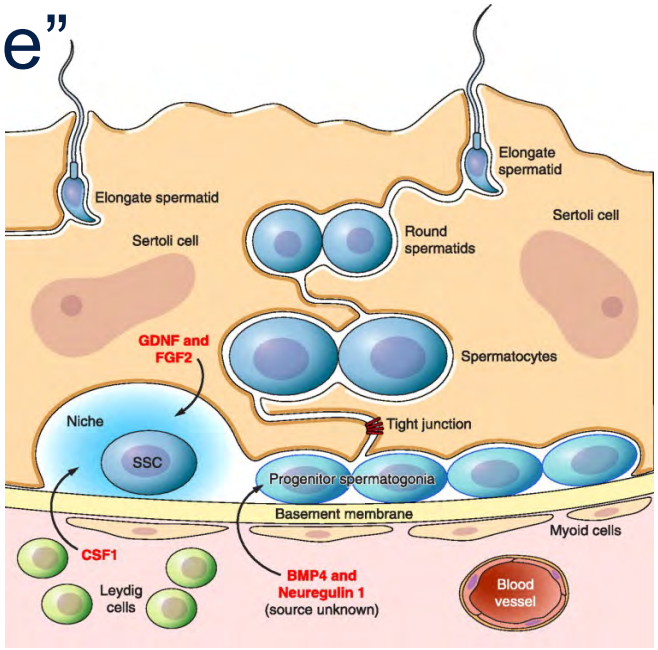
# Fertility Preservation for Pre-Pubertal Boys: Options and Challenges




1. **Autologous sperm stem cell transplantation**
   - Successful many species including monkeys
   - Natural conception or IVF/ICSI
2. ***In vitro* maturation of sperm stem cells**
   - Start with small amount source material
   - Will require IVF/ICSI

# Spermatogenesis

- Ideal supportive environment “niche”
  - Sertoli cells
  - Leydig cells
  - Mesenchymal cells
  - Myoid cells



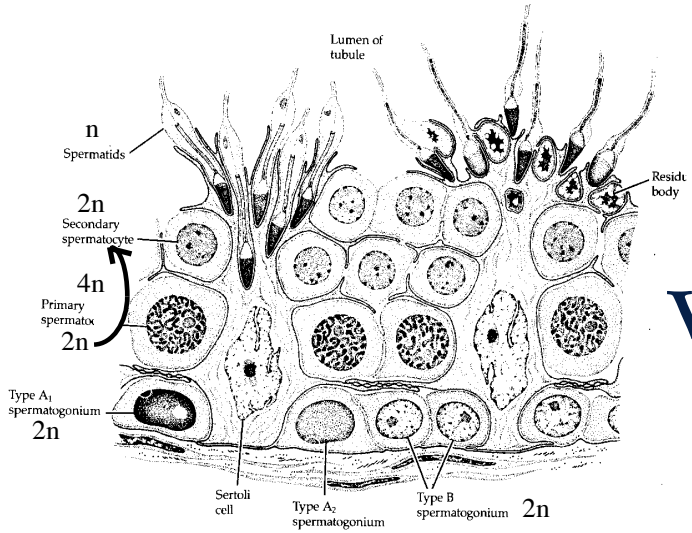

Oatley et al, 2012

- Spermatogonial stem cells (SSCs)

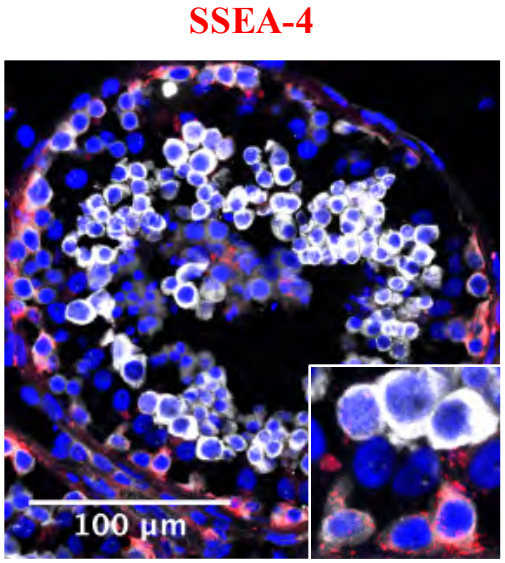
- Intact hypothalamus-pituitary-testis axis

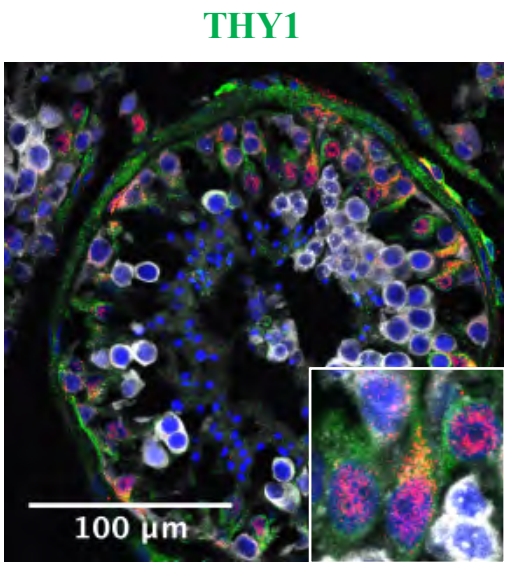
# Identification of Sperm Stem Cells (SSC)

- H&E staining (kill cells) vs. surface marker sorting (cells alive)
- H&E: self-renewing (Ad) and differentiating (Ap) spermatogonia
- Flow cytometry (FACS): SSEA4+ populations enriched for SSC; Sertoli cells within Thy-1

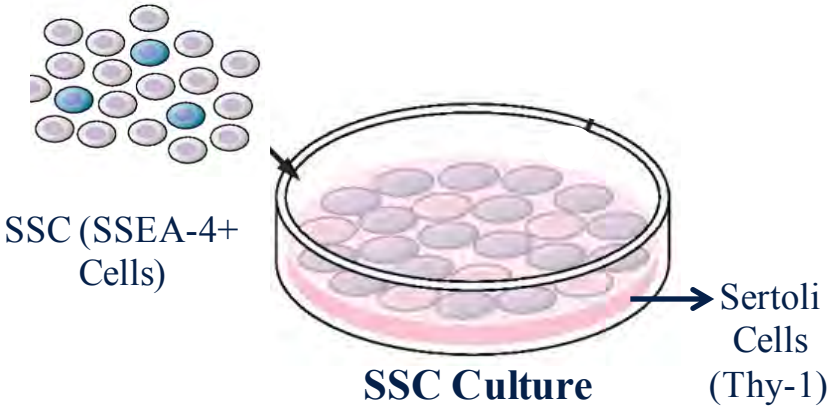


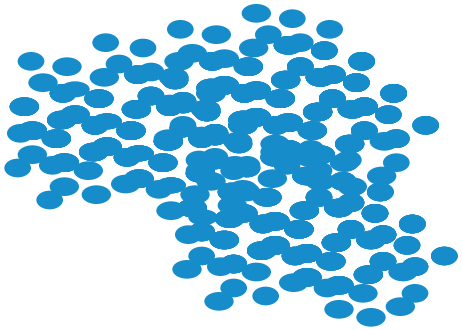

Vs.



# SSC Culture



**Normal growth SSC (SSEA-4 cells)**


- Smith JF, Yango P, Altman E, et al. Testicular niche required for human spermatogonial stem cell expansion. Stem cells translational medicine. 2014
- Altman E, Yango P, Moustafa R, Smith JF, Klatsky PC, Tran ND. Characterization of human spermatogonial stem cell markers in fetal, pediatric, and adult testicular tissues. Reproduction. 2014;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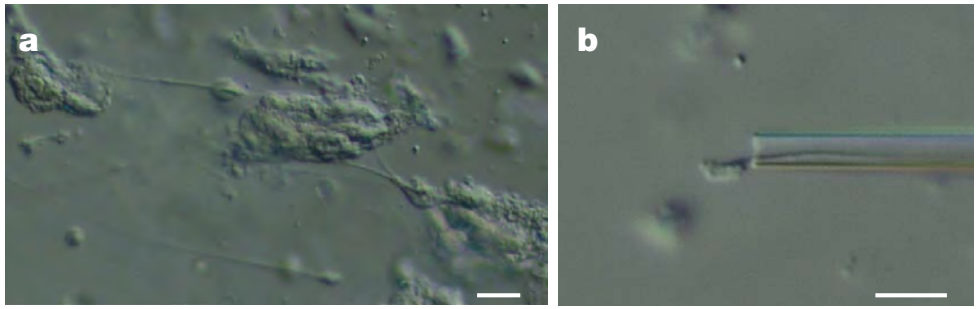
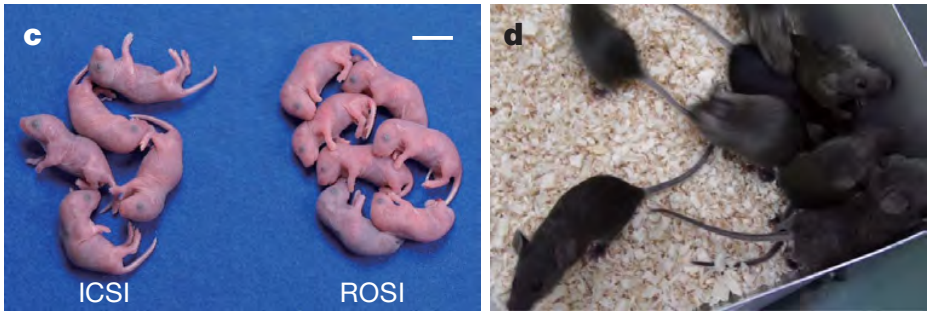
# *In vitro* maturation of neonatal tissue

- Newborn mouse testicular tissue turned into sperm in mice







- Spermatozoa used for ICSI, fertilization occurred
- Live pups born
- Litters bred and 2nd generation born
- Tissue cryopreserved, thawed, and mature sperm identified


- Sato T *et al. In vitro production of functional sperm in cultured neonatal mouse testes. Nature 2011;471:504-7.*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# Clinical and Research Challenges

- Logistical: rapid referral, coordination; access to FP specialists
- Technical
  - How big of a biopsy to take? 5%, orchiectomy?
- Basic/translational:
  - Culture techniques: Differentiate SSC to sperm
  - SSC transplantation in humans
  - Many more…
- Knowledge
  - Many patients and providers not aware of FP options
- Health policy
  - Insurance coverage

• Wyns C, Curaba M, Petit S, et al. Management of fertility preservation in prepubertal patients: 5 years' experience at the Catholic University of Louvain. Hum Reprod. 2011

# Male Fertility Preservation Take Home Points

- Collaboration between services (social work, oncology, nursing, cryo lab, urology); refer early
- Sperm or tissue banking prior to therapy
- Experimental testicular biopsy fertility preservation for pre-pubertal boys / transgender females
- Form individualized plans based on each clinical situation
- Work together to achieve solutions to challenges

UCSF
University of California
San Francisco

# Questions?

# Practical aspects of ovarian tissue cryopreservation in the pediatric population

Leslie Coker Appiah, M.D.

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No financial disclosures or conflicts of interest.

# Objectives

- Identify appropriate candidates for OTC
- Understand essential steps in setting up an OTC program
- Describe the technical and surgical aspects of OTC
- Understand the process for providing ovarian tissue cryopreservation in collaboration with an external center

# Case

- 9 yo pre-pubertal female with Fanconi anemia
- Symptomatic with bleeding and bruising for 1 year
- Bone marrow biopsy consistent with Fanconi anemia
- Plan for stem cell transplant
- Gynecology consulted for fertility preservation counseling
- Ovarian tissue cryopreservation performed
- Menstrual suppression not recommended as pre-pubertal

# Outline

a. Indications for OTC
    i. Chemo/radiation
    ii. Non-malignant disorders
    iii. Sex Diversity
    iv. Predisposition to POI
b. Setting up OTC program
c. Medical and surgical techniques
d. Collaboration with external centers

# Indications for Ovarian Tissue Cryopreservation

- High risk of gonadal failure from chemotherapy, radiation or surgical procedures for cancer treatment
- Nonmalignant disorders treated with immunosuppression or stem cell transplant
- Individuals with gender and sex diversity
- Genetic predisposition to accelerated follicular loss

# Clinical Ascertainment of Health Outcomes Among Adults Treated for Childhood Cancer

Melissa M Hudson et.al., JAMA. 2013;309(22):2371-2381

**Prevalence of Endocrine/Reproductive Late Effects in At-Risk Populations Following Exposure-Based Screening**

| Potential Late Effect | Screening test | Exposure Status | Number at risk[a] | Diagnosis before SJLIFE N | (%) | 95% CI | Diagnosis related to SJLIFE N | (%) | 95% CI | Diagnosis after SJLIFE N | (%) | 95% CI | Overall Prevalence N | (%) | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Primary ovarian failure | Menstrual history, FSH, Estradiol | Alkylating agents Radiation to female reproductive system | 553 | 44 | (8.0) | [5.8-10.5] | 20 | (3.6) | [2.2-5.5] | 1 | (0.2) | [0.0-1.0] | 65[h] | (11.8) | [9.2-14.7] |
| Male germ cell dysfunction[d,e] | Semen sample analysis | Alkylating agents Radiation to male reproductive system | 328 | 9 | (2.7) | [1.3-5.1] | 209 | (63.7) | [58.3-68.9] | 0 | (0.0) | | 218[i] | (66.4) | [61.1-71.6] |
| Leydig cell failure[d] | Morning testosterone, LH | Alkylating agents Radiation to male reproductive system | 574 | 25 | (4.4) | [2.8-6.4] | 37 | (6.4) | [4.6-8.8] | 4 | (0.7) | [0.2-1.8] | 66[j] | (11.5) | [9.0-14.4] |

- Health outcomes in 1,713 survivors median age 32 yrs (18-60 yrs)
- Prevalence of primary ovarian failure 12% in at risk females

**Cyclophosphamide Equivalent Dose Calculation.** The CED is calculated using the following equation: CED ($mg/m^2$) = 1.0 (cumulative cyclophosphamide dose ($mg/m^2$)) + 0.244 (cumulative ifosfamide dose ($mg/m^2$)) + 0.857 (cumulative procarbazine dose ($mg/m^2$)) + 14.286 (cumulative chlorambucil dose ($mg/m^2$)) + 15.0 (cumulative BCNU dose ($mg/m^2$)) + 16.0 (cumulative CCNU dose ($mg/m^2$)) + 40 (cumulative melphalan dose ($mg/m^2$)) + 50 (cumulative Thio-TEPA dose ($mg/m^2$)) + 100 (cumulative nitrogen mustard dose ($mg/m^2$)) + 8.823 (cumulative busulfan dose ($mg/m^2$)).

| Alkylating agent | Cumulative dose (mg/m2) | | |
|---|---|---|---|
| Cyclophosphamide | | | |
| Ifosfamide | | | |
| Procarbazine | | | |
| Chlorambucil | | | |
| BCNU | | | |
| CCNU | | | |
| Melphalan | | | |
| Thiotepa | | | |
| Nitrogen Mustard | | | |
| Busulfan | | | |
| | | | |
| Cyclophosphamide Equivalent Dose Score = | | | 0 mg/m2 |

http://oncofertility.northwestern.edu/sites/oncofertility.northwestern.edu/files/ced_calculator.xlsx

# Estimating Risk

**TABLE IV. Rate Ratios for Non-Surgical Premature Menopause: Multiple Poisson Regression Model**

| | CED | | | AAD | | |
|---|---|---|---|---|---|---|
| Variable | RR | 95% CI | *P*-value | RR | 95% CI | *P*-value |
| Age | **1.14** | **1.09–1.20** | **<0.001** | **1.13** | **1.07–1.19** | **<0.001** |
| Minimum ovarian dose | | | | | | |
| Other cancers | | | | | | |
| None | 1.00 | | | 1.00 | | |
| 1–99 cGy | 2.96 | 0.92–9.50 | 0.069 | **4.25** | **1.18–15.26** | **0.027** |
| ≥100 cGy | **11.68** | **3.59–38.04** | **<0.001** | **16.77** | **4.55–61.88** | **<0.001** |
| Hodgkin lymphoma | | | | | | |
| None | **13.86** | **4.04–47.57** | **<0.001** | **9.88** | **1.65–59.24** | **0.012** |
| 1–99 cGy | **10.04** | **3.40–29.65** | **<0.001** | **12.73** | **3.55–45.57** | **<0.001** |
| ≥100 cGy | **10.76** | **3.32–34.91** | **<0.001** | **10.73** | **2.70–42.64** | **<0.001** |
| CED ($mg/m^2$) | | | | | | |
| 0 | 1.00 | | | | | |
| >0–<4,000 | 0.56 | 0.07–4.27 | 0.578 | | | |
| ≥4,000–<8,000 | **2.74** | **1.13–6.61** | **0.025** | | | |
| ≥8,000 | **4.19** | **2.18–8.08** | **<0.001** | | | |
| AAD tertile | | | | | | |
| 0 | | | | 1.00 | | |
| 1–2 | | | | 2.09 | 0.97–4.51 | 0.060 |
| 3 | | | | **4.99** | **2.53–9.84** | **<0.001** |

CED, Cyclophosphamide Equivalent Dose; AAD, Alkylating Agent Dose score; RR, rate ratio; CI, confidence interval; values shown in bold are statistically significant.

Green et al. Pediatr Blood Cancer. 2014;61:53-67

# Gonadotoxic Risk: >80% risk of loss of reproductive potential

- Alkylating-intensive chemotherapy
  - any treatment regimen containing procarbazine
  - busulfan cumulative dose >600 mg/m2
  - cyclophosphamide equivalent dose (CED) ≥ 7,500 mg/m2
  - alkylating chemotherapy conditioning prior to SCT
- Whole abdomen/pelvic irradiation to ovaries
  - ≥15 Gy pre-pubertal, >10 Gy post-pubertal, >6 Gy adult
- Whole abdomen/pelvic irradiation to uterus ≥30 Gy
- Total body irradiation and cranial radiation ≥30 Gy

Metzger ML. J Clin Oncol; 31(9), 2013

# Subfertility/Infertility Risk

| High risk > 80% | Medium Risk >20 and <80% | Low Risk < 20% |
|---|---|---|
| Conditioning for BMT | AML | ALL |
| Hodgkin's: w/ alkylating agents | Hepatoblastoma | Wilms' tumor |
| Soft-tissue sarcoma: metastatic | Osteosarcoma | Soft-tissue sarcoma: stage I |
| Ewing's sarcoma: metastatic | Ewing's sarcoma: non-metastatic | Retinoblastoma |
| | Soft-tissue sarcoma: stage II/III | Germ-cell tumors (fertility sparing) |
| | Neuroblastoma | |
| | Non-Hodgkin lymphoma | |
| | Hodgkin's: alternating alkylator tx | |

# Gonadotoxicity of Newer Agents

- Paclitaxel, docetaxel (taxanes used in AC protocols)
- Oxaliplatin
- Irinotecan
- Bevacizumab
- Cetuximab
- Trastuzumab
- Erlotinib
- Imatinib

# Determinants of Gonadotoxicity

- Patient related factors
  - age
  - gender
- Treatment related factors
  - type and cumulative dose of chemotherapy
  - dose and site of radiation
  - type of surgery performed

# Differences in Sexual Differentiation

- Incongruence among the chromosomal, gonadal or phenotypic sex of an individual

- Risks to future biologic potential
  - abnormal gonadal development
  - gonadectomy for risk of malignancy
  - abnormal hormone production
  - potential discordance between gonadal type and gender identity

## Serum Levels of Anti-Müllerian Hormone as a Marker of Ovarian Function in 926 Healthy Females from Birth to Adulthood and in 172 Turner Syndrome Patients

- 926 controls
  - 788 between ages 0 and 20 years
  - 148 between the ages of 20.1-69 years
- 172 Turner syndrome (45X, various karyotype, 45X/46XX)

| Subjects ages 0-25 years | 45X (40) | Various (28) | 45X/46XX (10) |
|---|---|---|---|
| % AMH in reference range | 15% (6) | 43% (12) | 100% (10) |
| AMH (median; range) pmol/l | <2; 2-11 | 3; <2-33 | 16; 8-58 |
| Subjects ages 25-69 years | All chromosomal variations n= 88 | | |
| % AMH in reference range | 6% (5) | | |
| AMH (median; range) pmol/l | > 2 | | |

Hagen et al., J Clin Endocrinol Metab, Nov 2010, 95(11):5003–5010

**Fertility Preservation in Females with Turner Syndrome: A Comprehensive Review and Practical Guidelines**

K Oktay[1,2], G Bedoschi[1,2], K Berkowitz[3], R Bronson[4], B Kashani[5], P McGovern[5], L Pal[6], G Quinn[7,8], and K Rubin[9]

- Early identification of TS patients with ovarian reserve
- Salvage existing viable oocytes
- Pre-pubertal girls
  - sufficient ovarian reserve (AMH > 2 ng/ml)
    - serial serum AMH to delay intervention to post-puberty
    - ovarian tissue cryopreservation if AMH falls to < 2 ng/ml
    - oocyte cryopreservation at a post-pubertal age
  - insufficient reserve (AMH ≤ 2 ng/ml)
    - ovarian tissue cryopreservation
- Post-pubertal girls
  - recommend fertility preservation regardless of the initial AMH

Oktay al., JPAG. 2016 October ; 29(5): 409–416

**45,X/46,XY mixed gonadal dysgenesis: A case of successful sperm extraction.**

Flannigan RK[1], Chow V[2], Ma S[3], Yuzpe A[2].

- Mixed gonadal dysgenesis
  - Few case reports of successful paternity in phenotypic males
  - No reports of extraction of immature oocytes for IVM in phenotypic females
  - No reports of stimulation of ovarian follicles

Sugawara 2012;25(4):96-9
Flannigan 2014;8(1-2)e:108-10

# Stem cell transplant: Non-oncologic conditions

| Anemia | Autoimmune conditions | Other |
|---|---|---|
| Aplastic anemia<br>Fanconi's<br>Diamond Blackfan | Multiple sclerosis | Severe combined immuno-deficiency |
| Sickle-cell anemia | Systemic sclerosis | Wiskott-Aldrich disease |
| Thalassemia | Systemic lupus erythematosus | Metabolic storage defects<br>Mucopolysaccaridoses<br>Amyloidosis<br>Gaucher's disease |
| | Rheumatoid arthritis | |

Pandey. Radiol Clin N Am 2016;54:375-396

# World Professional Association for Transgender Health (WPATH)

- *Gender nonconformity* - extent to which a person's gender identity, role, or expression differs from the cultural norms prescribed for people of a particular sex

- *Gender dysphoria* - discomfort or distress caused by a discrepancy between person's gender identity and sex assigned at birth

- True prevalence unknown

- Treatment for gender dysphoria  may or may not involve a change in gender expression or body modifications

# Physical Interventions for Gender Dysphoria

- Hormonal minimization of existing secondary sexual characteristics
- Maximum feminization/masculinization

| Fully reversible | Partially reversible | Irreversible |
|---|---|---|
| GnRHa | Estrogen | Surgery |
| Medroxyprogesterone | Testosterone | |
| Spironolactone | | |
| Combined oral contraceptives | | |

# Effects of Medical Intervention on Fertility

- Estrogen:
  - decreased testicular volume
  - poor semen quality
  - azoospermia with possible reversal
- Testosterone:
  - reversible amenorrhea without follicle depletion
  - pregnancies reported in FTM individuals on or after testosterone
- Puberty blockers
  - prepubertal or pubertal adolescents many never develop reproductive function in their natal sex

De Roo et al. 2016, Wallace et al 2014

## Fertility options in transgender people

Chloë De Roo[a], Kelly Tilleman[a], Guy T'Sjoen[b] and Petra De Sutter[a]

- Oocyte and embryo cryopreservation standard options
- Family building may require  gestational surrogacy
- Ovarian and testicular cryopreservation investigational options and may occur at time of genital reconstructive surgery
- Physical Barriers
  - FTM patient - vaginal examination & invasive procedures
  - MTF patient - masturbation, semen production & storage; testicular sperm extraction/aspiration

de Roo et al., Inter Rev Psych2016; vol 28, no. 1, 112-119

# Outline

a. Indications for OTC
   i. Chemo/radiation
   ii. Non-malignant disorders
   iii. Sex Diversity
   iv. Predisposition to POI
b. Setting up OTC program
c. Medical and surgical techniques
d. Collaboration with external centers

# Ovarian Tissue Cryopreservation

Livebirth after orthotopic transplantation of cryopreserved ovarian tissue

J Donnez, M M Dolmans, D Demylle, P Jadoul, C Pirard, J Squifflet, B Martinez-Madrid, A Van Langendonckt

**Live birth after autograft of ovarian tissue cryopreserved during childhood**

**Isabelle Demeestere[1,2,*], Philippe Simon[3], Laurence Dedeken[4], Federica Moffa[1,6], Sophie Tsépélidis[1,2,7], Cecile Brachet[5], Anne Delb**

News

**Woman gives birth to baby using ovary frozen in her childhood in 'world first'**

share

14 DECEMBER 2016

86 successful births and 9 ongoing pregnancies worldwide in women transplanted with frozen-thawed ovarian tissue: focus on birth and perinatal outcome in 40 of these children

- 95 total children worldwide
- Age range from adolescence to mid 30's
- Mean gestational age 39 for 40 of the patients with follow-up
- Half of singletons conceived naturally; twins by IVF
- Suggest that OTC is becoming an established fertility preservation method and should no longer be considered experimental

Jensen et al. J Assist Reprod Genet (2017) 34: 325

# Transplantations of frozen-thawed ovarian tissue demonstrate high reproductive performance and the need to revise restrictive criteria

Dror Meirow, M.D.,[a,b] Hila Ra'anani, M.D.,[a,b] Moran Shapira, M.D.,[a,b] Masha Brenghausen, Ph.D.,[a,b] Sanaz Derech Chaim, B.Sc.,[a] Sarit Aviel-Ronen, M.D., Ph.D.,[c] Ninette Amariglio, Ph.D.,[d] Eyal Schiff, M.D.,[b] Raoul Orvieto, M.D.,[a,b] and Jehoshua Dor, M.D.[a,b]

- 20 patients underwent auto-transplantation
- Ages 14 through 39 years at cryopreservation
- 15 hematologic malignancies and 5 solid tumors
- 10 patients treated with non-sterilizing chemotherapy before harvesting
- 5.6 year average time after tissue cryopreservation
- Mean age at transplantation 34 years

# Transplantations of frozen-thawed ovarian tissue demonstrate high reproductive performance and the need to revise restrictive criteria

Dror Meirow, M.D.,[a,b] Hila Ra'anani, M.D.,[a,b] Moran Shapira, M.D.,[a,b] Masha Brenghausen, Ph.D.,[a,b] Sanaz Derech Chaim, B.Sc.,[a] Sarit Aviel-Ronen, M.D., Ph.D.,[c] Ninette Amariglio, Ph.D.,[d] Eyal Schiff, M.D.,[b] Raoul Orvieto, M.D.,[a,b] and Jehoshua Dor, M.D.[a,b]

- 16 patients primary ovarian failure
- 4 patients had ovarian function but were infertile
  - 2 with lab work consistent with ovarian insufficiency
  - 2 aged 45
- 93% endocrine recovery rate
- 53% conception rate
- 32% delivery rate

# Ovarian tissue transplantation

**Patient characteristics and pre/posttransplantation endocrine profiles.**

| Patient | Diagnosis | Pre-OTCP chemotherapy[a] | No. of children | Age (y) OTCP | Age (y) Tx | Ovarian function before Tx: Menses | Ovarian function before Tx: FSH (mIU/mL) | Ovarian function before Tx: $E^2$ (pmol/L) | Ovarian function after Tx: Menses[b] | Ovarian function after Tx: FSH (mIU/mL) | Ovarian function after Tx: $E^2$ (pmol/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No previous chemotherapy | | | | | | | | | | | |
| 5 | CML | – | 0 | 19 | 27 | No | 116.4 | <37 | Yes (6M) | 7.2 | 147 |
| 5[c] | CML | – | 0 | 21 | 28 | No | 44 | 174 | Yes (4M) | 100 | 360 |
| 7 | Hodgkin's | – | 0 | 19 | 35 | No | 87 | <100 | Yes | 12.4 | 107 |
| 9 | Breast cancer | – | 1 | 37 | 45 | Yes | 8.4 | 100 | Yes | 5.7 | 177 |
| 10 | NHL | – | 0 | 39 | 41 | No | 99 | <100 | Yes (1M) | 96 | <100 |
| 11 | Hodgkin's | – | 0 | 32 | 36 | No | 54.6 | <73.4 | Yes | 27.6 | 126 |
| 12 | Breast cancer | – | 0 | 33 | 38 | Yes | 4.8 | 858 | Yes | 6.8 | 380 |
| 13 | NHL | – | 1 | 34 | 42 | No | 30 | 100 | Yes (6M) | 26.4 | <100 |
| 15 | Hodgkin's | – | 0 | 37 | 40 | No | 41.8 | 137 | Yes | 7.8 | 645 |
| 17 | Breast cancer | – | 0 | 37 | 45 | Yes | 6.8 | 232 | Yes | 14 | <185 |
| 18 | Ewing's sarcoma | – | 0 | 24 | 28 | No | 107 | <70 | Yes | 24.3 | <100 |
| Previous chemotherapy | | | | | | | | | | | |
| 1 | NHL | VACOP_B, MINE/ESHAP | 1 | 30 | 32 | No | 100 | <70 | Yes | 7 | 342 |
| 2 | NHL | VACOP_BX6 | 1 | 38 | 42 | No | 120 | <100 | Yes | 11 | 220 |
| 3 | Hodgkin's | ABVDX4 | 1 | 31 | 36 | Yes | 10.3 | 380 | Yes | 9.5 | 99 |
| 4 | Hodgkin's | ABVDX6 | 1 | 24 | 27 | No | 81 | <70 | Yes | 12.2 | <73.4 |
| 6 | Hodgkin's | ABVD | 0 | 23 | 26 | No | 60.7 | 146 | Yes | 13.8 | <73.4 |
| 8 | Ewing's sarcoma | VCAIE | 0 | 14 | 21 | No | 60 | 69 | No | 94 | <100 |
| 14 | Hodgkin's | ABVDX6 | 0 | 29 | 32 | No | 108 | 70 | Yes | 27 | 288 |
| 16 | Hodgkin's | ABVDX6 | 0 | 32 | 36 | No | 33.1 | 99 | Yes | 16 | 95 |
| 19 | Hodgkin's | ABVDX5, MOPPX1 | 0 | 23 | 28 | No | 68 | 70 | Yes | 44 | 134 |
| 20 | AML | Doxorubicin + ARA-C | 0 | 19 | 31 | No | 37 | 180 | Yes | 29 | 70 |

*Note:* AML = acute myeloid leukemia; CML = chronic myelogenous leukemia; $E_2$ = estradiol; FSH = follicle-stimulating hormone; NHL = non-Hodgkin's lymphoma; OTCP = ovarian tissue cryopreservation; Tx = transplantation.

[a] VACOP_B = etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone, bleomycin; MINE/ESHAP = mesna, ifosfamide, mitoxantrone, etoposide/teniposide, methylprednisolone, high-dose cytarabine, cisplatin; ABVD = doxorubicin, bleomycin, vinblastine, dacarbazine; MOPP = mechlorethamine, vincristine, procarbazine, vincristine; VCAIE = vincristine, cyclophosphamide, arabinosidecytosine, idarubucin.

[b] Numbers in brackets represent duration of menses in case menses ceased during follow-up observation.

[c] Results after the second transplantation.

**Methods for detection of tissue involvement with cancer cells.**

| Cancer | Patients (n) | Histology | Immunohistochemistry | Molecular biology | SCID mice transplantation |
|---|---|---|---|---|---|
| Hodgkin's disease | 9 | 9 | 9 | | |
| Non-Hodgkin's lymphoma | 4 | 4 | 4 | | |
| Breast cancer | 3 | 3 | 3 | | |
| Ewing's sarcoma | 2 | 2 | 2 | 1 | |
| Chronic myeloid leukemia | 1 | 1 | | 1 | |
| Acute myeloid leukemia | 1 | 1 | 1 | 1 | 1 |

*Meirow. Ovarian transplantation in 20 cancer survivors. Fertil Steril 2016*

# Outcomes of transplantations of cryopreserved ovarian tissue to 41 women in Denmark

- Ovarian tissue cryopreservation initiated in Denmark in 2000
- 800 patients have undergone ovarian tissue cryopreservation
- Annual activity of 13-14 cases per million inhabitants per year
- 53 transplantations to 41 patients over 10 years
- Among 32 women with a pregnancy-wish, 24 clinical pregnancies and 10 (31%) had a child/children
- Transplanted ovarian tissue may last 10 years

Jensen et al. Hum. Reprod. 2015;0(0):1-8

**Pediatric and Teen Ovarian Tissue Removed for Cryopreservation Contains Follicles Irrespective of Age, Disease Diagnosis, Treatment History, and Specimen Processing Methods.**

Duncan FE[1], Pavone ME[1], Gunn AH[1], Badawy S[2], Gracia C[3], Ginsberg JP[4], Lockart B[5], Gosiengfiao Y[5], Woodruff TK[1].

- 24 patients s/p ovarian tissue cryopreservation
- No previous treatment and low and high risk treatment
- Oncologic and non-oncologic diagnoses
- 10/24 underwent removal of cortical strips vs oophorectomy
- Primordial and/or early-activated primary follicles in all samples
- Small pre-antral follicles identified in patients who had not received oncologic treatments

Duncan et al. J Adolesc Young Adult Oncol. 2015 Dec 1; 4(4): 174–183

**The immature human ovary shows loss of abnormal follicles and increasing follicle developmental competence through childhood and adolescence.**

Anderson RA[1], McLaughlin M, Wallace WH, Albertini DF, Telfer EE.

- Presence of primordial follicles does not guarantee that cryopreserved ovarian tissue will have sufficient ovarian potential for future function.

- Demonstrated that human pre-pubertal ovaries contain a high proportion of abnormal non-growing follicles that have a reduced ability to grow *in vitro*.

Anderson et al. Hum Reprod. 2014 Jan;29(1):97-106.

# Essential elements in setting up an OTC program

Departmental and Institutional Support

- Business Manager
- Oncology physician champions
- Hospital policy
- Patient Navigator (RN or other)
  - Identify patients for consult
  - Coordinate visits, procedures, follow-up, research participation
- Advanced practice provider (optional)
  - Consults
  - Development of written educational materials

# Essential elements in setting up an OTC program

Equipment and Space

# Outline

a. Indications for OTC
   i. Chemo/radiation
   ii. Non-malignant disorders
   iii. Sex Diversity
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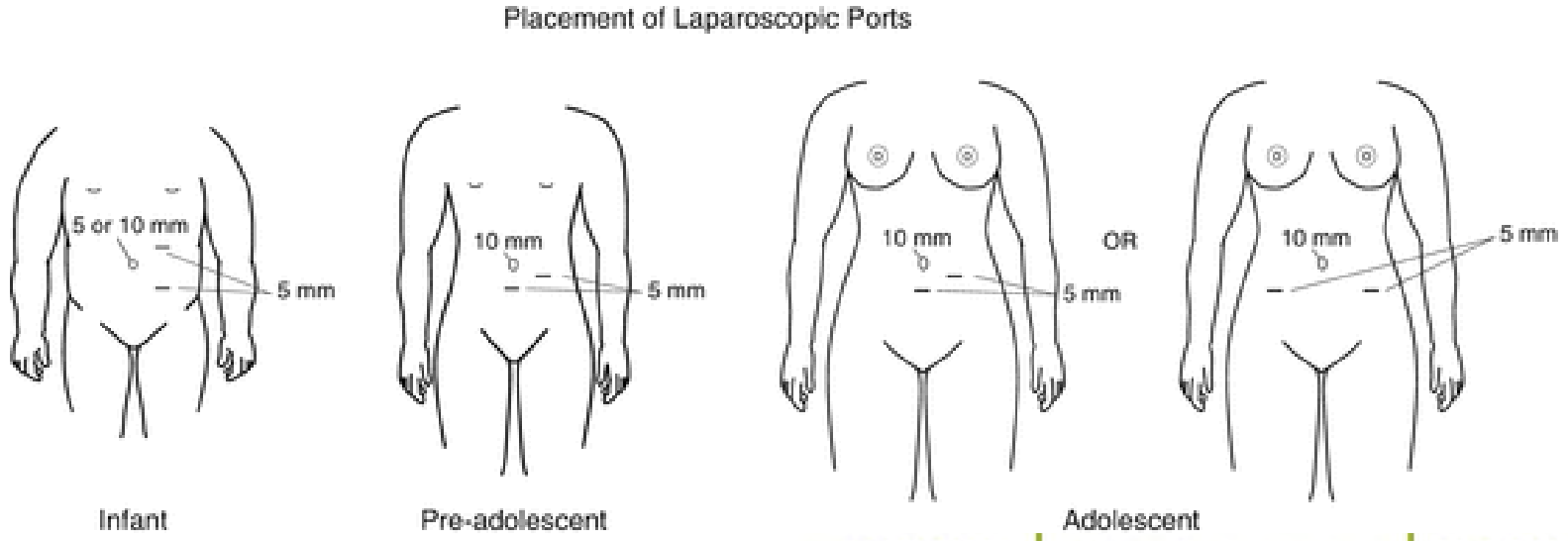
b. Setting up OTC program
c. Medical and surgical techniques
d. Collaboration with external centers

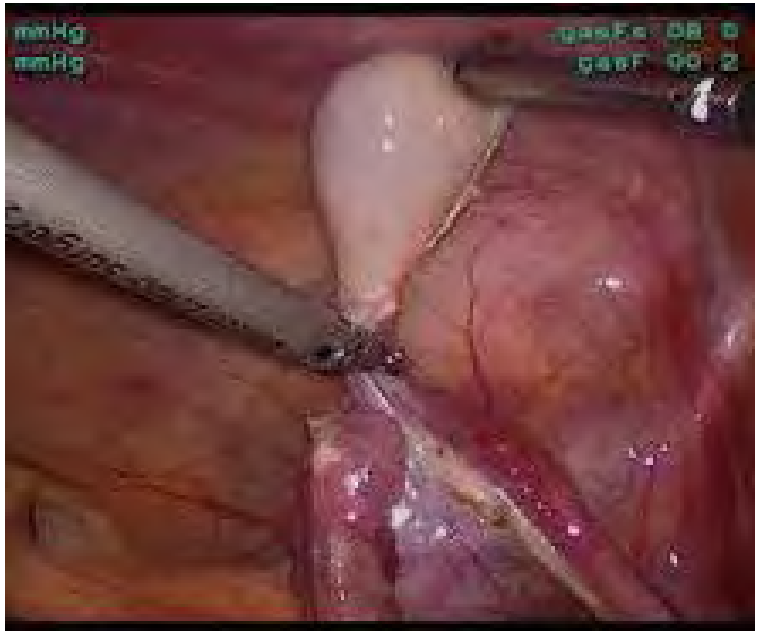
# Technical Aspects of OTC

- Laparoscopy ideal however laparotomy feasible if related to oncologic resection
- Bundle with cancer related procedure to minimize anesthesia
- Port placement determined by patient size/age
- Right ovary typically most accessible with left-sided ports
- Suprapubic or lower quadrant ports also utilized



# Technical Aspects of OTC

Oophorectomy



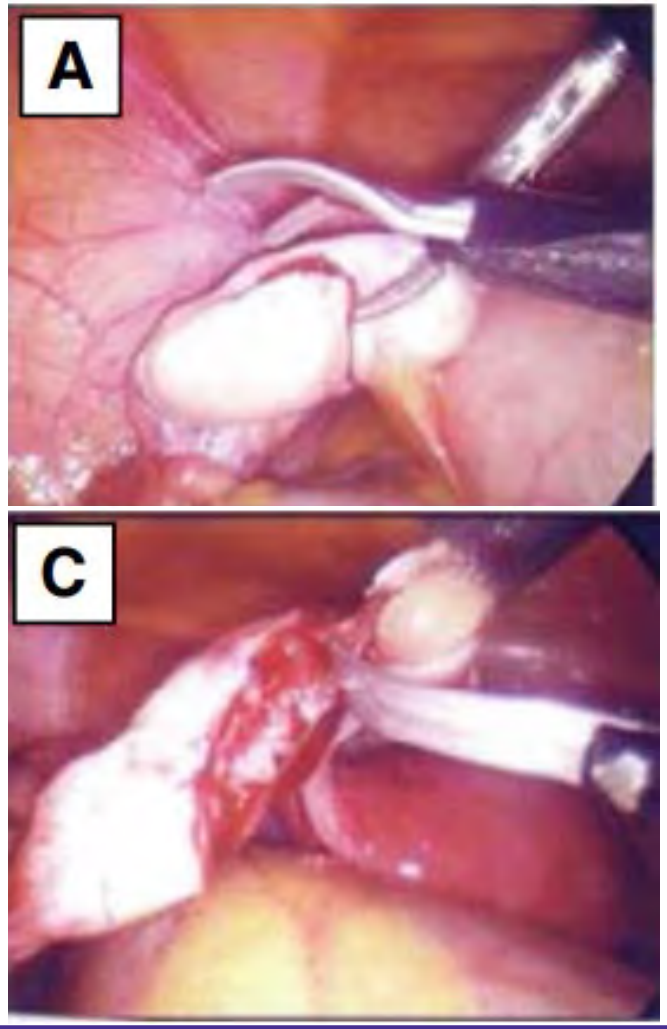
- Allows more cortical tissue for future use

- Select ovary without cyst or corpus luteum
- Minimize manipulation of ovary by grasping uteroovarian ligament – “no touch technique”
- Transect uteroovarian ligament → mesovarium → infundibulopelvic ligament
- Transect fallopian tube at isthmus in infant and pre-pubertal girls due to narrow mesovarium

Corkum et al. Am Journal of Surg 214 2017

# Technical Aspects of OTC

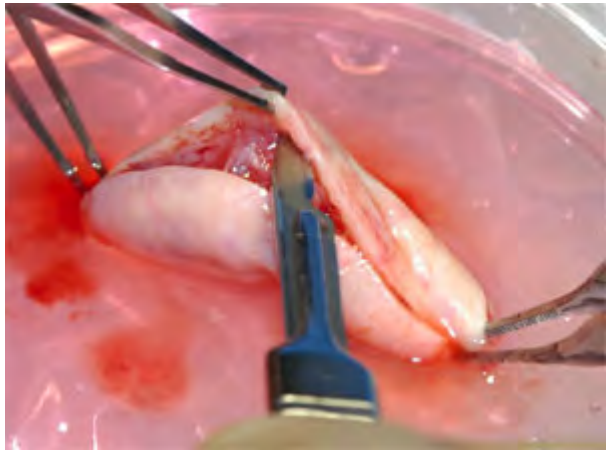
## Cortical Biopsy

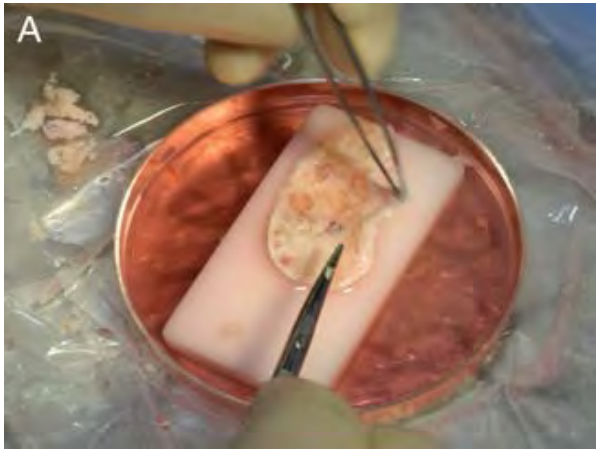



- Select ovary without cyst or corpus luteum
- Minimize manipulation of ovary by grasping uteroovarian ligament – “no touch technique”
- Cold scissors to transect longitudinal strips of ovary
- Cautery, argon beam, thrombin products for anticoagulation
- Allows potential recovery of remaining ovary

Gracia et al. J Assist Reprod Genet 2012

# Technical Aspects of OTC



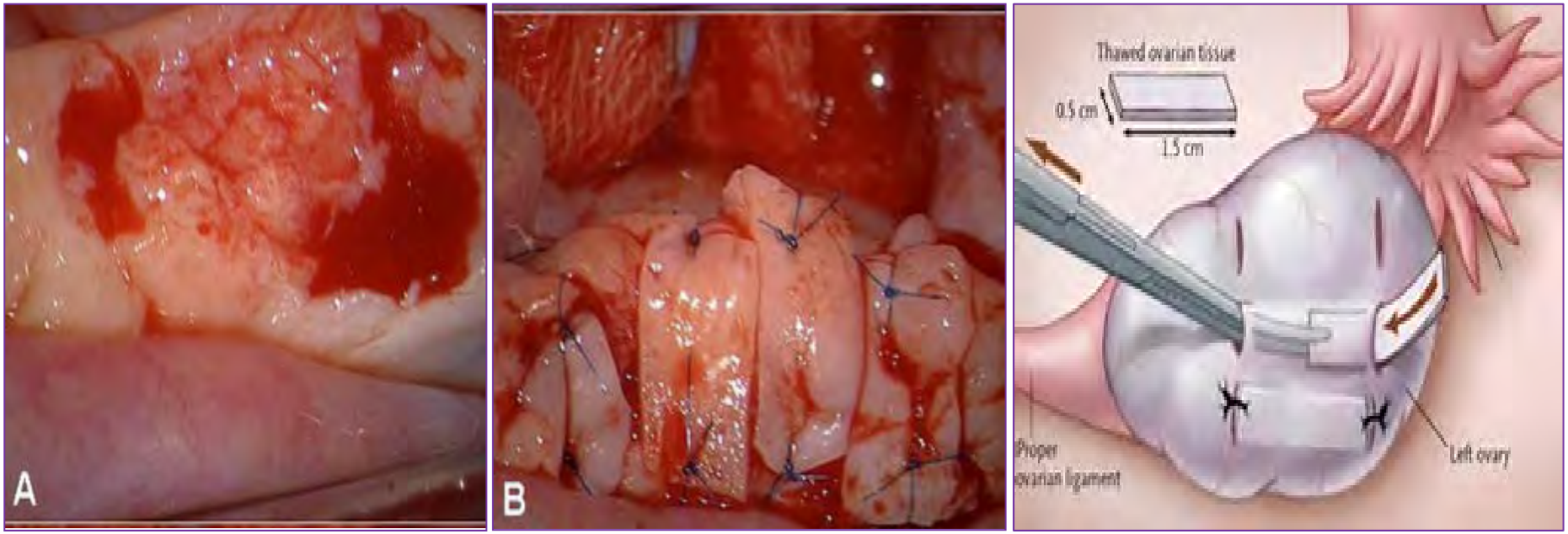



- Ovarian cortex contains primordial and primary follicles
- Cortex transected into 1.0 cm x 0.5 x 0.2 cm strips for freezing
- Slow-freeze technique
- Vitrification
- May be stored indefinitely to date

# Orthotopic Transplantation: Ovarian Fossa

Donnez et al. Frontiers in Bioscience 2012

# Orthotopic Transplantation: Contralateral Ovary




Donnez J et al. Hum. Reprod. Update 2006;12:519-535

# Heterotopic Transplantation

**First reported clinical pregnancy following heterotopic grafting of cryopreserved ovarian tissue in a woman after a bilateral oophorectomy.**

Stern CJ[1], Gook D, Hale LG, Agresta F, Oldham J, Rozen G, Jobling T.

- 21 yo s/p bilateral oophorectomy for granulosa cell tumor
- OTC prior to the second surgery with histological analysis
- Desired transplantation 7 years later post histologic reevaluation
- Grafts to pelvic sidewalls and anterior abdominal wall under peritoneum without pregnancy after transfer
- Second graft to anterior abdominal wall 2 years later
- Stimulation, retrieval, ICSI, embryo transfer and twin delivery

Stern et al. Hum Reprod. 2013 Nov;28(11):2996-9.

# In Vitro Maturation

**First pregnancy and live birth resulting from cryopreserved embryos obtained from in vitro matured oocytes after oophorectomy in an ovarian cancer patient.**

Prasath EB[1], Chan ML, Wong WH, Lim CJ, Tharmalingam MD, Hendricks M, Loh SF, Chia YN.

- 21 yo s/p interval bilateral oophorectomy for bilateral serous carcinoma of the ovary
- OTC performed at second surgery
- All visible follicles aspirated
- ICSI followed by 2 embryo transfer
- Delivery of healthy infant
- Several reports of live birth after IVM of growing follicles
- No reports of live birth after IVM of primordial follicles

# Procedural Steps: Prior to OR day

- **Notify long term storage site of OTC procedure.**
- **Notify surgeon performing port/central line to coordinate the procedure.**
- **Notify Pathology/Cell Therapy Lab to confirm feasibility of date of procedure.**

- **Notify OR staff – preferable to perform procedure at the same location each time; Surgery Center or Main OR.**
- **Notify Special Chemistry to obtain "kit" for infectious disease blood draw.**
- **Notify regulatory team of ovarian tissue cryopreservation procedure if under IRB.**

# Procedural Steps: Day of Procedure

- **Surgery team to pick-up the cooler, media and tubes for blood draw from Cell Therapy Lab (CTL) 30 minutes prior to procedure start.**
- **OR staff to notify CTL when the patient is entering the operating room and when laparoscopy begins.**
- **Blood draw for infectious disease testing to be performed by anesthesia team at case start (3 purple tubes and 1 red tube).1 purple tube to be sent with ovary to CTL.**
- **Port/central placement/bone marrow biopsy to occur first as sterile procedure (30-45 minutes).**

# Procedural Steps: Day of Procedure

- **Surgeon to call out time that ovary is transected; to be recorded in medical record and/or research paperwork.**
- **Ovary should be removed per protocol and placed in holding media with purple tube of blood to be given to pathology/CTL.**
- **Ovary is processed in CTL or pathology per protocol.**
- **Pathology may attain sample for histologic assessment.**
- **May proceed with cancer treatment w/in 24 - 72 hours.**

# Cell Therapy Lab/Pathology

- **CTL/pathology processes tissue and plasma (purple top).**
- **CTL/pathology stores tissue with plasma in liquid nitrogen vapor phase until ID results return.**
- **CTL/pathology transports tissue with plasma in liquid nitrogen dewar supplied by long term storage facility once ID results return.**

# Contacts: Ovarian biopsy/excision

- Pathology
- Operating Room Director
- Special Chemistry
- Cell Therapy Lab
- Regulatory/Finance
- Professional Billing
- Contact for philanthropic funds

# Outline

a. Indications for OTC
   i. Chemo/radiation
   ii. Non-malignant disorders
   iii. Sex Diversity
   iv. Predisposition to POI
b. Setting up OTC program
c. Medical and surgical techniques
d. Collaboration with external centers

# Collaborating with an External Site:
## Andrology/Embryology Lab
## Regional OTC site

- **Surgeon to pick-up the cooler, media, tubes from external site.**
- **Port/central line to occur first as sterile procedure.**
- **Blood draw for ID testing (optional) to be performed by anesthesia team at case start (3 purple tubes and 1 red tube). 1 purple tube to be sent with ovary for long term storage.**

- **Pathologist notified of case start time to be present at resection for gross assessment and possible tissue sampling for histology.**
- **Surgeon to call out time that ovary is transected, to be recorded.**
- **Ovary removed per protocol and placed in holding media.**

# Collaborating with an External Site:
## Andrology/Embryology Lab
## Regional OTC site

- **Ovary (in holding media) placed in cooler with purple tube of blood and handed to OR assistant to transfer to courier.**
- **Ovary is transferred to external site for processing and transfer to long term storage facility.**
- **May proceed with cancer treatment within 24-72 hours.**

# Take Home Points: OTC

- 100 births worldwide to date with a 28-32% birth rate.
- The only fertility preservation option for pre-pubertal females.
- An option for individuals at risk of infertility from cancer, non-malignant c
- R dered after non-sterilizing doses of chemotherapy.
- Restoration of hormonal function is a benefit of transplantation.
- IVM is investigational technology that may obviate need for transplantation.
- Fertility centers and embryology labs may serve as regional sites.

*"It Takes a Village"*


Thank You
The Ohio State University
Wexner Medical Center
The James Cancer Hospital and Solove Research Institute