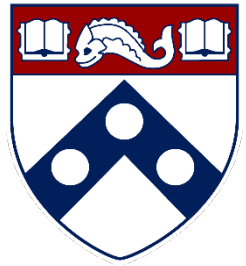
Fellow Education Day 2019

Wendy Vitek, MD
University of Rochester

Divya K. Shah, MD, MME
University of Pennsylvania



Course Objectives

- Describe currently available options for female and male fertility preservation including recent advances in embryo, oocyte, ovarian tissue, 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.

Time	Topic	Speaker
08:00-08:15	Introduction to Course and Learning Teams	
08:15-09:00	Fertility Preservation in the Male	Jim Smith, MD, MS – Urologist and Director of Male Reproductive Health, University of California, San Francisco (UCSF)
09:05-9:15	Introduction to Team Based Learning	
09:20-10:20	Team based learning: Adult male and early reproductive female	Divya Shah, MD, MME – Reproductive Endocrinologist, University of Pennsylvania Wendy Vitek, MD – Reproductive Endocrinologist and Director of Fertility Preservation, University of Rochester
10:25-11:10	Fertility preservation in breast cancer patients: issues of timing	Jacqueline Jeruss, M.D., Ph.D. – Surgical Oncologist and Director, Breast Care Center, University of Michigan
11:15-12:15	Lunch	
12:20-01:05	Practical aspects of ovarian tissue cryopreservation in the pediatric population	Leslie Appiah, MD – Pediatric and Adolescent Gynecologist and Chief, Division of General Obstetrics & Gynecology, The University of Colorado Denver
01:10-02:10	Team based learning: Late reproductive and pre-pubertal female	Divya Shah, MD, MME – Reproductive Endocrinologist and Infertility Specialist, University of Pennsylvania Wendy Vitek, MD – Reproductive Endocrinologist and Infertility Specialist, Director of Fertility Preservation, University of Rochester
02:15-03:00	Oncofertility State of the Union: advances from the past 5 years and projections for the next 5 years	Teresa Woodruff, PhD – Director Women's Health Research Institute, Chief, Division of Obstetrics and Gynecology-Fertility Preservation, Northwestern University

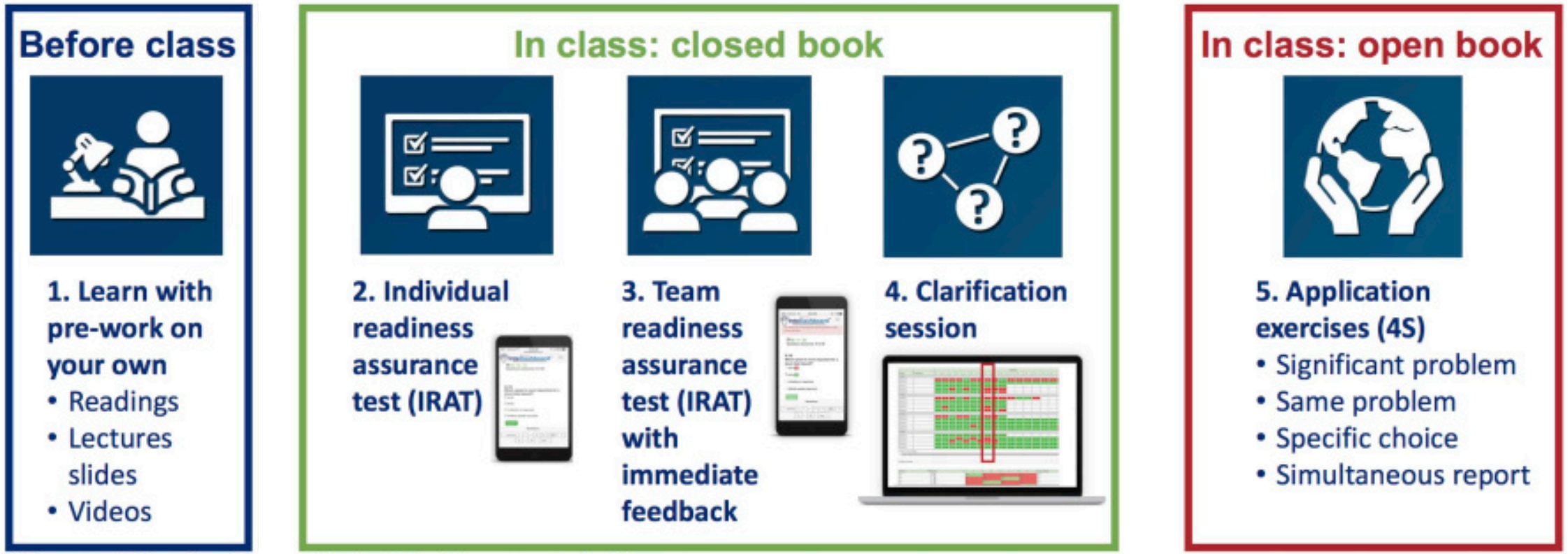
Team Introductions

Jim Smith, MD, MS

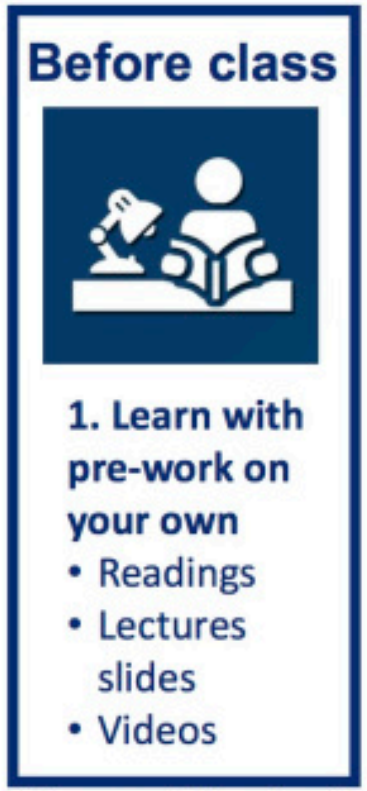
- Associate Professor, Department of Urology
- Director of Male Reproductive Health
- University of California, San Francisco

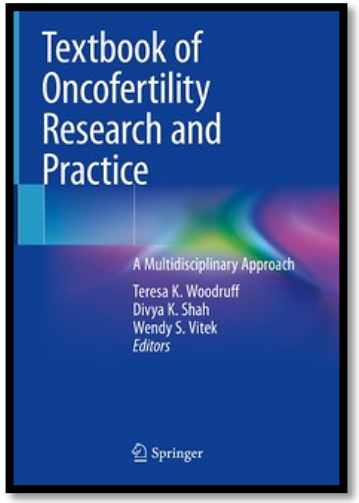
Time	Topic	Speaker
08:00-08:15	Introduction to Course and Learning Teams	
08:15-09:00	Fertility Preservation in the Male	Jim Smith, MD, MS – Urologist and Director of Male Reproductive Health, University of California, San Francisco (UCSF)
09:05-9:15	Introduction to Team Based Learning	
09:20-10:20	Team based learning: Adult male and early reproductive female	Divya Shah, MD, MME – Reproductive Endocrinologist, University of Pennsylvania Wendy Vitek, MD – Reproductive Endocrinologist and Director of Fertility Preservation, University of Rochester
10:25-11:10	Fertility preservation in breast cancer patients: issues of timing	Jacqueline Jeruss, M.D., Ph.D. – Surgical Oncologist and Director, Breast Care Center, University of Michigan
11:15-12:15	Lunch	
12:20-01:05	Practical aspects of ovarian tissue cryopreservation in the pediatric population	Leslie Appiah, MD – Pediatric and Adolescent Gynecologist and Chief, Division of General Obstetrics & Gynecology, The University of Colorado Denver
01:10-02:10	Team based learning: Late reproductive and pre-pubertal female	Divya Shah, MD, MME – Reproductive Endocrinologist and Infertility Specialist, University of Pennsylvania Wendy Vitek, MD – Reproductive Endocrinologist and Infertility Specialist, Director of Fertility Preservation, University of Rochester
02:15-03:00	Oncofertility State of the Union: advances from the past 5 years and projections for the next 5 years	Teresa Woodruff, PhD – Director Women's Health Research Institute, Chief, Division of Obstetrics and Gynecology-Fertility Preservation, Northwestern University

Principles of Team Based Learning



Before class preparation





Clinical experience

Application Exercise

In class: open book

5. Application exercises (4S)

- Significant problem
- Same problem
- Specific choice
- Simultaneous report

In-Class Activities (4 S's)

- **Significant Problems**. Teams work on a relevant, significant problem.
- **Same Problem**. Teams work on the same problem.
- **Specific Choice**. Teams required to make a specific choice.
- **Simultaneous Report**. Teams report simultaneously.

CASE #1:

A 17-year-old transwoman presents for fertility preservation. She recalls dressing up as a female as a child. In middle school, she started to feel like she wanted to change the way people referred to her gender, but "pushed that away and ignored it for a while."

About two years ago, she started thinking again about wanting to change her body and her presentation.

She started working with a therapist and has been treated for anxiety and depression with duloxetine. She has started presenting as female when she goes out socially. She is uncomfortable masturbating but will do so to facilitate tucking. She identifies as pansexual and has had oral and vaginal sex with male and female partners since age 15.

She would like to be a mother in the future and would consider adoption. She is anxious to start gender affirming hormone therapy with spironolactone and estrace with the goals of having less body hair, body fat redistribution and breast development.

Question 1:

Which of the following would you recommend as the best approach to fertility preservation?

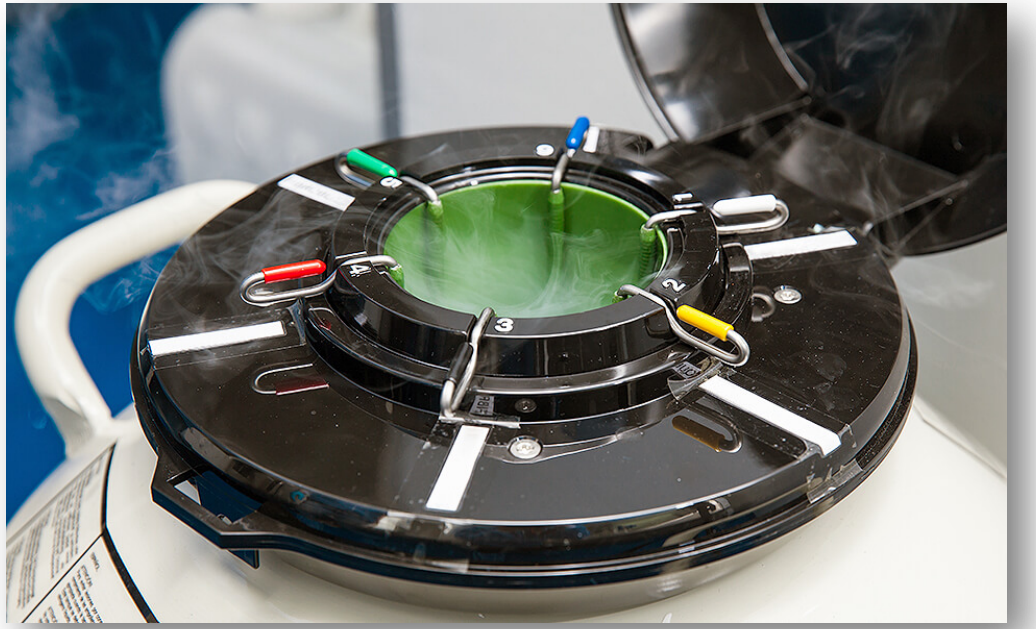
A. Start gender affirming hormone therapy and revisit fertility when ready to conceive
B. Sperm bank via masturbation
C. Sperm bank via electroejaculation
D. Sperm bank via testicular sperm extraction

Question 2:

Given the patient's age, which of the following steps should be taken?

A. Obtain consent/assent from the patient for sperm banking
B. Obtain parental consent for sperm banking
C. Obtain parent consent for pornographic material in the collection room to assist with collection
D. Provide pornographic material from the collection room in the absence of parental consent

The sample revealed a total motile count of 2.1 million prior to cryopreservation with a post thaw total motile count of 0.1 million. She has 6 vials banked.



Question 3:

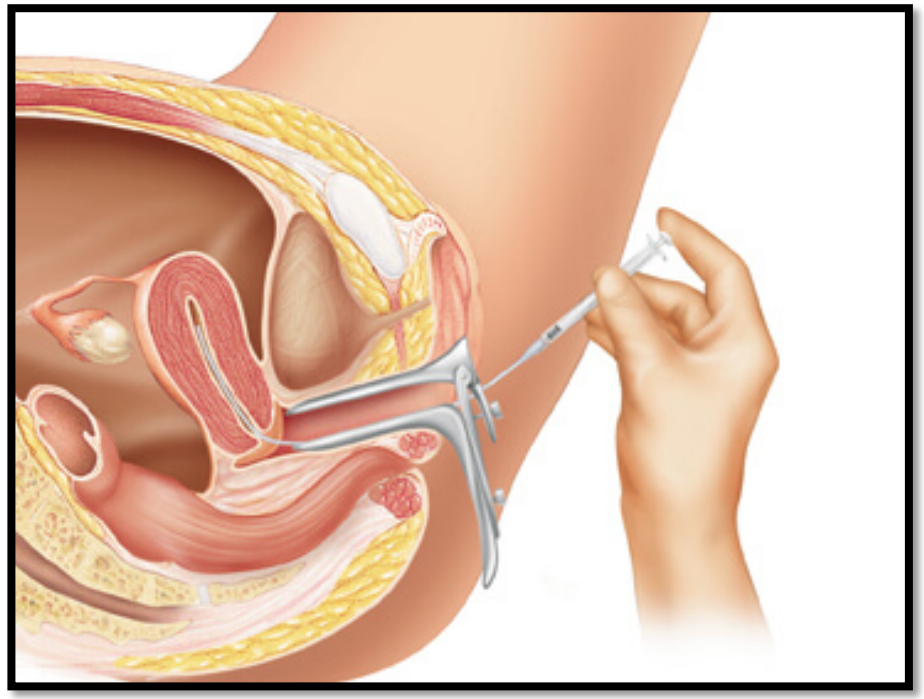
What are possible explanations for her severe oligospermia?

A. Transwomen demonstrate higher incidence of oligospermia than cisgender sperm bankers
B. Nondisclosed hormone therapy
C. Altered testicular function related to tucking and tight clothes

She presents at age 27 with her partner who is a 25-year-old cis female. The patient is maintained on estrace 3 mg twice daily and spironolactone 100 mg twice daily.

Her labs reveal an estradiol of 154 pg/ml, free testosterone 1.28 ng/dl and total testosterone LC-MS/MS 61 ng/dl.

Her partner has regular cycles and has never tried to conceive. They would like to attempt intrauterine insemination.



Question 4:

How would you counsel the couple regarding their options?

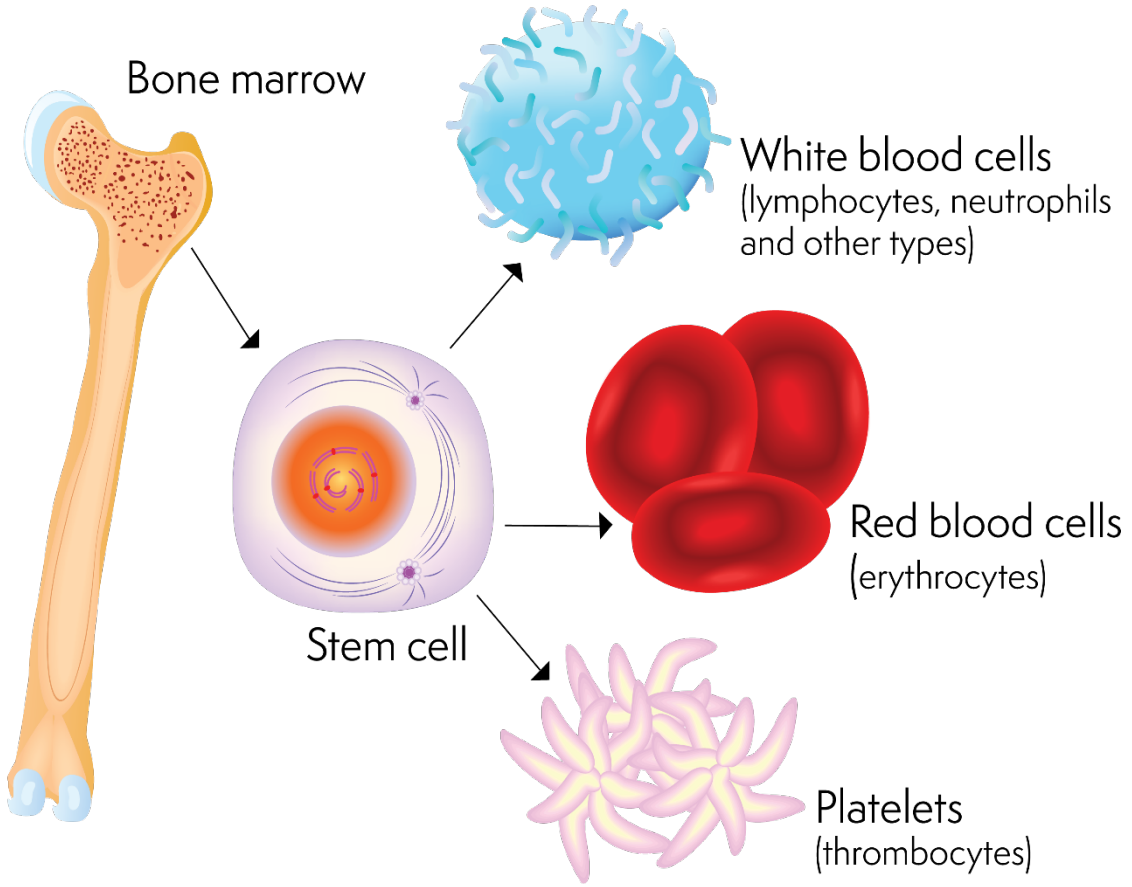
A. Attempt sperm collection now for IVF/ICSI with fresh autologous sperm
B. Stop estrace, spironolactone and tucking and attempt sperm collection in 3 months
C. Pool the 6 frozen vials of sperm for one insemination
D. Recommend insemination with donor sperm
E. Recommend IVF/ICSI with cryopreserved autologous sperm

CASE #2:

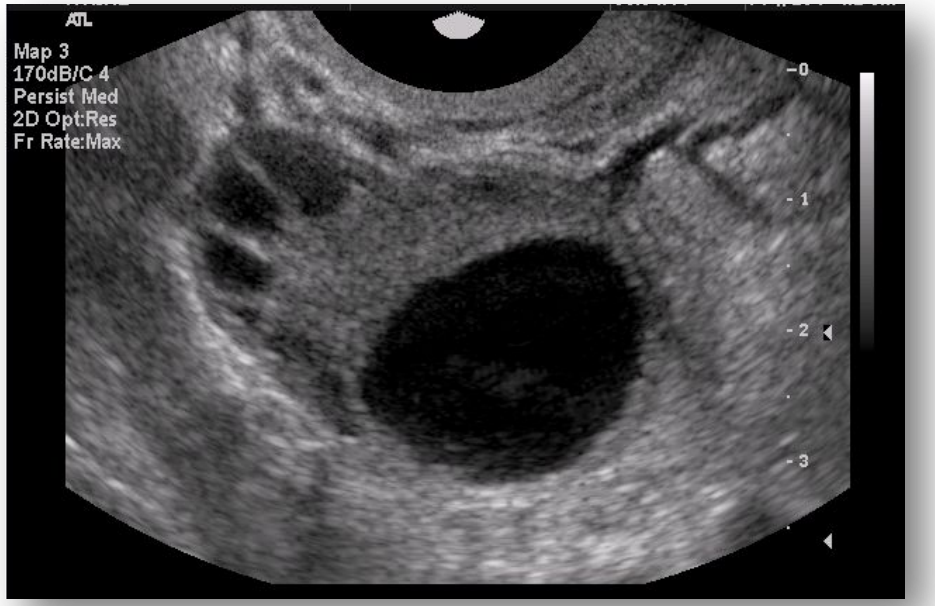
A 17-year-old girl presents is scheduled to undergo hematopoietic stem cell transplant for non-Hodgkin's lymphoma.

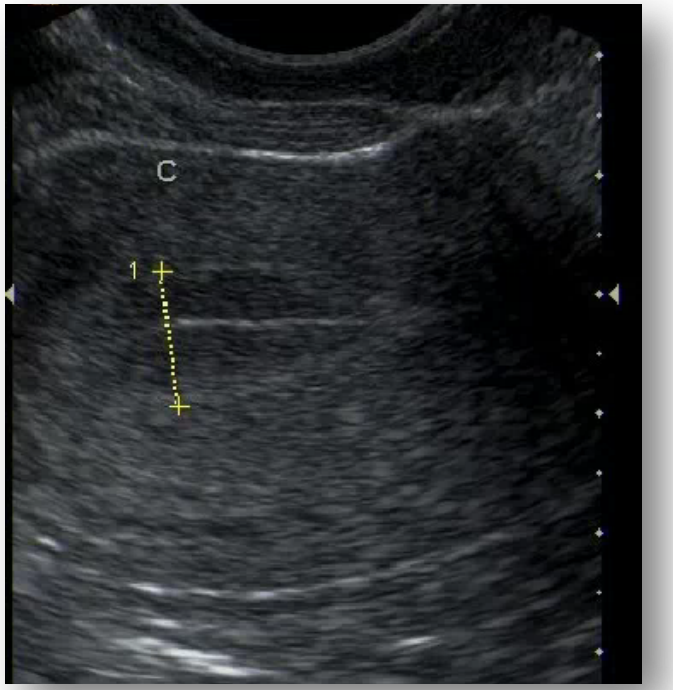
Her Oncology team has discussed the risk of subsequent infertility and would like to proceed with therapy as soon as possible. The patient has expressed interest in discussing fertility preservation.

She underwent menarche at age 12 and has never been sexually active. She has regular cycles q 30 days. LMP was two weeks ago.



She has been seen and counseled with both parents presents and gives her assent for oocyte cryopreservation. A pelvic ultrasound performed at the time of initial consultation demonstrates a 18 mm follicle on her right ovary and a 12 mm trilaminar endometrium. The antral follicle count is > 20 on each ovary. Labs are as follows: estradiol 254 pg/ml, LH 17.4 IU/L, progesterone 1.3 ng/ml.





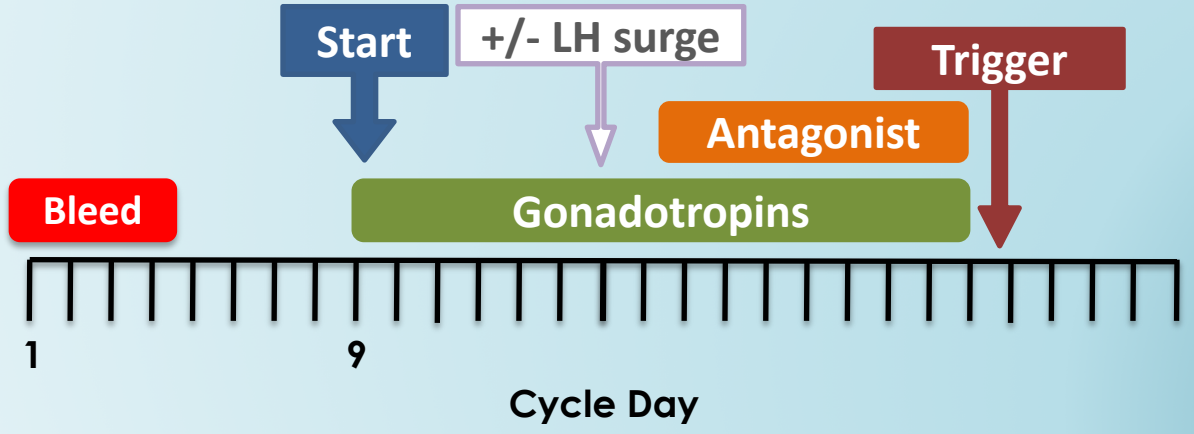
Question 1:

Given the patient's clinical presentation, which option(s) would you select for ovarian stimulation?

A. Await menses before starting gonadotropins. Add a GnRH antagonist once the lead follicle reaches 12-14 mm.
B. Start gonadotropins now. Add a GnRH antagonist once another lead follicle reaches 12-14 mm.
C. Await ovulation before starting low dose leuprolide in the luteal phase. Await withdrawal bleed before starting gonadotropins.
D. Trigger ovulation with HCG before starting gonadotropin stimulation. Add a GnRH antagonist once a lead follicle reaches 12-14 mm.
E. Await ovulation before starting gonadotropin stimulation. Add a GnRH antagonist once a lead follicle reaches 12-14 mm

Late follicular phase: random start option #1

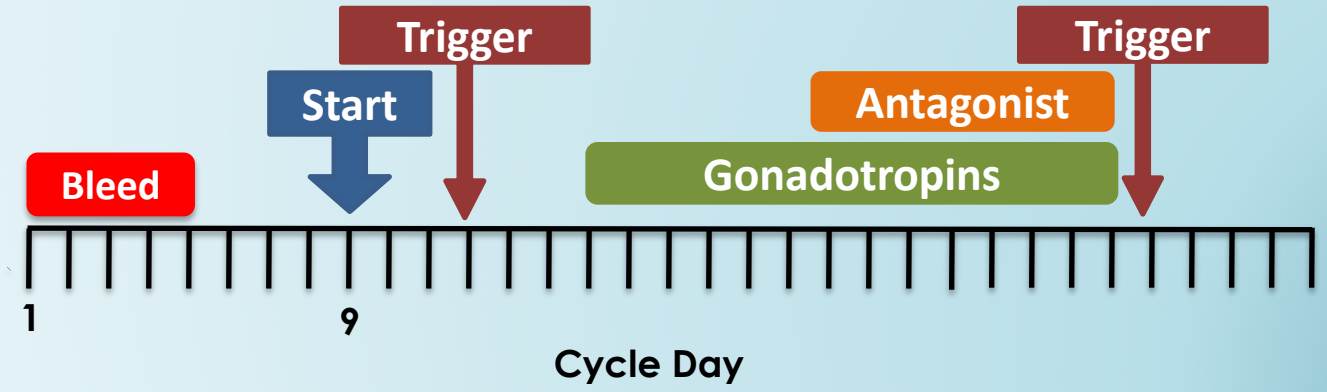
1. Start gonadotropins on day of presentation.
2. Add GnRH antagonist when secondary cohort following lead follicle reaches 12 mm regardless of size of dominant follicle.
3. Disregard any spontaneous LH surge.
4. Trigger when lead follicles in secondary cohort reach 18 mm.



Cakmak H, et al., 2013

Late follicular phase: random start option #2

1. Trigger ovulation when dominant follicle reaches 18 mm.
2. Start gonadotropins 2-3 days after trigger.
3. Add GnRH antagonist when lead follicle reaches 12-14 mm.
4. Trigger when lead follicles reach 18 mm.



Cakmak H, et al., 2013

The patient undergoes an IVF stimulation cycle with a robust response as shown here.

TD	Date	Serology					Medication (Record daily dose in UNITS, or by MASS)									Ov. Vol.		Follicle		
		E2	S1	S2	S3	S4	cc	GnRH a/ant	hMG	FSH	E2	hCG	Other1	Other2	Other3	Rt.	Lt.	Rt.	Lt.	Count
			LH	PRG	FSH	HCG		Ganirelix Acetate	Menopur	Follistim		Novarel	OCP							
2	Sun.Jun. 05, 16								150	150										
3	Mon.Jun. 06, 16								150	150										
4	Tue.Jun. 07, 16	245.0	1.37	0.43					150	150										
5	Wed.Jun. 08, 16								150	150										
6	Thu.Jun. 09, 16	695.0	0.53	0.5				250	150	150								21	23	
7	Fri.Jun. 10, 16							250	150	150										
8	Sat.Jun. 11, 16	1334.0	1.05	0.64				250	150	150								20	10	
9	Sun.Jun. 12, 16							250	150	150										
10	Mon.Jun. 13, 16	2921.0	0.5	1.12				250	75	150								31	25	
11	Tue.Jun. 14, 16	3874.0	0.43	1.15				250										30	25	
12	Wed.Jun. 15, 16																			

31/31

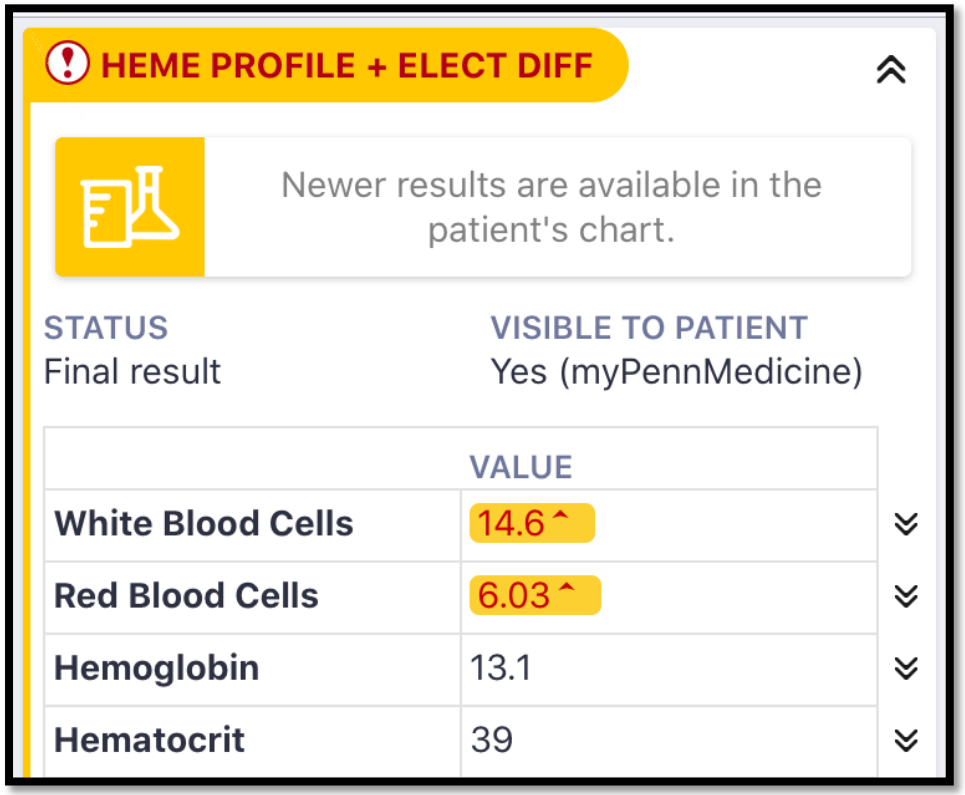
TD	Date	Follicles (sizes in mm.)	
		Right	Left
4	Tue.Jun. 07, 16		
5	Wed.Jun. 08, 16		
6	Thu.Jun. 09, 16	8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 12	8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 10, 12, 12
7	Fri.Jun. 10, 16		
8	Sat.Jun. 11, 16	8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 10, 12, 12, 12, 14, 14, 15, 16	8, 10, 10, 12, 12, 12, 14, 14, 14, 16
9	Sun.Jun. 12, 16		
10	Mon.Jun. 13, 16	8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 10, 12, 12, 12, 12, 12, 14, 15, 15, 16, 16, 17, 18	8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 10, 10, 10, 12, 12, 12, 12, 14, 15, 17, 17, 17, 19
11	Tue.Jun. 14, 16	8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 12, 12, 12, 12, 14, 15, 15, 16, 18, 18, 18, 19, 20, 20, 21	8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 10, 12, 12, 12, 12, 14, 15, 16, 16, 19, 20, 20, 21

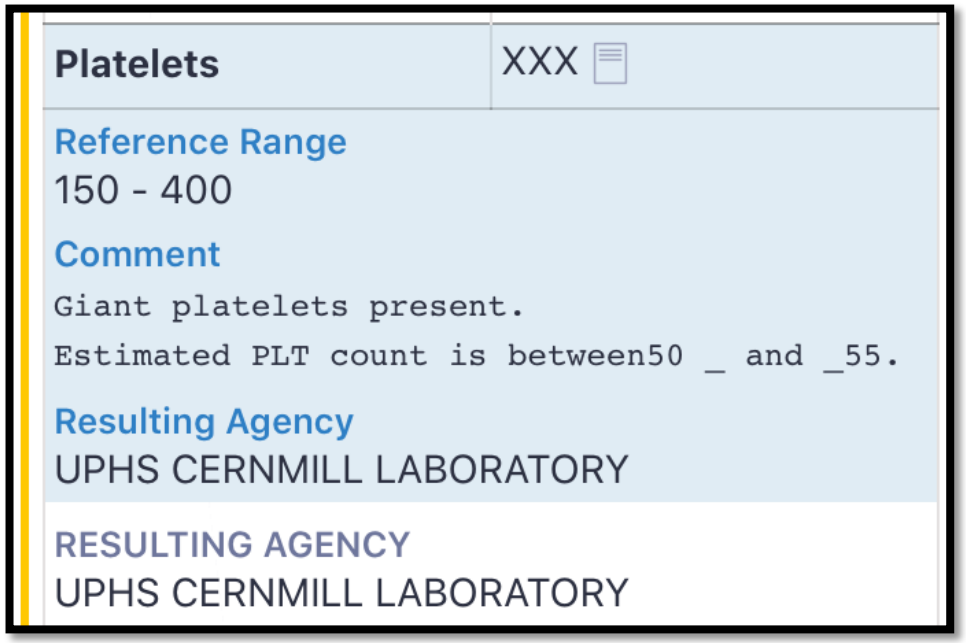
Question 3:

What agent would you recommend to trigger an “LH surge”?

A. 5000 units HCG
B. 10,000 units HCG
C. Leuprolide only
D. Leuprolide + 1000 units HCG

The patient triggers with leuprolide and her egg retrieval is scheduled for 8am two days later. A baseline CBC drawn by her oncology team the next day is suggestive of immune thrombocytopenia with an estimated platelet count between 50,000 and 55,000.





Question 4:

What would you recommend for management?

A. Admit for platelet transfusion prior to egg retrieval
B. Proceed with egg retrieval
C. Initiate a course of oral steroids
D. Cancel egg retrieval

The patient is started on oral steroids and proceeds with egg retrieval. Four days later she calls with severe nausea and vomiting, reporting that she cannot keep down liquids. Her labs are shown here:

HEME PROFILE + ELECT DIFF

STATUS	VISIBLE TO PATIENT
Final result	Yes (myPennMedicine)

SPECIMEN TYPE	SPECIMEN SOURCE
Blood	Blood

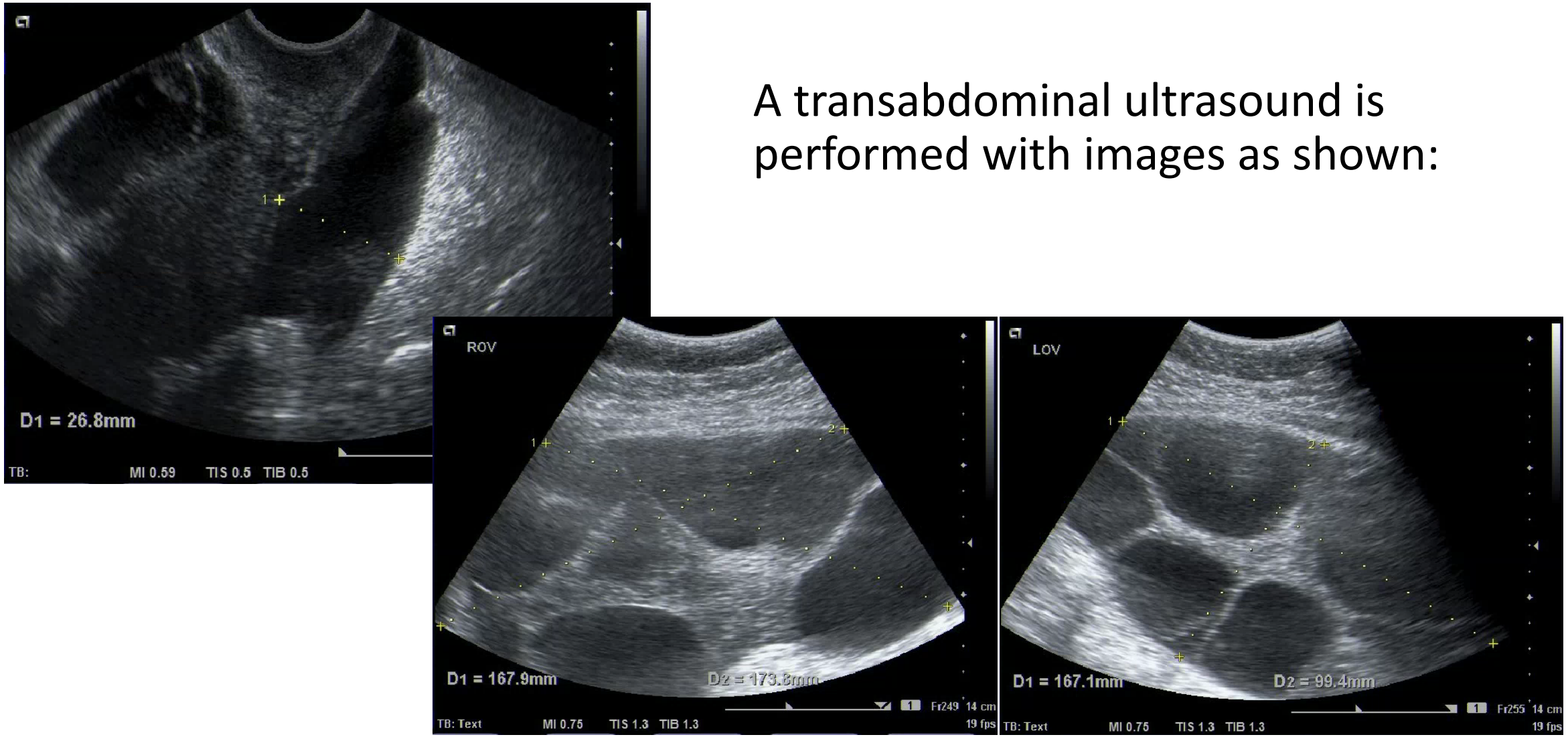
	VALUE
White Blood Cells	19.1 ^
Red Blood Cells	6.37 ^
Hemoglobin	13.8
Hematocrit	42

BASIC METABOLIC PANEL

Newer results are available in the patient's chart.

STATUS	VISIBLE TO PATIENT
Final result	No (Blocked)

	VALUE
Glucose	82
Urea Nitrogen	13
Creatinine	1.10 ^
Sodium	135 ⌄
Potassium	4.7
Chloride	102
Carbon Dioxide	19 ⌄
Anion Gap	14 ^
Calcium	8.6 ⌄



A transabdominal ultrasound is performed with images as shown:

Question 4:

What would you recommend for management?

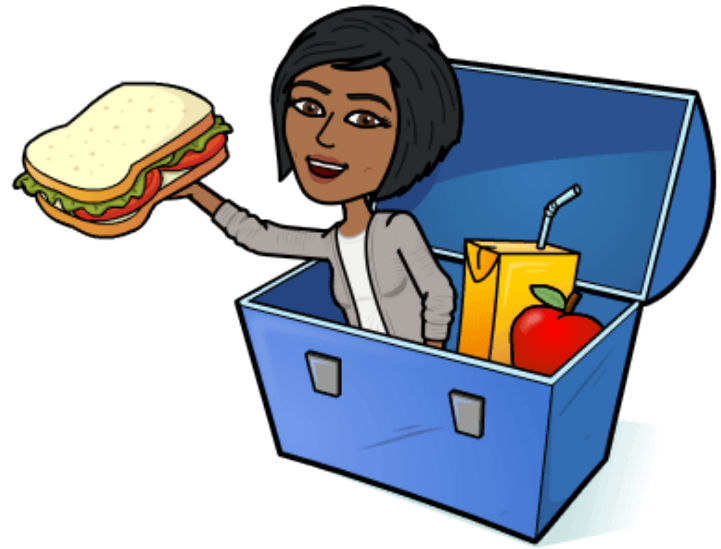
A. Low-molecular-weight heparin
B. Diuretics
C. IV boluses of normal saline
D. Cabergoline
E. Paracentesis

Time	Topic	Speaker
08:00-08:15	Introduction to Course and Learning Teams	
08:15-09:00	Fertility Preservation in the Male	Jim Smith, MD, MS – Urologist and Director of Male Reproductive Health, University of California, San Francisco (UCSF)
09:05-9:15	Introduction to Team Based Learning	
09:20-10:20	Team based learning: Adult male and early reproductive female	Divya Shah, MD, MME – Reproductive Endocrinologist, University of Pennsylvania Wendy Vitek, MD – Reproductive Endocrinologist and Director of Fertility Preservation, University of Rochester
10:25-11:10	Fertility preservation in breast cancer patients: issues of timing	Jacqueline Jeruss, M.D., Ph.D. – Surgical Oncologist and Director, Breast Care Center, University of Michigan
11:15-12:15	Lunch	
12:20-01:05	Practical aspects of ovarian tissue cryopreservation in the pediatric population	Leslie Appiah, MD – Pediatric and Adolescent Gynecologist and Chief, Division of General Obstetrics & Gynecology, The University of Colorado Denver
01:10-02:10	Team based learning: Late reproductive and pre-pubertal female	Divya Shah, MD, MME – Reproductive Endocrinologist and Infertility Specialist, University of Pennsylvania Wendy Vitek, MD – Reproductive Endocrinologist and Infertility Specialist, Director of Fertility Preservation, University of Rochester
02:15-03:00	Oncofertility State of the Union: advances from the past 5 years and projections for the next 5 years	Teresa Woodruff, PhD – Director Women's Health Research Institute, Chief, Division of Obstetrics and Gynecology-Fertility Preservation, Northwestern University

Jacqui Jeruss, MD, PhD

- Associate Professor, Departments of Surgery, Pathology, and Biomedical Engineering, University of Michigan
- Director, Breast Care Center
- Director, Breast Surgical Oncology Fellowship


TIME FOR
LUNCH
TIME FOR



Time	Topic	Speaker
08:00-08:15	Introduction to Course and Learning Teams	
08:15-09:00	Fertility Preservation in the Male	Jim Smith, MD, MS – Urologist and Director of Male Reproductive Health, University of California, San Francisco (UCSF)
09:05-9:15	Introduction to Team Based Learning	
09:20-10:20	Team based learning: Adult male and early reproductive female	Divya Shah, MD, MME – Reproductive Endocrinologist, University of Pennsylvania Wendy Vitek, MD – Reproductive Endocrinologist and Director of Fertility Preservation, University of Rochester
10:25-11:10	Fertility preservation in breast cancer patients: issues of timing	Jacqueline Jeruss, M.D., Ph.D. – Surgical Oncologist and Director, Breast Care Center, University of Michigan
11:15-12:15	Lunch	
12:20-01:05	Practical aspects of ovarian tissue cryopreservation in the pediatric population	Leslie Appiah, MD – Pediatric and Adolescent Gynecologist and Chief, Division of General Obstetrics & Gynecology, The University of Colorado Denver
01:10-02:10	Team based learning: Late reproductive and pre-pubertal female	Divya Shah, MD, MME – Reproductive Endocrinologist and Infertility Specialist, University of Pennsylvania Wendy Vitek, MD – Reproductive Endocrinologist and Infertility Specialist, Director of Fertility Preservation, University of Rochester
02:15-03:00	Oncofertility State of the Union: advances from the past 5 years and projections for the next 5 years	Teresa Woodruff, PhD – Director Women's Health Research Institute, Chief, Division of Obstetrics and Gynecology-Fertility Preservation, Northwestern University

Leslie Appiah, MD

- Associate Professor and Chief
- Division of General Obstetrics & Gynecology
- Director, Fertility Preservation & Reproductive Late Effects Program
- The University of Colorado Denver

- Pediatric and Adolescent Gynecology
- Children’s Hospital Colorado

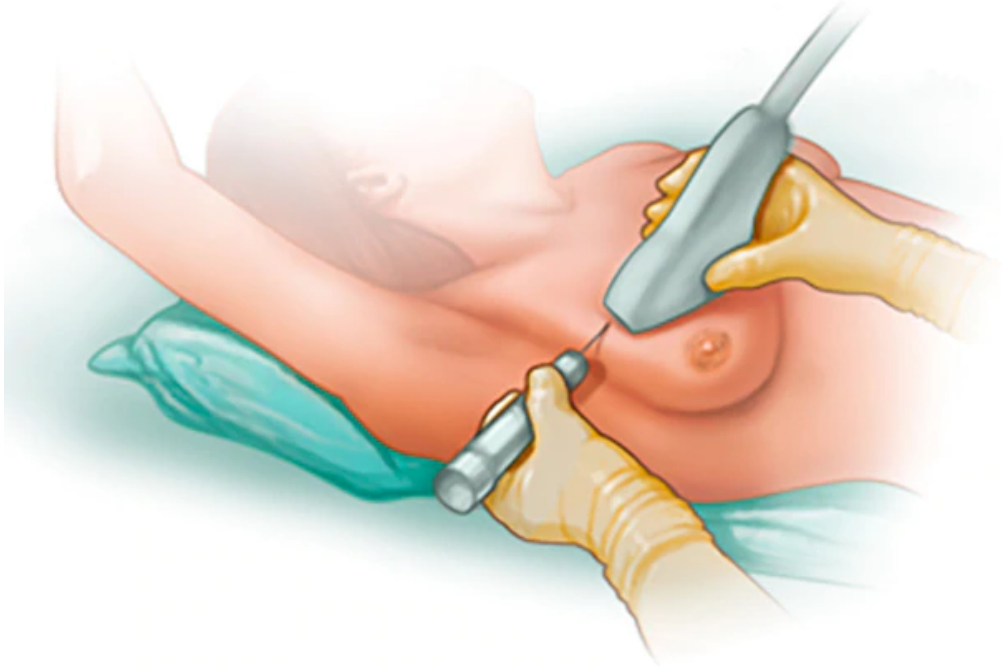
Time	Topic	Speaker
08:00-08:15	Introduction to Course and Learning Teams	
08:15-09:00	Fertility Preservation in the Male	Jim Smith, MD, MS – Urologist and Director of Male Reproductive Health, University of California, San Francisco (UCSF)
09:05-9:15	Introduction to Team Based Learning	
09:20-10:20	Team based learning: Adult male and early reproductive female	Divya Shah, MD, MME – Reproductive Endocrinologist, University of Pennsylvania Wendy Vitek, MD – Reproductive Endocrinologist and Director of Fertility Preservation, University of Rochester
10:25-11:10	Fertility preservation in breast cancer patients: issues of timing	Jacqueline Jeruss, M.D., Ph.D. – Surgical Oncologist and Director, Breast Care Center, University of Michigan
11:15-12:15	Lunch	
12:20-01:05	Practical aspects of ovarian tissue cryopreservation in the pediatric population	Leslie Appiah, MD – Pediatric and Adolescent Gynecologist and Chief, Division of General Obstetrics & Gynecology, The University of Colorado Denver
01:10-02:10	Team based learning: Late reproductive and pre-pubertal female	Divya Shah, MD, MME – Reproductive Endocrinologist and Infertility Specialist, University of Pennsylvania Wendy Vitek, MD – Reproductive Endocrinologist and Infertility Specialist, Director of Fertility Preservation, University of Rochester
02:15-03:00	Oncofertility State of the Union: advances from the past 5 years and projections for the next 5 years	Teresa Woodruff, PhD – Director Women's Health Research Institute, Chief, Division of Obstetrics and Gynecology-Fertility Preservation, Northwestern University

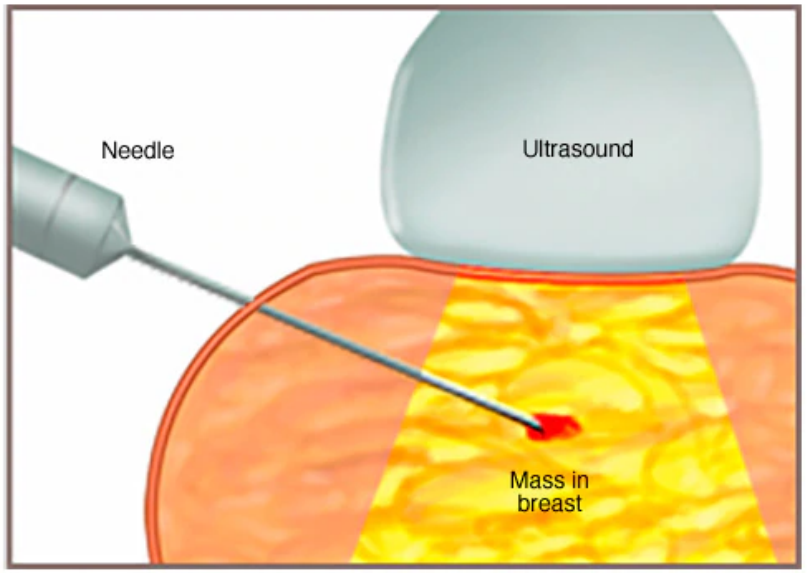
CASE #3:

A 38-year-old G0 palpated a left breast mass. Mammography revealed a 2-cm spiculated mass and ultrasound guided needle biopsy was performed. Pathology revealed an invasive ductal carcinoma that was ER/PR positive and HER-2/neu negative.

Given a strong family history of breast cancer, she underwent genetic testing and was found to carry a *BCRA* mutation.

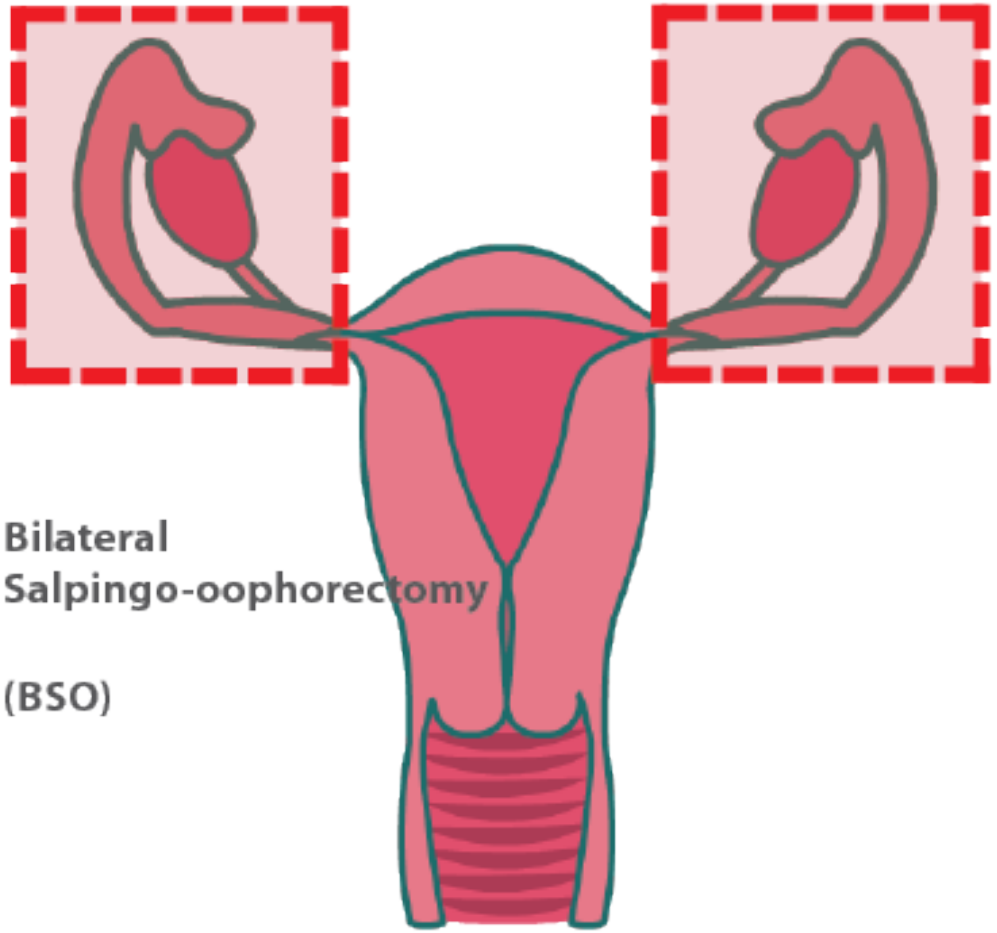
She underwent a left modified radical mastectomy (positive lymph nodes) and right prophylactic mastectomy with immediate reconstruction. She was diagnosed with clinical Stage IIB breast cancer.





Based on oncotype testing, adjuvant chemotherapy with adriamycin and cyclophosphamide followed by paclitaxel for 4 cycles has been recommended. She will also receive radiation therapy to the left axilla and hormone therapy with Tamoxifen for 10 years. She has been counseled regarding bilateral salpingo-oophorectomy at the completion of childbearing.

She presents to discuss fertility preservation. She is in a committed relationship but is not married.



Question 1: Given the patient's clinical presentation, which option(s) would you select for fertility preservation and why?

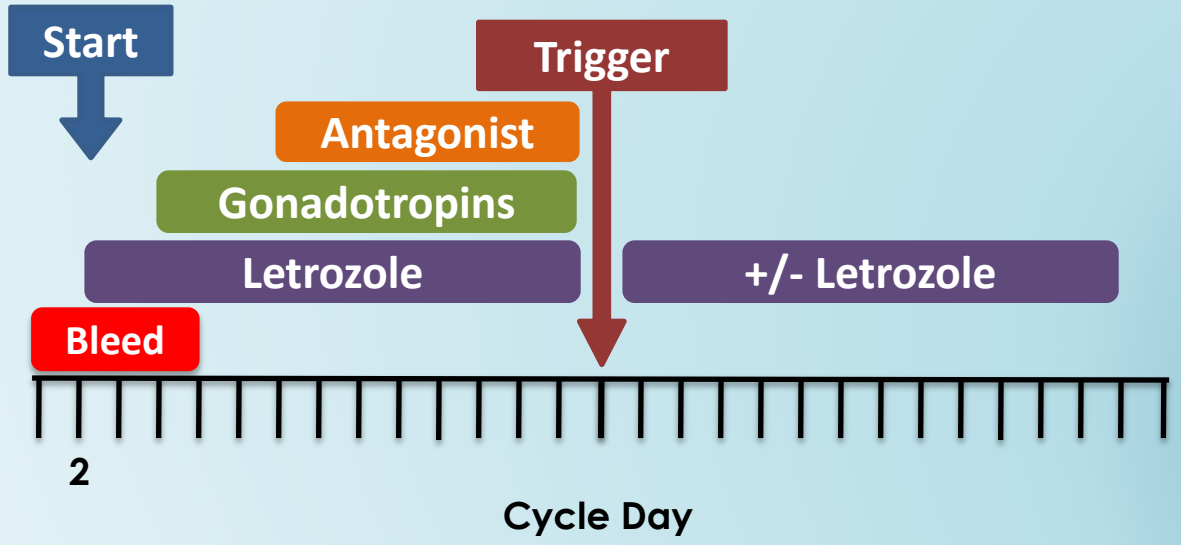
A. Immediate controlled ovarian stimulation with subsequent oocyte cryopreservation
B. Immediate controlled ovarian stimulation with subsequent embryo cryopreservation
C. Immediate controlled ovarian stimulation with subsequent embryo cryopreservation with preimplantation genetic testing (PGT-M) for BRCA mutation
D. Ovarian tissue cryopreservation with bilateral salpingo-oophorectomy
E. Leuprolide prior to and during chemotherapy

Question 2: If you advised immediate controlled stimulation and the patient is her early follicular phase, which option(s) would you select for ovarian hyperstimulation and why?

A. Starting gonadotropins in the early follicular phase and add a GnRH antagonist once the lead follicle reaches 12-14 mm
B. Starting letrozole on the second day of the menstrual cycle and continue until trigger, add gonadotropin 2 days after letrozole initiation, trigger with HCG once 2 follicles reach 19-20 mm
C. Starting letrozole on the second day of the menstrual cycle and continue until trigger, add gonadotropin 2 days after letrozole initiation, trigger with luprolide acetate once 2 follicles reach 19-20 mm
D. Start or restart letrozole for 5-7 days post retrieval or until the estradiol level is <50 pg/ml

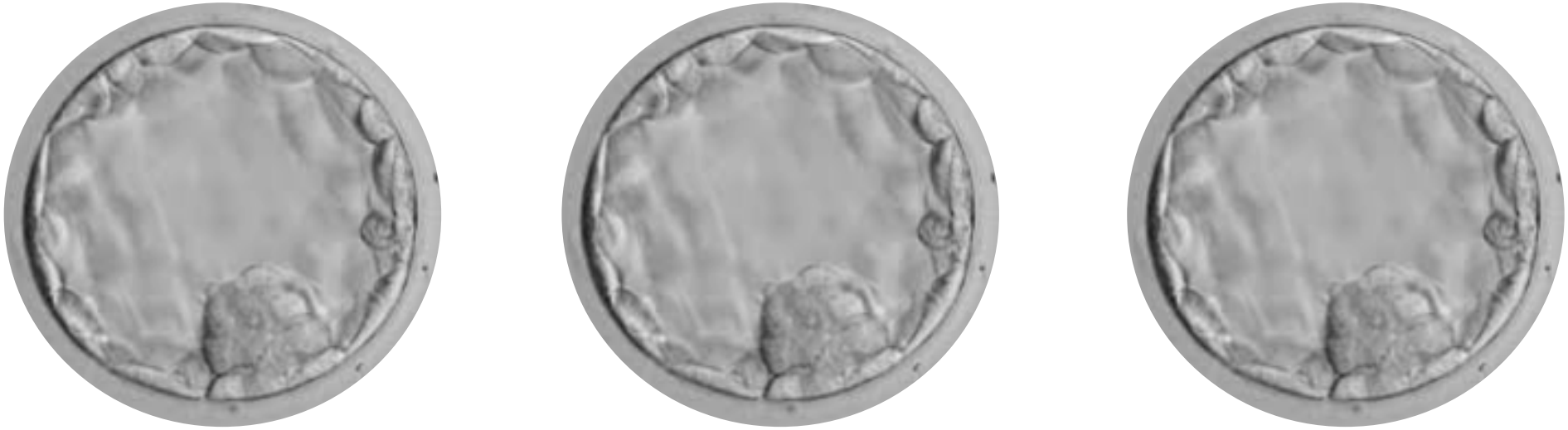
Stimulation for estrogen-sensitive tumors: letrozole

1. Start letrozole on cycle day 2-3.
2. Start gonadotropins 2 days later.
3. Add GnRH antagonist when lead follicle reaches 12-14 mm.
4. Trigger when lead follicles reach 19-20 mm.
5. Restart letrozole after retrieval and continue until estradiol <50 pg/mL.



Oktay K, et al., 2006

She undergoes embryo cryopreservation without preimplantation genetic testing and is able to freeze 3 high quality blastocysts. She presents for follow up 2 years later and appears to be disease free. She is taking Tamoxifen daily. She and her partner recently married. They are ready to start a family.



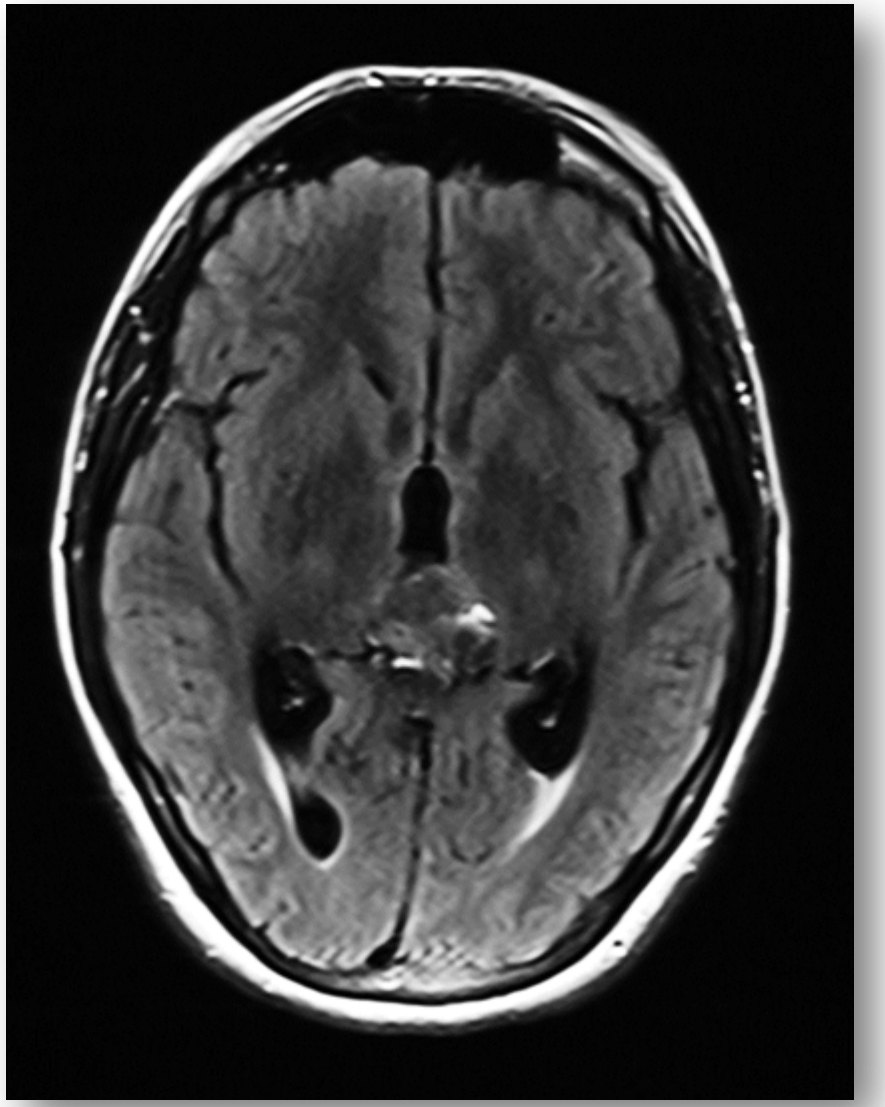
Question 3: Which option(s) would you select for pregnancy and why?

A. Discontinue Tamoxifen for two months prior to attempting natural conception; plan to restart Tamoxifen after pregnancy
B. Continue Tamoxifen for 3 more years before discontinuing, attempting natural conception, and restarting after
C. Continue Tamoxifen and recommend gestational carrier
D. Perform a natural cycle frozen embryo transfer
E. Perform a medicated or programmed frozen embryo transfer

CASE #4:

A 12-year-old girl presents with diplopia and is found to have a pineal tumor (primitive neuroectodermal tumor or PNET) on head CT. She underwent craniotomy two weeks ago. Her subsequent treatment plan includes craniospinal radiation therapy and cyclophosphamide chemotherapy. Her Oncology team requests a consultation for fertility preservation.

She presents with her mother. She is premenarchal and has not yet experienced breast development. She has no other past medical or past surgical history other than what is described above. Her medications include amlodipine and marinol. She has never been sexually active.



Question 1: Given the patient's clinical presentation, which option(s) would you select for fertility preservation and why?

A. Immediate controlled ovarian stimulation with subsequent oocyte cryopreservation
B. Laparoscopic ovarian transposition
C. Ovarian tissue cryopreservation
D. Laparoscopic ovarian transposition with concurrent ovarian tissue cryopreservation

Question 2: If you advised ovarian transposition, how would you perform the procedure? Why?

A. Cut utero-ovarian ligament, transfix ovary inferomedially to uterosacral ligament
B. Cut utero-ovarian ligament and fallopian tube, transfix ovary to lateral pelvic sidewall above level of ischial spines
C. Cut utero-ovarian ligament and fallopian tube, tunnel ovary through peritoneal window and transfix to lateral pelvic sidewall above level of ischial spines
D. Cut utero-ovarian ligament, transfix ovary superiorly to uterine fundus

- *Suppose the patient was undergoing pelvic radiation therapy for localized rectal cancer with no plans for chemotherapy.*

Question 3: Which option(s) would you select for fertility preservation and why?

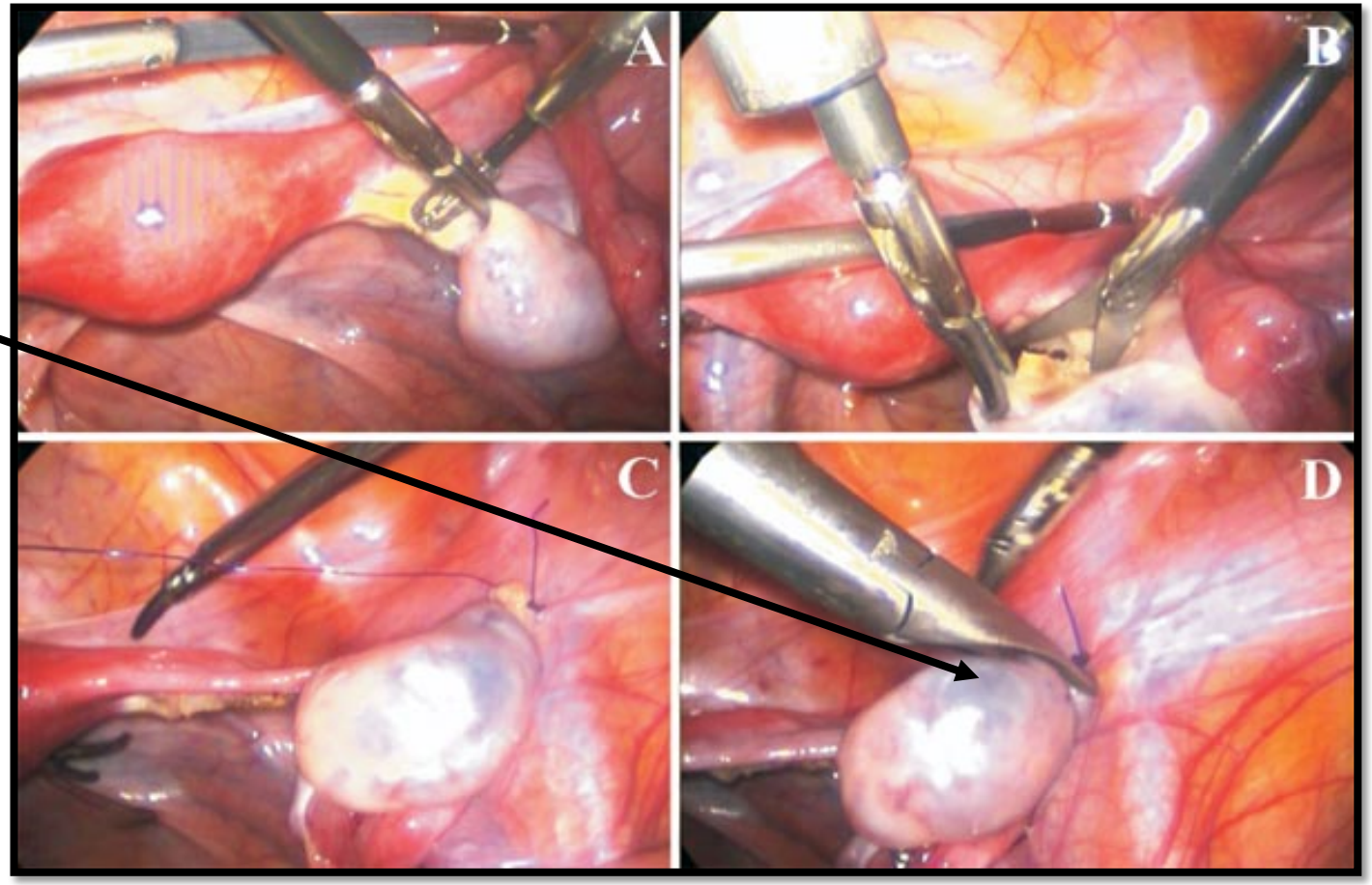
A. Immediate controlled ovarian stimulation with subsequent oocyte cryopreservation
B. Laparoscopic ovarian transposition
C. Ovarian tissue cryopreservation
D. Laparoscopic ovarian transposition with concurrent ovarian tissue cryopreservation

Question 4: How would you now perform the ovarian transposition procedure and why?

A. Cut utero-ovarian ligament, transfix ovary inferomedially to uterosacral ligament
B. Cut utero-ovarian ligament and fallopian tube, transfix ovary to lateral pelvic sidewall above level of ischial spines
C. Cut utero-ovarian ligament and fallopian tube, tunnel ovary through peritoneal window and transfix to lateral pelvic sidewall above level of ischial spines
D. Cut utero-ovarian ligament, transfix ovary superiorly to uterine fundus

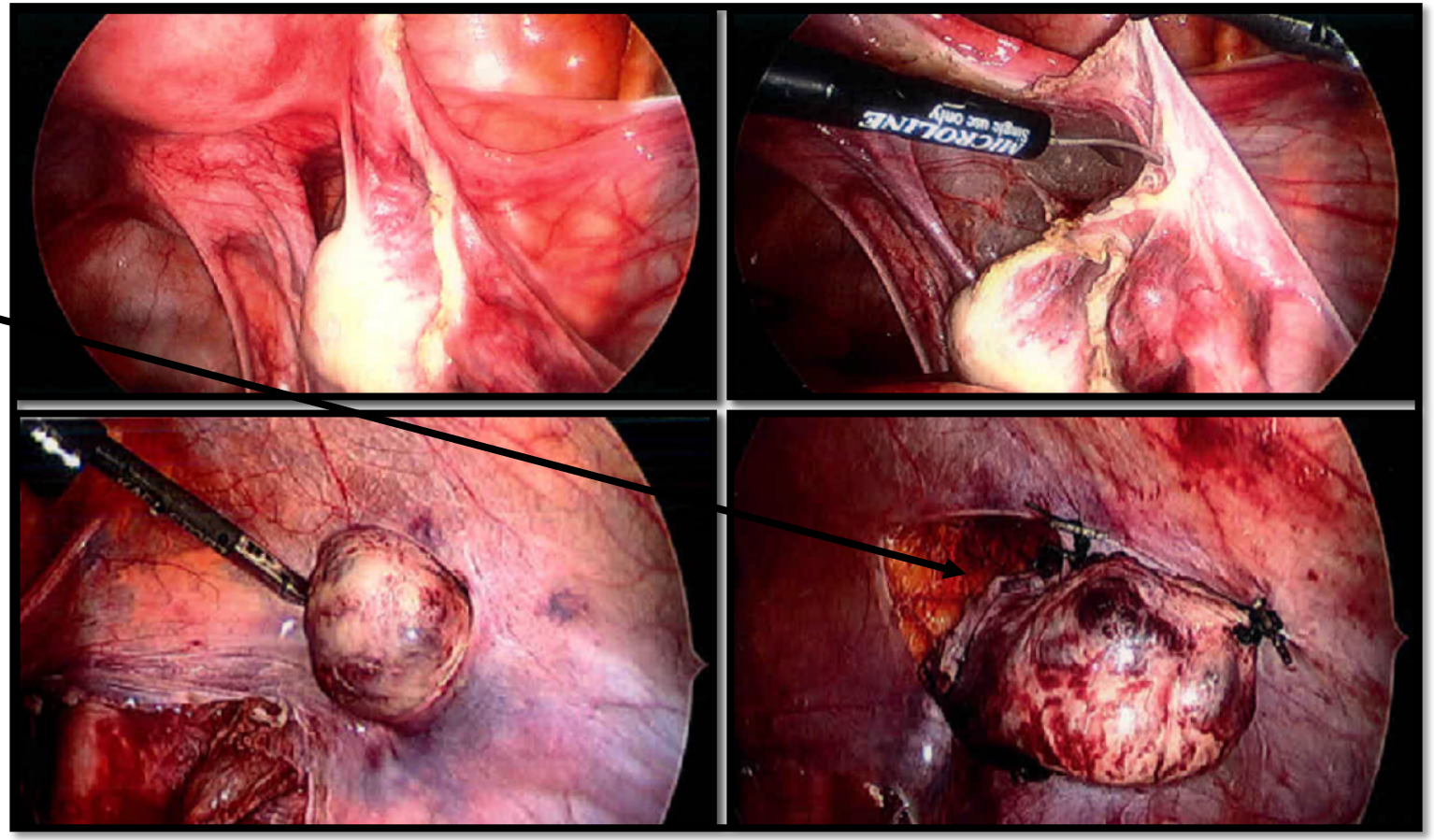
Ovarian transposition: superior transposition

To pelvic sidewall above pelvic brim

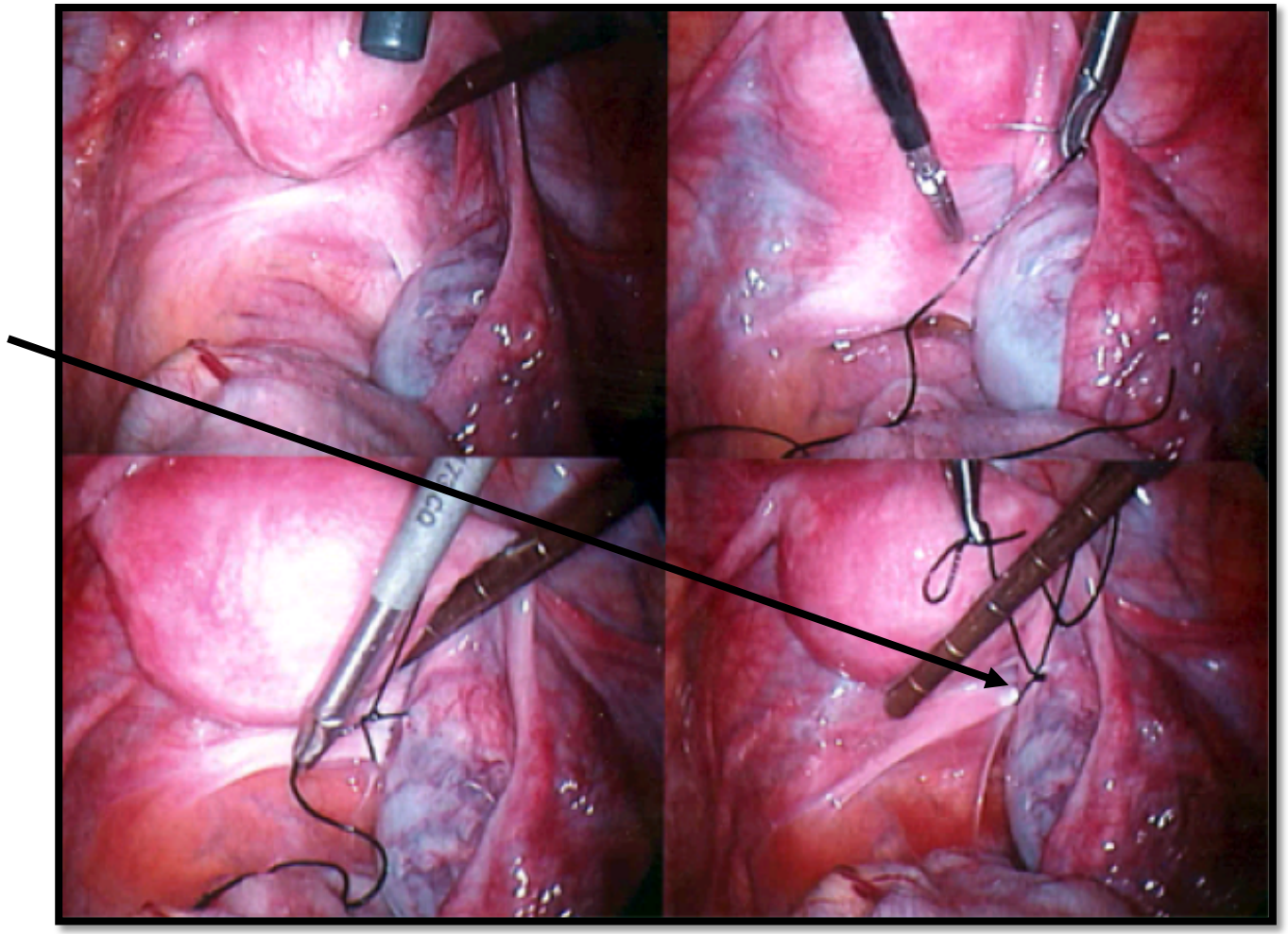


Ovarian transposition: superior transposition

Through peritoneal window to pelvic sidewall above pelvic brim



Ovarian transposition: inferior transposition



- *Suppose the patient described above initially presented with leukemia. Her oncology team plans induction chemotherapy in the near future and has counseled her that there is a high likelihood of her requiring a future hematopoietic stem cell transplant.*

Question 5: Which option would you select for fertility preservation and why?

A. Immediate controlled ovarian stimulation with subsequent oocyte cryopreservation
B. Laparoscopic ovarian transposition
C. Ovarian tissue cryopreservation
D. Laparoscopic ovarian transposition with concurrent ovarian tissue cryopreservation
E. None of the above

Question 6: If you advised ovarian tissue cryopreservation, when would you advise the patient to undergo this procedure and why?

A. Before induction chemotherapy
B. After induction chemotherapy but before hematopoietic stem cell transplant
C. After hematopoietic stem cell transplant

Time	Topic	Speaker
08:00-08:15	Introduction to Course and Learning Teams	
08:15-09:00	Fertility Preservation in the Male	Jim Smith, MD, MS – Urologist and Director of Male Reproductive Health, University of California, San Francisco (UCSF)
09:05-9:15	Introduction to Team Based Learning	
09:20-10:20	Team based learning: Adult male and early reproductive female	Divya Shah, MD, MME – Reproductive Endocrinologist, University of Pennsylvania Wendy Vitek, MD – Reproductive Endocrinologist and Director of Fertility Preservation, University of Rochester
10:25-11:10	Fertility preservation in breast cancer patients: issues of timing	Jacqueline Jeruss, M.D., Ph.D. – Surgical Oncologist and Director, Breast Care Center, University of Michigan
11:15-12:15	Lunch	
12:20-01:05	Practical aspects of ovarian tissue cryopreservation in the pediatric population	Leslie Appiah, MD – Pediatric and Adolescent Gynecologist and Chief, Division of General Obstetrics & Gynecology, The University of Colorado Denver
01:10-02:10	Team based learning: Late reproductive and pre-pubertal female	Divya Shah, MD, MME – Reproductive Endocrinologist and Infertility Specialist, University of Pennsylvania Wendy Vitek, MD – Reproductive Endocrinologist and Infertility Specialist, Director of Fertility Preservation, University of Rochester
02:15-03:00	Oncofertility State of the Union: advances from the past 5 years and projections for the next 5 years	Teresa Woodruff, PhD – Director Women's Health Research Institute, Chief, Division of Obstetrics and Gynecology-Fertility Preservation, Northwestern University

Teresa Woodruff, PhD

- Director, Women's Health Research Institute
- Chief, Reproductive Biology Research, Department of Obstetrics and Gynecology
- Thomas J. Watkins Memorial Professor of Obstetrics and Gynecology

Appreciation

Please fill out the post-course questionnaire and evaluation!

UCSF
University of California
San Francisco

UCSF School
of Medicine

UCSF Department of
Urology

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

James F. Smith, MD MS
Director, Male Reproductive Health
Associate Professor, Department of Urology

Disclosure

- Fellow, inc: advisory panel

Outline

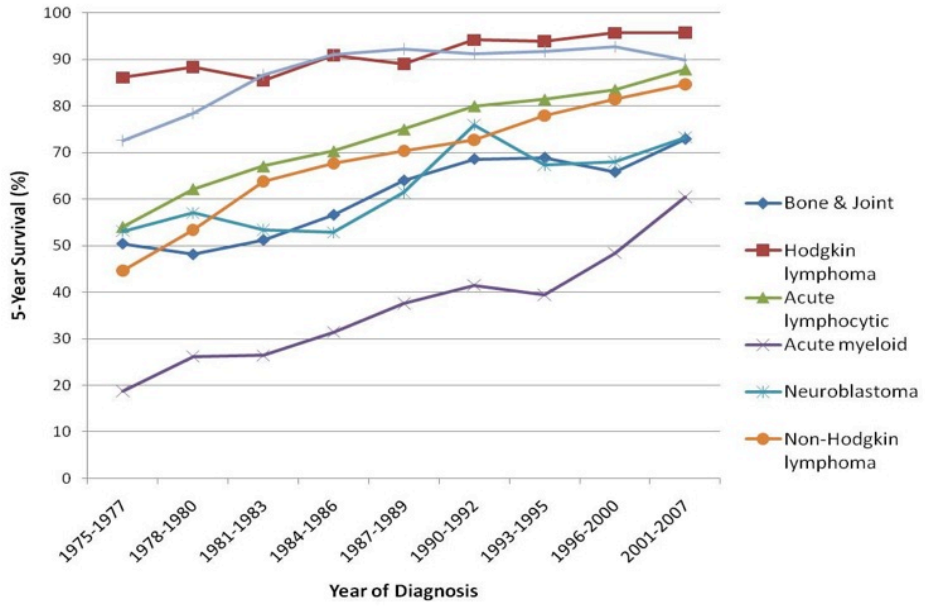
- Background
- Spermatogenesis overview
- Pre-pubertal fertility preservation (experimental)
- Post pubertal fertility preservation (Standard of care, complexities)
- Chemotherapy: reproductive risk and safety concerns

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
  - 4,000+ boys annually
  - Reproduction one of top concerns after survival
  - Adult fertility preservation



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

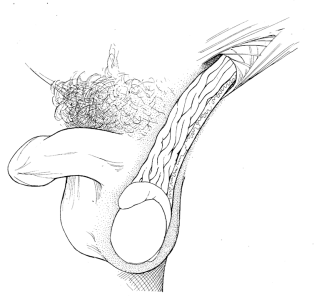
UCSF

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?

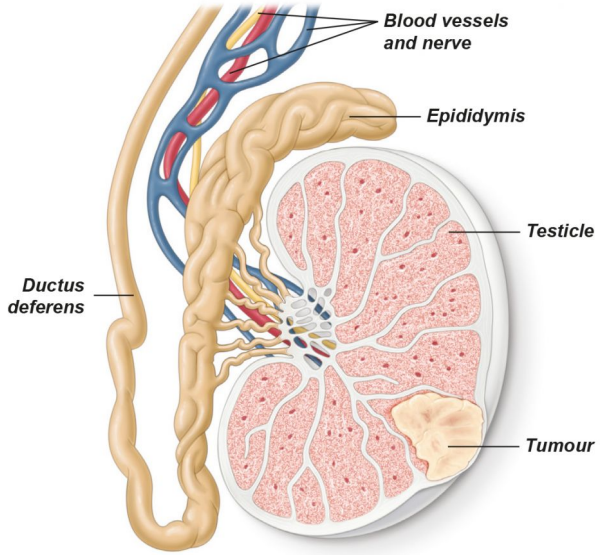


	Pre-testicular	Testicular/Sperm	Post-Testicular
Medical	B-thalassemia	Cancer (of any type)	Epididymitis
	Pituitary tumor	Chemo-, radiation therapy	Cystic Fibrosis
	Sickle cell anemia	Fevers, heat, exposures	CBAVD / UAVD
	Kallman's	Orchitis (mumps)	Cord injury/Spina Bifida
		Klinefelter's, varicocele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

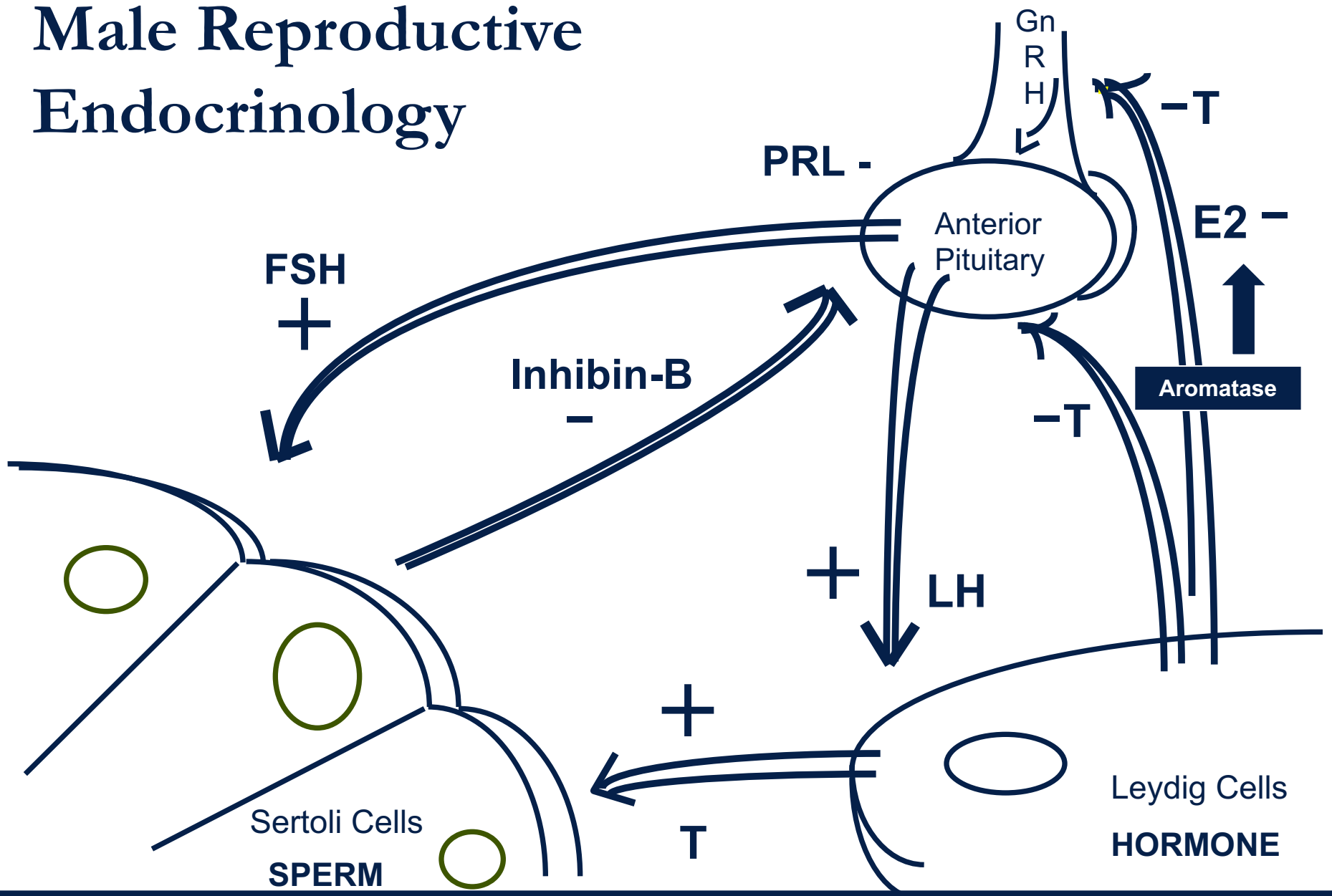
- Timing of intercourse: Sperm survives max 5-7 days; Oocytes 12-24 hours
- Length of time trying: 85% couples conceive 12 months trying
- Lubricants

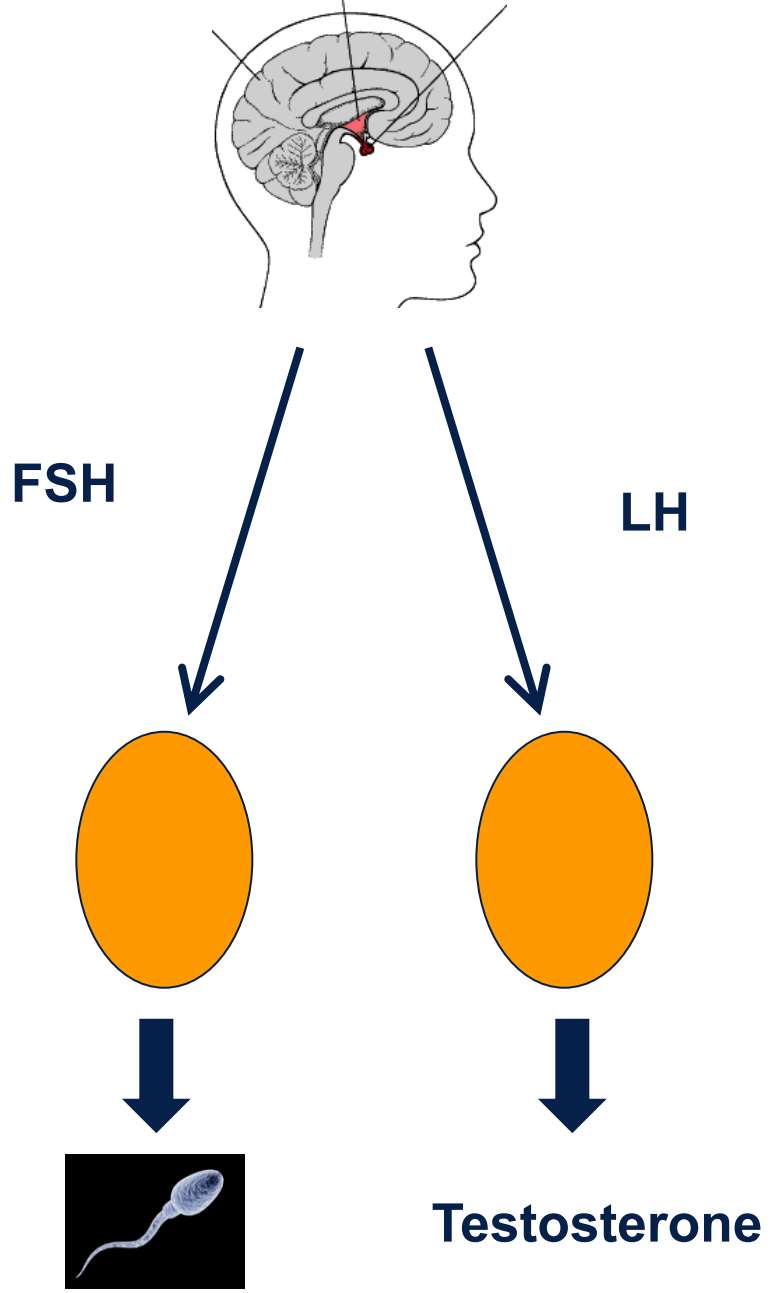
29 y/o testicular cancer

- Desire for fertility after testicular cancer treatment. Right orchiectomy 3 years ago. Did not bank sperm.
- High risk for recurrence and received BEP x3.
- Spouse is 27 y/o G0 regular cycles.
  - Now, FSH 37, T 350, LH 15; SA x 2 virtual azoospermia (100-300 nonmotile sperm present)
  - What is the diagnosis?
    - Obstruction?

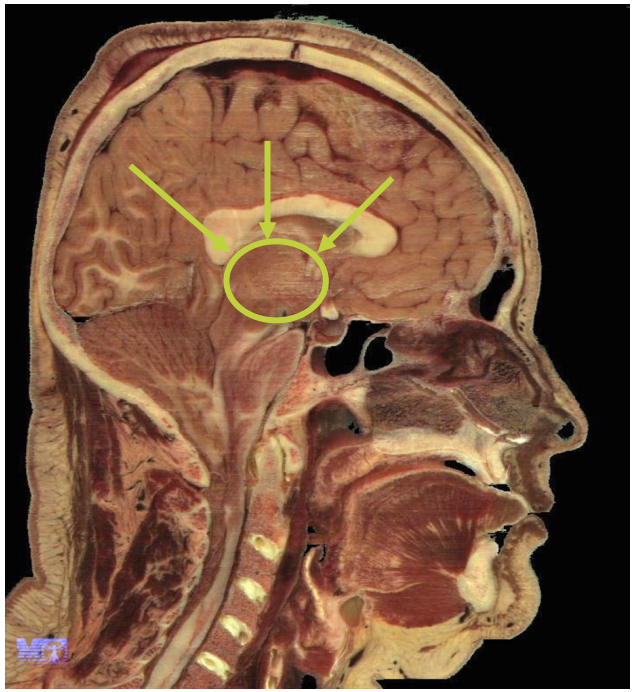


Male Reproductive Endocrinology




FSH
LH
Testosterone

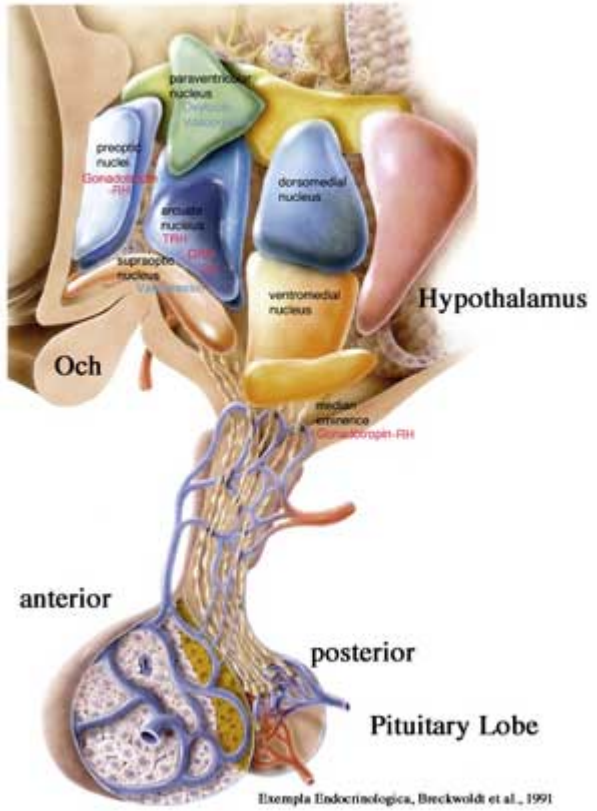
Hormonal Control of Male Reproductive System: Hypothalamic-Pituitary-Gonadal Axis



Hypothalamus:

- 4 gram clump of neuronal cell bodies below ventricle.
- Input from various brain centers.
- Pulse generator for cyclical secretion of HPG axis hormones.
- Pulsatile release of GnRH stimulates LH and FSH secretion from pituitary.
- $T_{1/2}$ of GnRH is 5-7 minutes.

Hormonal Control of Male Reproductive System: Hypothalamic-Pituitary-Gonadal Axis



Pituitary:

-Releases peptides LH and FSH

-Pituitary hormones are pulsatile

-Prolactin secreted independently

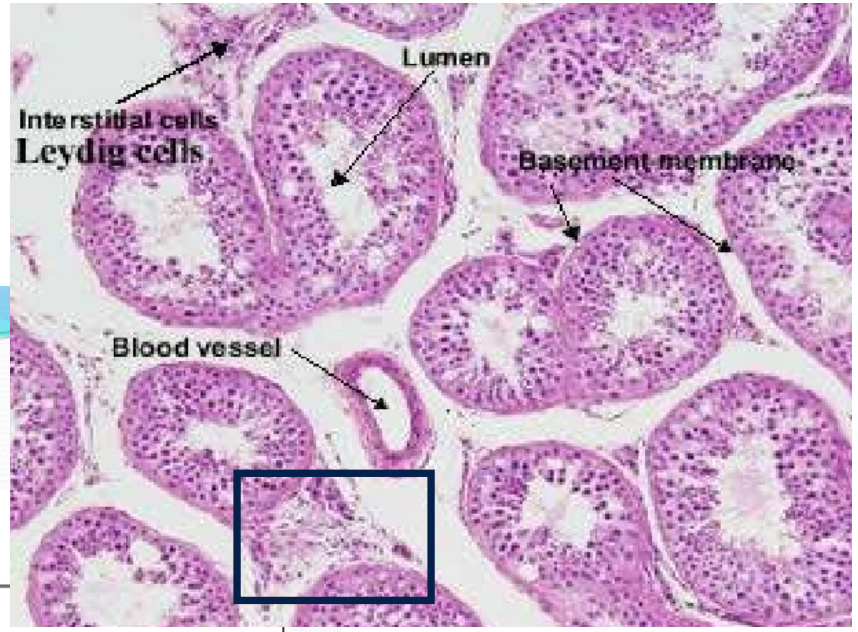
-LH acts on Leydig cells:

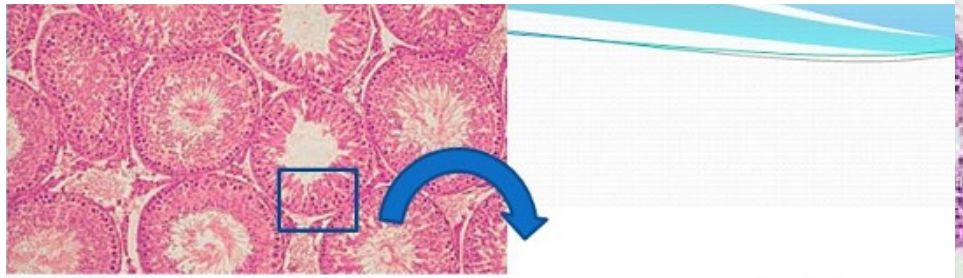
Testosterone

-FSH acts on Sertoli cells:

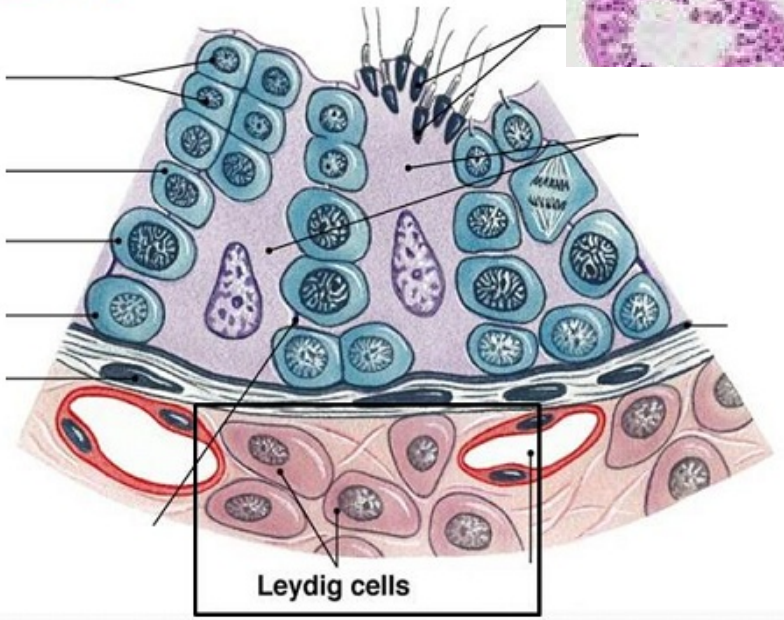
Needed for puberty and normal spermatogenesis

Leydig cells

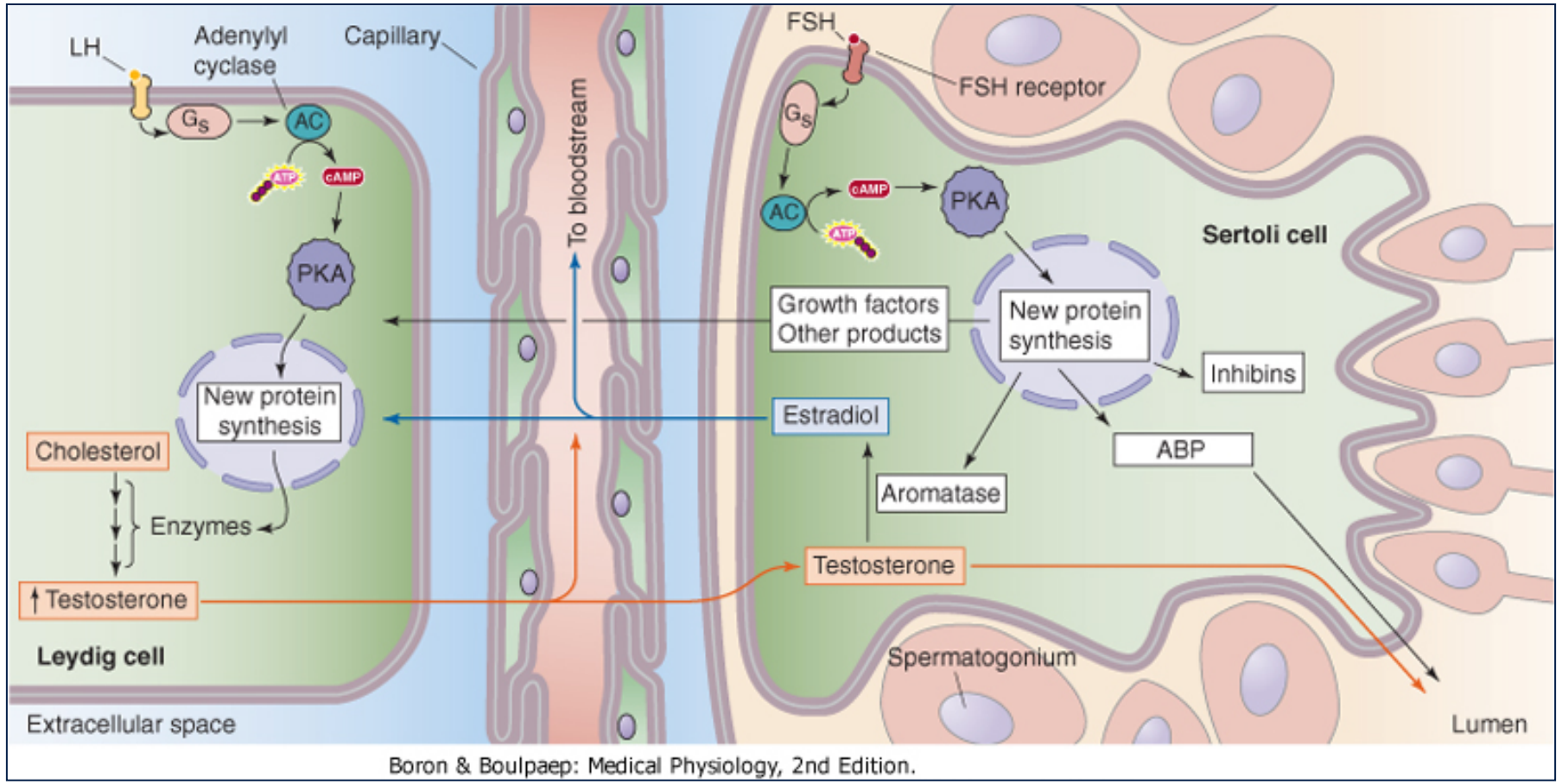




Leydig cells
produce
testosterone



Effects of gonadotropins on the Leydig and Sertoli cell compartments



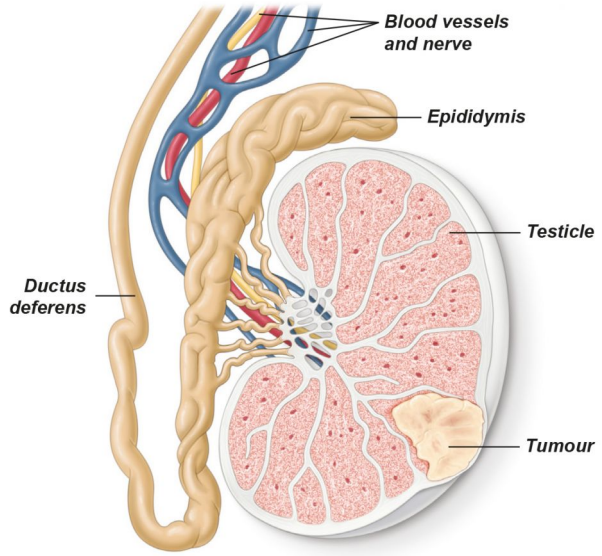
Boron & Boulpaep: Medical Physiology, 2nd Edition.

Characteristic Endocrine Profiles among Sub-Fertile / Infertile Men

	T	FSH	LH	PRL	Inhib B
Normal	NL	NL	NL	NL	High
1° testis failure	NL / Low	High	NL / High	NL	Low
Hypogonadotropic hypogonadism	Low	Low	Low	NL	Nl/ Low
Hyperprolactinemia	Low	Low	Low	High	Nl/low

29 y/o testicular cancer

- How do standard chemotherapies affect sperm quality?



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

Sonmezer M, Oktay K. Fertility preservation in female patients. Hum Reprod Update 2004;10:251-66.
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

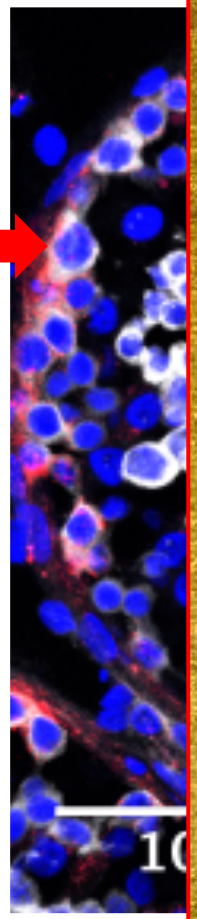
Radiation, Hypogonadism, and Male Infertility

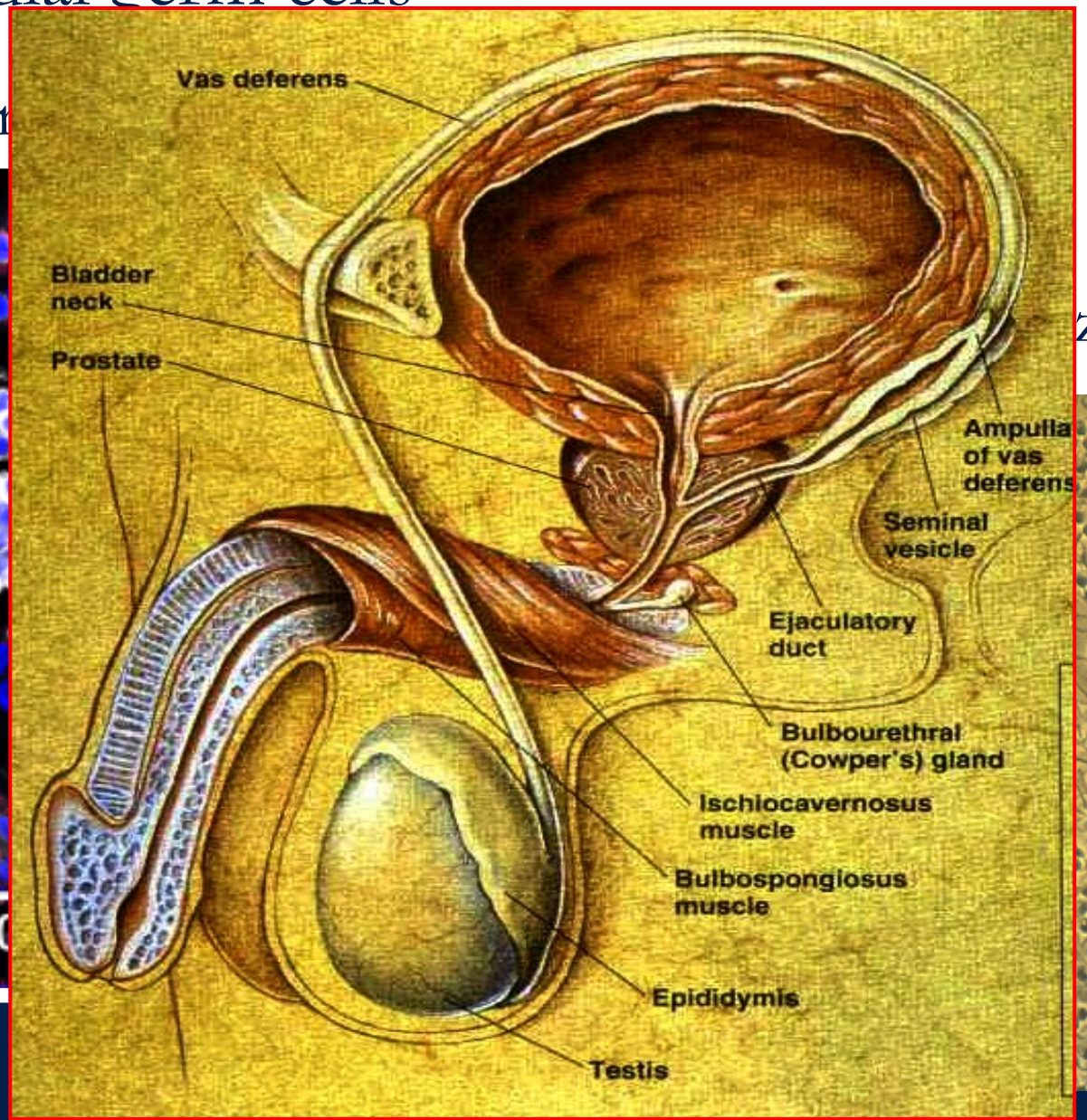
- Location (pelvis, gonads, whole body) of exposure
- Gonad dose
  - Azoospermia temporary if <3 Gy
  - Azoospermia permanent if >3 Gy
- Endocrine function:
  - Leydig cells preserved if < 12 Gy exposure (increased LH in some)
  - Hypogonadism if gonadal exposure > 20 Gy (pre-pubertal)
  - Hypogonadism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

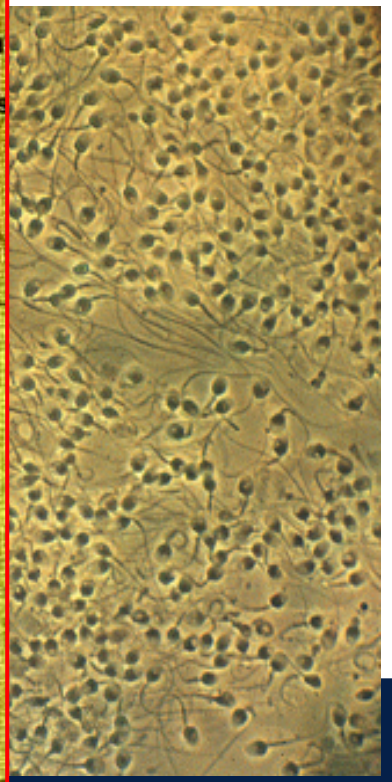
Spermatogenesis: production of sperm from primordial germ cells

Sper

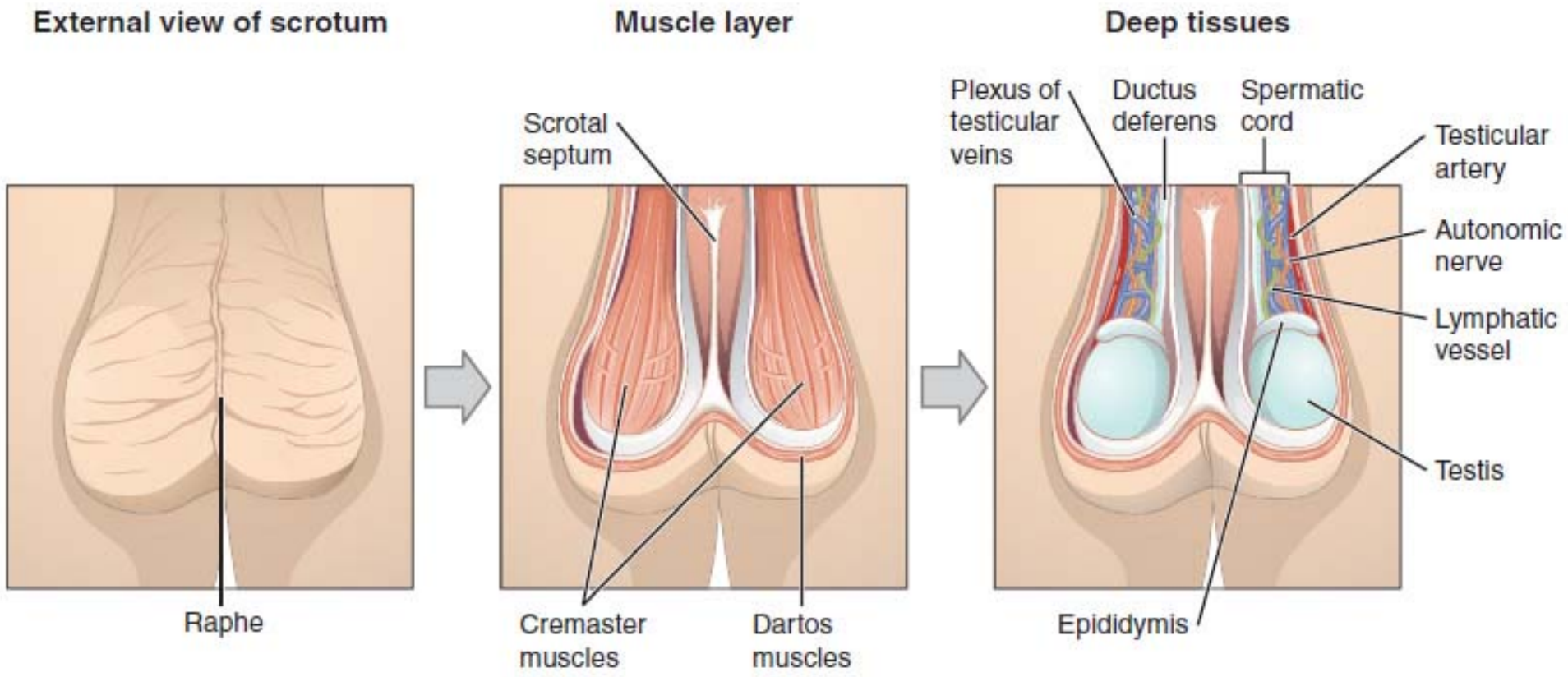




zoa

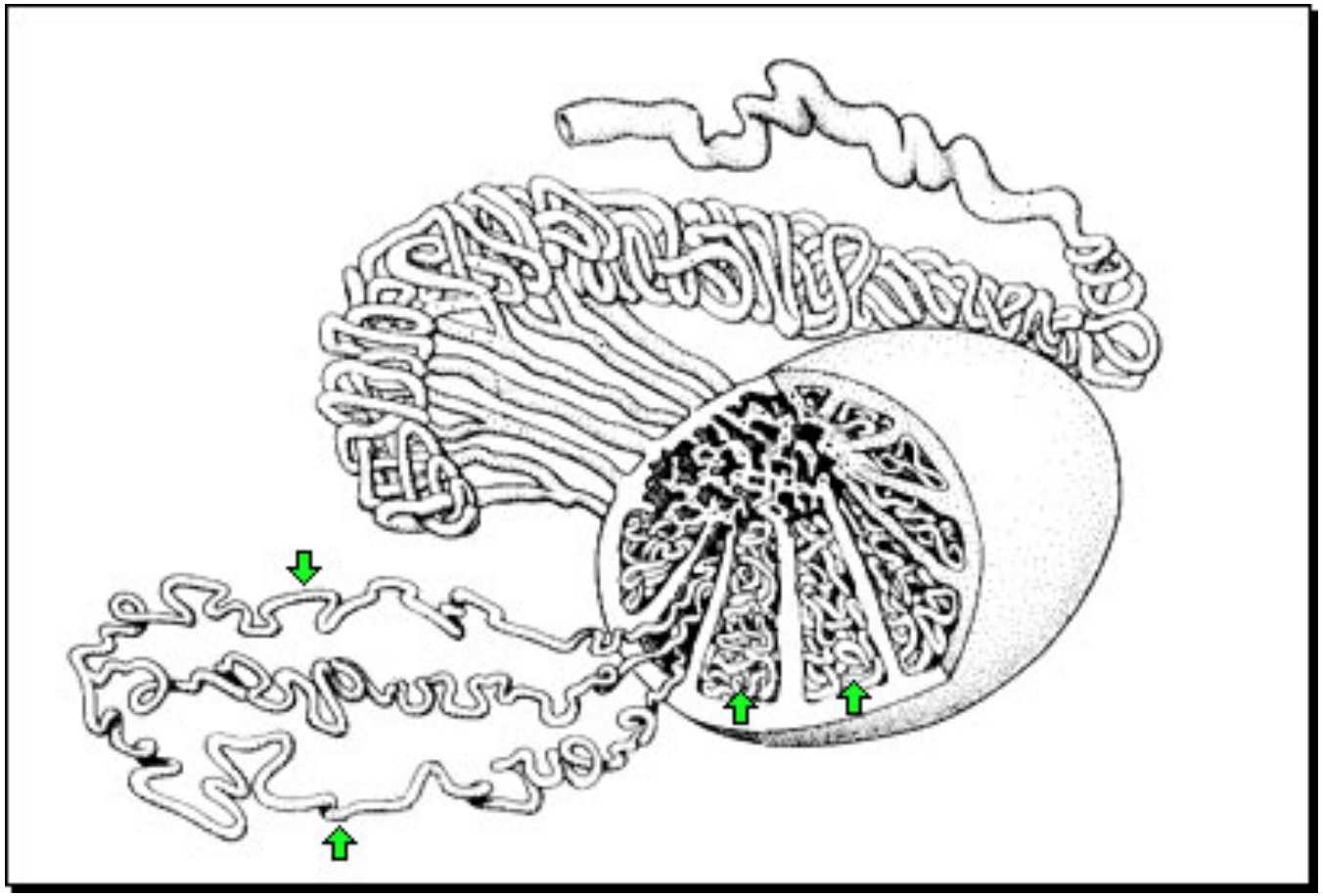


Male Reproductive Anatomy



Testicular framework

Seminiferous tubules



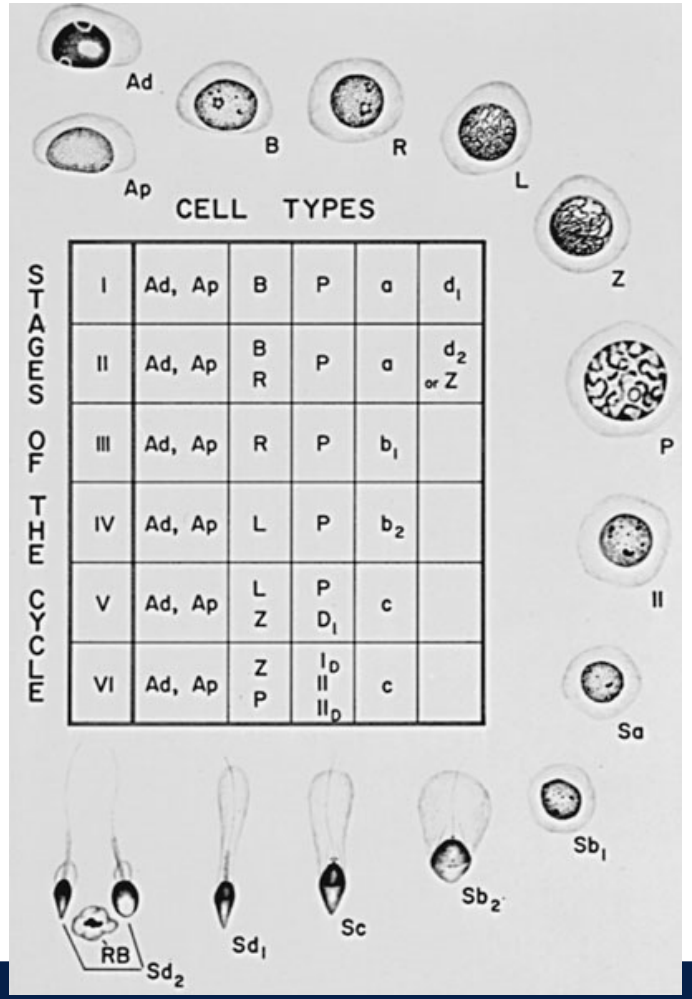
http://trc.ucdavis.edu/mjguinan/apc100/modules/Reproductive/mammal/images/testis02.jpg

Spermatogenesis

1. Mitotic division
2. Meiotic division

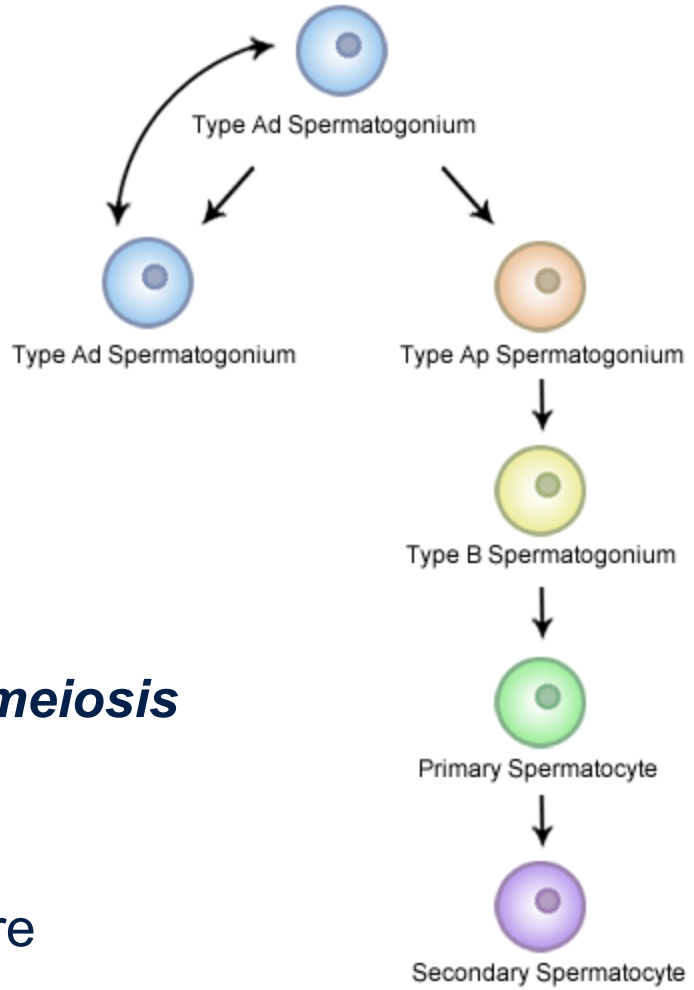
Spermatogenesis

3. Cellular remodeling- Spermiogenesis

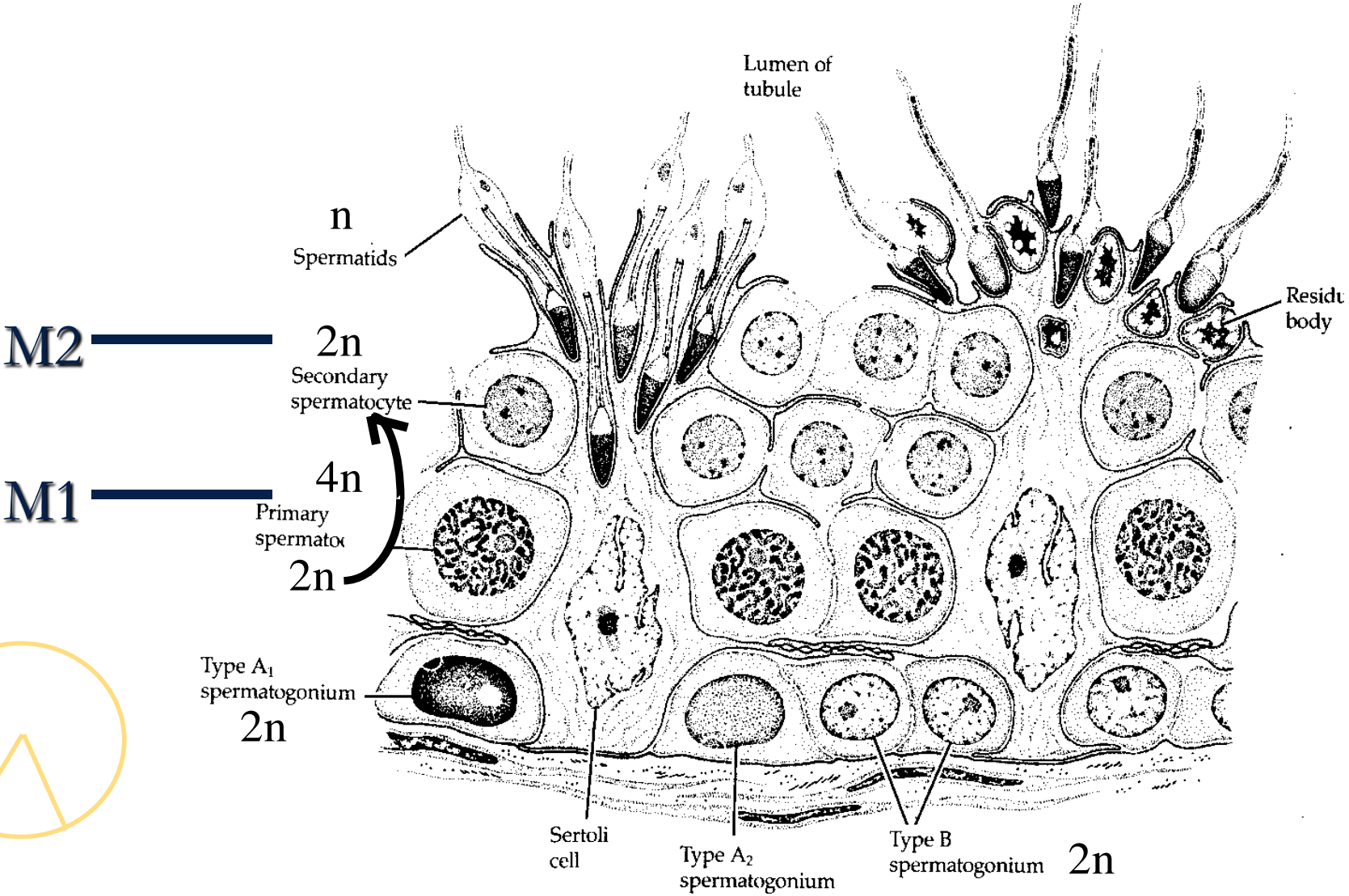


Two Main Types of Spermatogonia

- **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



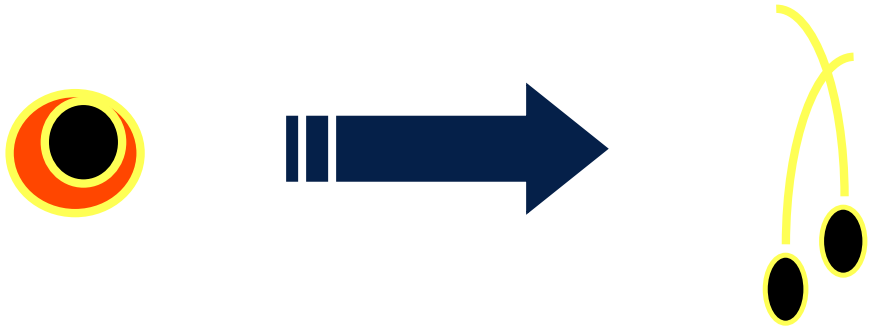
Spermatogenesis- Meiosis in the Testis

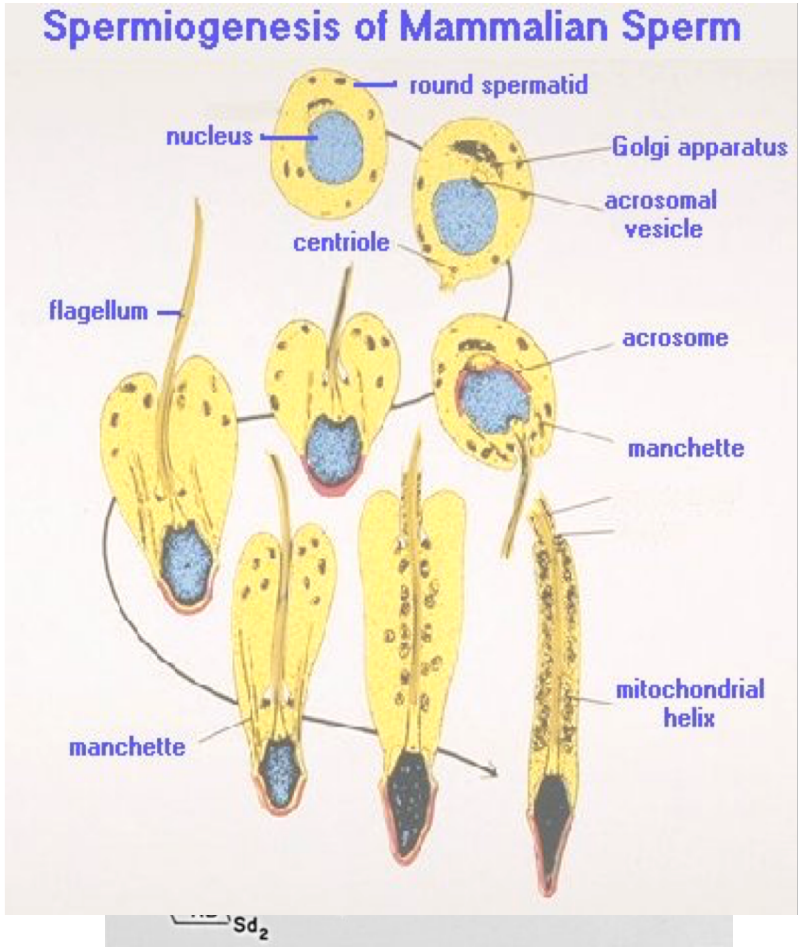


Mruk DD, Cheng CY. The Mammalian Blood-Testis Barrier: Its Biology and Regulation. Endocrine reviews. 2015;36(5):564-591. doi:10.1210/er.2014-1101.

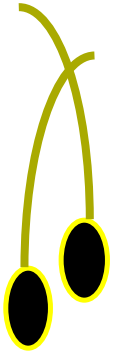
Spermiogenesis

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



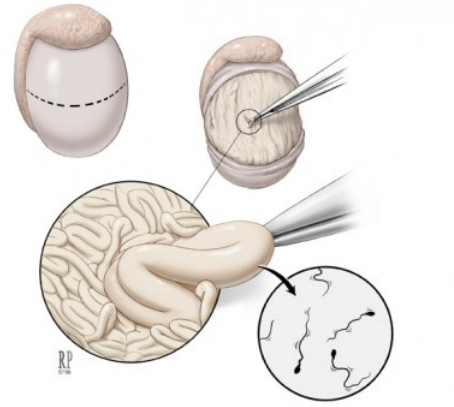


Spermatogenesis Summary



- 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 Virtual Azoospermia



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

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

Micro-TESE: Outcomes

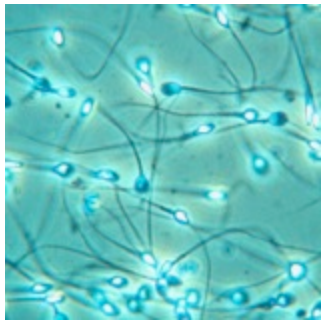
- Sperm retrieved in 37% of patients overall.
- Hypospermatogenesis positively associated with success.
- Cyclophosphamide exposure negatively associated with success.
- 50% clinical pregnancy rate
- 42% live birth rate

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

Indication	No.	Total Patients (%)	Sperm Retrieved?		Retrieval Rate (%)
			Yes	No	
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?

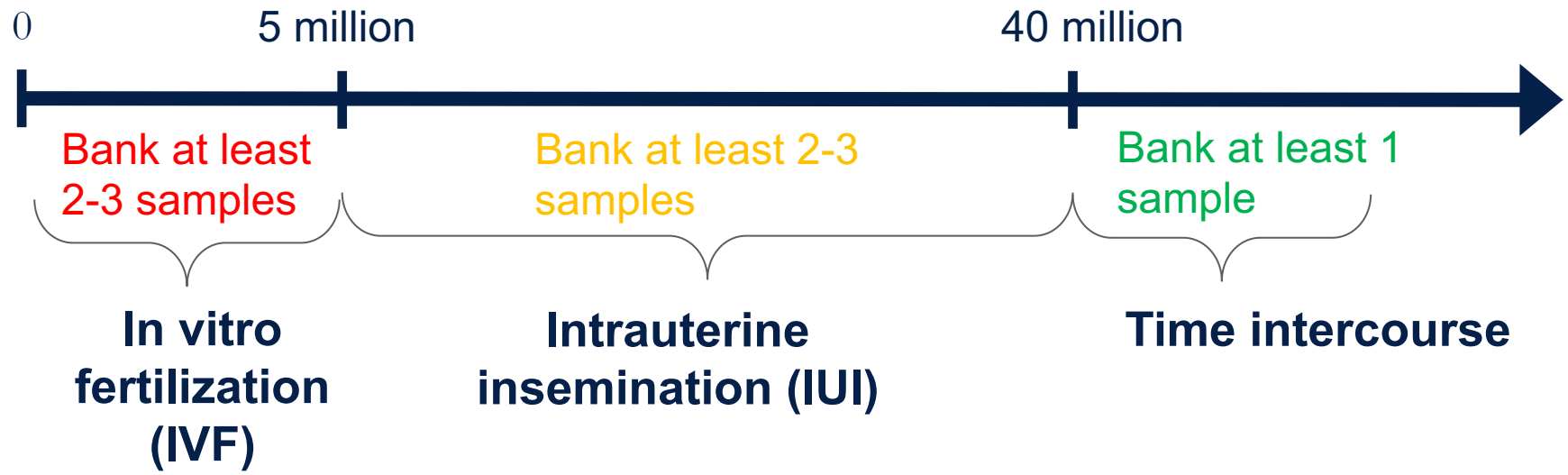
Cancer and Fertility Preservation Pre-Chemo



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?

Cryopreservation Strategy by Total Motile Count

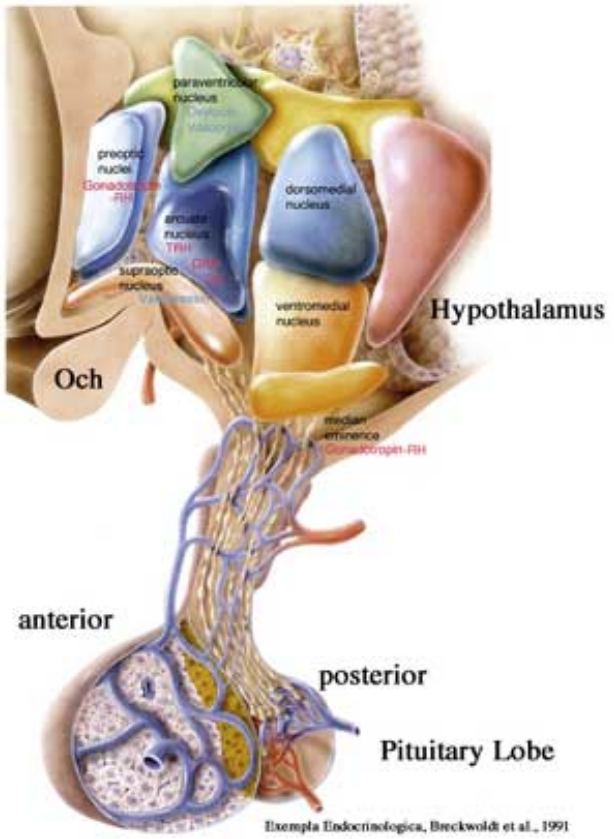


Why not bank before treatment (males)?

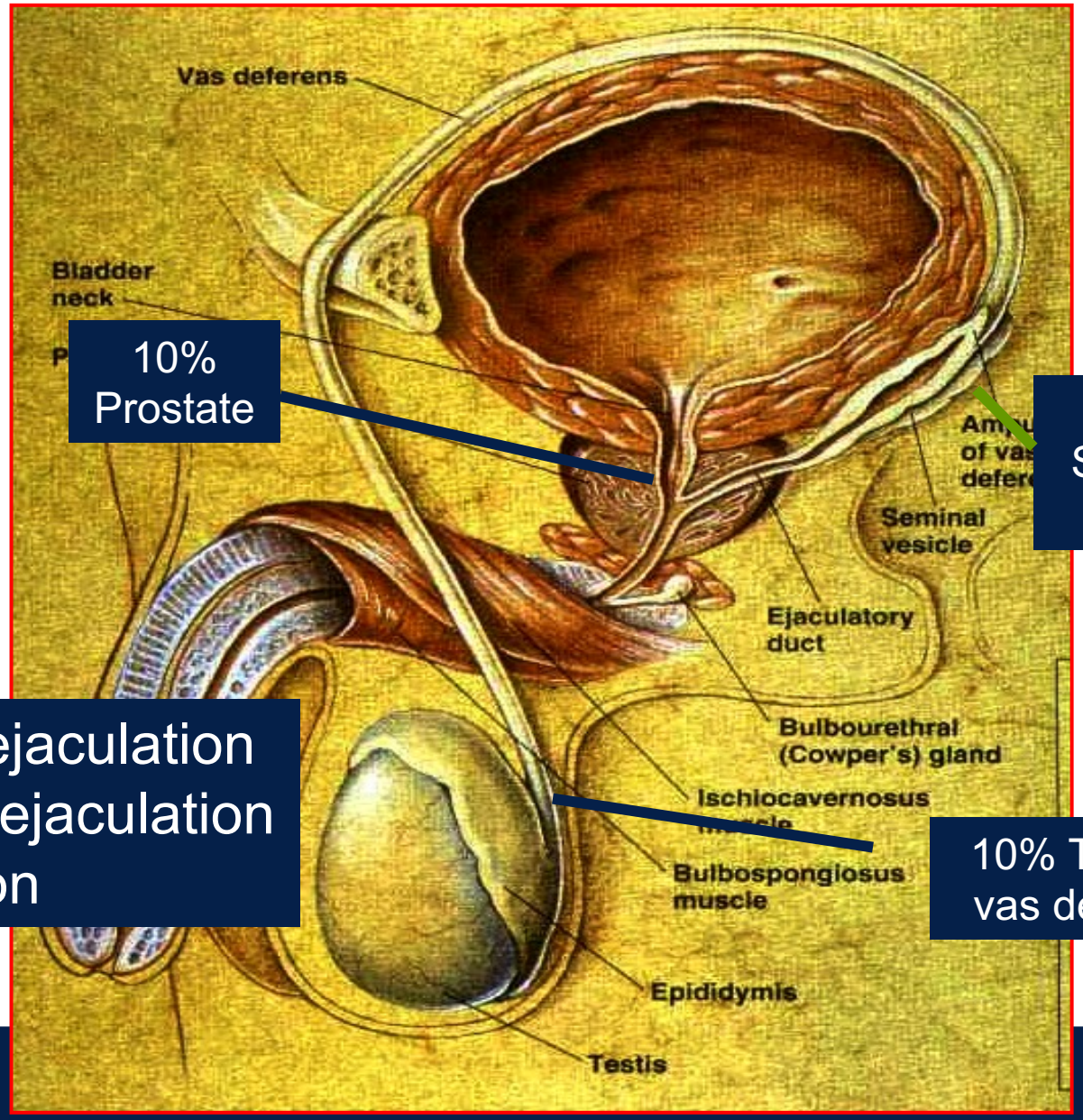
- Belief that wouldn't need it. Perception of small fertility threat
- Not enough time before starting therapy
- Banking too expensive
- Infrastructure for preservation not accessible
- Oncology / surgeons not referring patient (Knowledge gap)
- Preservation experimental
- Significant regret about not banking

35 y/o testicular cancer recurrence

- Orchiectomy 4 years ago. Received 20 Gy retroperitoneum that time. Developed retroperitoneal recurrence and underwent RPLND 3 years ago. Couple had first child spontaneously 5 years ago. Now returns to clinic desiring another child.
- What fertility testing do you do?
  - FSH 6.5, T 525, LH 8.1, inhibin B 150
  - SA with PEU:
    - No ejaculate; urine no sperm
  - Diagnosis?
  - What fertility options do we have?



Anatomy- The Ejaculate



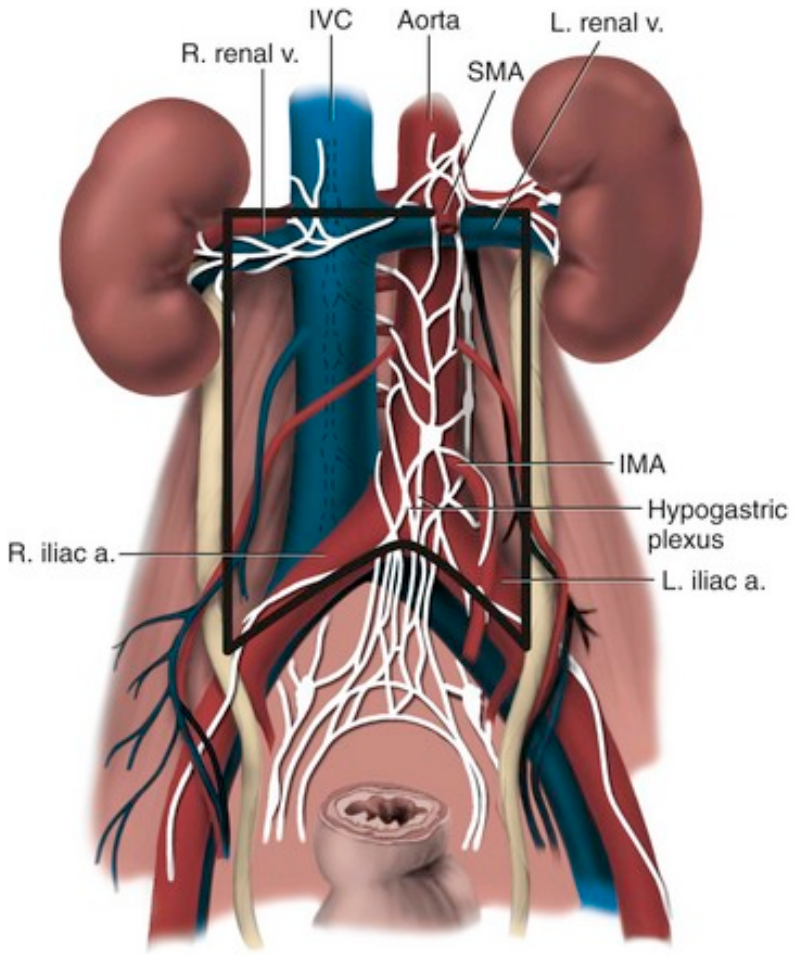
10% Prostate

80% Seminal vesicle

Premature ejaculation
Retrograde ejaculation
Anejaculation

10% Testicle, vas deferens

Ejaculation



Hypogastric plexus

Prostate, SV, Vas

T12-L2

Emission

Perineal muscles, Bladder neck

Pudendal nerve

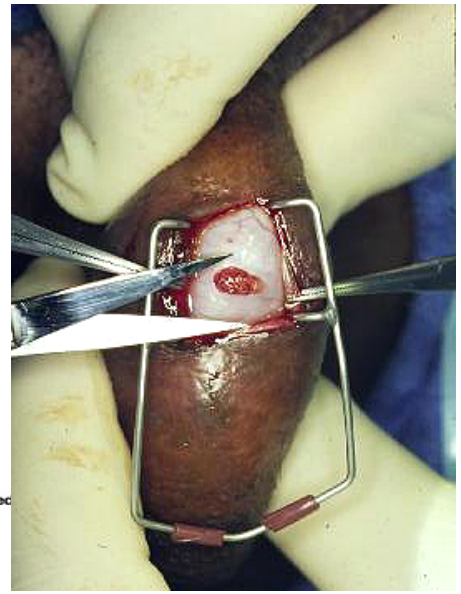
S2-S4

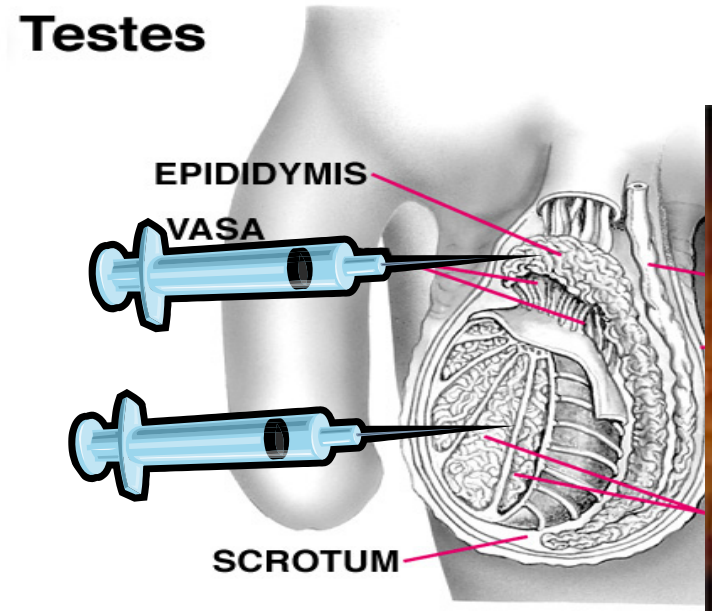
Ejaculation

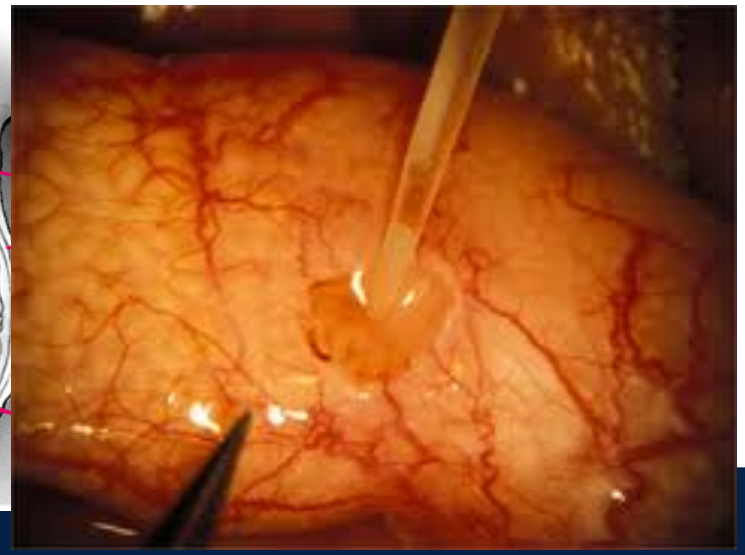
Orgasm

Sperm Aspiration or Extraction (OA)

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



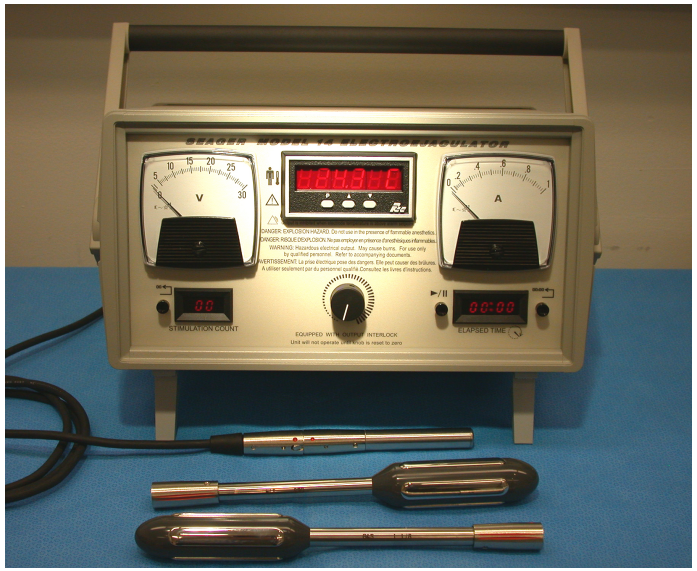




Electroejaculation (EEJ)

- 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

45 yo male presents to the urologist with complaints of infertility for five years.

-At 35, he and his wife conceived without difficulty and have a 10 year-old son. They desire a second child.

-He has a history of rectal cancer (stage III) s/p low anterior resection at 40 years of age at outside hospital.

-He received FOLFOX.

45 y/o, cancer

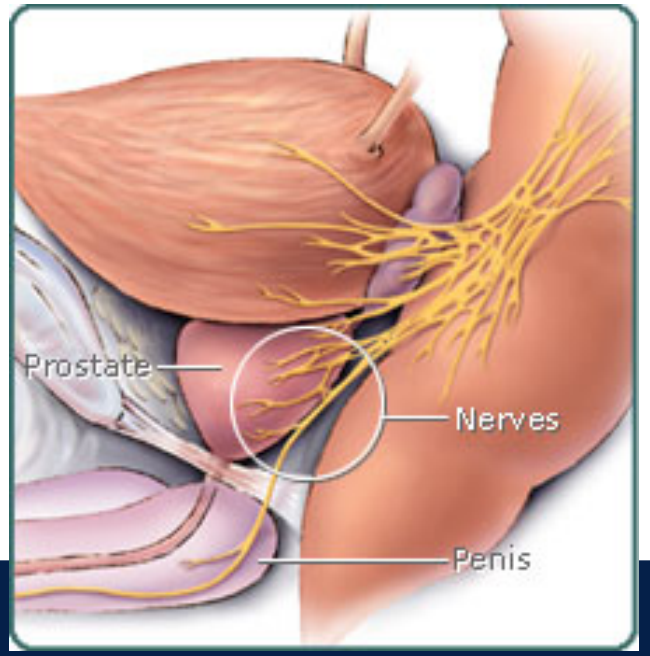
-He has no evidence of disease (NED).

-He was not offered sperm cryopreservation prior to therapy.

-Semen analysis x 2 shows no antegrade ejaculate.

-He confirms next that he has not had any antegrade ejaculations since the low anterior resection procedure.

-Next step(s)?



45 y/o, cancer

-Post Ejaculate Urinalysis (PEU): 50 mL urine, no sperm.

-Serum hormone testing reveals:

-FSH 15 mIU/mL

-Testosterone 250ng/dL

-LH 12.5 ng/dL

- Normal Prolactin and Estradiol levels

-Diagnosis?

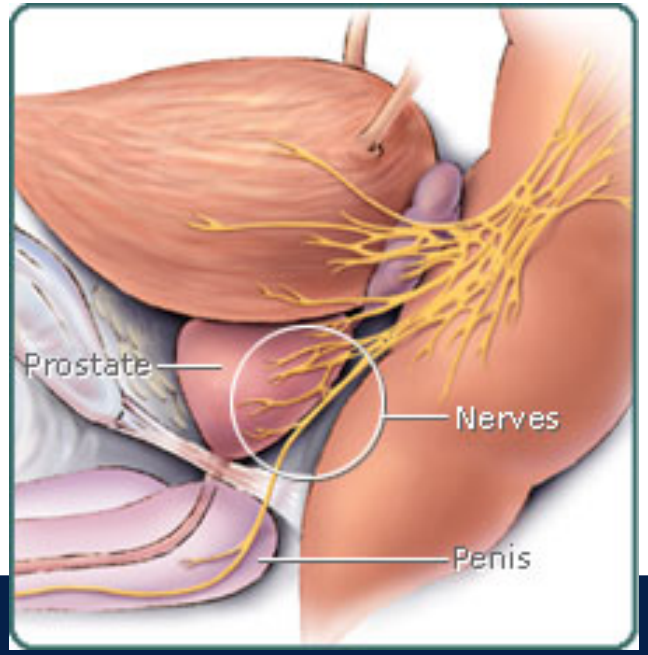
-What are his fertility options?

45 y/o, cancer

-He proceeds with micro-TESE and sperm found is used to successfully fertilize his wife's eggs for IVF. She is pregnant.

-He reports that since his low anterior resection surgery 5 years ago, his erections have been very weak.

-What options does he have to improve his erections?

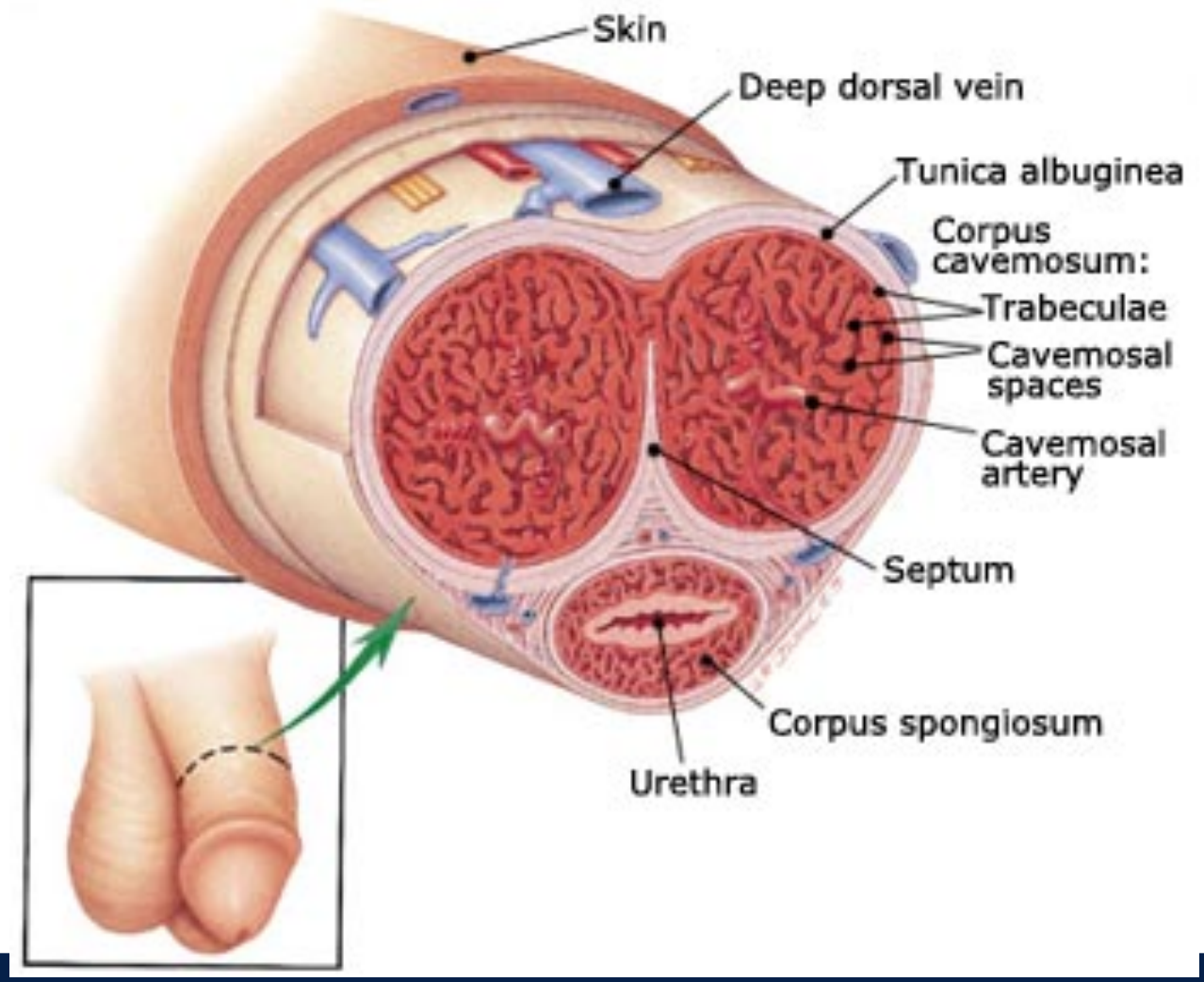


Definition of ED

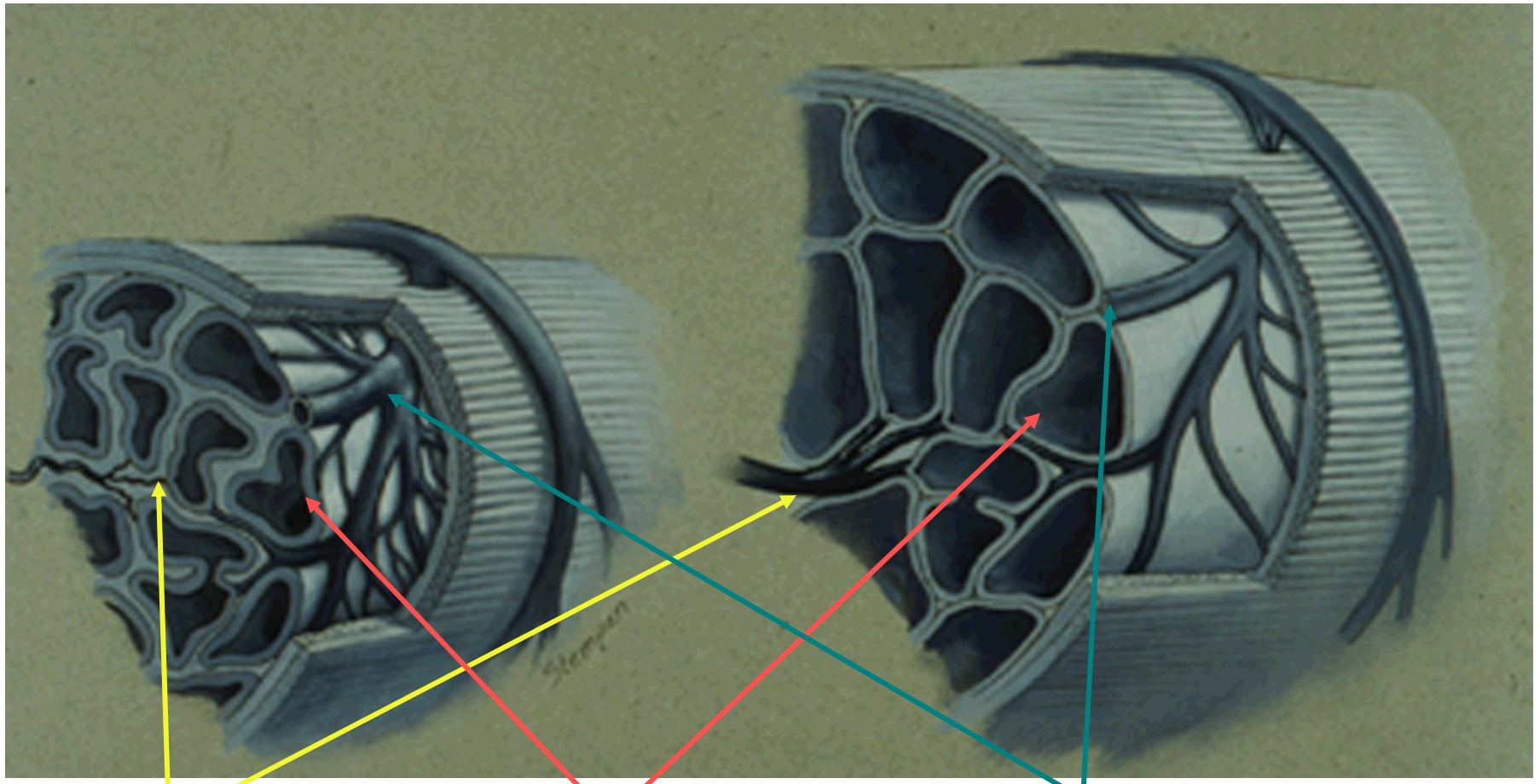
"…the consistent or recurrent inability of a man to attain and/or maintain a penile erection sufficient for sexual performance"

Recommendations of the 1st International Consultation on Erectile Dysfunction. In: Jardin A, et al, eds. *Erectile Dysfunction*. Plymouth, United Kingdom: Health Publication, Ltd; 2000:711-726.

Penile Anatomy



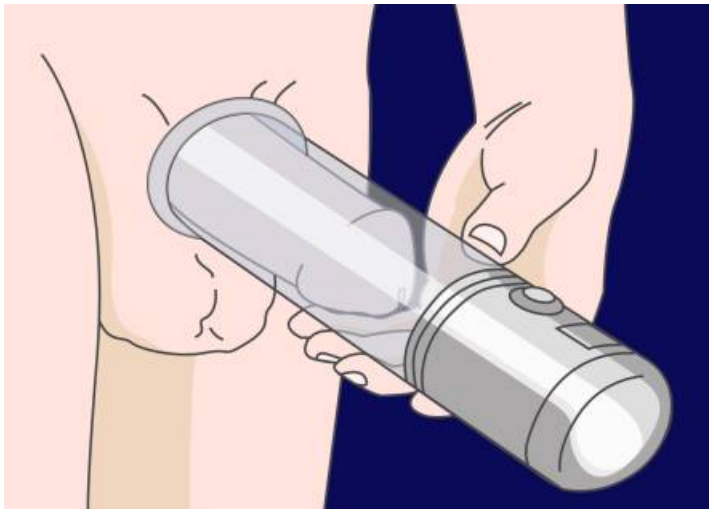
Mechanism of Erection



Therapy for ED

- 1st Line:
  - PDE5 inhibitors, sexual therapy (for select cases)
- 2nd Line:
  - Vacuum Erection Device, intraurethral suppository (“MUSE”), intracavernosal injections (“Bimix” or “Trimix”)
- 3rd Line:
  - Penile prosthesis

Vacuum Erection Device

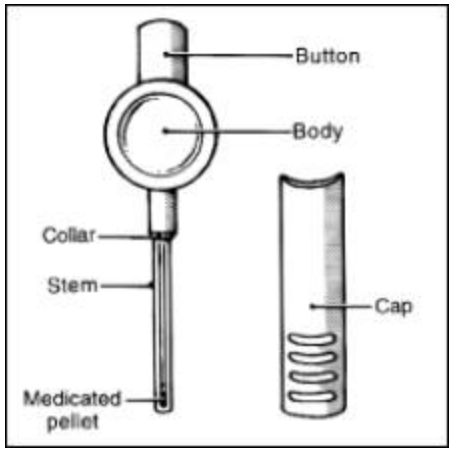


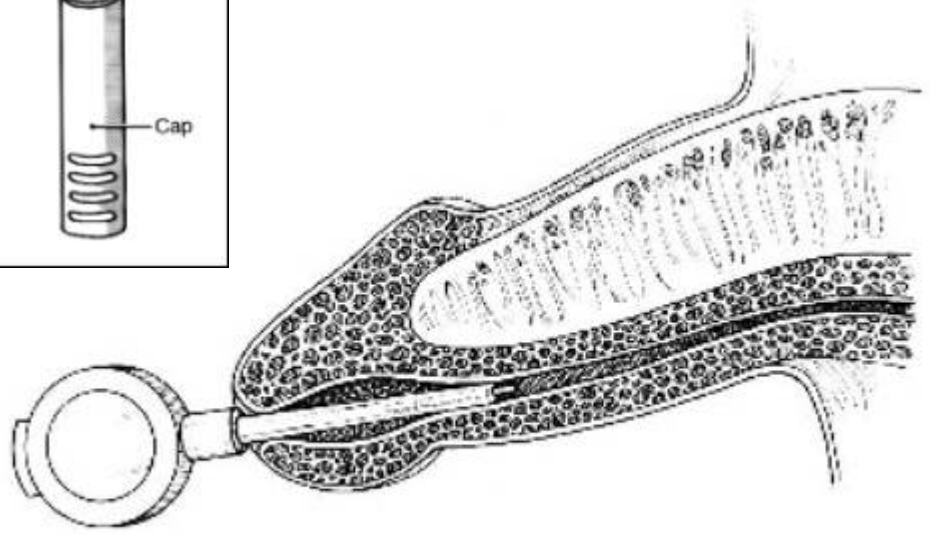
- Requires manual dexterity, instruction is necessary
- 30-minute maximum duration of constriction is advised to prevent penile ischemia
- Precautions necessary in patients on anticoagulant therapy or those easily bruised
- Effective in 60-70%

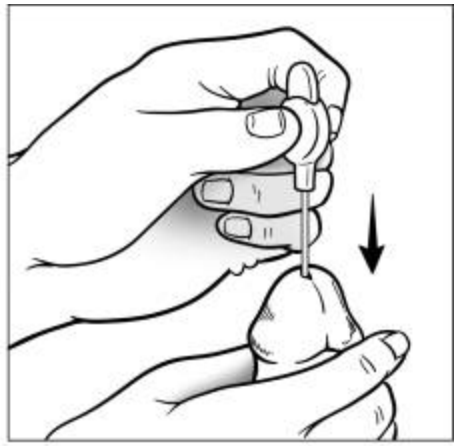
Levine, Urol Clin North Am. 2001
Montague et al, AUA Clinical Guidelines Panel on Erectile Dysfunction. J Urol. 1996

Intraurethral Alprostadil (MUSE)

Smooth muscle–relaxing urethra suppository mimics physiology of erection (PGE_1)

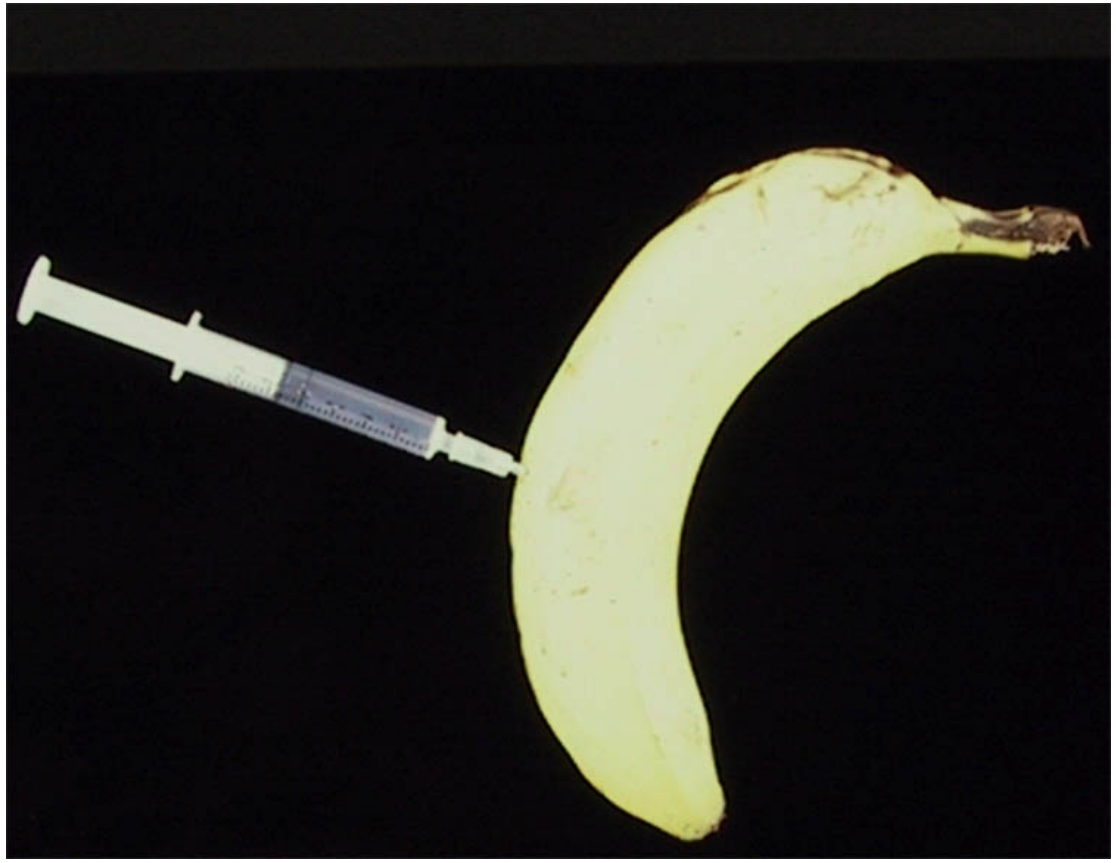






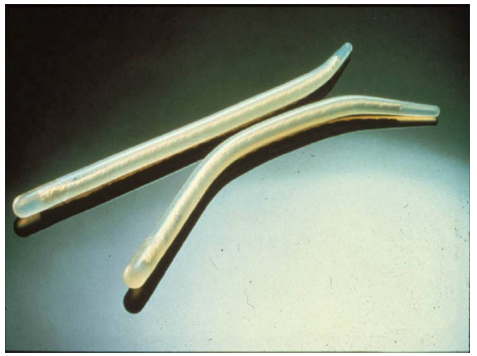
Intracavernosal Injection Therapy

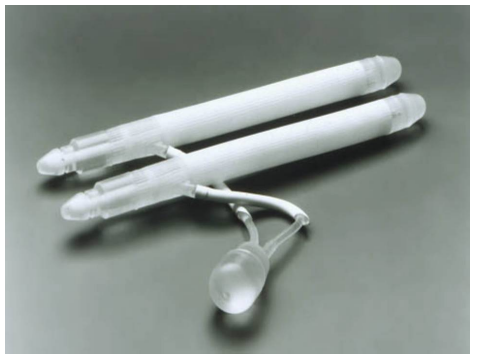
"Bimix" "Trimix"
papaverine, phentolamine, prostaglandin E1

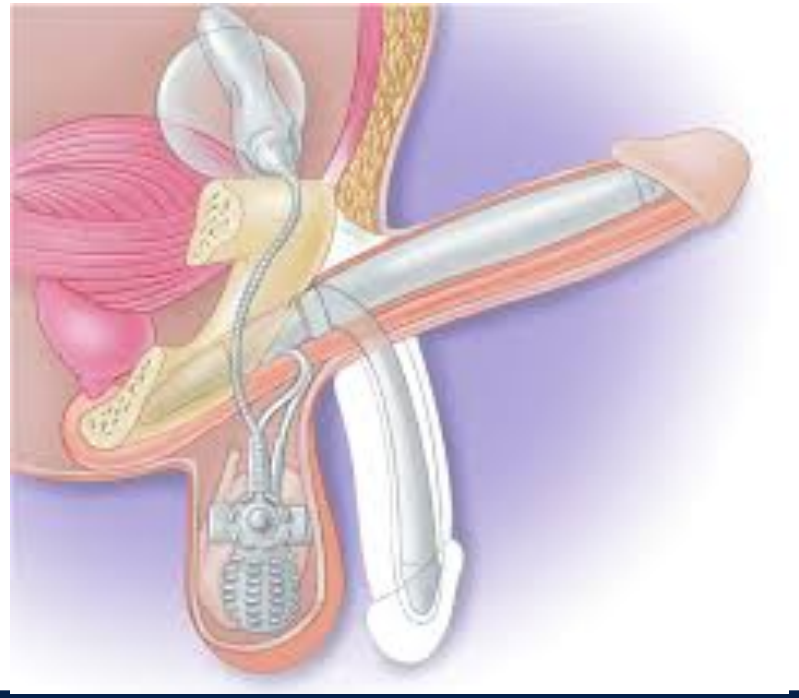


Penile Implant

- Lack of efficacy or dissatisfaction with other modalities
- High satisfaction with IPP
- 90-120-minute surgery, 23hr stay







3 y/o boy with brain tumor

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

3 y/o boy with brain tumor

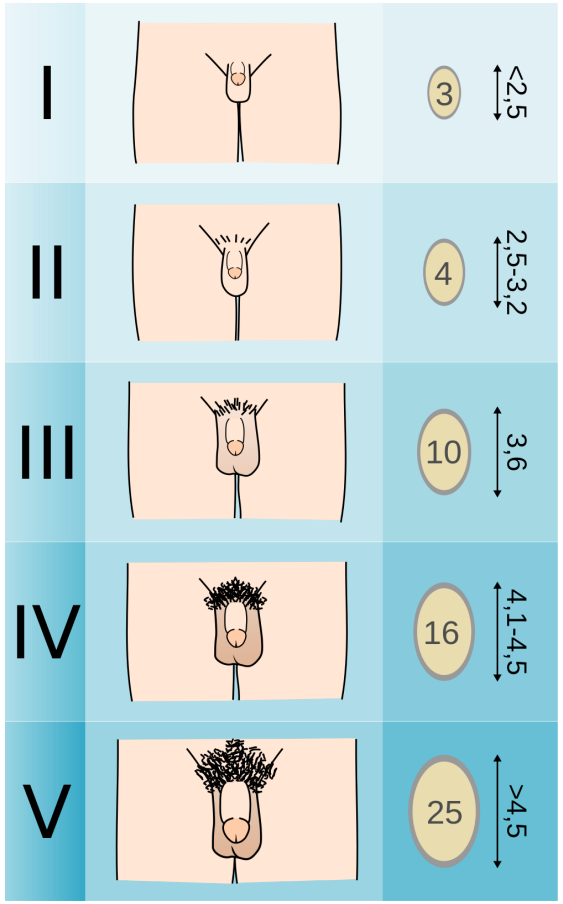
He will be receiving a lumbar puncture and central line catheter next week

-His parents (a UCSF electrophysiology cardiologist and psychologist) ask you about the risk of infertility associated with the planned therapeutic regimen.

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

-What can we tell them?

Clinical Assessment and Development of Spermatogenesis



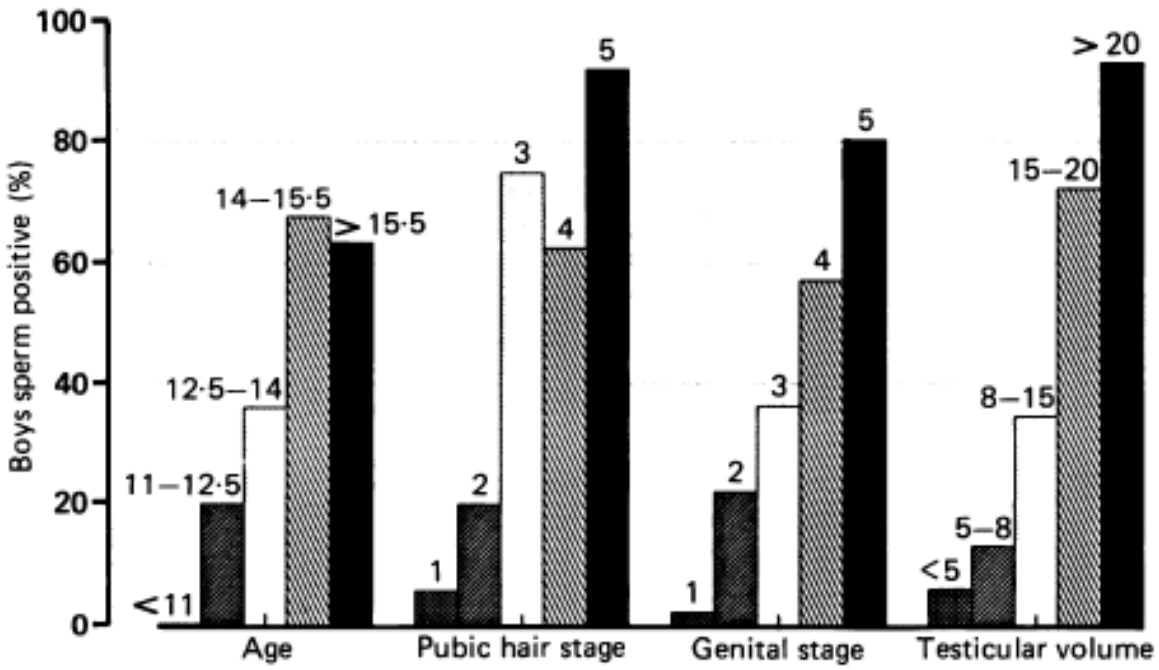


Figure 2 Overall cumulative probability of spermaturia according to age, Tanner stages for pubic hair (1–5) and genitals (1–5), and testicular volume (ml) (n=129).

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

3 y/o boy with brain tumor

the Oncofertility® Consortium AT NORTHWESTERN UNIVERSITY

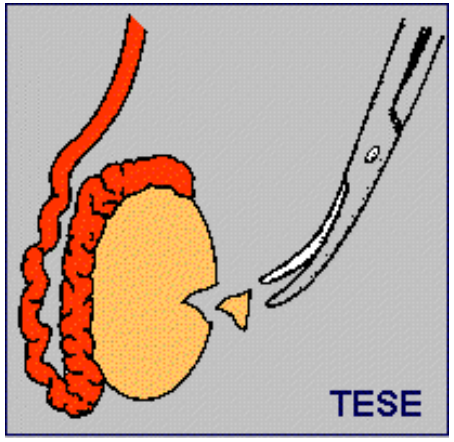
You perform an open testicular biopsy concurrent with his lumbar puncture and central line placement

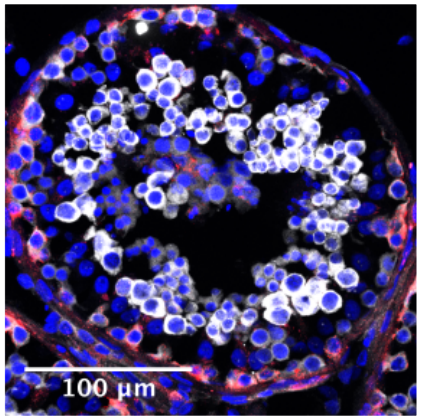
-What do you tell them about cost and insurance coverage?

-They ask about future possible uses for this tissue…

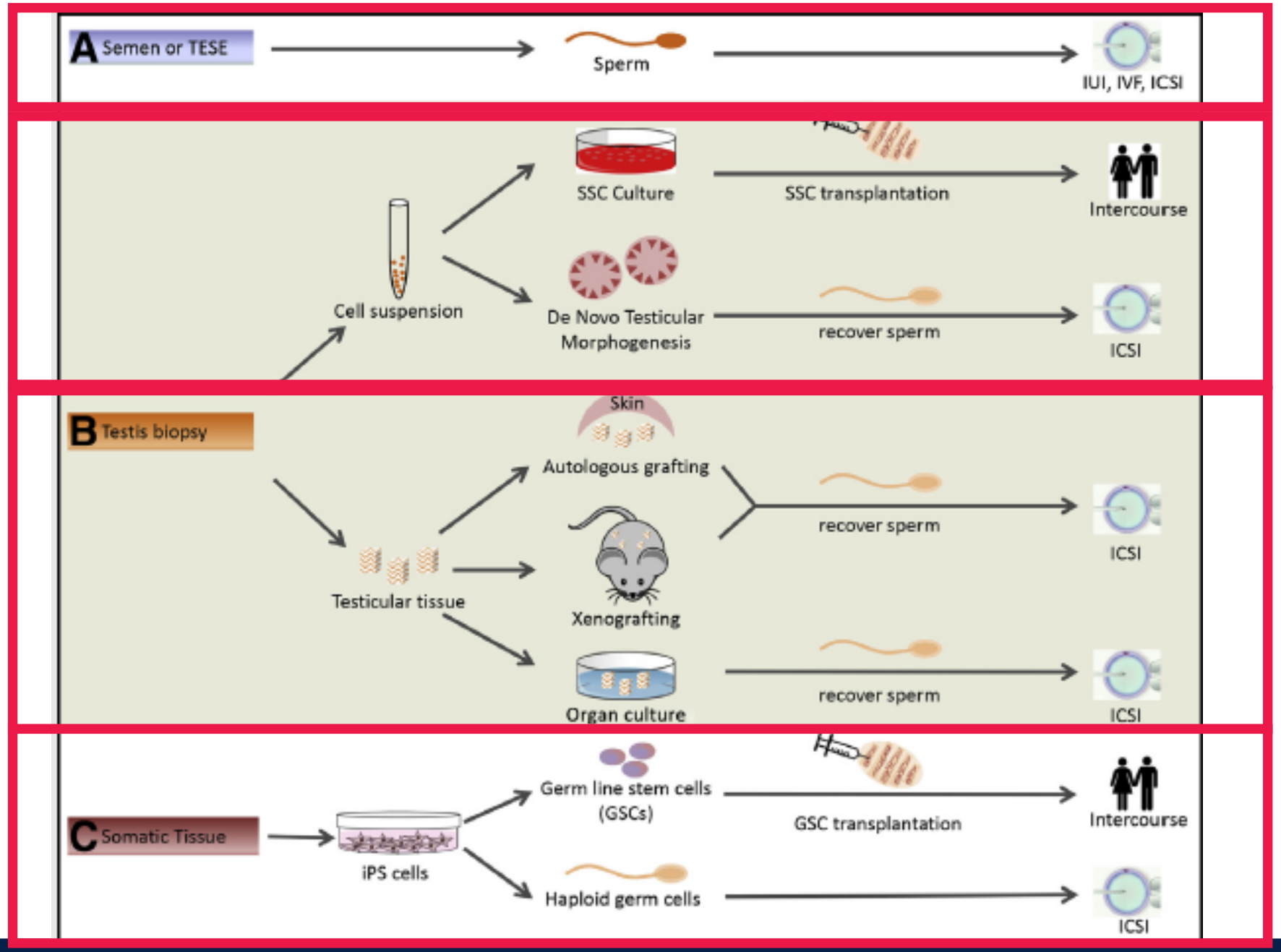
UCSF

Testicular Sperm Stem Cell Extraction (Pedi-TeSE)





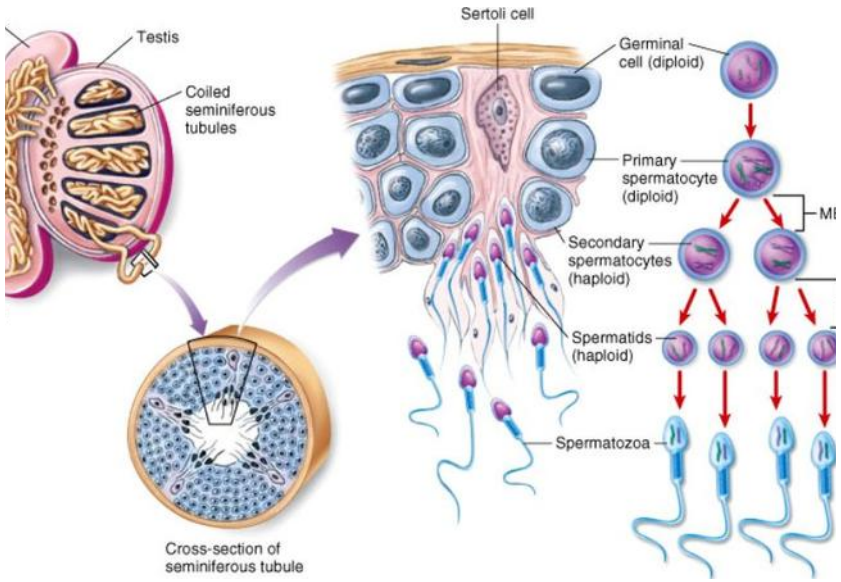
- 30 minute surgical procedure, combined with other procedures
- Testicular tissue removed, cryopreserved.
- Sperm stem cells, no sperm in pre-pubertal boys
- **Ethical considerations**



Gassei K, Orwig KE. Experimental methods to preserve male fertility and treat male factor infertility. Fertil Steril. 2016 Feb;105(2):256-66. doi: 10.1016/j.fertnstert.2015.12.020. Epub 2015 Dec 30. Review.

UCSF

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

How is this similar or different for transgender children? Post-pubertal transgender women?

- Facing sterilizing treatment, however…
- Is there a rush to do surgery?
  - Prepubertal trans-girls suppressed with Lupron (leuprolide)
  - Gonads suppressed at any age
- Need to make decision when undergo orchiectomy
- Post-pubertal patients often use spironolactone + estrogen rather than Lupron

13 y/o transgender girl

The mother of a 13 yo transgender girl calls you about fertility preservation her daughter. Since age 9, she's been taking Lupron. She's planning bottom surgery with bilateral orchiectomy. Tanner stage 2 with 4ml testicles bilaterally.

-How do common medications given to suppress testosterone affect spermatogenesis?

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

13 y/o transgender girl

- At time of orchiectomy, dissected tissue.
- Secondary spermatocytes present
- Cryopreserved tissue

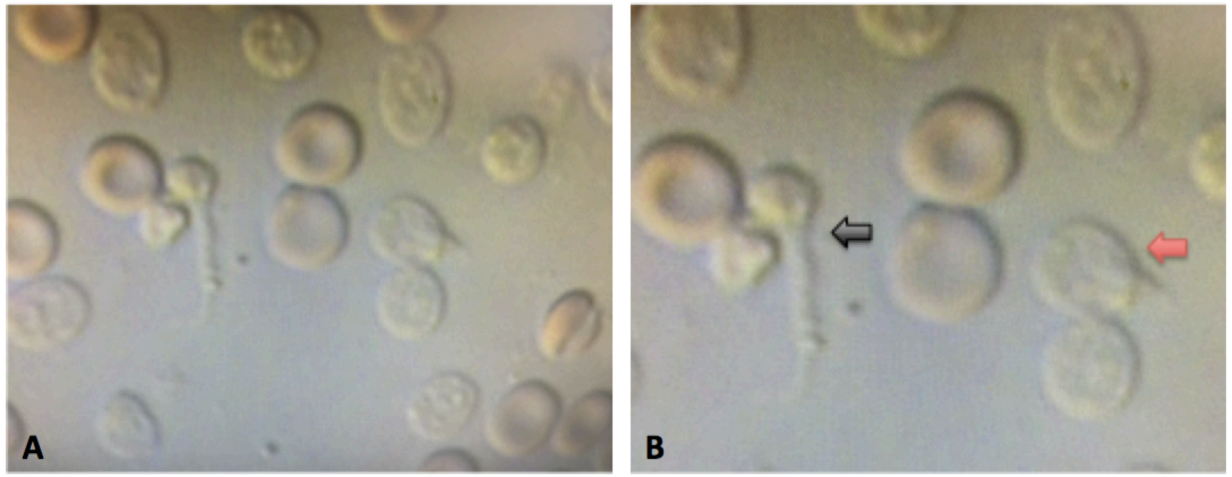


Figure 1. 10x light microscopy of TESE specimen A) 10x B) Late spermatid (black arrow) early spermatid(red arrow)

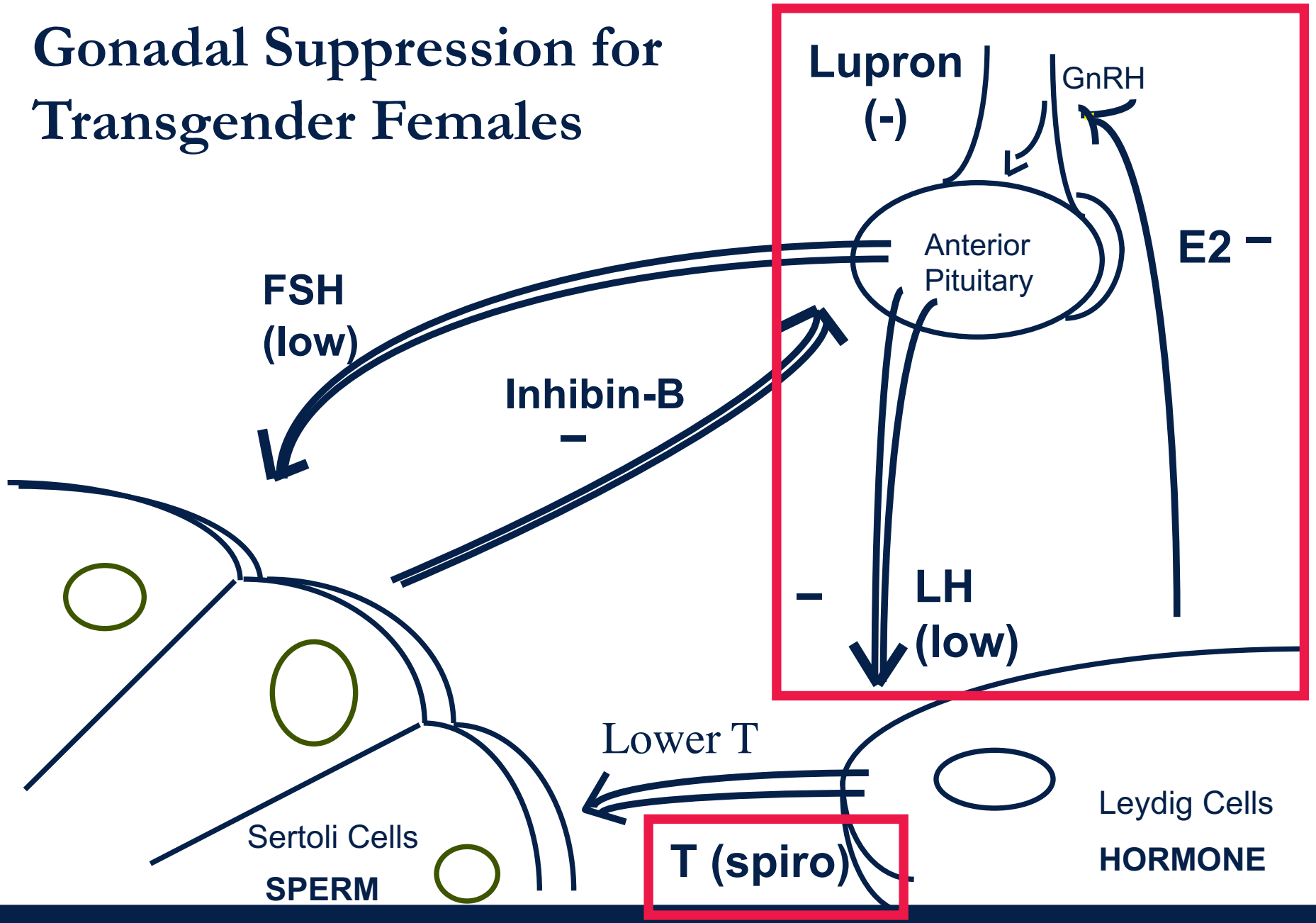
What about FP options for transgender adolescents and young adults?

- 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?**
- **Does long term estrogen, leuprolide, spironolactone suppress spermatogenesis through non-hormonal mechanisms?**

Adeleye A, Reid G, Mok Lin E, Smith JF. Fertility Preservation for Transgender Females after Gender Affirming Treatment is Effective. Urology 2018

Gonadal Suppression for Transgender Females



Sperm Cryopreservation for Transgender Females

- N=28 transgender females (natal males)
- 43 semen specimens, 10 collected after discontinuing therapy for at least 3 months
- On therapy vs. off therapy:
  - Semen volume 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 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. Urology 2018.

Fertility Preservation & Restoration Take Home Points

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

UCSF
University of California
San Francisco

Questions?

Practical aspects of ovarian tissue cryopreservation in the pediatric population

Leslie Coker Appiah, M.D.

Associate Professor
Division Chief General Obstetrics and Gynecology
Director, Fertility Preservation and Reproductive Late Effects Program
The University of Colorado Denver | Anschutz Medical Campus
Pediatric and Adolescent Gynecology
Nationwide Children's Hospital
Denver, CO

November 11, 2019

Financial Disclosure and Conflicts of Interest: None

Objectives

- Identify appropriate candidates for Ovarian Tissue Cryopreservation (OTC)
- Explain technical and surgical aspects of OTC
- Describe collaborating as a regional OTC referral center
- Discuss considerations for ovarian tissue transplantation

Outline

a. Indications and stratifying risk
    i. Malignancy
    ii. Non-malignant disorders
    iii. Sex diversity
    iv. Predisposition to POI
b. Surgical and laboratory aspects
c. Collaborating as a regional OTC referral center
d. Considerations for ovarian tissue transplantation (OTT)

Case

- 20 yo G0 with stage IIA nodular sclerosing Hodgkin's Lymphoma (HL)
- Treated with ABV therapy w/o dacarbazine
- Disease progression noted at end of therapy PET
- Plan for chemotherapy with rituximab, ifosfamide, carboplatin and etoposide (R-ICE) followed by autologous stem cell transplant (ASCT)
- Counseled regarding risk of oocyte freezing with proximity to chemotherapy

Case

- AMH 3.08 ng/dl, FSH 4.9 mIU/ml, E2 99 pg/ml
- Elected ovarian tissue cryopreservation (OTC)
- Stand alone laparoscopic removal of right ovary
- Ovary sent for processing at external REI lab
- Nexplanon for contraception and GnRHa initiated
- Proceeded with ASCT with BEAM

Indications for Ovarian Tissue Cryopreservation

- Malignant disorders:
  - Insufficient time for ovulation induction and/or IVF
  - Pre- and post pubertal females at high risk of gonadal failure
  - Absence of previous high gonadotoxic chemotherapy
- Nonmalignant disorders treated with immunosuppression or SCT
- Individuals with gender and sex diversity

Clinical Ascertainment of Health Outcomes Among Adults Treated for Childhood Cancer

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.

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 >20 Gy

Gonadotoxicity of Newer Agents

- Oxaliplatin
- Irinotecan
- Bevacizumab – 30% rate of primary ovarian insufficiency
- Cetuximab
- Trastuzumab
- Erlotinib
- Imatinib

Subfertility/Infertility Risk		
Low Risk < 20%	Medium Risk >30 and <70%	High risk > 80%
ALL	AML	BMT Conditioning
Wilms' tumor	Breast	Whole body irradiation
Soft-tissue sarcoma: stage I	Osteosarcoma	Pelvic/testicular irradiation
Retinoblastoma	Ewing's sarcoma: non-metastatic	Hodgkin: alkylator
Germ-cell tumors (fertility sparing)	Soft-tissue sarcoma: stage II/III	Soft-tissue sarcoma: stage IV
CNS tumor: surgery and irradiation < 24 Gy	Neuroblastoma	Metastatic Ewing's sarcoma
	Non-Hodgkin lymphoma	
	Hodgkin: alternating alkylator tx	

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		

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

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

Stem cell transplant: Non-oncologic conditions

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

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

Fertility options in transgender people

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

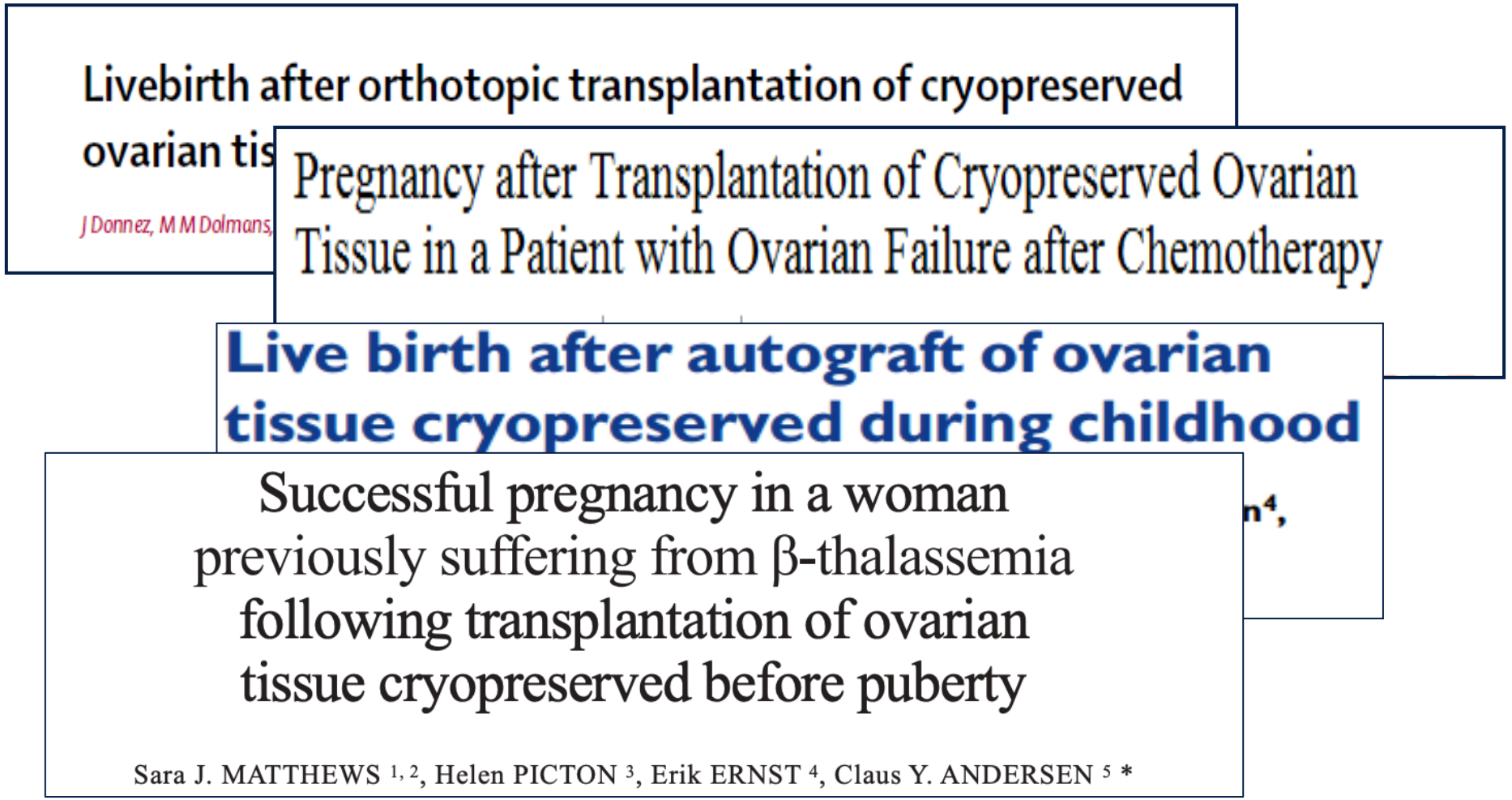
- Oocyte and embryo cryopreservation standard options
- Family building may require gestational surrogacy
- Ovarian and testicular cryopreservation investigational options and may occur at time of genital reconstructive surgery
- Physical Barriers
  - FTM patient - vaginal examination & invasive procedures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

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

Fertility Preservation Methods

Standard	Success rates	Investigational
Mature oocyte Cryopreservation	35 - 50%	Immature oocyte cryopreservation
Sperm cryopreservation	57%	Ovarian tissue freezing
Embryo cryopreservation	40%	Testicular tissue freezing
Ovarian transposition	60-90%	GnRHa ovarian suppression
Ovarian shielding	75-80%	Artificial ovary

Ovarian Tissue Cryopreservation



Matthews SJ et al. Minerva Ginecol. 2018; 70(4):432–435

Poirot C. et al Lancet 2012;379:588

Ernst E et al. Eur J Cancer 2013;49:911–4


REVIEW ARTICLE

Edward W. Campion, M.D., *Editor*

Fertility Preservation in Women

Jacques Donnez, M.D., Ph.D., and Marie-Madeleine Dolmans, M.D., Ph.D.

- 130 children born worldwide
- 4700+ cryopreserved tissues with 360 transplantations
- Age range **pre-pubertal*** to mid 30's
- Half of singletons conceived naturally, otherwise IVF
- 29 - 36% delivery rate
- Recommend OTC no longer experimental for post-pubertal patients

Donnez et al. NEJM; 2017;377(17):1657-1665
Jensen et al. J Assist Reprod Genet (2017) 34: 325
Shenfield et al. Hum Reprod Open. 2017
Gellert et al. J Assist Reprod Genet. 2018

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*.

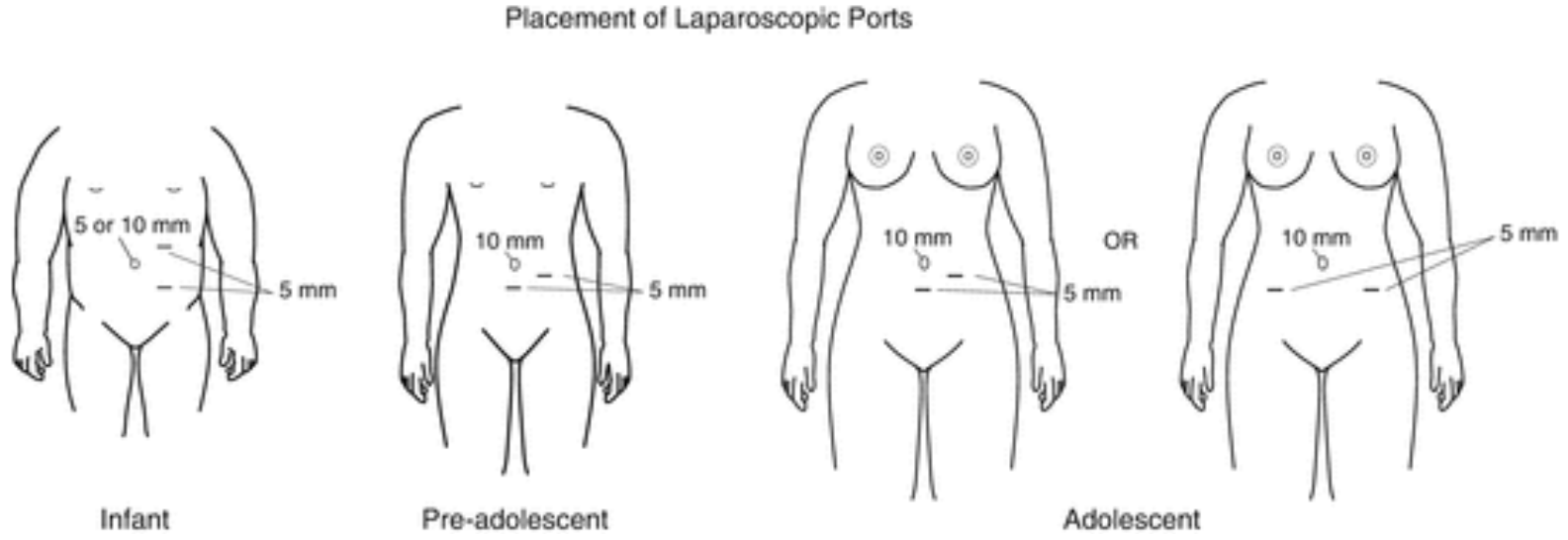
Anderson et al. Hum Reprod. 2014 Jan;29(1):97-106.

Outline

a. Indications and stratifying risk
    i. Malignancy
    ii. Non-malignant disorders
    iii. Sex diversity
    iv. Predisposition to POI
b. Surgical and laboratory aspects
c. Collaborating as a regional OTC referral center
d. Considerations for ovarian tissue transplantation (OTT)

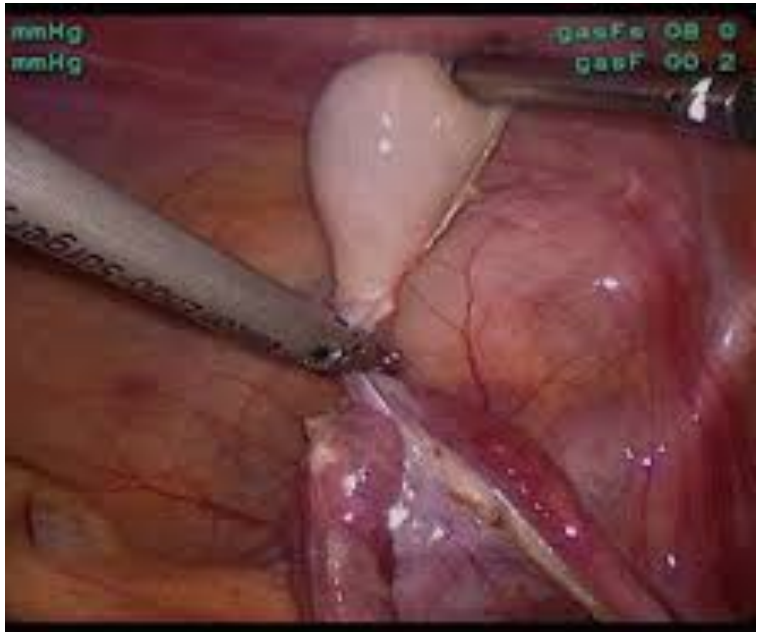
Technical Aspects of OTC

- 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



Technical Aspects of OTC

Oophorectomy

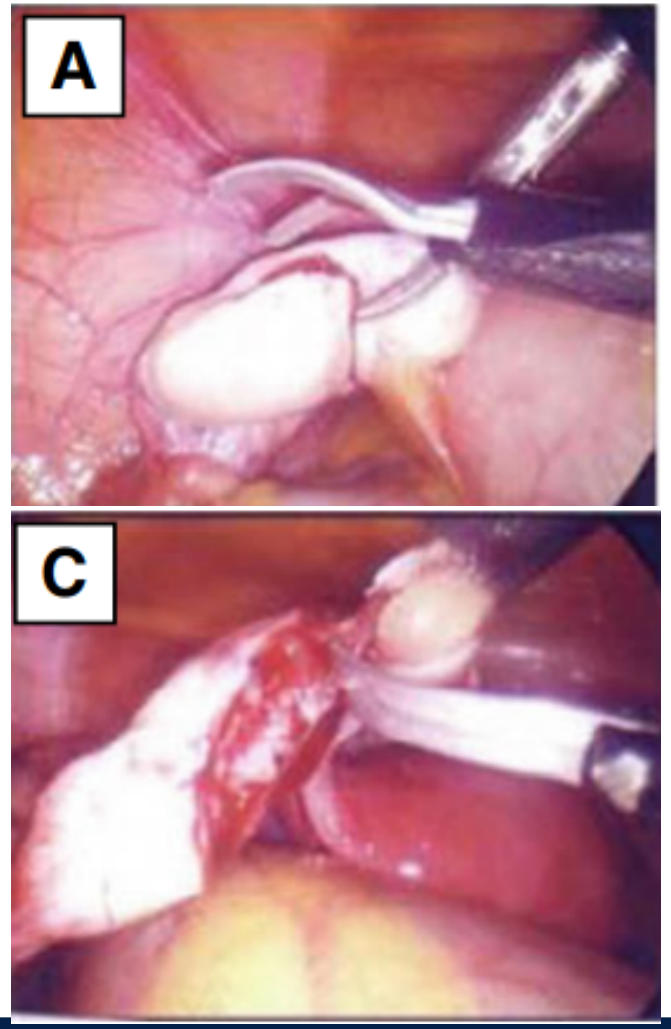


- Allows more cortical tissue for future use

- 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

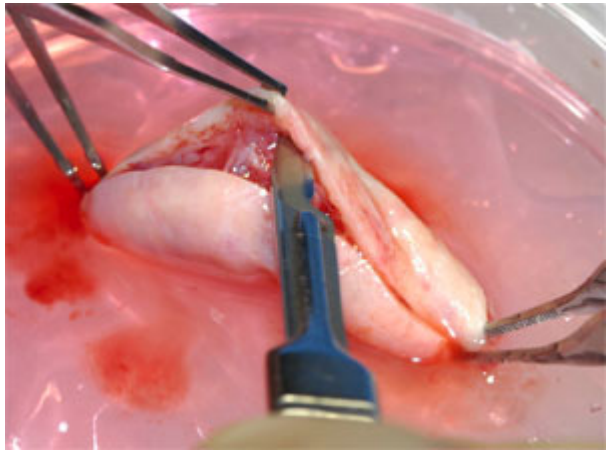
Technical Aspects of OTC

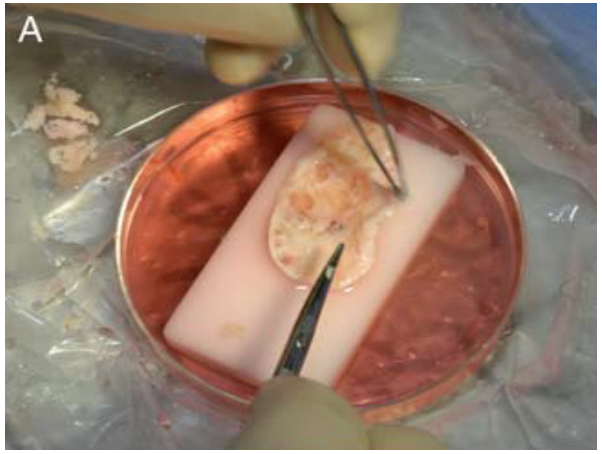
Cortical Biopsy

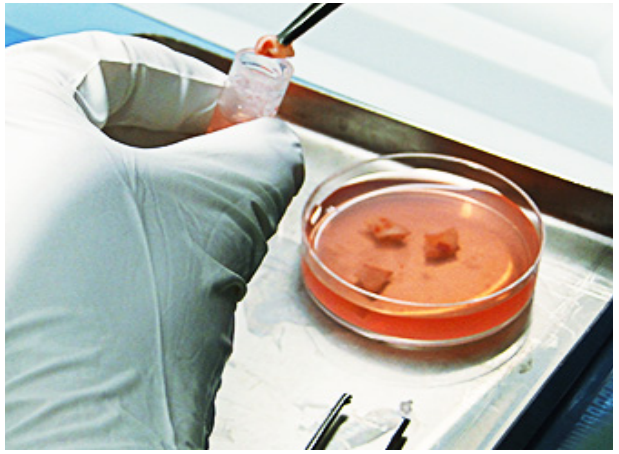


- Select ovary without cyst or corpus luteum
- Minimize manipulation of ovary by grasping uteroovarian ligament – “no touch technique”
- Cold scissors to transect longitudinal strips of ovary
- Cautery, argon beam, thrombin products for anticoagulation
- Allows potential recovery of

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

Outline

a. Indications and stratifying risk
    i. Malignancy
    ii. Non-malignant disorders
    iii. Sex diversity
    iv. Predisposition to POI
b. Surgical and laboratory aspects
c. Collaborating as a regional OTC referral center
d. Considerations for ovarian tissue transplantation (OTT)

Collaborating as a Regional Referral 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**

- **Surgeon to call out time that ovary is transected, to be recorded**
- **Ovary removed per protocol and placed in holding media**
- **Portion of ovary containing cortex and medulla sent to pathology for gross and histologic assessment**

Collaborating as a Regional Referral Site:

- **Ovary in media placed in cooler with purple tube of blood and handed to OR assistant to transfer to courier**
- **Courier transfers ovary to external site for processing and transfer to long term storage facility**
- **Patient proceeds with cancer treatment within 1-5 days**

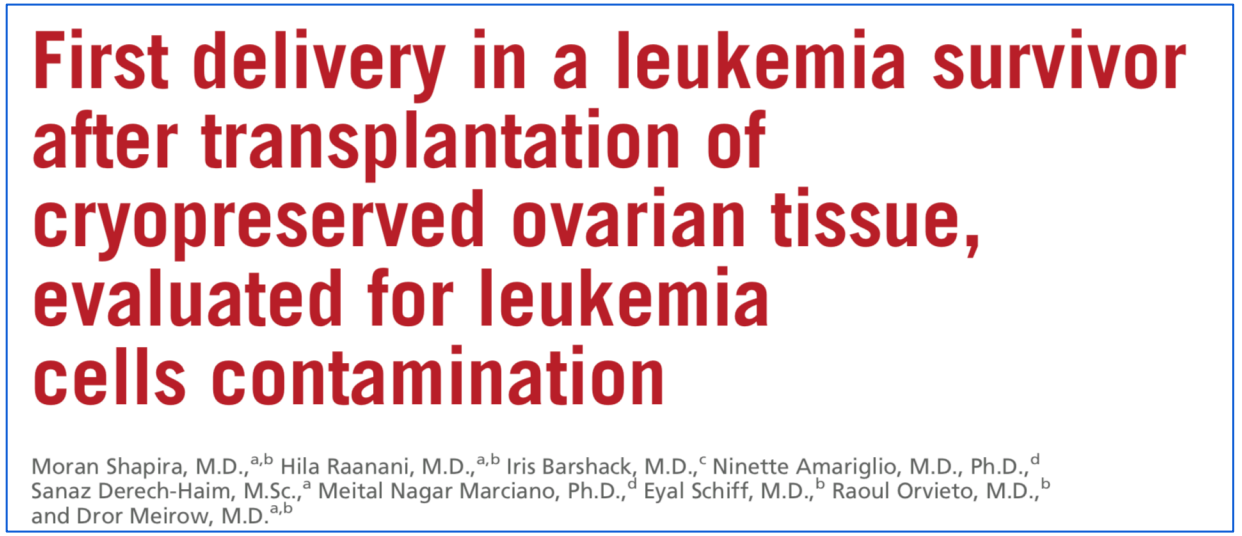
Outline

a. Indications and stratifying risk
    i. Malignancy
    ii. Non-malignant disorders
    iii. Sex diversity
    iv. Predisposition to POI
b. Surgical and laboratory aspects
c. Collaborating as a regional OTC referral center
d. Considerations for ovarian tissue transplantation (OTT)

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]

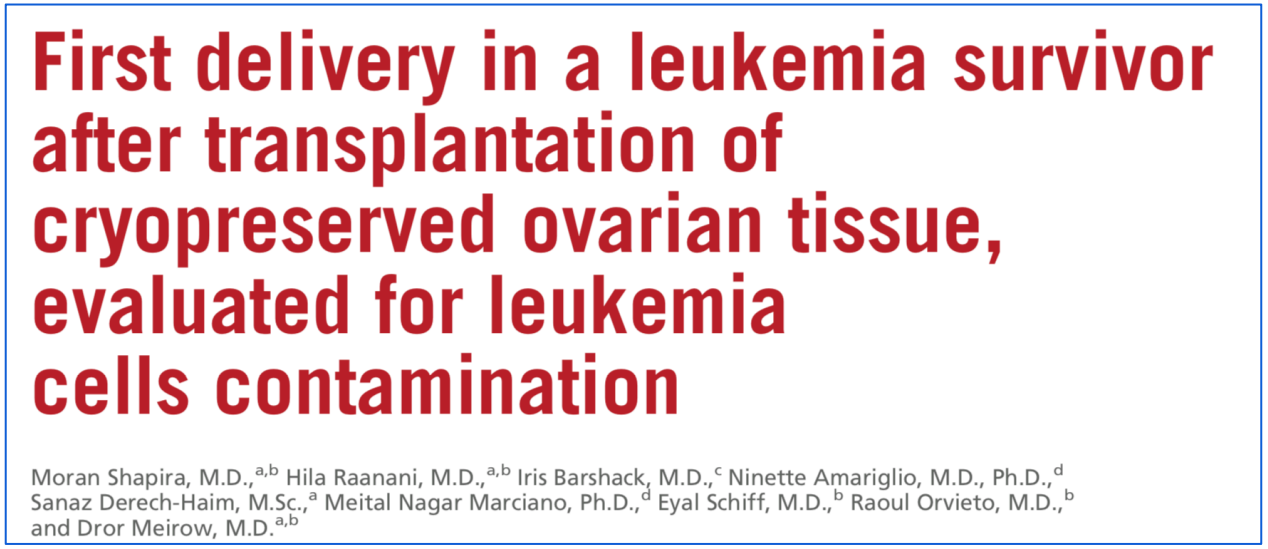
- 20 patients underwent auto-transplantation
- Ages 14 through 39 years at cryopreservation
- 10 patients with non-sterilizing chemotherapy before harvest
- Tissue transplanted average 5.6 years later
  - 16 patients with primary ovarian failure
  - 93% endocrine recovery and 32% delivery rate



First delivery in a leukemia survivor after transplantation of cryopreserved ovarian tissue, evaluated for leukemia cells contamination

Moran Shapira, M.D.,[a,b] Hila Raanani, M.D.,[a,b] Iris Barshack, M.D.,[c] Ninette Amariglio, M.D., Ph.D.,[d] Sanaz Derech-Haim, M.Sc.,[a] Meital Nagar Marciano, Ph.D.,[d] Eyal Schiff, M.D.,[b] Raoul Orvieto, M.D.,[b] and Dror Meirow, M.D.[a,b]

- 19-year-old with history of AML s/p OTC prior to BMT
- Histology and immunohistochemistry negative for leukemia cells
- Xenotransplantation to three SCID mice
- Followed for six months - no macroscopic/microscopic signs of leukemia

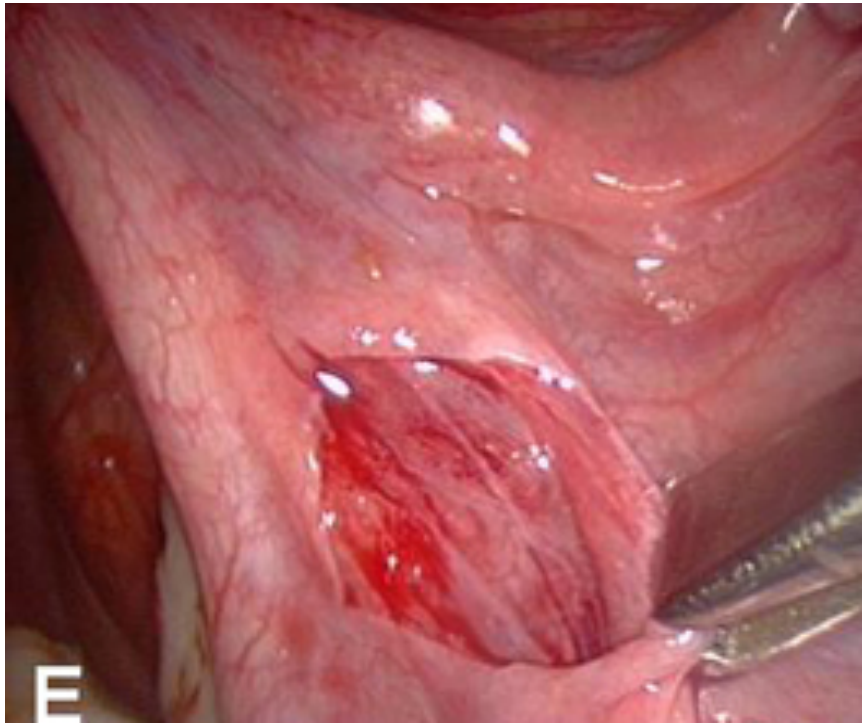


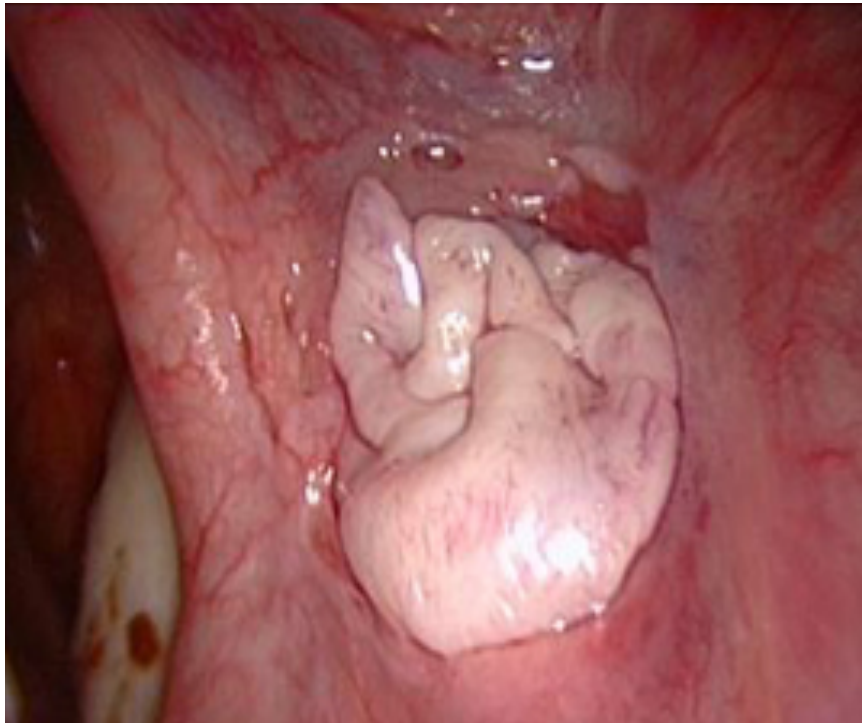
First delivery in a leukemia survivor after transplantation of cryopreserved ovarian tissue, evaluated for leukemia cells contamination

Moran Shapira, M.D.,[a,b] Hila Raanani, M.D.,[a,b] Iris Barshack, M.D.,[c] Ninette Amariglio, M.D., Ph.D.,[d] Sanaz Derech-Haim, M.Sc.,[a] Meital Nagar Marciano, Ph.D.,[d] Eyal Schiff, M.D.,[b] Raoul Orvieto, M.D.,[b] and Dror Meirow, M.D.[a,b]

- FISH for disease-specific gene rearrangement below probe cut-off
- Next-gen gene sequencing panel implicated in myeloproliferative disorders revealed no significant molecular event
- Orthotopic transplantation followed by OS, IVF, and delivery of healthy newborn
- Patient leukemia free more than 2 years since transplantation

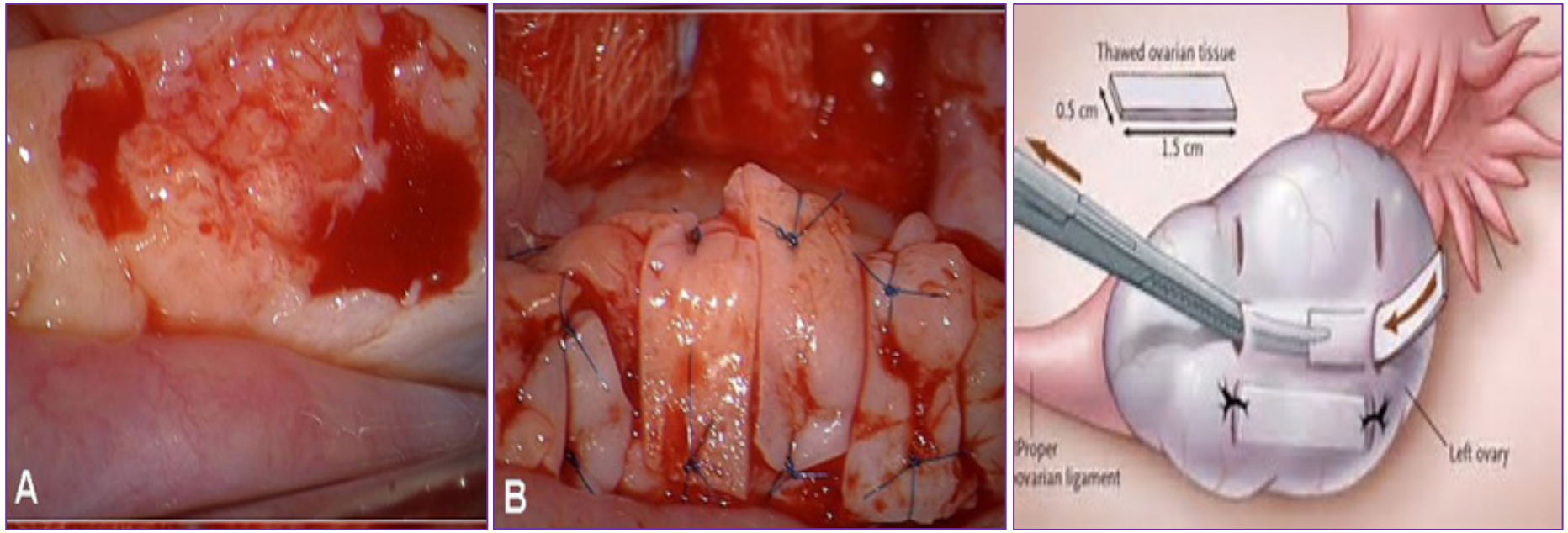
Orthotopic Transplantation: Ovarian Fossa





Donnez et al. Frontiers in Bioscience 2012

Orthotopic Transplantation: Contralateral Ovary



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

Heterotopic Transplantation

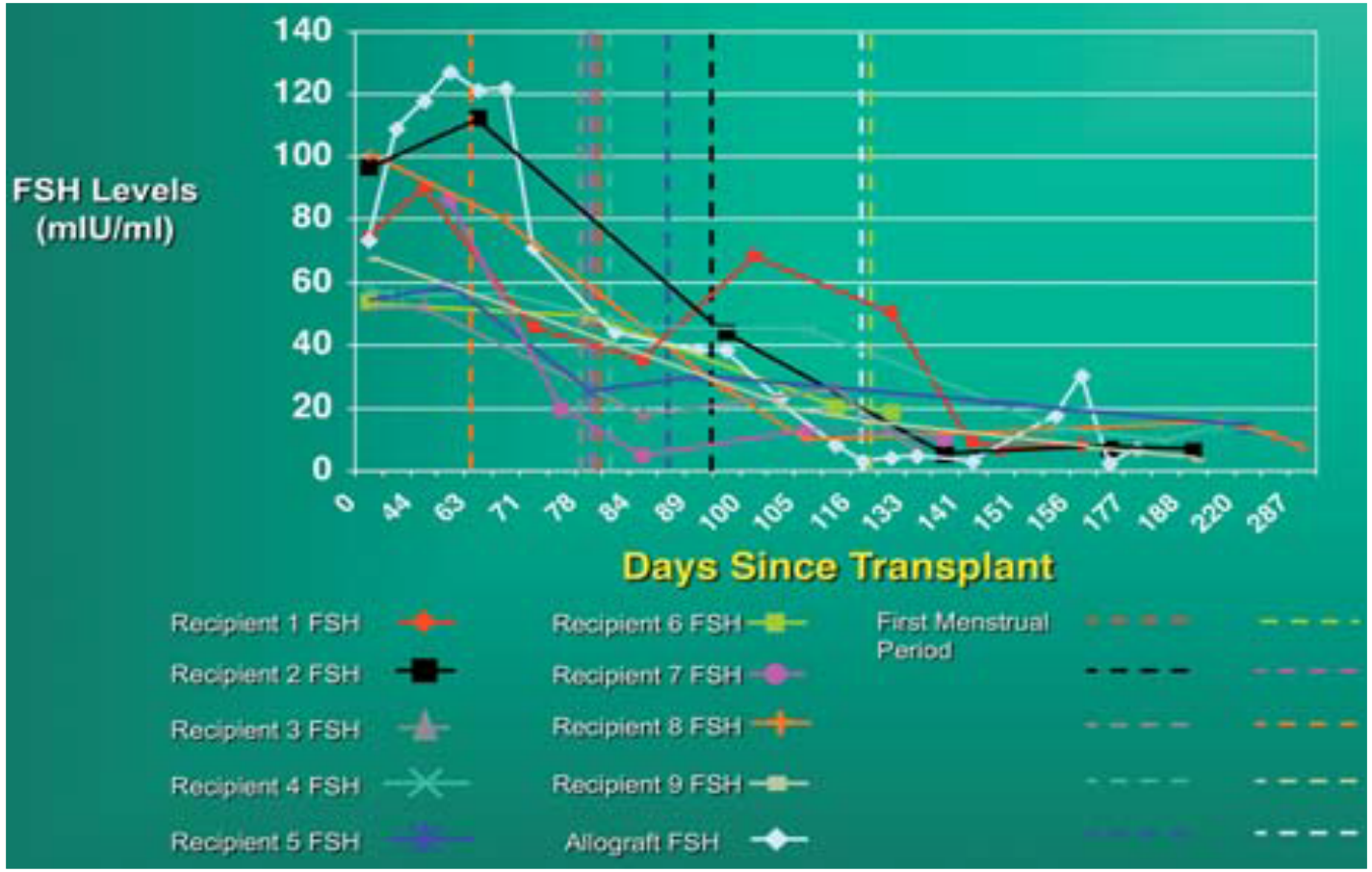
First reported clinical pregnancy following heterotopic grafting of cryopreserved ovarian tissue in a woman after a bilateral oophorectomy.

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.

Restoration of Hormonal Function



Silber. Ovarian cryopreservation and transplantation for fertility preservation. MHR 2012

Limitations to Ovarian Tissue Transplantation

- Revascularization may take up to 5 days
- Loss of 50 – 90% of follicles at transplantation due to ischemia and hypoxia
- Transplantation of tissue containing malignant cells
  - Triple-wash of follicles shown to remove leukemia cells
- Concern for complication - rate less than 1%

Baird et al. Arch Gynecol Obstet. 2017;2954:1033-1039
Shikanov et al. *Tissue Eng Part A*. 2011;1723–24:3095-3104
Corkum et al. Am J Surg. 2017;214:695-700
Beckman et al. Arch Gynecol Obstet. 2017;2954:1033-1039

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.

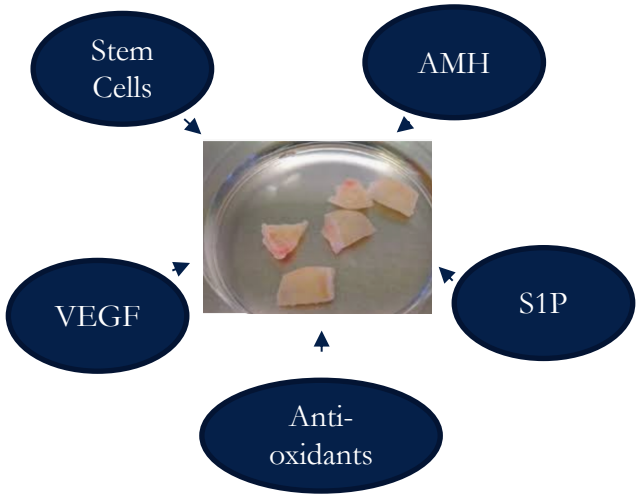
Prasath EB[1], Chan ML, Wong WH, Lim CJ, Tharmalingam MD, Hendricks M, Loh SF, Chia YN.

- 21 yo s/p interval bilateral oophorectomy for bilateral serous carcinoma
- All visible follicles aspirated after oophorectomy and OTC performed
- 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

Human Reproduction, Vol.29, No.2 pp. 276–278, 2014
Uzelac. Fertil Steril 2015;104:1258–60. 2015

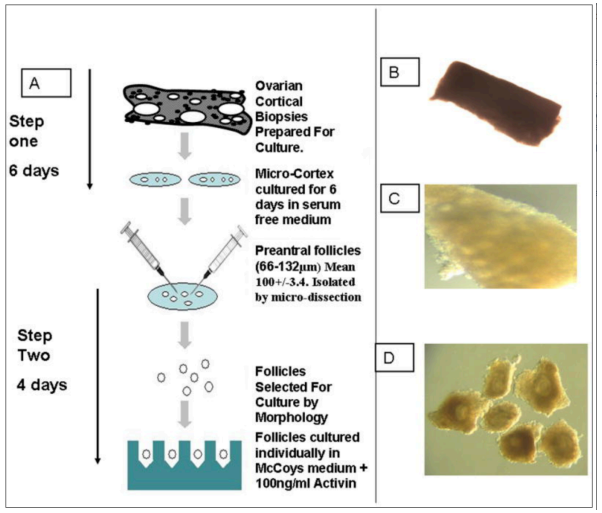
Methodologies to Optimize the Potential of Cryopreserved Tissue

Additives



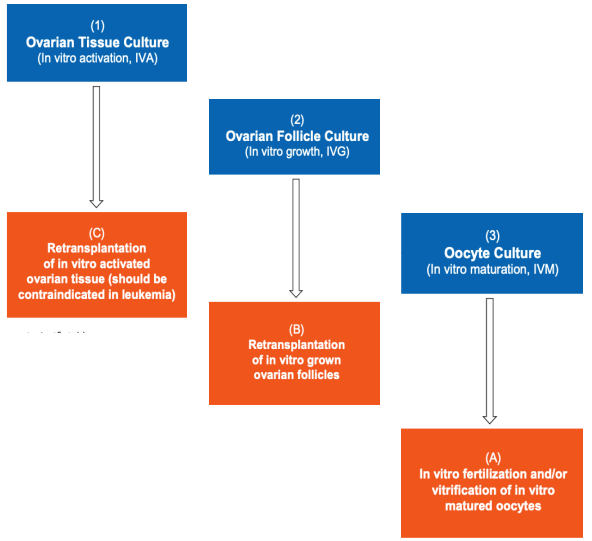
Takae and Suzuki. Reprod Med Biol. 2019 Apr 8;18(3):217-224

In Vitro Growth of Primordial Follicles



Telfer and Zelinski. Fertil Steril. 2013 May;99(6):1523-33

Artificial Ovary



Salama and Woodruff. Acta Obstet Gynecol Scand. 2019;98:659–664

Take Home Points: OTC

- 130 births worldwide to date with a 29-41% birth rate
- Only fertility preservation option for pre-pubertal females
- Option for malignant and non-malignant conditions with fertility risk
- Transplantation may be considered after non-sterilizing chemotherapy

Take Home Points: OTC

- Restoration of hormonal function is a benefit of transplantation
- IVM may obviate need for transplantation in patients at high risk of re-seeding
- Regional fertility centers and embryology labs improve access to care for ovarian tissue cryopreservation

Thank you