# Current and New Approaches for Fertility Preservation in Challenging Patients

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# How to make a baby

(what are the building blocks?)

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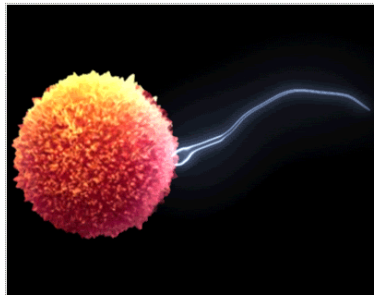

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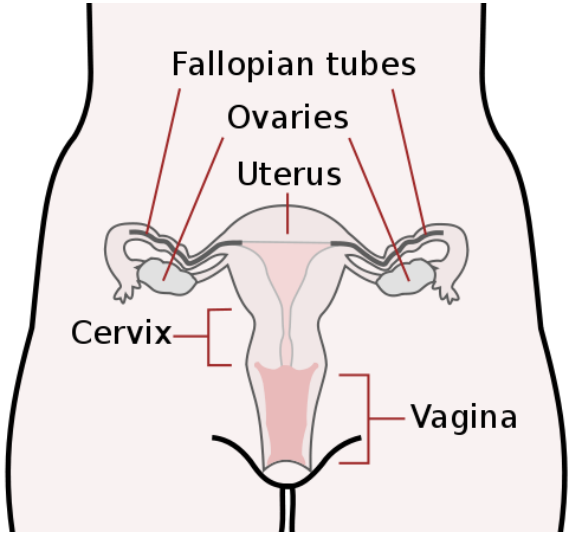
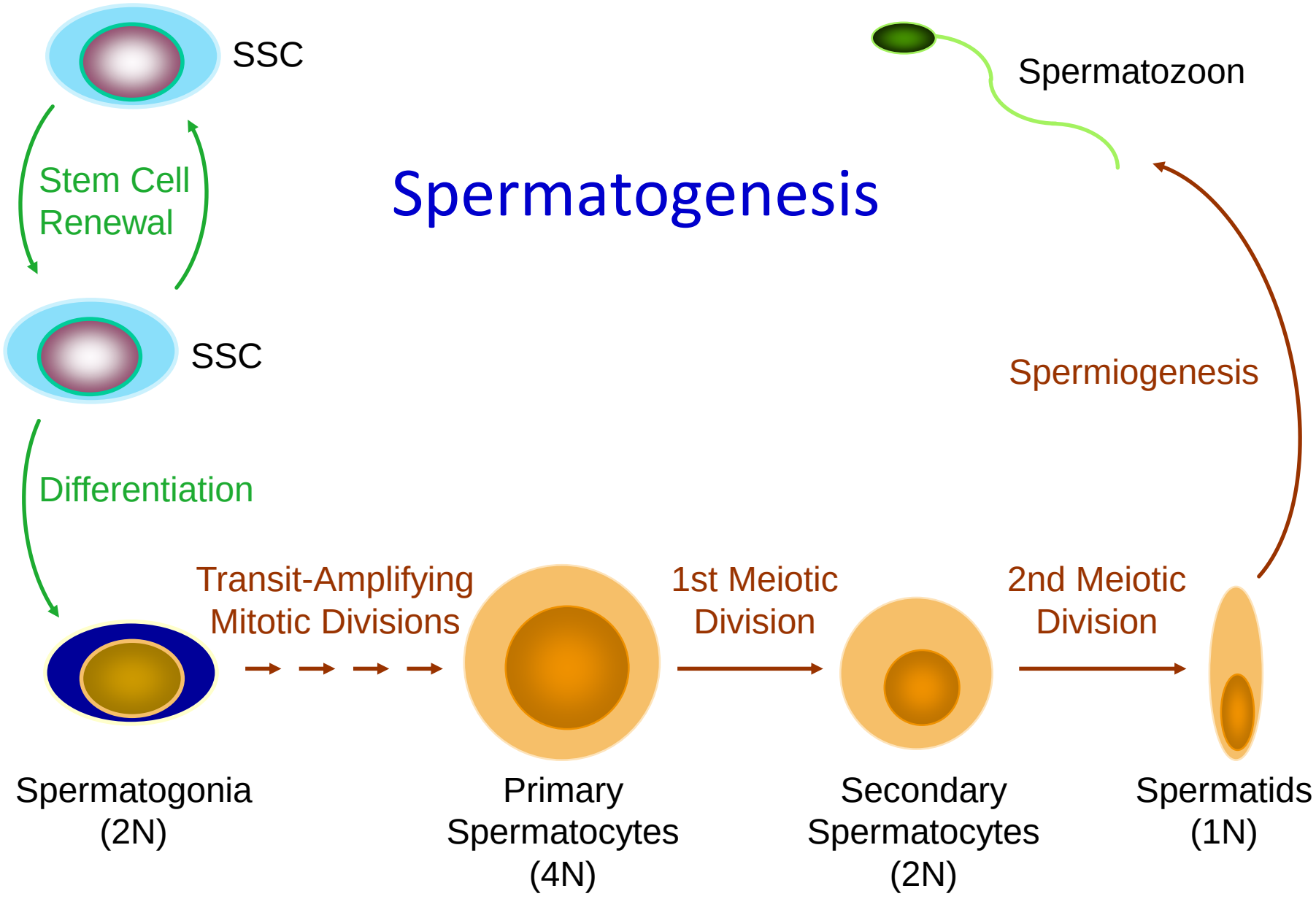
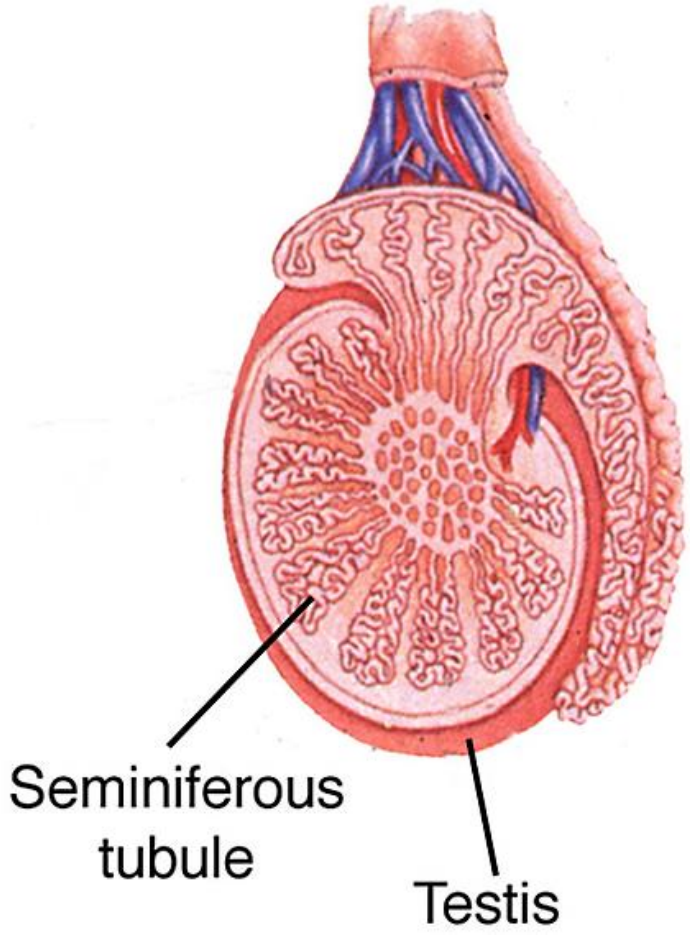
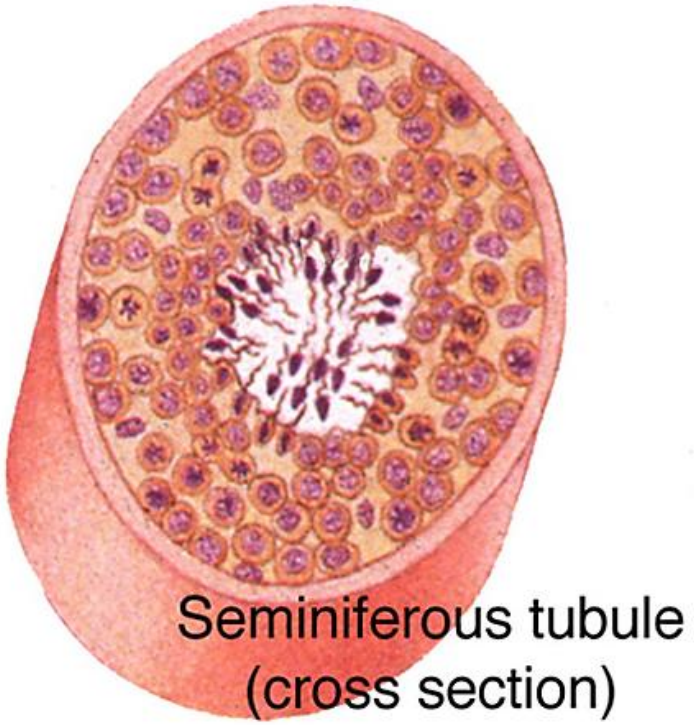





# Spermatogenesis

# Anatomy of the Testis



# Normal Semen Parameters (WHO)

- Volume: ~1.5 milliliters semen per ejaculate
- Sperm count: 15 million sperm per milliliter of ejaculate
- Total sperm per ejaculate: ~40 million

Cooper et al., Human Reproduction Update, 2010, 16:231-245

# Male Infertility

- Infertility affects 10-15% of couples in the US
- A male factor is the cause in 30-40% of cases
- A female factor is the cause in 30-40% of cases
- Half of infertility is idiopathic in nature and there are no treatments for 75% of cases

# Fertility after Cancer

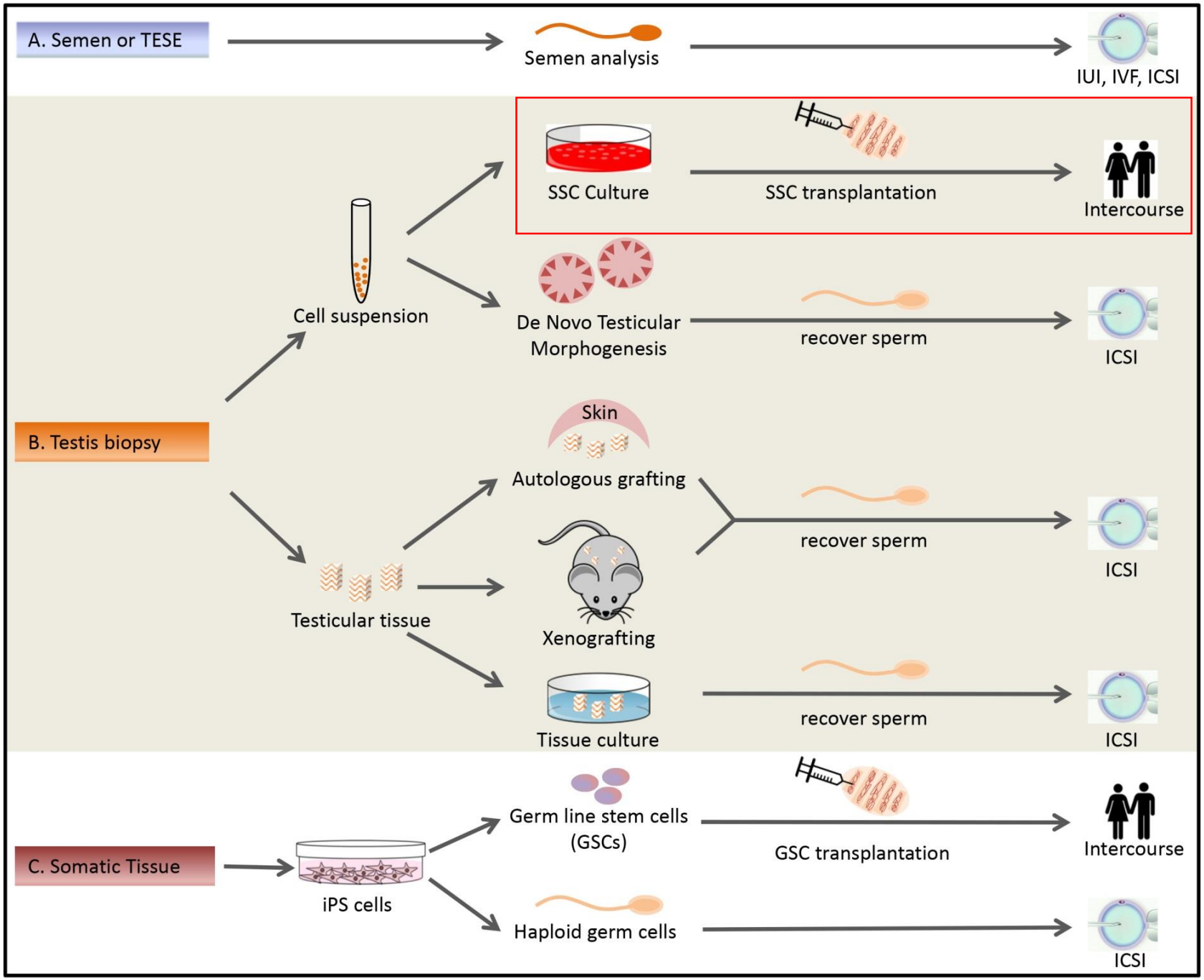
- Chemotherapy and radiation treatments for cancer or other conditions can cause permanent infertility
- Fertility status affects cancer survivor quality of life
- Adult women and men can cryopreserve eggs, sperm or embryos, which can be used in the future to achieve pregnancy
- These options are not available to preadolescent boys and girls who are not producing mature eggs or sperm
- The five year survival rate for childhood cancer patients is 85% (SEER)
- Prepubertal boys have spermatogonial stem cells in their testes that are poised to initiate sperm production at puberty
- Several academic centers in the US and abroad are preserving testicular tissue for boys in anticipation that SSCs can be used in the future to restore fertility

# Why Does it Matter?

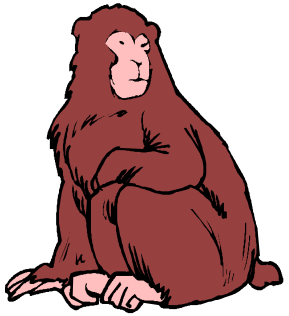
- The summed incidence of chemotherapy or radiation-induced male infertility that cannot be treated with existing reproductive technologies each year in the United States is over 4000.
  - ✓1813 adult male cancer survivors who did not freeze semen
  - ✓1874 childhood cancer survivors (boys) receiving high risk treatments
  - ✓500 children receiving HSC transplants for non-malignant conditions
- Testicular tissues have already been frozen for over 1000 patients (mostly children) worldwide.

# Standard and Experimental Options to Preserve and Restore Male Fertility

Gassei and Orwig *Fertil Steril* 2016



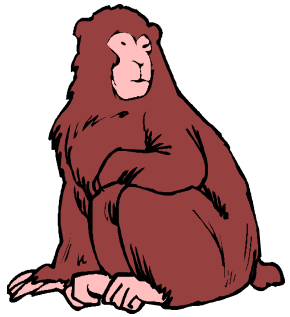
# Monkey model of cancer survivorship



Biopsy


Cryopreservation

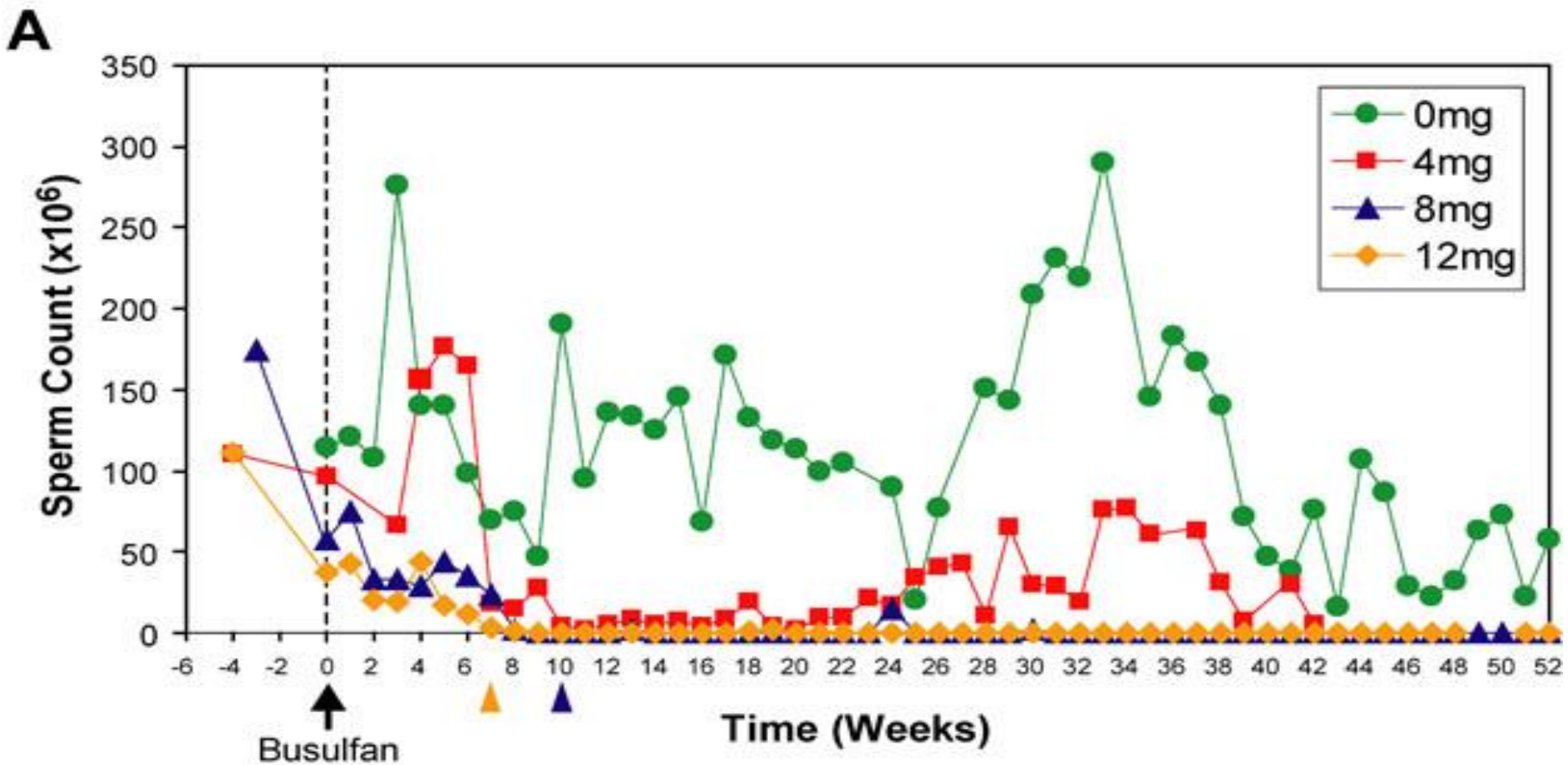
Chemotherapy



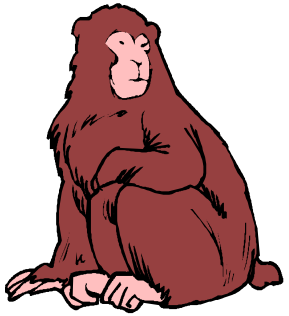
Semen analysis

Hermann et al., *Stem Cells*, 2007

# Spermatogenic deficits



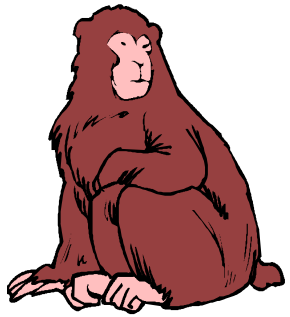
# Autologous SSC Transplantation



Biopsy


Cryogenic Storage

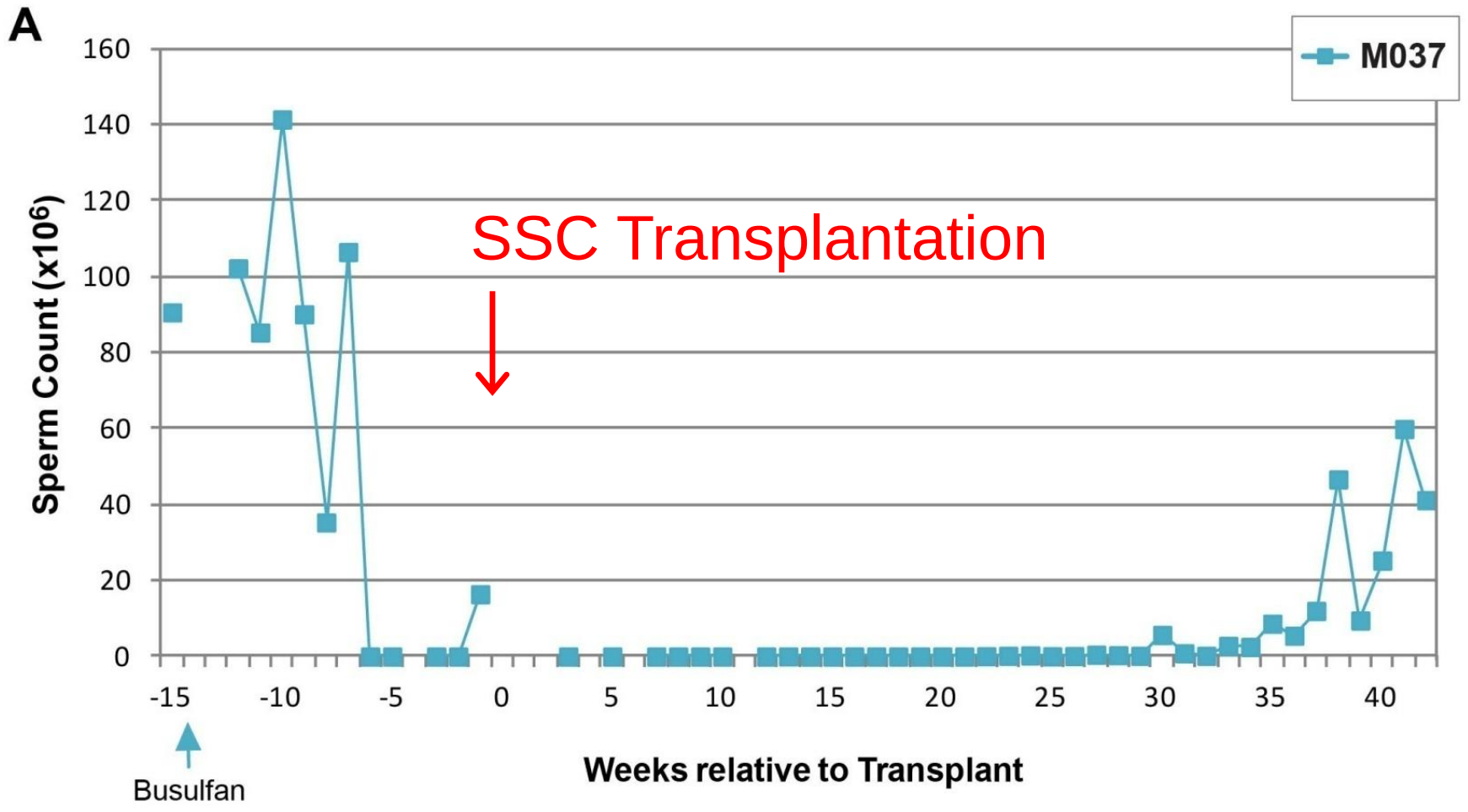
Chemotherapy



Autologous Transplant

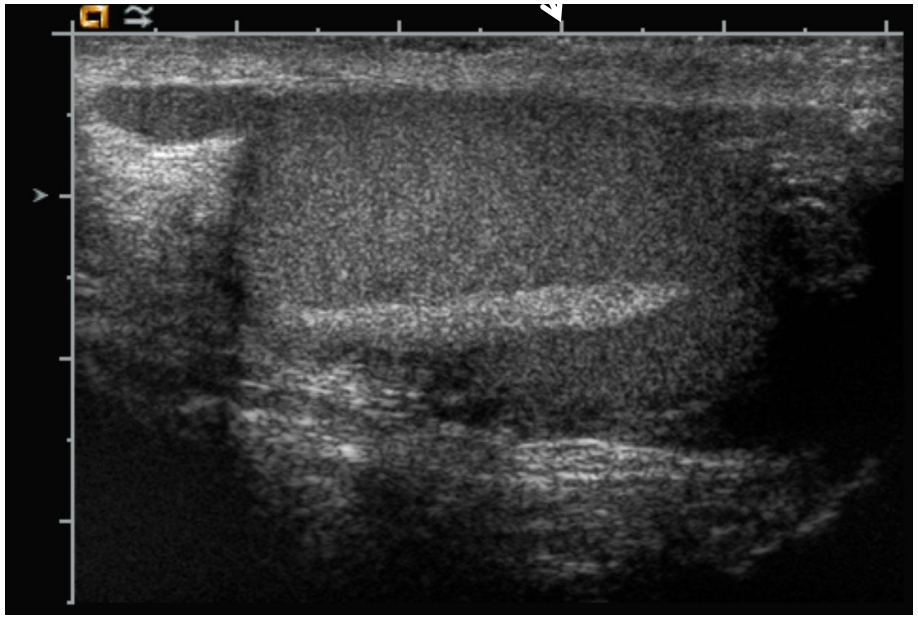
# Ultrasound-guided rete testis injection

# Regeneration of Spermatogenesis

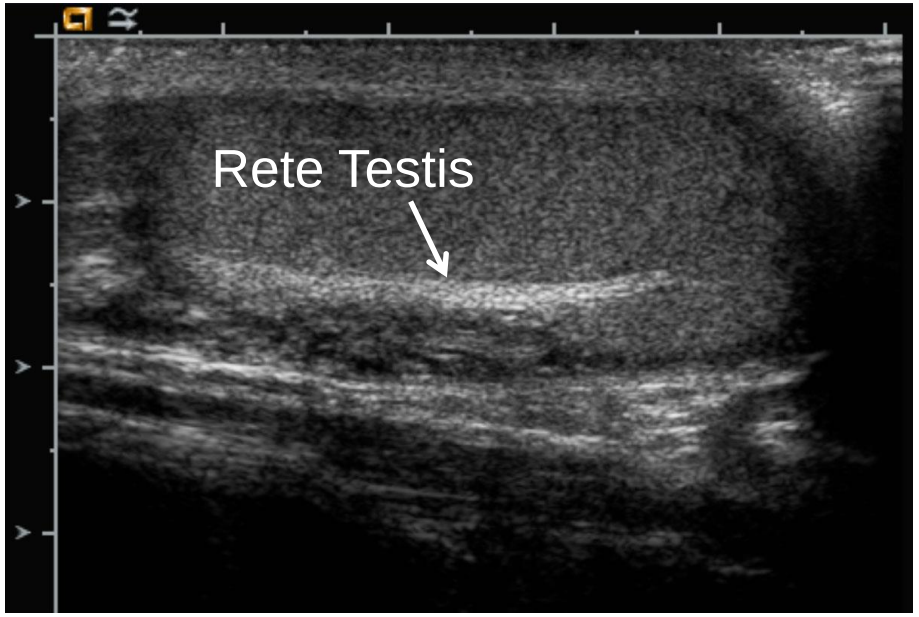



Hermann et al., *Cell Stem Cell*, 2012

# Spermatogonial stem cell transplantation is technically feasible in humans.



16 yr old boy



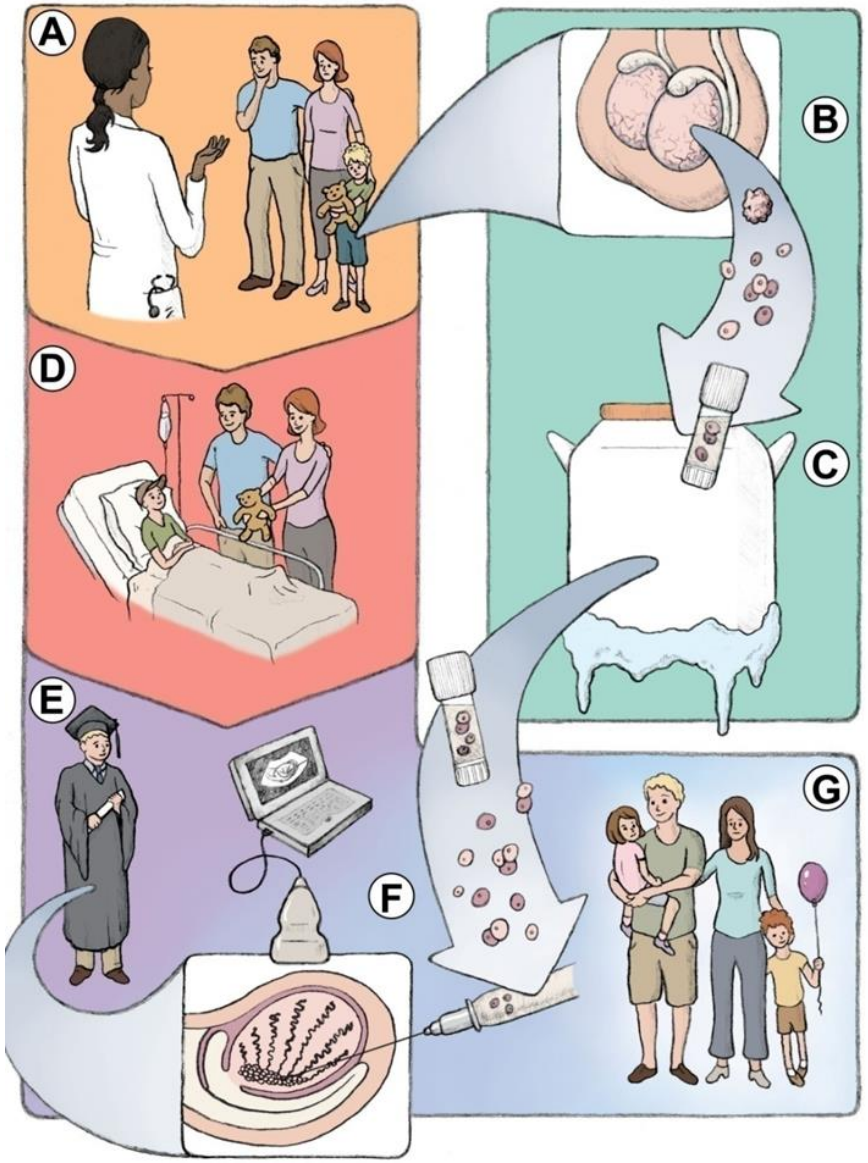

17 yr old boy

Should we be cryopreserving testicular tissue for prepubertal patients now because we anticipate that new stem cell therapies will be available in the future?

Ultrasound images courtesy of Richard Yu, Children's Hospital of Boston

# Fertility Preservation Program in Pittsburgh

(www.fertilitypreservationpittsburgh.org)




## Cryopreserved since 2011

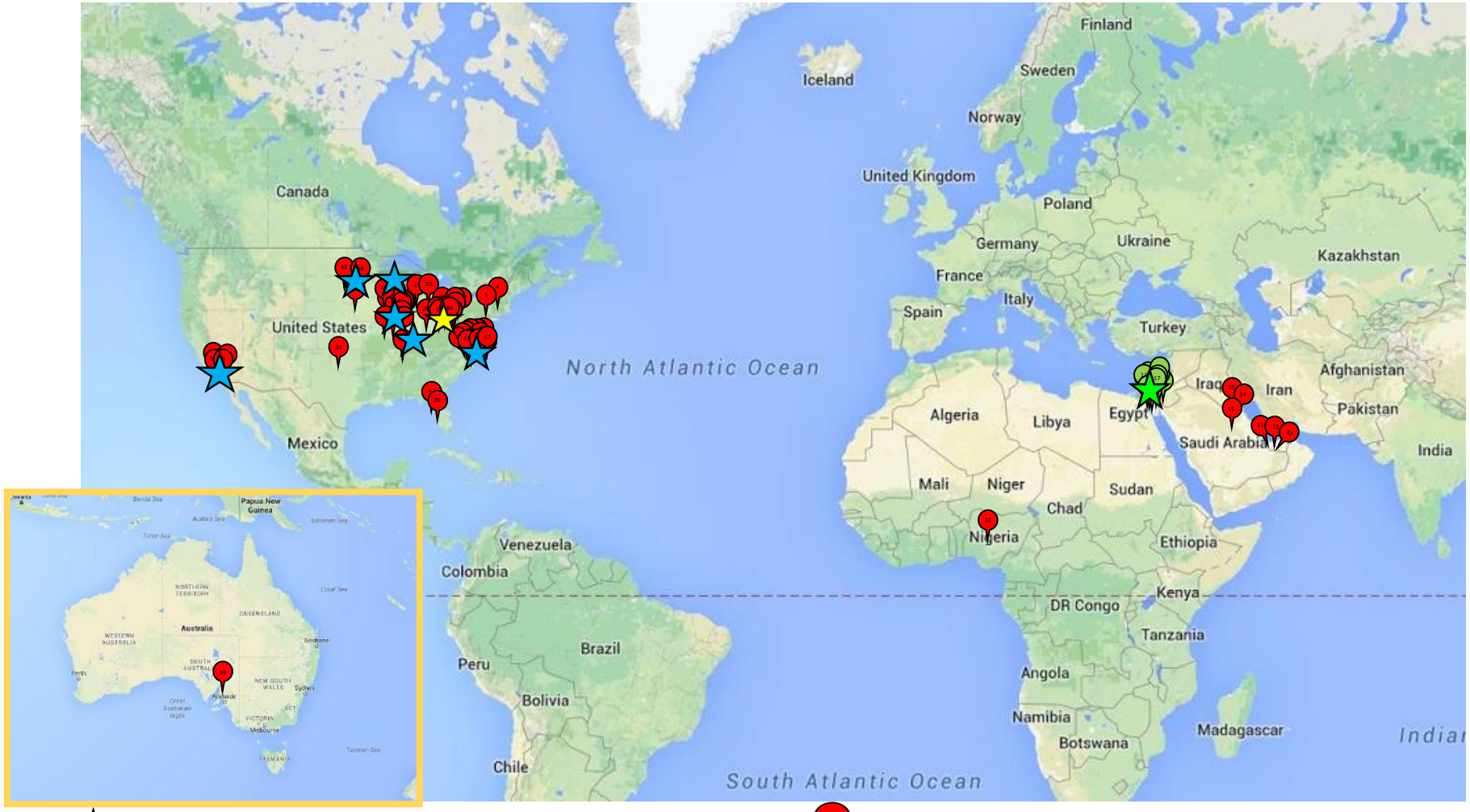
- Testicular tissue: 85 boys
- Ovarian tissue: 19 girls/women
- Approved to recruit patients at satellites sites nationwide

## Our Mission

- Educate Patients and Physicians
- Provide fertility preservation options
- Pioneer new technologies and translate them to the clinic
- Train the next generation of FP experts

# Our National/International Impact

## Testicular Tissue Cryopreservation in the US and Abroad




Pittsburgh Coordinating Center (32 patients)

Patients (85 total)

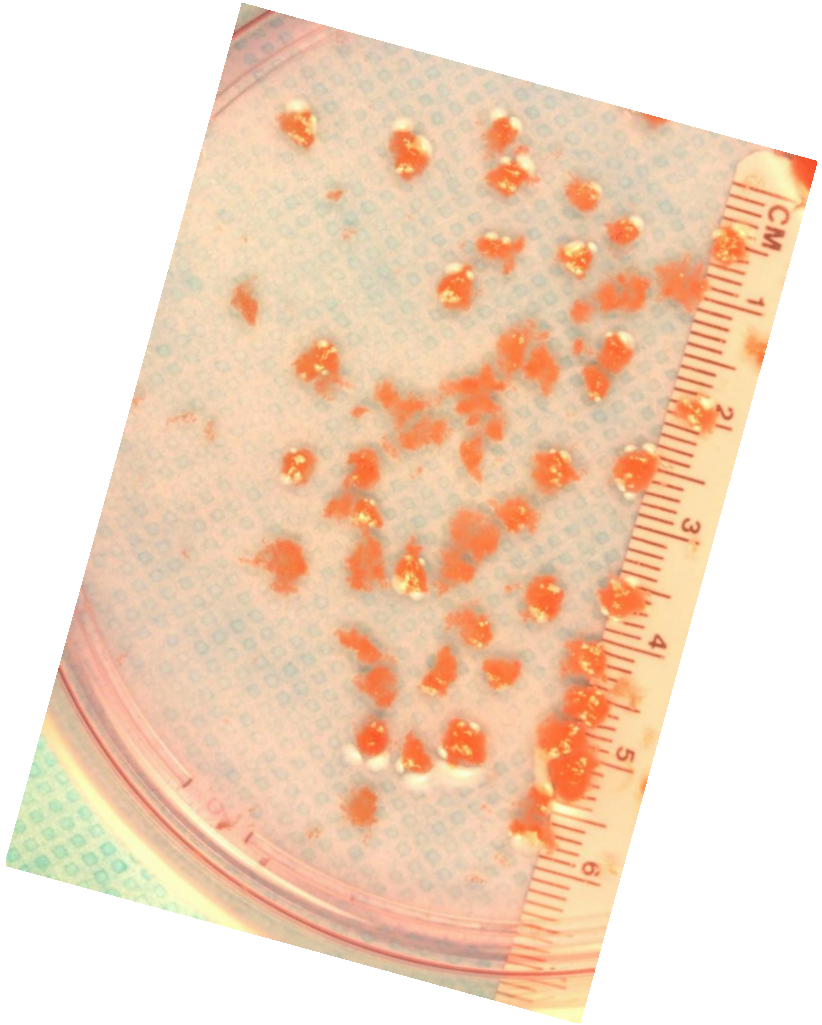
Coordinated Centers: Washington DC (11), Chicago (15), LA (2), Cincinnati (1), Mayo (7), Milwaukee (0)

Collaborators: Ben-Gurion University of the Negev, Be'er Sheva, Israel (17 patients)

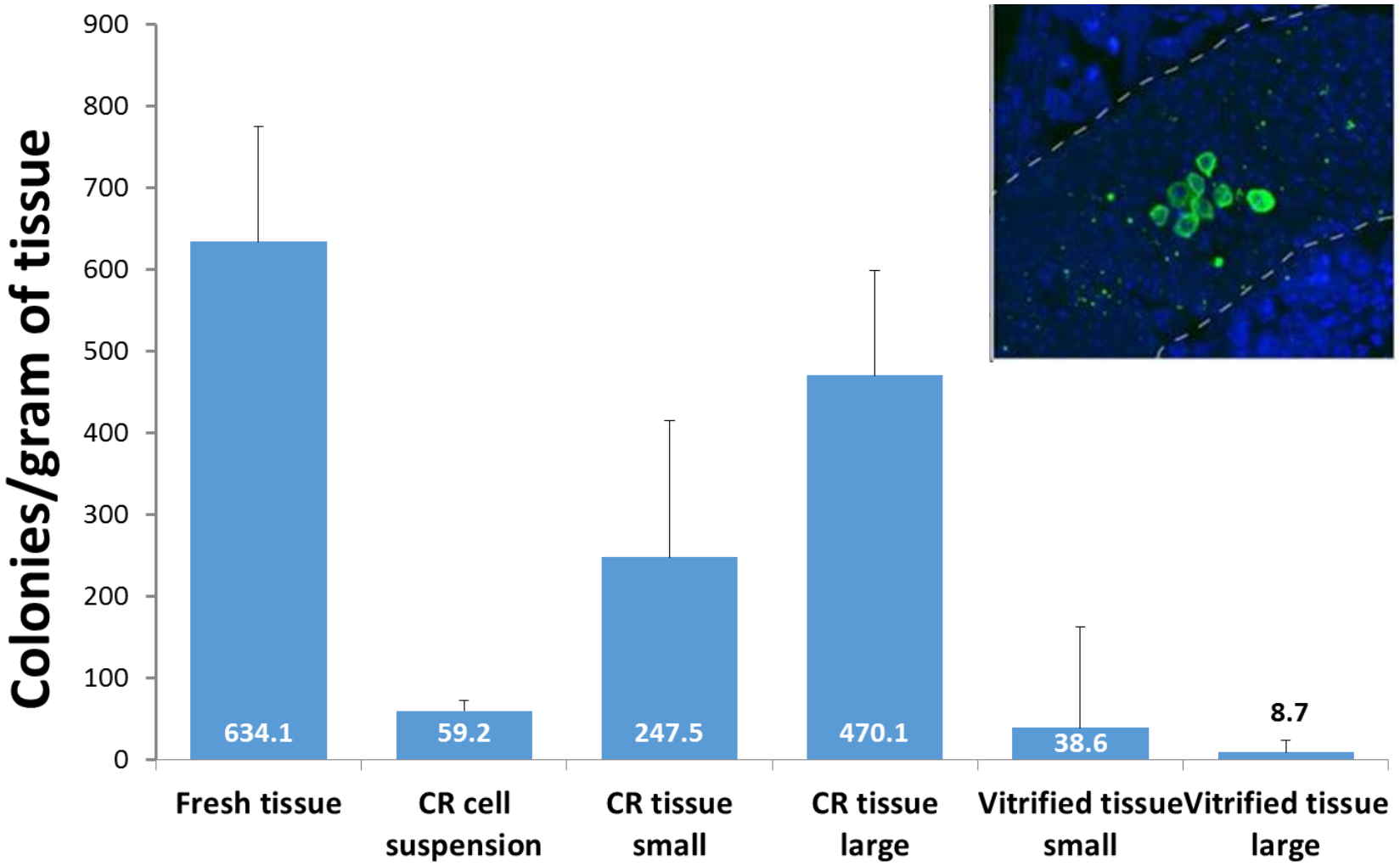
# Challenges to SSC transplantation for cancer survivors

- Majority of patients are not informed about fertility risks and options for preserving fertility
  - ✓ Multidisciplinary discussions
- Small biopsies from prepubertal patients may contain few stem cells
  - ✓ Culture
- Risk of reintroducing cancer into a survivor
- Optimize cryopreservation
  - ✓ Cell suspension versus tissue pieces; slow freeze versus vitrification

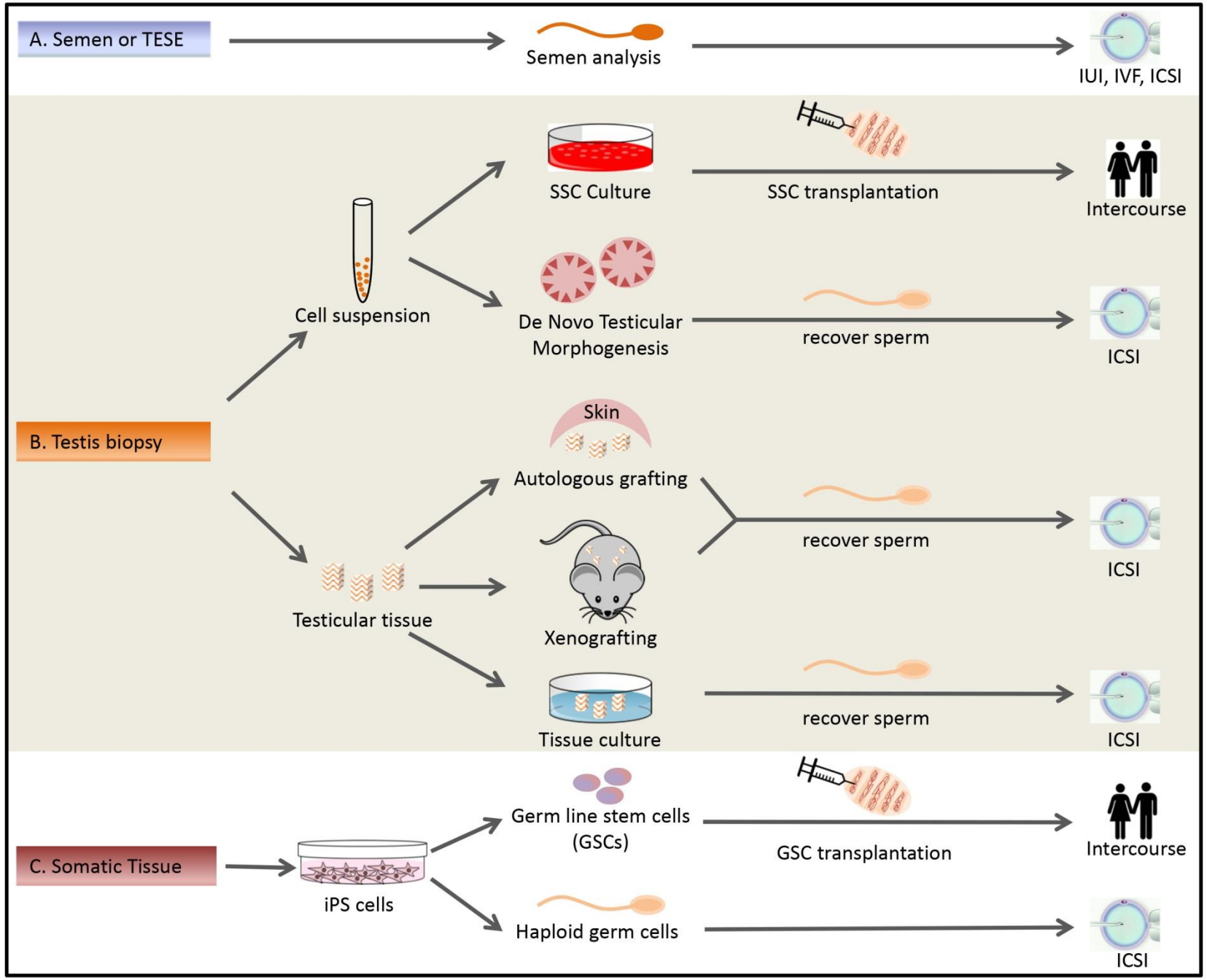
# Cells or Tissue?



# Recovery of colonizing spermatogonia from frozen/thawed tissue vs. cells




Valli et al., in Preparation




Gassei and Orwig *Fertil Steril* 2016

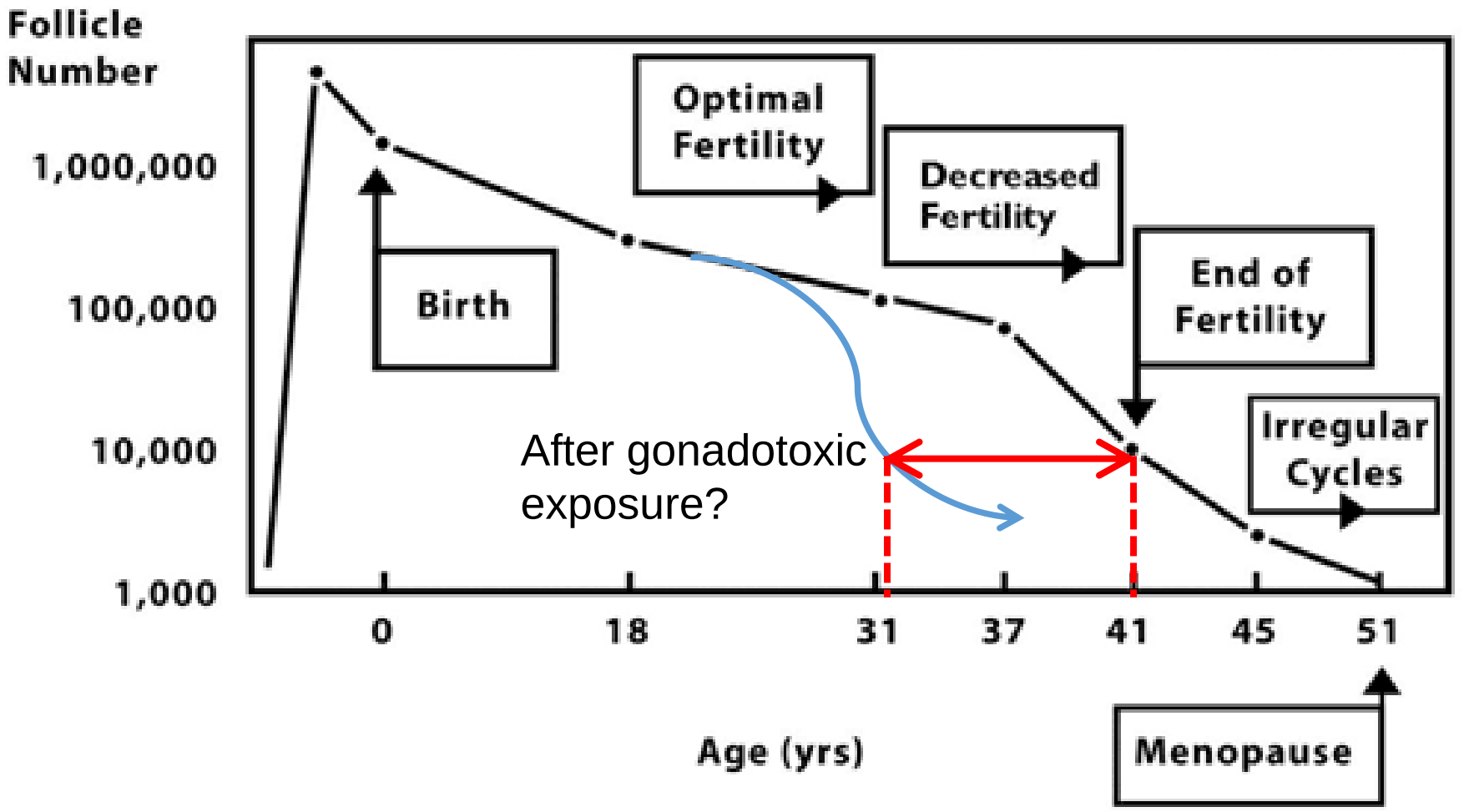
# Cryopreservation Method

- Spermatogonial stem cells can be frozen using the same conditions used for somatic cell lines (Brinster RL, Science, 2002)
  - ✓Permeating cryoprotectant (e.g., DMSO, EG) and non-permeating substance (Human serum albumin, sucrose)
  - ✓Slow programmed freezing followed by LN2 (-196°C)
  - ✓Rodent SSCs remain functional for at least 14 years after freezing (Wu et al., Human Reprod, 2012)
- Testicular tissue
  - ✓Slow programmed freezing followed by LN2 (-196°C) (Keros et al., 2005 & 2007; Wyns et al., 2007; Ginsberg et al., 2010; Sadri-Ardekani et al., 2009 & 2011)
  - ✓Vitrification (Jahnukainen et al., 2007; Zeng et al., 2009; Baert et al., 2013)
  - ✓No consensus on best freezing method for testicular tissue

Women (girls) are more complicated than men (boys)

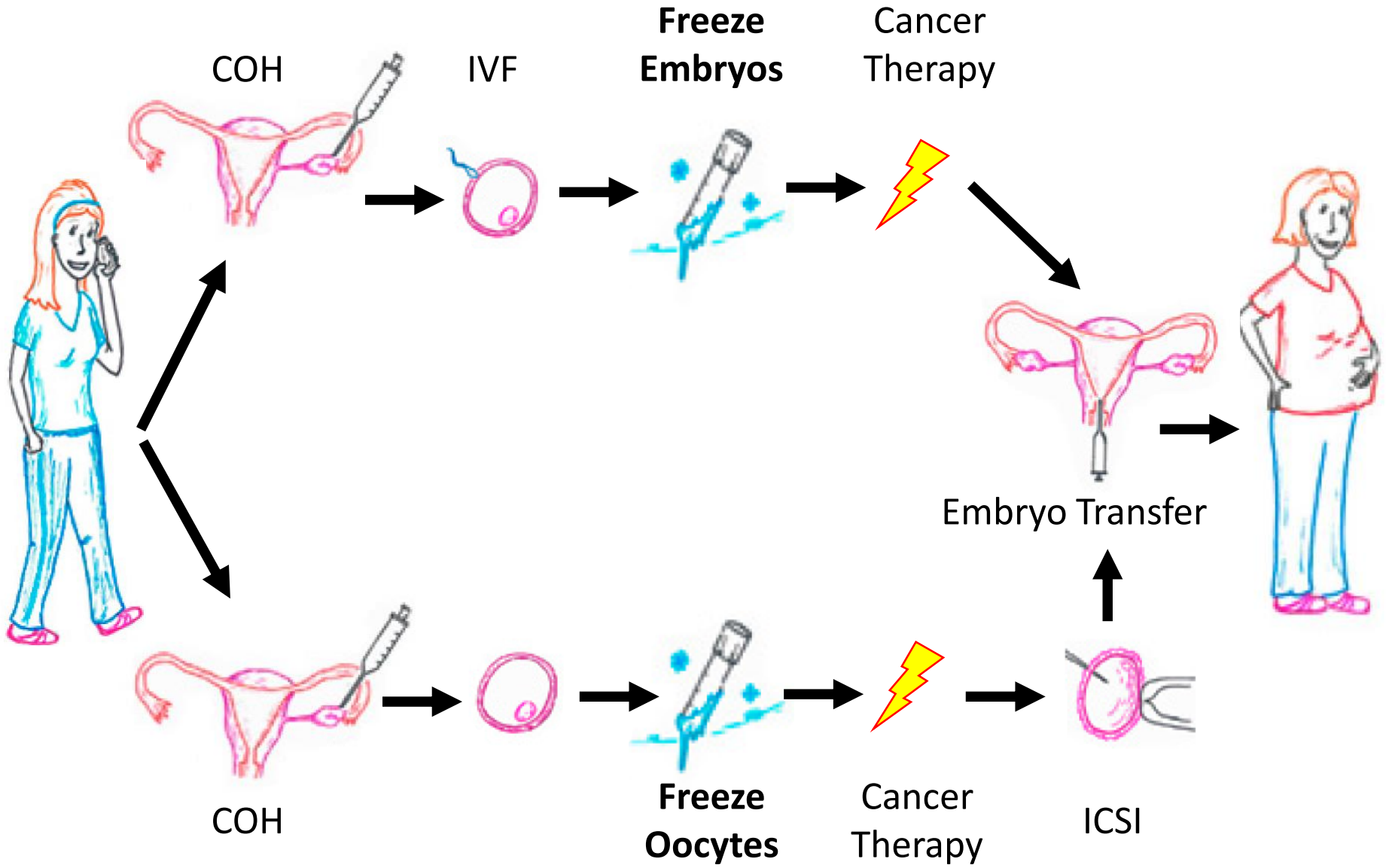
Men (boys) are simpler than women (girls)

# Risk of Premature Ovarian Insufficiency

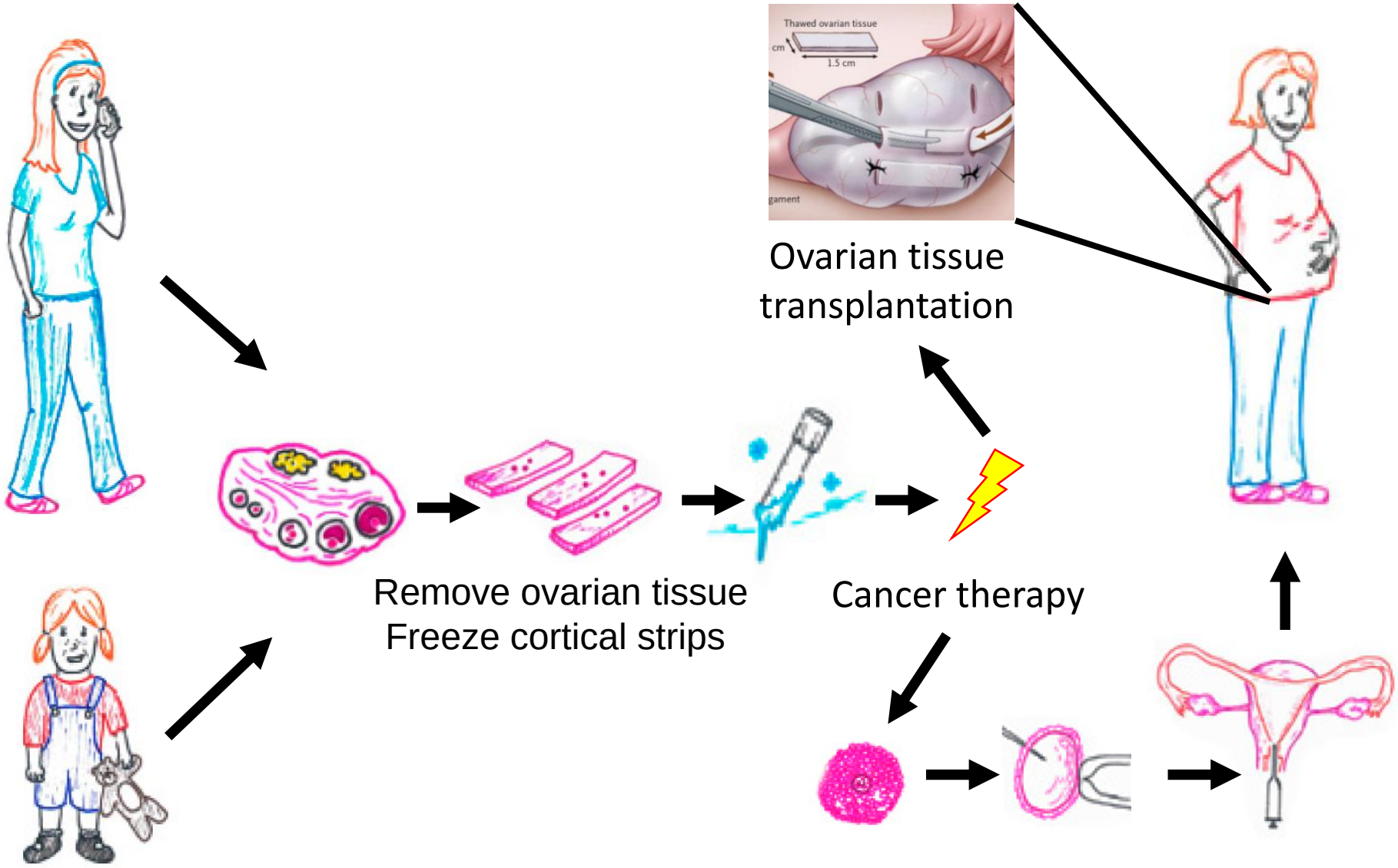


The key for women and girls is to preserve eggs in the ovary because we may not be able to Regenerate eggs after the toxic insult

# Fertility Preservation Options for Women



# Ovarian Tissue Cryopreservation



# Miracle Second Baby: Ovarian Tissue Transplant

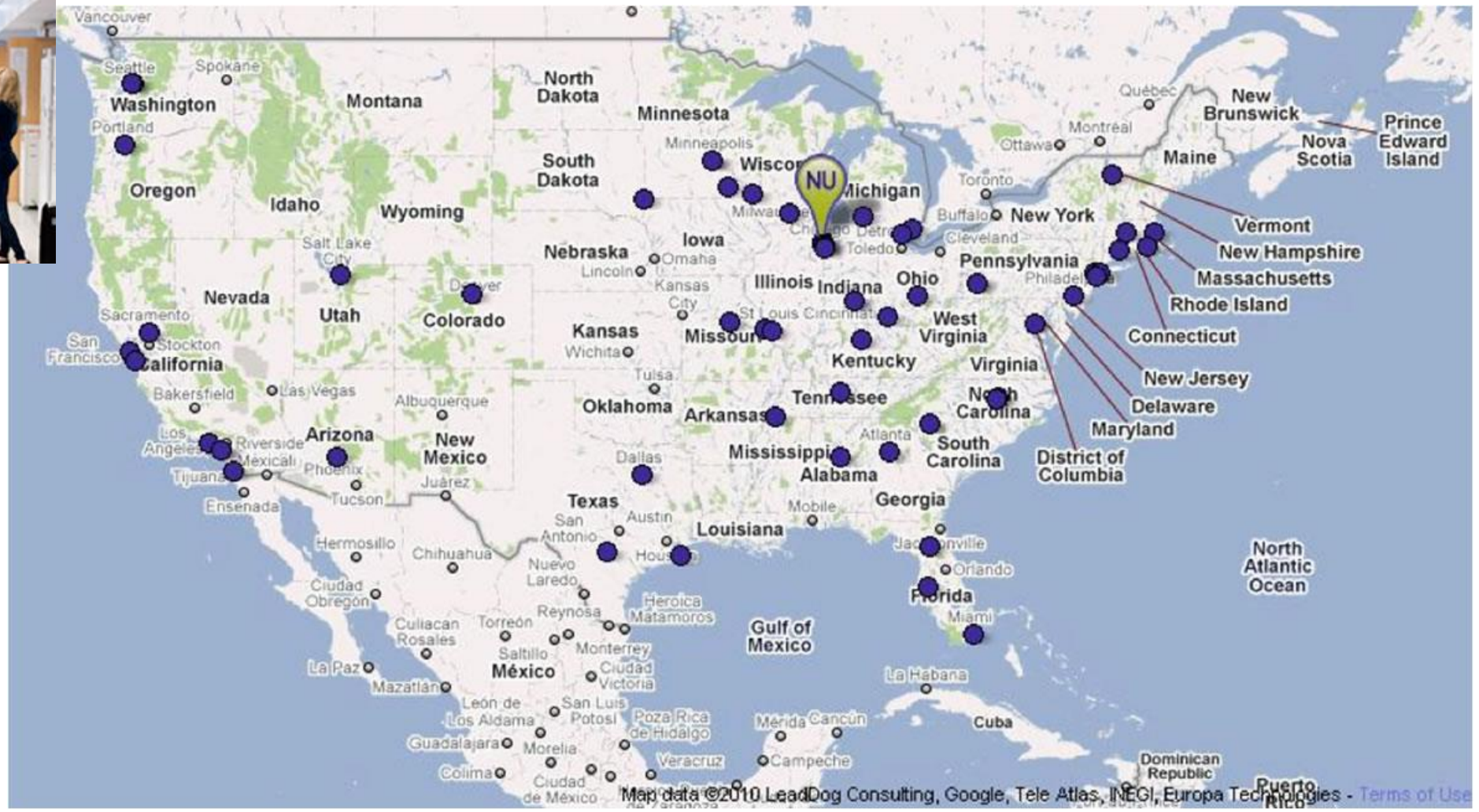
- Mrs. Stinne Bergholdt, Denmark: Early menopause at age 27 after successful treatment for Ewing's Sarcoma
- Two children following ovarian tissue transplantation
- Over 100 live births from ovarian tissue transplant
- Israel is transitioning ovarian tissue freezing to standard of care

Risk of reintroducing cancer!

Ernst et al., *Hum Reprod* 2010

# National Physicians Cooperative

## Oncofertility Consortium



# Cryopreservation Method

- Slow Freezing (Hovatta O, Reprod Biomed Online, 2005)
  - ✓Permeating cryoprotectant (e.g., DMSO, EG) and non-permeating substance (Human serum albumin, sucrose)
  - ✓Slow programmed freezing followed by LN2 (-196°C)
  - ✓Tissue remains viable after freezing for at least 6 years (Donnez et al., Lancet, 2004)
- Vitrification (Gandolfi et al., Fertil Steril, 2006)
  - ✓Suspend tissue in high concentration cryopotectant for short time
  - ✓Plunge in LN2 (-196°C)
- Evidence may favor vitrification because it preserves ovarian stroma as well as follicles (Gandolfi et al., 2006; Isachenko et al., 2009; Keros et al., 2009; Silber SJ, 2012)

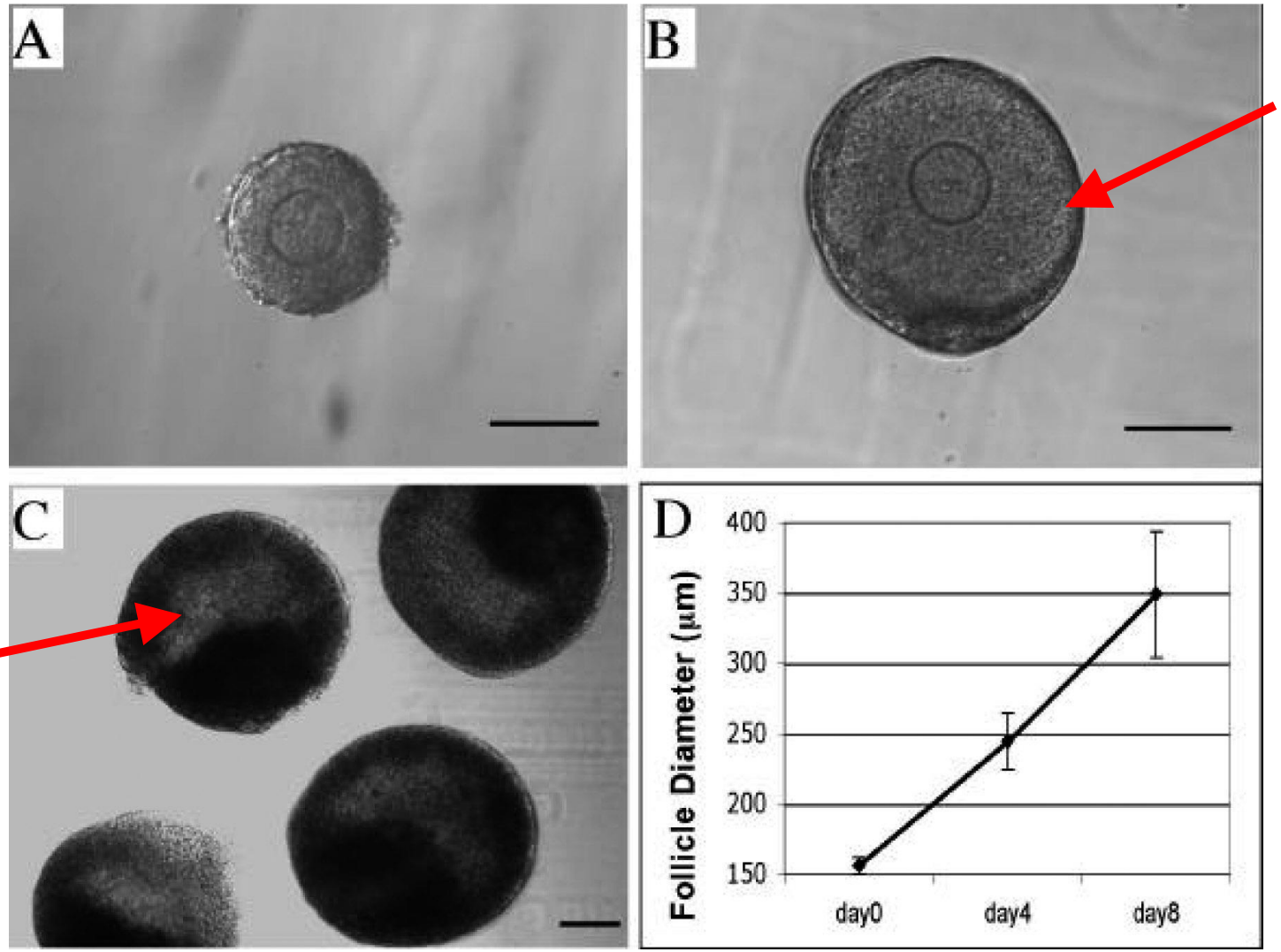
# Pros and Cons:
# Ovarian Tissue Cryopreservation/Transplantation

- Pros:
  - ✓Time
  - ✓Only option for girls
- Cons:
  - ✓Experimental
  - ✓Limited clinical experience or historical data on pregnancy outcomes
  - ✓Risk of reintroducing cancer into a survivor

# Alternatives to Ovarian Tissue Transplantation

- Less gonadotoxic treatment regimens
  - ✓ May reduce acute infertility, but risk of premature ovarian insufficiency (POI) should still be considered
  - ✓ *In vivo* assessments of gonadotoxicity required for new treatment regimens
- Ovarian protection
  - ✓ Lupron, G-CSF, Imatinib, everolimus, fingolimod, bevacizumab
- *In vitro* maturation
  - ✓ 1-step from secondary follicles
  - ✓ 2-step from primordial follicles

# In vitro Follicle development in Alginate matrix



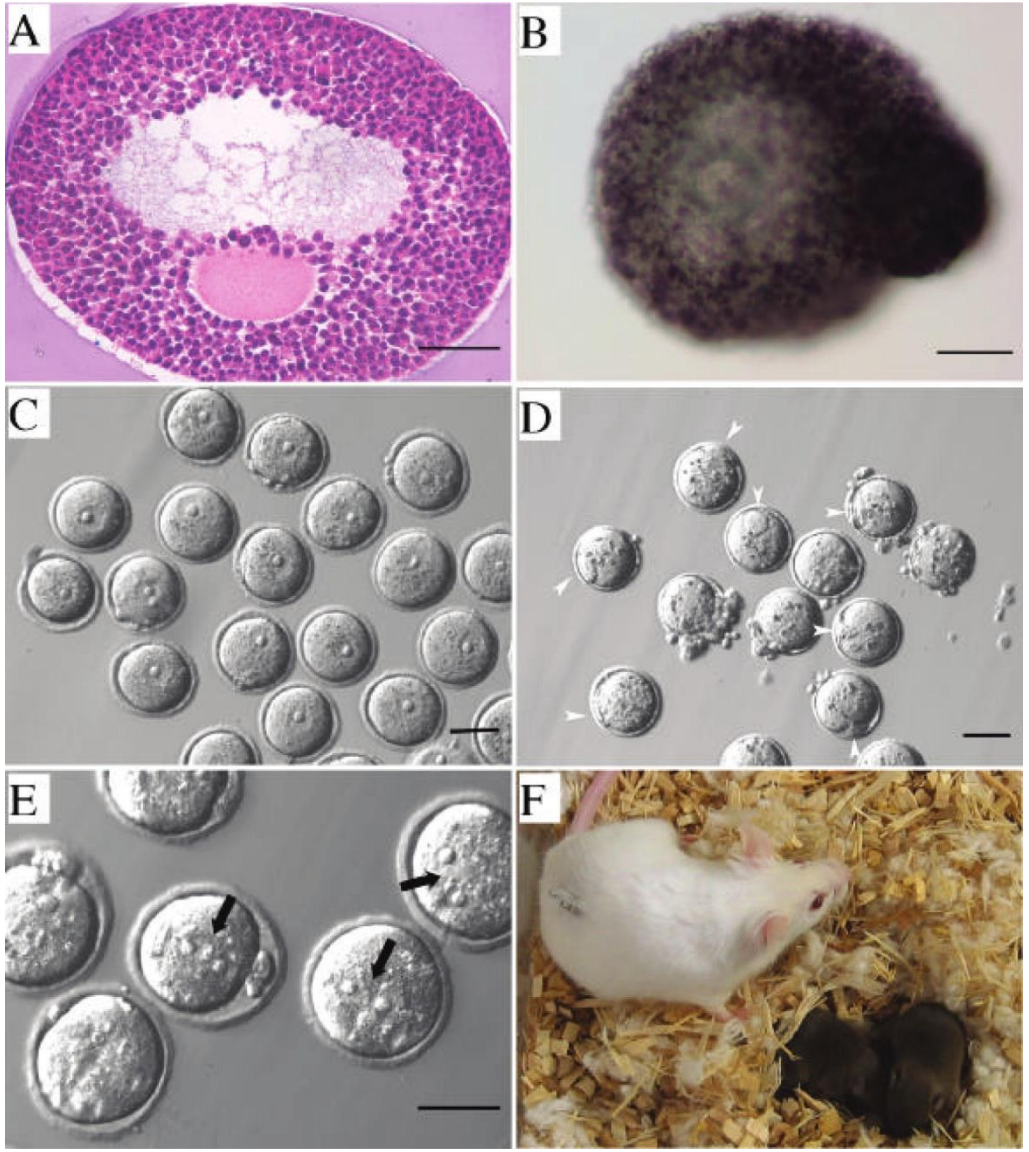

Granulosa cells clearly proliferate by 4 days in culture

Follicles developed antrums by 8 days in culture

Xu et al. (Woodruff), *Tiss Eng* 2006

# Babies!

Maintained meiotic arrest




Resumed meiosis with hCG – Polar bodies

Fertilization 2 pronuclei

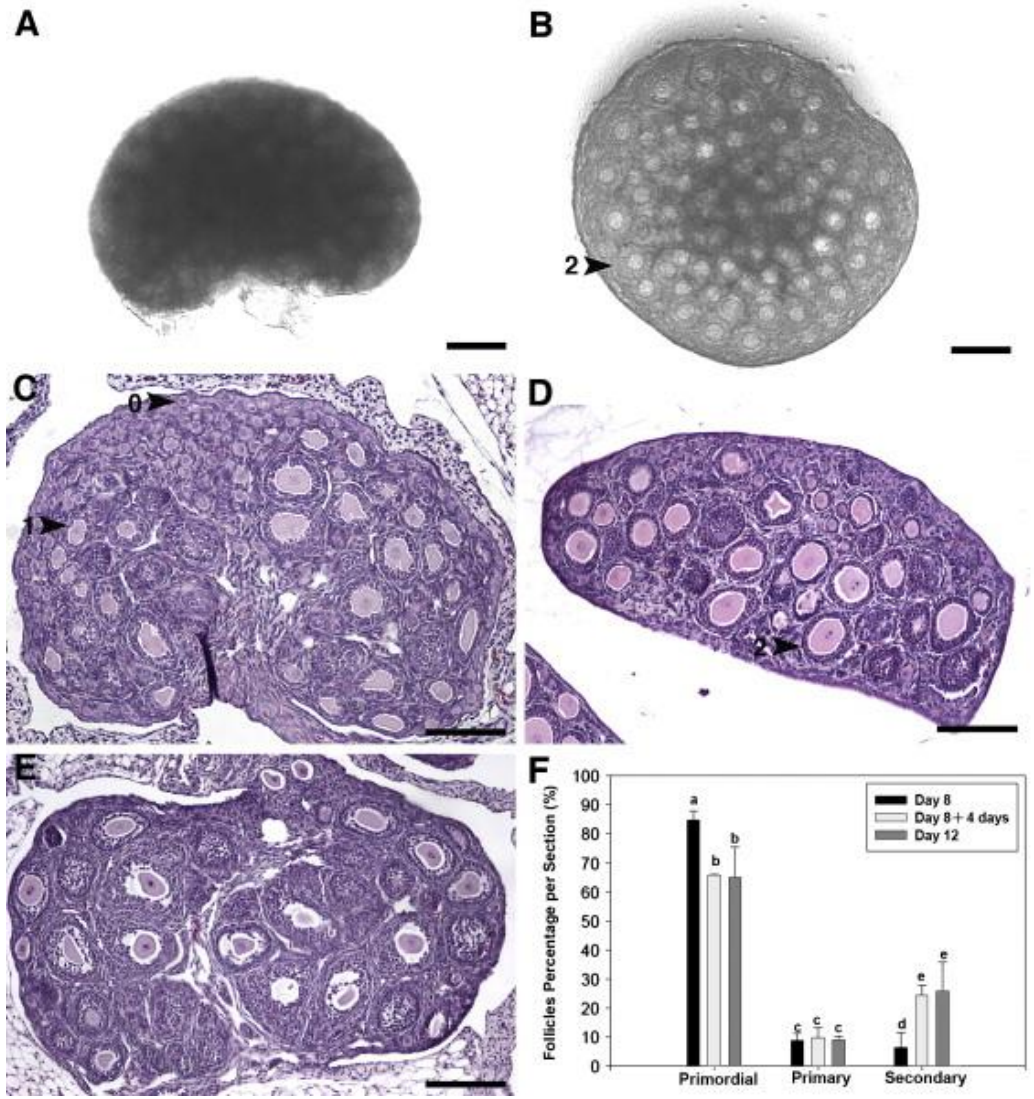
Xu et al. (Woodruff), *Tiss Eng* 2006

# Two-step follicle culture

8 day old mouse ovary

8 day old mouse ovary

12 day old mouse ovary



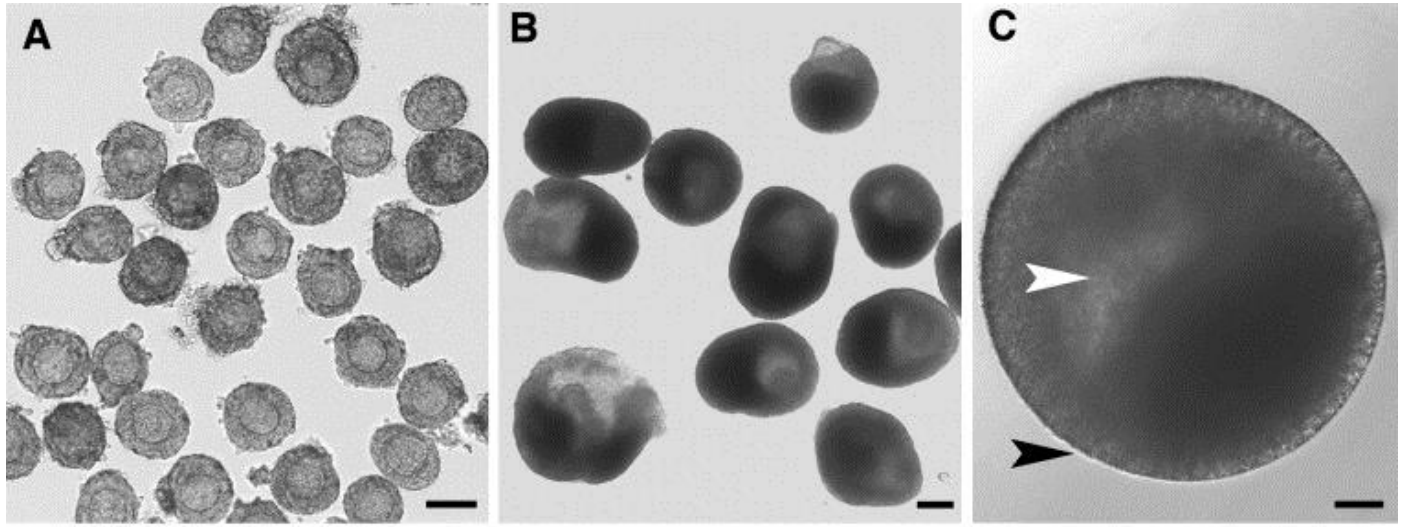

8 day old mouse ovary + 4 days in culture

8 day old mouse ovary + 4 days in culture

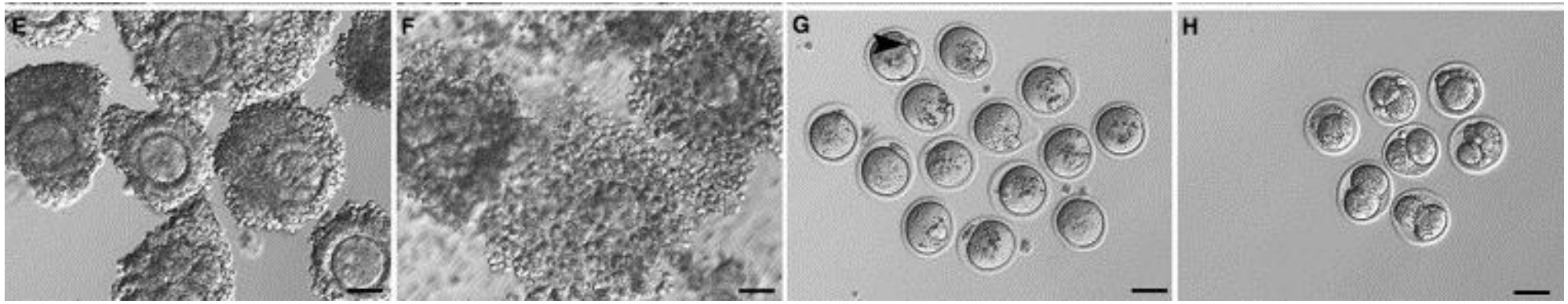
Jin et al. (Woodruff), *Fertil Steril* 2010

# Two Step Follicle Culture Produces Fertilization-Competent Eggs

Isolated secondary follicles from 4 day organ culture

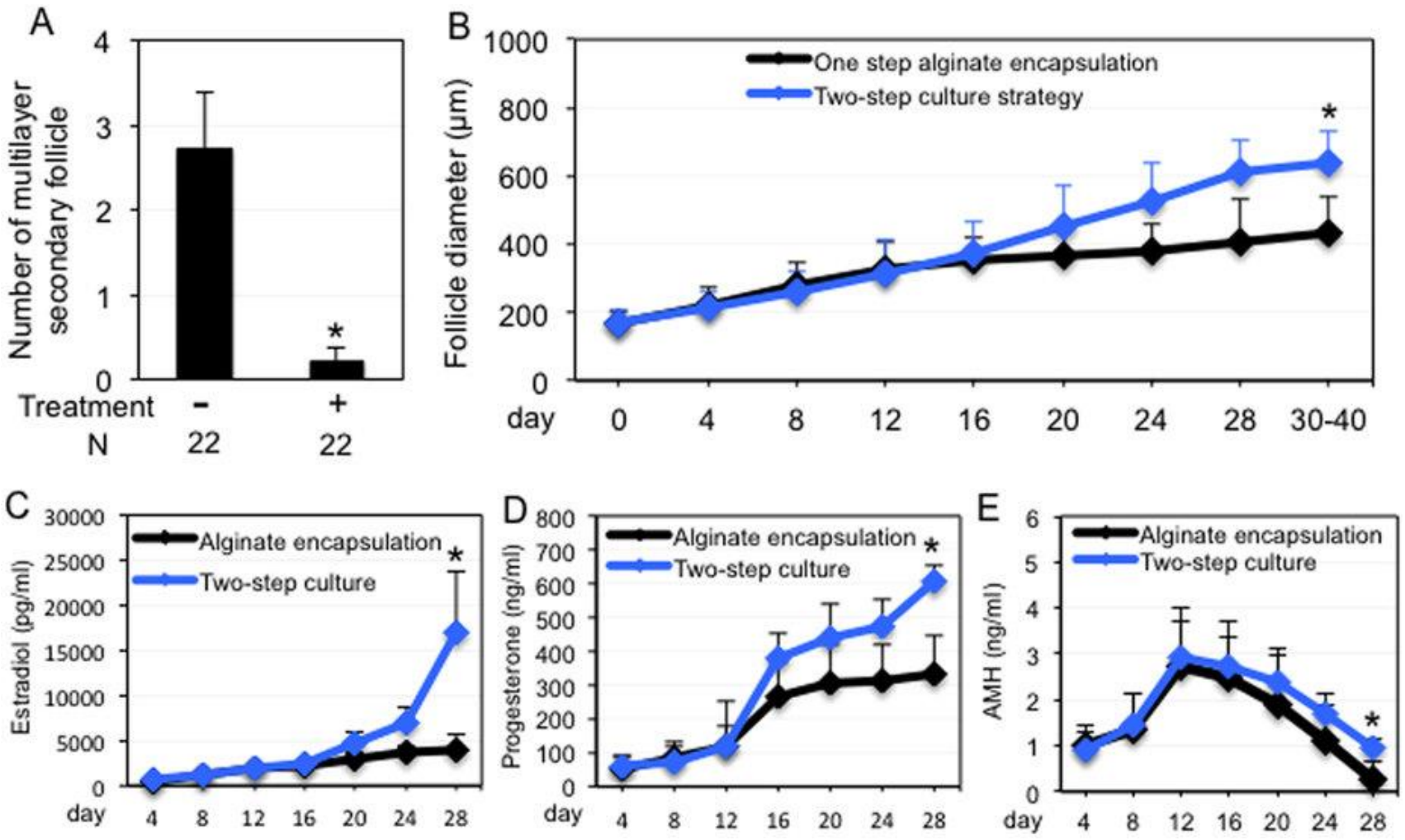



12 additional days in fibrin-alginate matrix



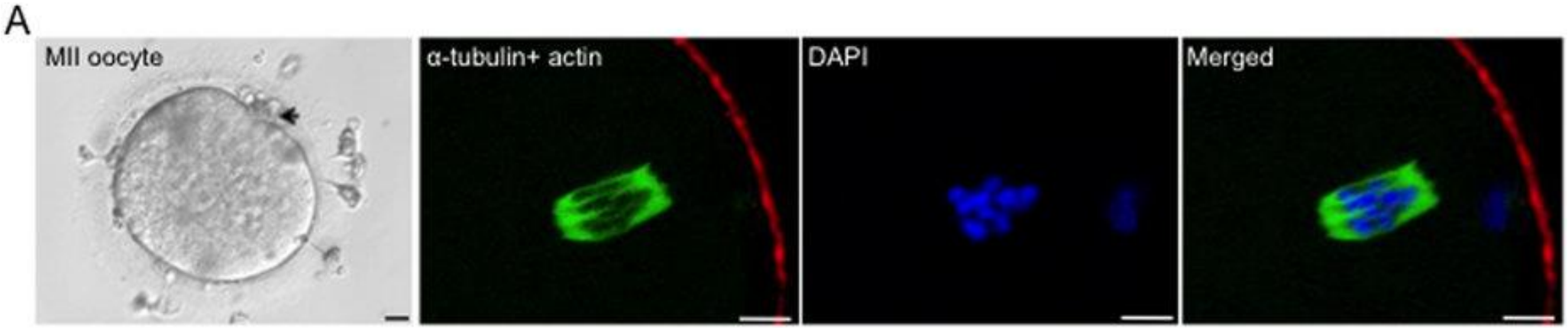

Jin et al. (Woodruff), *Fertil Steril* 2010

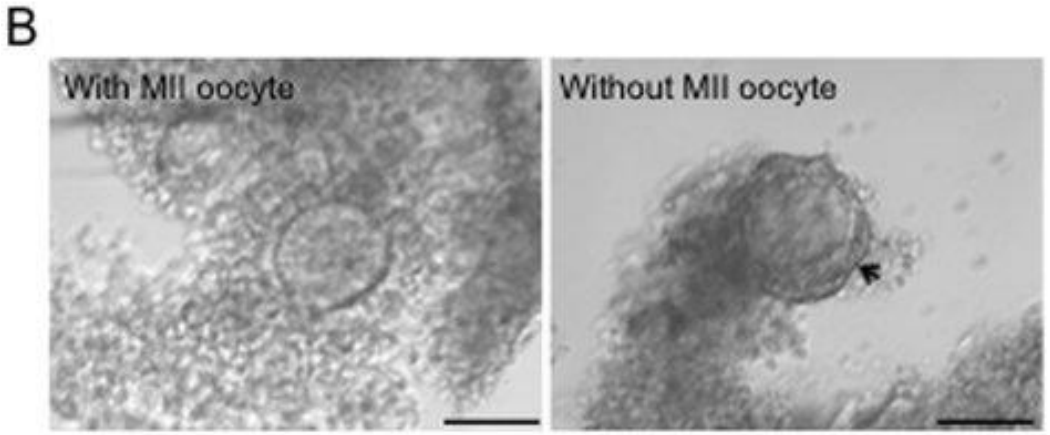
# In Vitro Maturation of Human Follicles

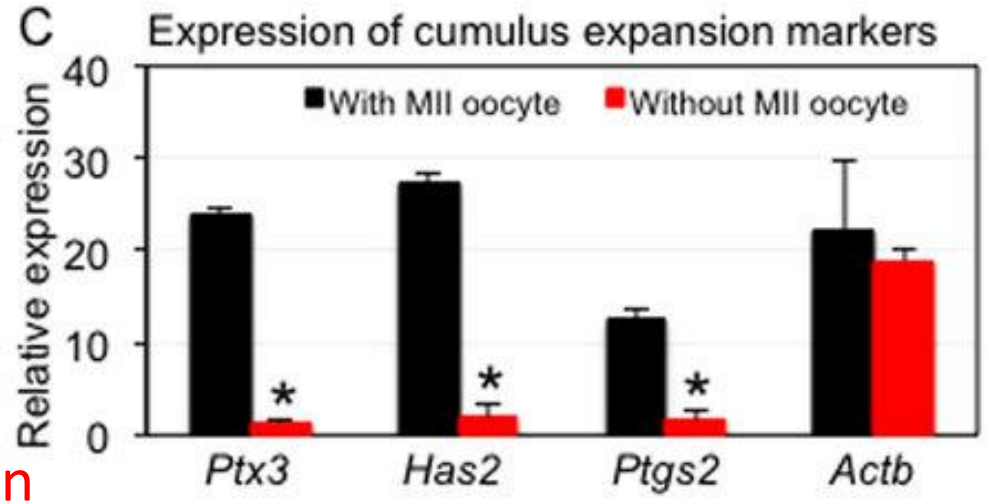



Xiao (Woodruff) et al., *Sci Rep* 2015

# In Vitro Maturation of Human Follicles (MII Oocytes!!)




Cumulus expansion with MII oocyte

No cumulus expansion with MII oocyte

Xiao (Woodruff) et al., *Sci Rep* 2015

# Take home messages

- There are established cell-based therapies to treat male and female infertility
  - ✓IUI, IVF, ICSI
- Men are from Mars, Women are from Venus
  - ✓Spermatogenesis is a stem cell-based system
  - ✓Women are born with a finite number of eggs
- There are standard of care and experimental cell-based therapies to preserve and restore fertility for women, men, girls and boys
  - ✓These options are available today and expanding
  - ✓One day it may be possible to produce mature sperm or eggs from skin or other somatic cells

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