Sperm and testicular tissue banking

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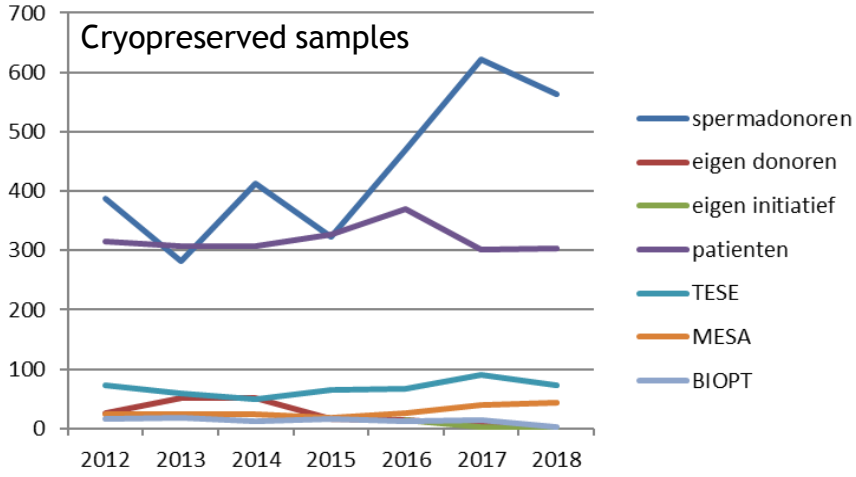
Clinical Sperm and testicular tissue bank in Amsterdam UMC location AMC

- From 1976
- ~130-175 new cases each year ejaculates
- ~30-50 new cases each year MESA
- ~100-150 new cases each year TESE (67% sperm banking)

- Total sperm storage AMC
  - >1.300 patients ages 12-62
  - > 3.000 ejaculates
  - >42.000 straws
- Total testicular tissue storage AMC
  - 96 patients age 0.5-15 years
  - > 300 straws



Differences in male and female gonads

The function of the testis is formation of gametes and hormones. There are striking differences between ovary and testis:

- Gonad compartments
- Development of gametes

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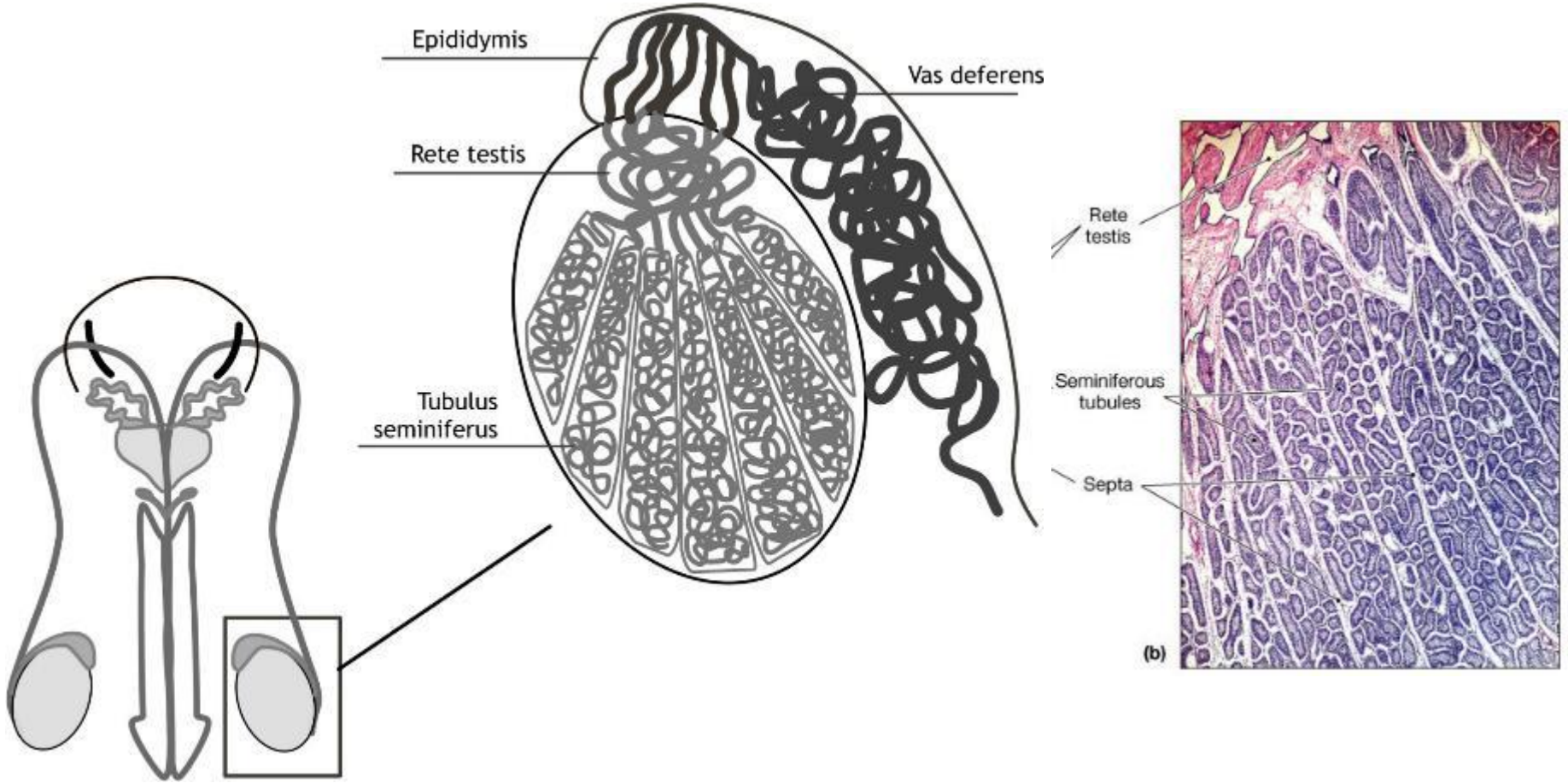
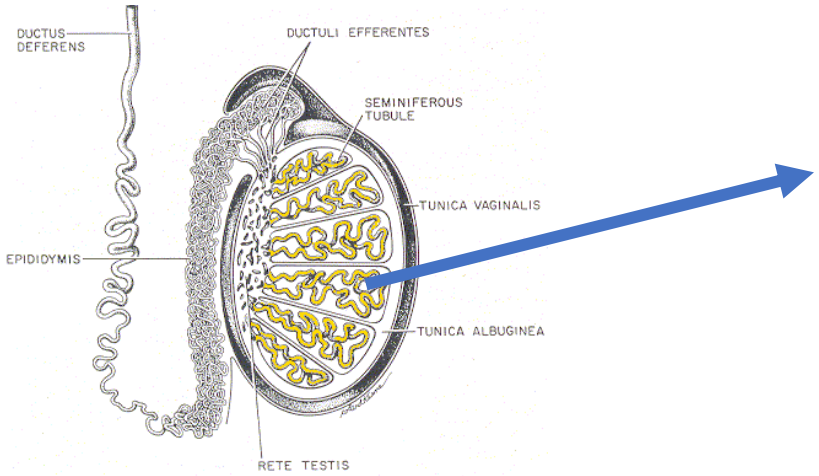
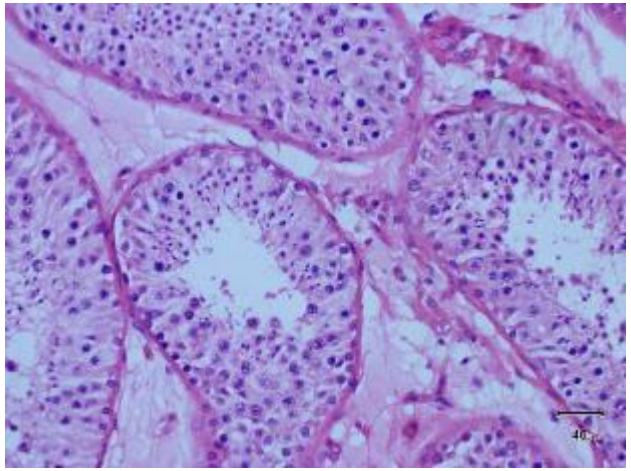
The testis: compartment

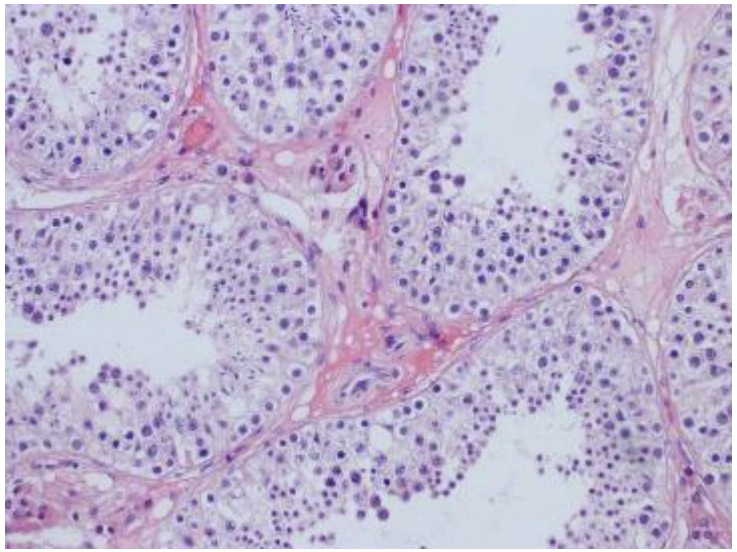


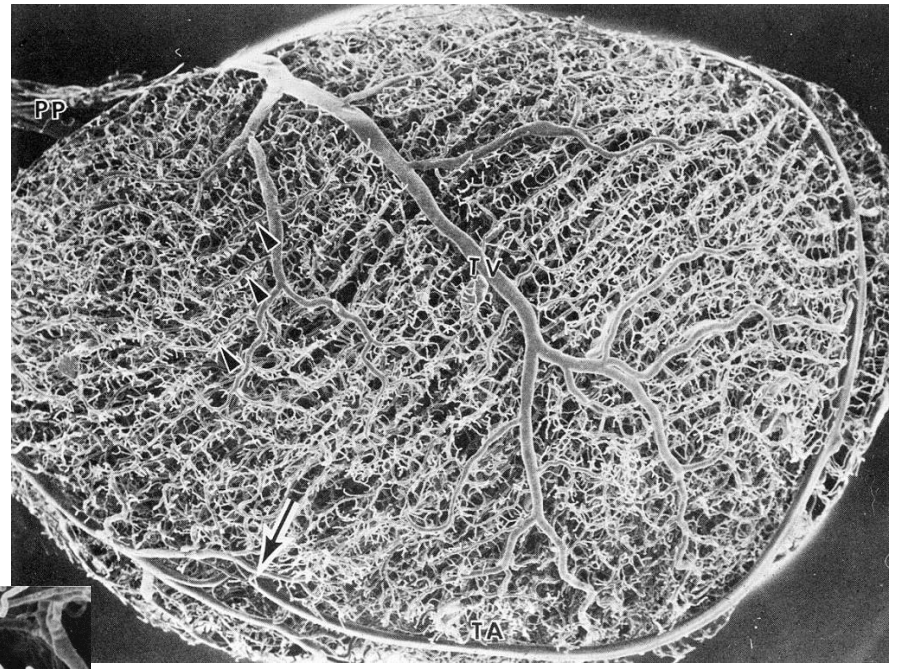
The testis: compartments

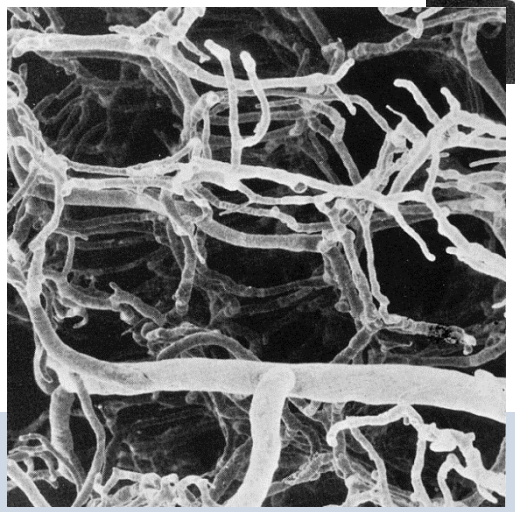




Dense vascularization





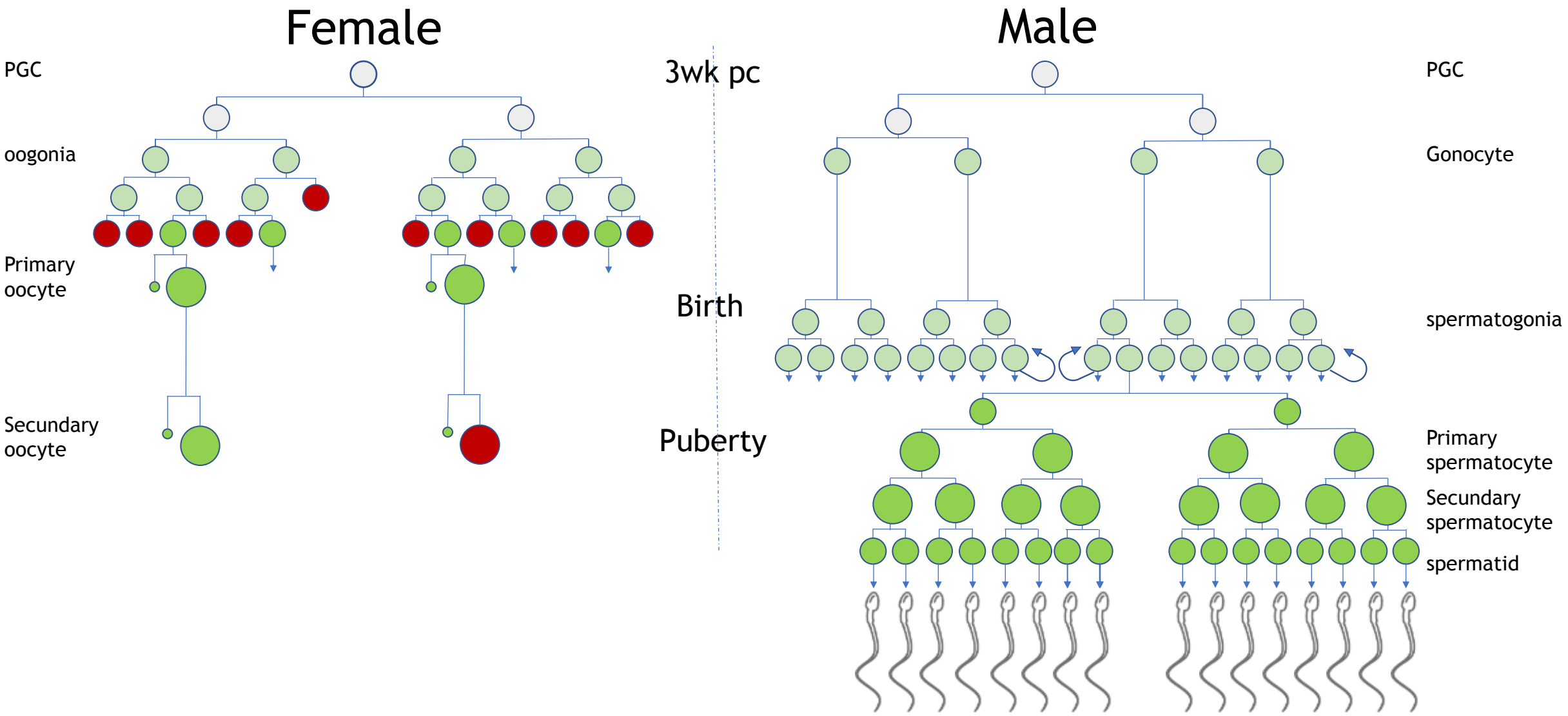


Differences in male and female gonads

The function of the testis is formation of gametes and hormones. There are striking differences between ovary and testis :

- Gonad compartments
- Development of gametes

Differences in gamete development

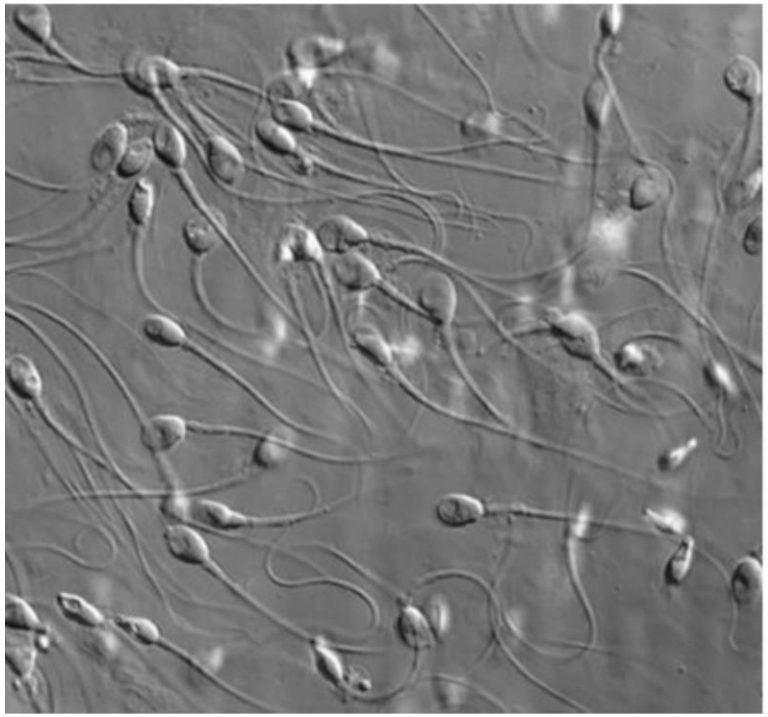


Purpose of cryopreserved Sperm or testicular tissue

Sperm or testicular tissue

- As source for clinical treatment in case of male and/or female subfertility
- Fertility preservation

Sperm obtained for subfertility treatment



For infertility treatement, sperm is the most used cell type for clinical treatment, including donor sperm or sperm from partner

Easy to obtain from ejaculate without any intervention

Easy to cryopreserve

- No pretreatment of sample
- In glycerol-based cryoprotectant
- In straws
- Directly in nitrogen vapour in vapour storage container

Clinical use of cryosperm form the ejaculate in infertility

From male donor in case of infertile partner or no partner

Treatment of choice

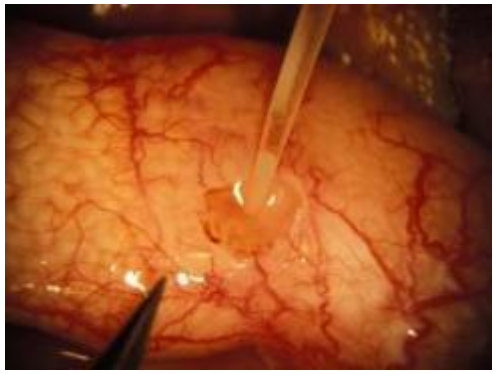
cervical insemination or intrauterin insemination (IUI)

- No or mild ovarian stimulation
- 15-20% chances of ongoing pregnancy per cycle

If no ejaculated sperm, retrieval from epididymis

MESA (Microsurgical Epididymal Sperm Aspiration)
Sperm from epididymis

From 1992
VUB



Sperm is collected in HTF-hepes/albuman buffer

Cryopreservation:

- In glycerol-based cryoprotectant
- In straws
- Directly in nitrogen vapour in vapour storage

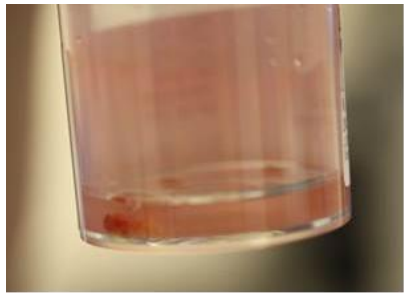
Treatment of choice for reproduction:
Intracytoplasmic sperm injection (ICSI)

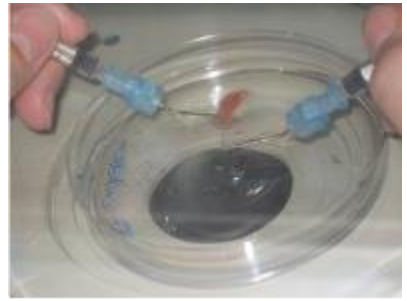
- Requires intervention to obtain sperm
- Ovarian hyper stimulation and ovum collection
- 25-30% chance of ongoing pregnancy per cycle

If no epididymal sperm, retrieval from testis

TESE (testicular sperm extraction)
Sperm from testis tissue

From 1993 VUB
From 2007 in AMC





Cryopreservation:

- Requires intervention to obtain biopsy
- Extraction of sperm from biopsy
- cryopreserved similar as MESA obtained sperm

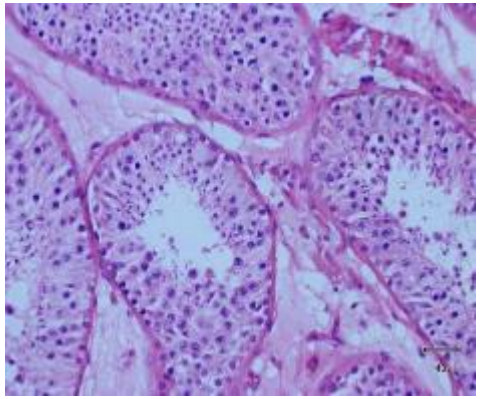
Treatment of choice for reproduction:
Intracytoplasmic sperm injection (ICSI)

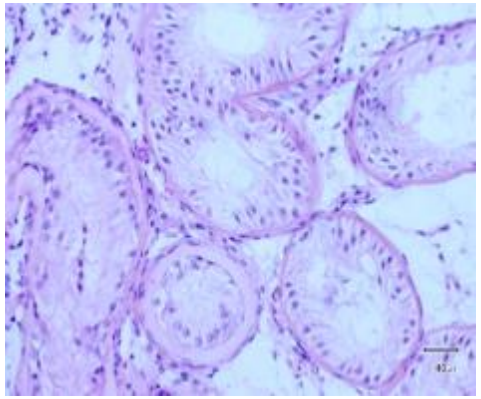
- Ovarian hyper stimulation and ovum collection
- 25-30% chance of ongoing pregnancy per cycle

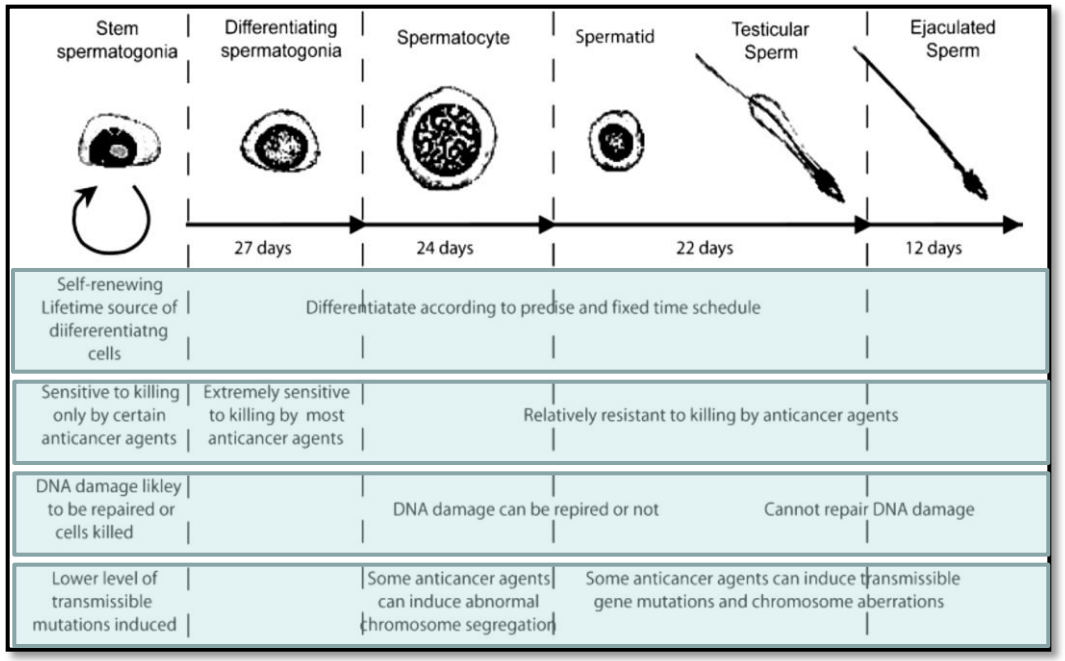
Sperm or testicular tissue obtained for fertility preservation

Patients with expected germ cell loss

Previous chemo- or radiotherapy



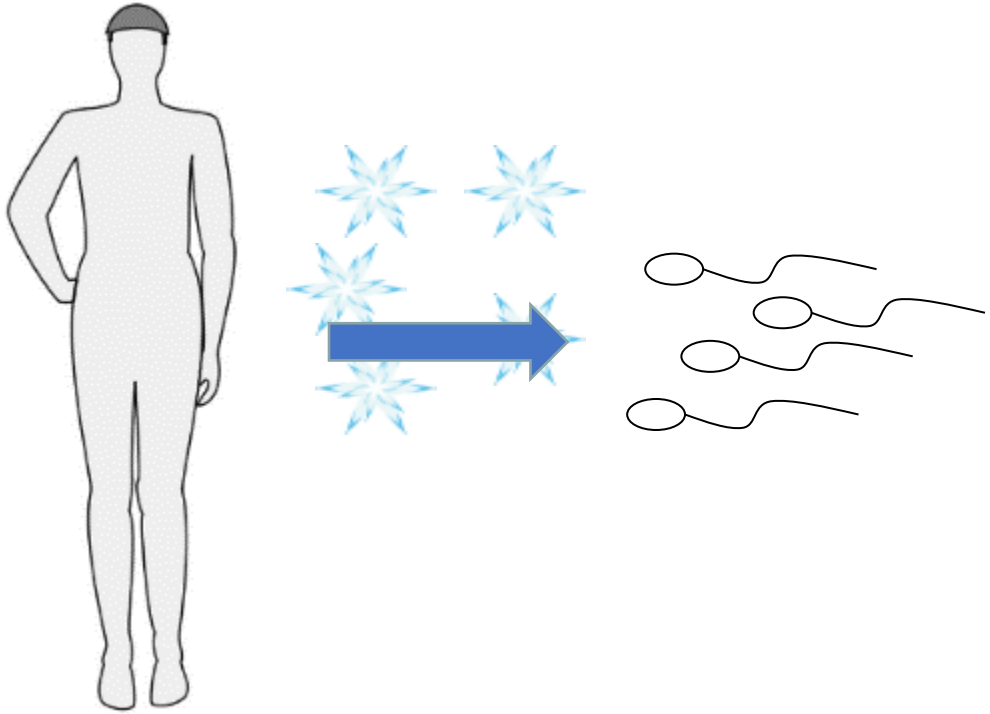




Meistrich Pediatr Blood Cancer 2009

Fertility preservation

Cryopreservatie of sperm before onset gonadotoxic treatment

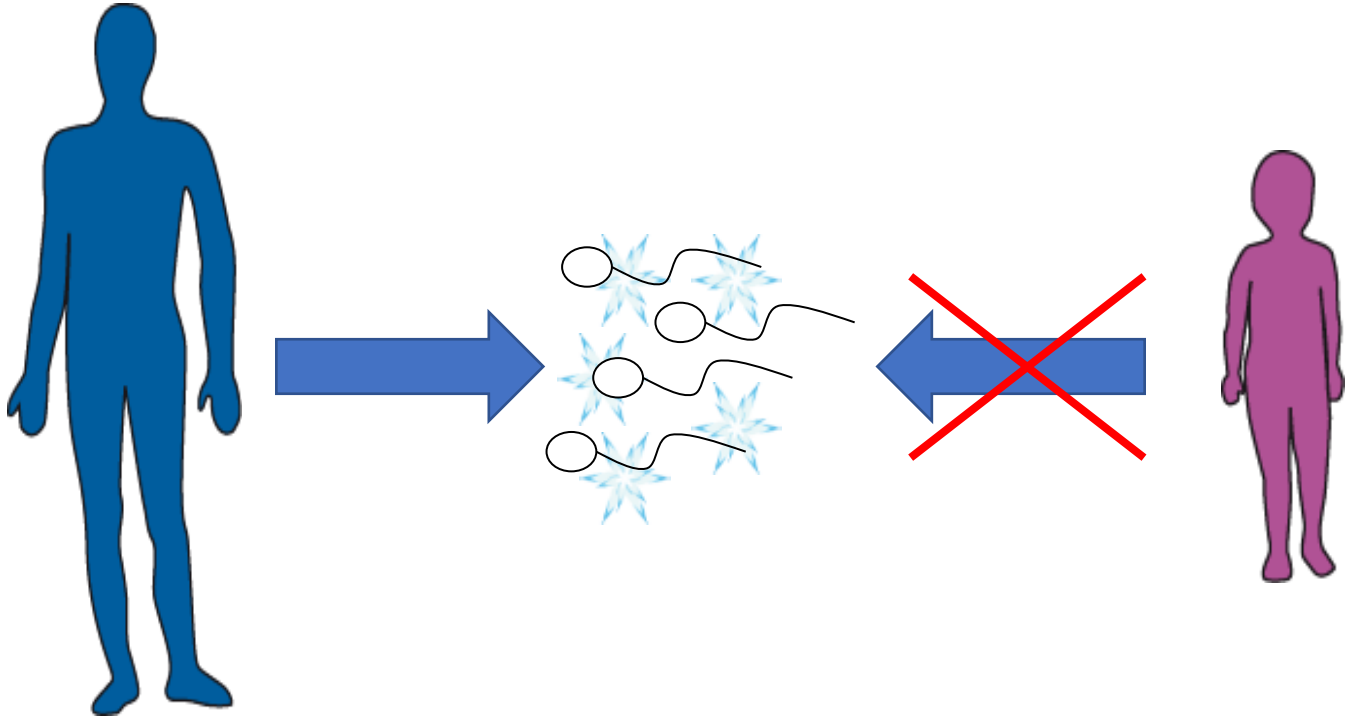


Sperm for fertility preservation obtained from:

- Ejaculate
- Epididymis
- Testis

Cryopreserved similar as for infertile men or donors

Fertility preservation

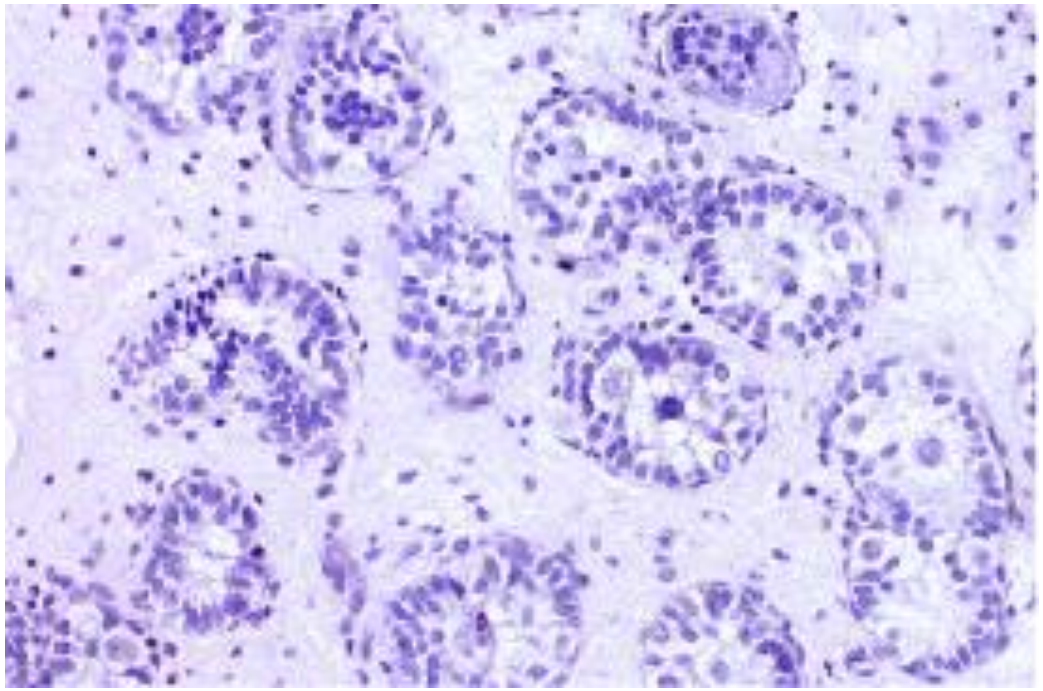


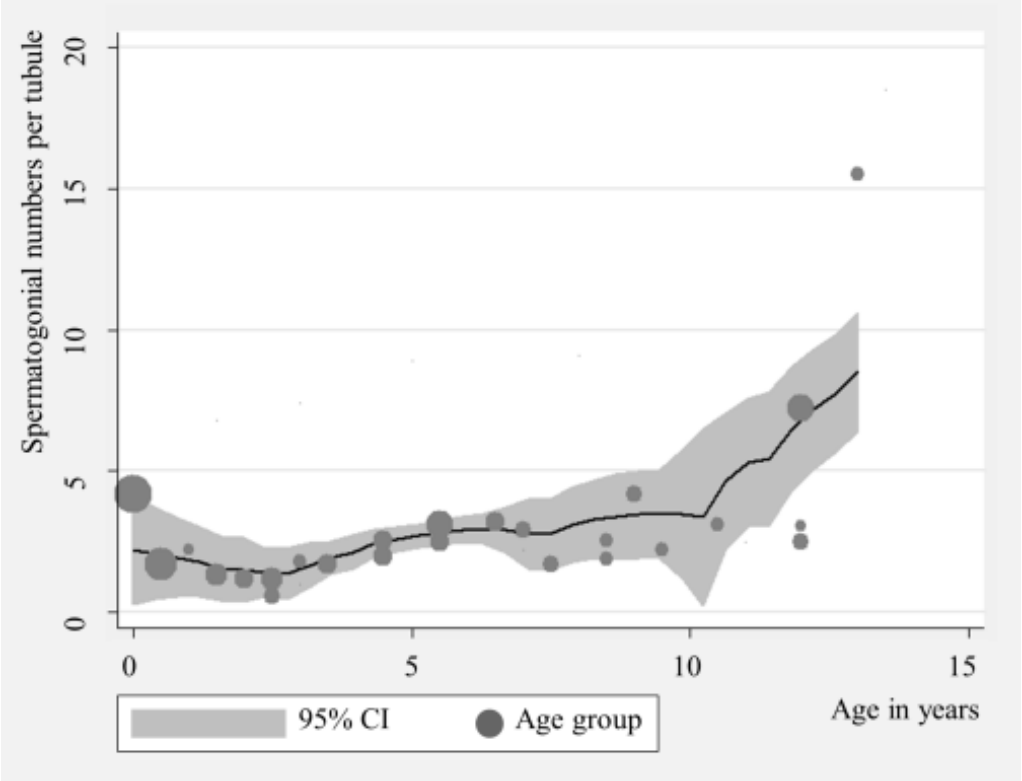
For prepubertal boys with cancer there is no means to preserve fertility with sperm

Blatt, et al., Med Pediatr Oncol. (1999), Wallace, et al., Lancet (2005)

Spermatogonial counts during prepubertal life

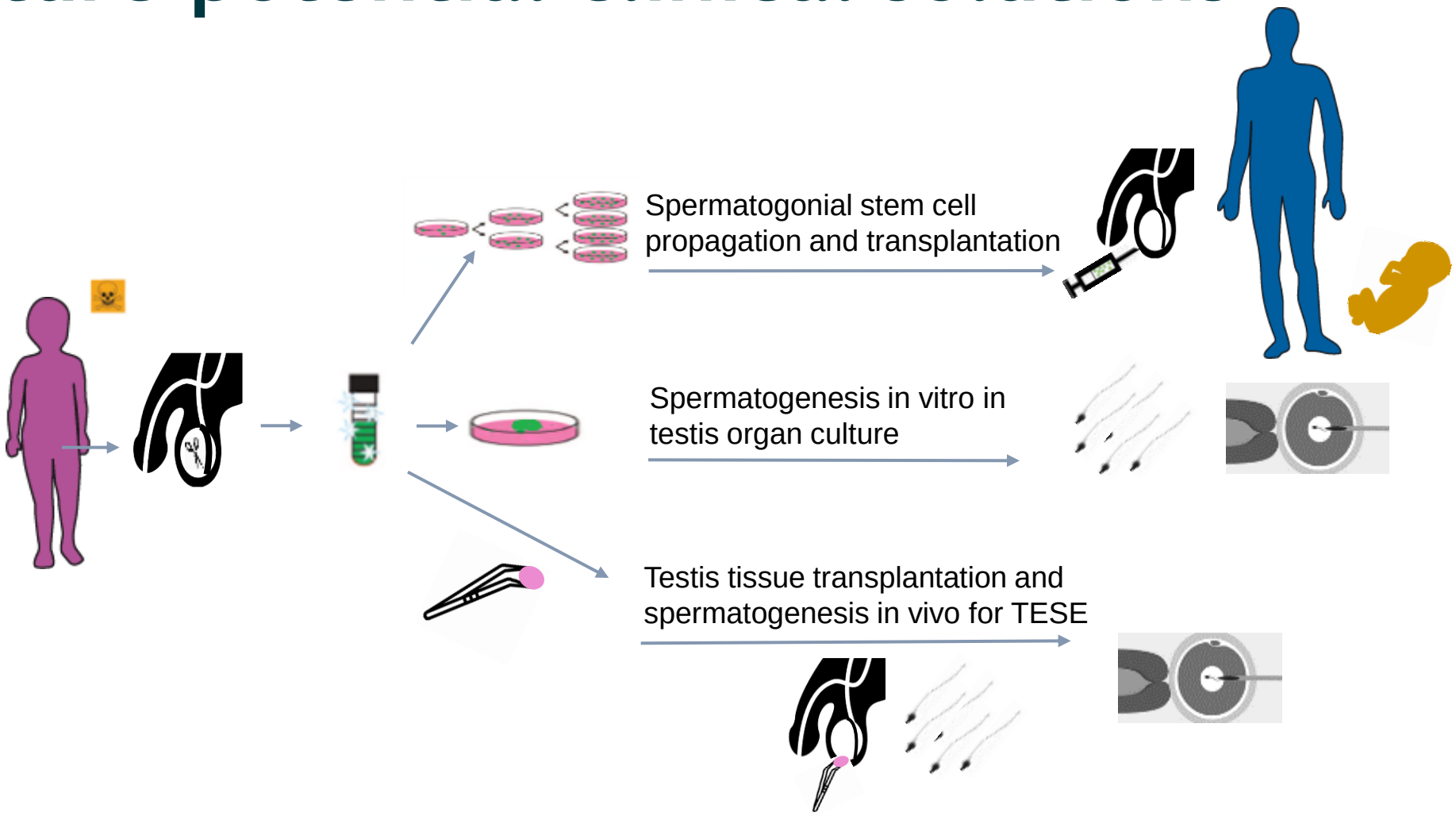
Polynomial meta-regression analyses on spermatogonial numbers per tubular cross section from four studies creating n=238





Masliukaite, Hagen et al., Fert Steril 2016

Future potential clinical solutions



Experience in the AMC

CCMO approval to cryopreserve testis biopsy from childhood cancer patients since 2009

Cryopreservation since 2011- 2018

- 96 patients (Ages 6 months – 15.5 years)
- Collection of biopsy (size ranging from 50-200µl)
- Collected tissue is cut in fragments of $3mm^3$
- Cryopreserving in 2-5 straws with each 10-20 fragments
- Controlled slow freezing in DMSO-based cryoprotectant (Keros et al., Hum Rep 2005)

Taking testis biopsy

Effect of biopsy surgery

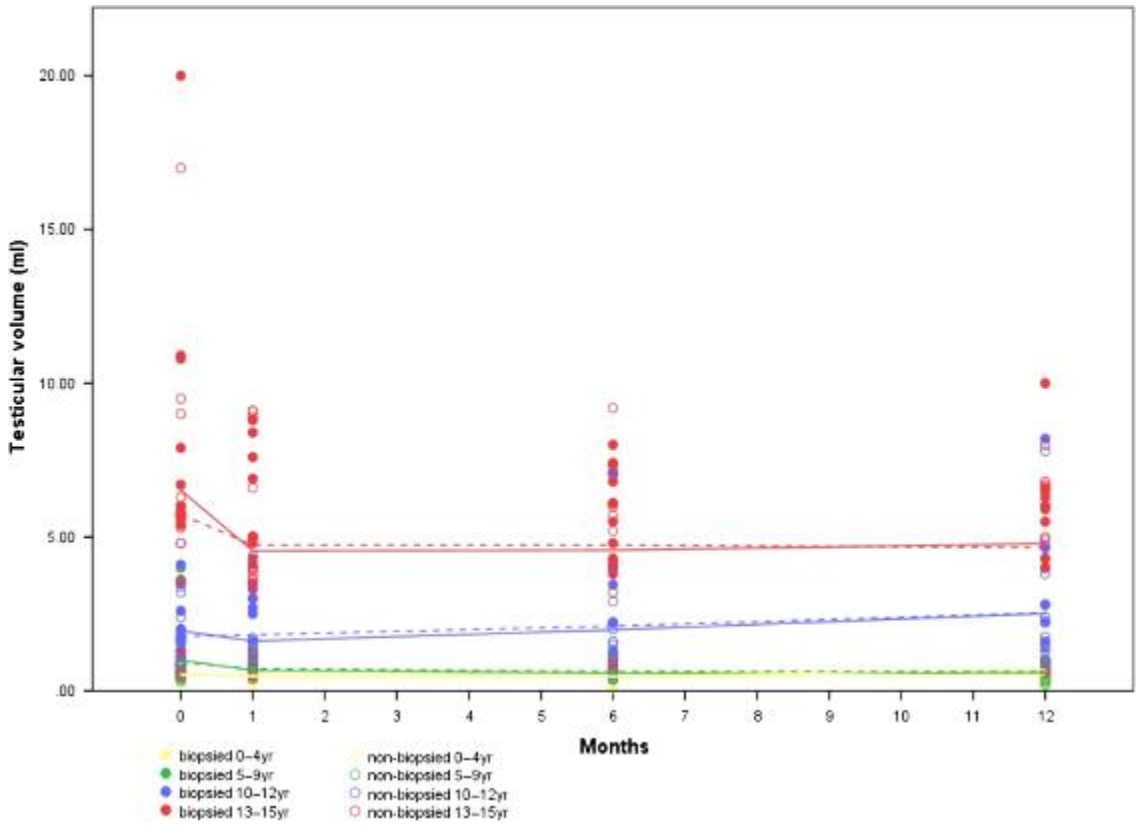


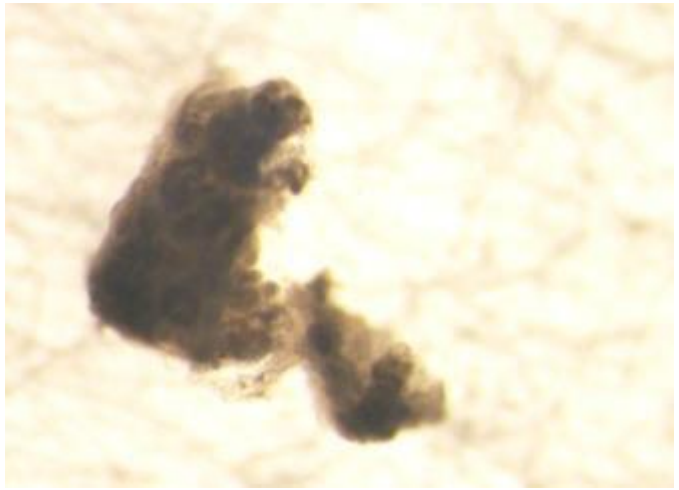
Table II Secondary outcomes.

Variable	Biopsied testis	
Acute adverse effects ($n = 78$)		
Post-operative bleeding, *n* (%)	1 (1.3%)	
Wound infection, *n* (%)	2 (2.6%)	
Variable	**Biopsied testis**	**Contralateral testis**
Aberrations at 1 month ($n = 64$)		
Calcifications, *n* (%)	2 (3.1%)	2 (3.1%)
Epididymal cyst, *n* (%)	3 (4.7%)	1 (1.6%)
Hydrocele, *n* (%)	4 (6.3%)	1 (1.9%)
Extra-testicular haematoma, *n* (%)	5 (7.8%)	
Intratesticular haematoma, *n* (%)	2 (3.1%)	
Fibrotic lesion, *n* (%)		
Aberrations at 6 months ($n = 58$)		
Calcifications, *n* (%)	2 (3.4%)	2 (3.4%)
Epididymal cyst, *n* (%)	2 (3.4%)	1 (1.7%)
Hydrocele, *n* (%)	2 (3.4%)	
Extra-testicular haematoma, *n* (%)		
Intratesticular haematoma, *n* (%)		
Fibrotic lesion, *n* (%)		
Aberrations at 12 months ($n = 55$)		
Calcifications, *n* (%)	1 (1.6%)	1 (1.6%)
Epididymal cyst, *n* (%)		1 (1.6%)
Hydrocele, *n* (%)	1 (1.6%)	2 (3.1%)
Extra-testicular haematoma, *n* (%)		
Intratesticular haematoma, *n* (%)		
Fibrotic lesion, *n* (%)	4 (6.3%)	

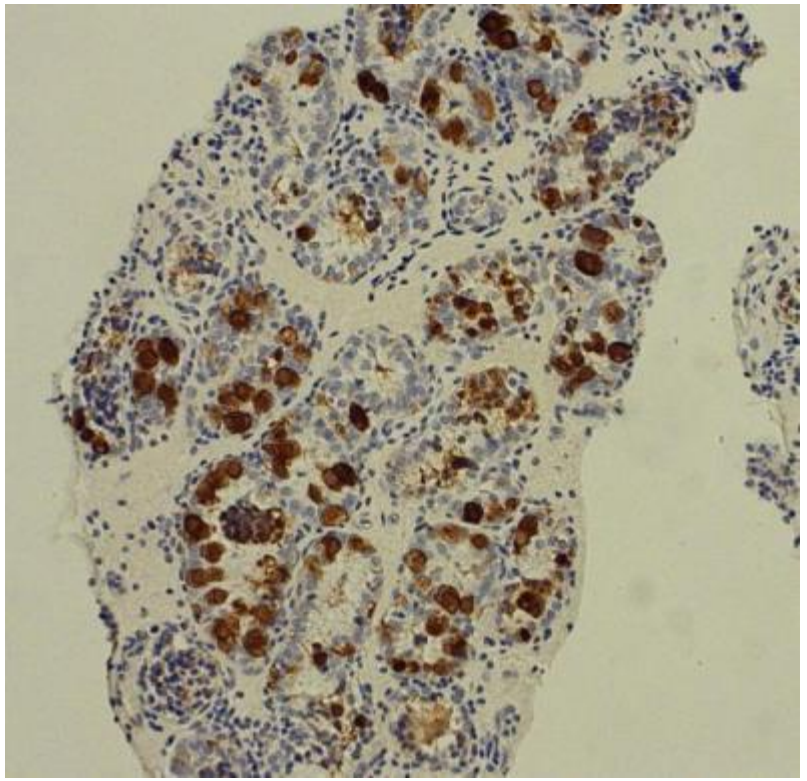
Development of testis is not hampered after biopsy for fertility preservation in prepubertal boys

Uijldert et al., Hum Rep 2017

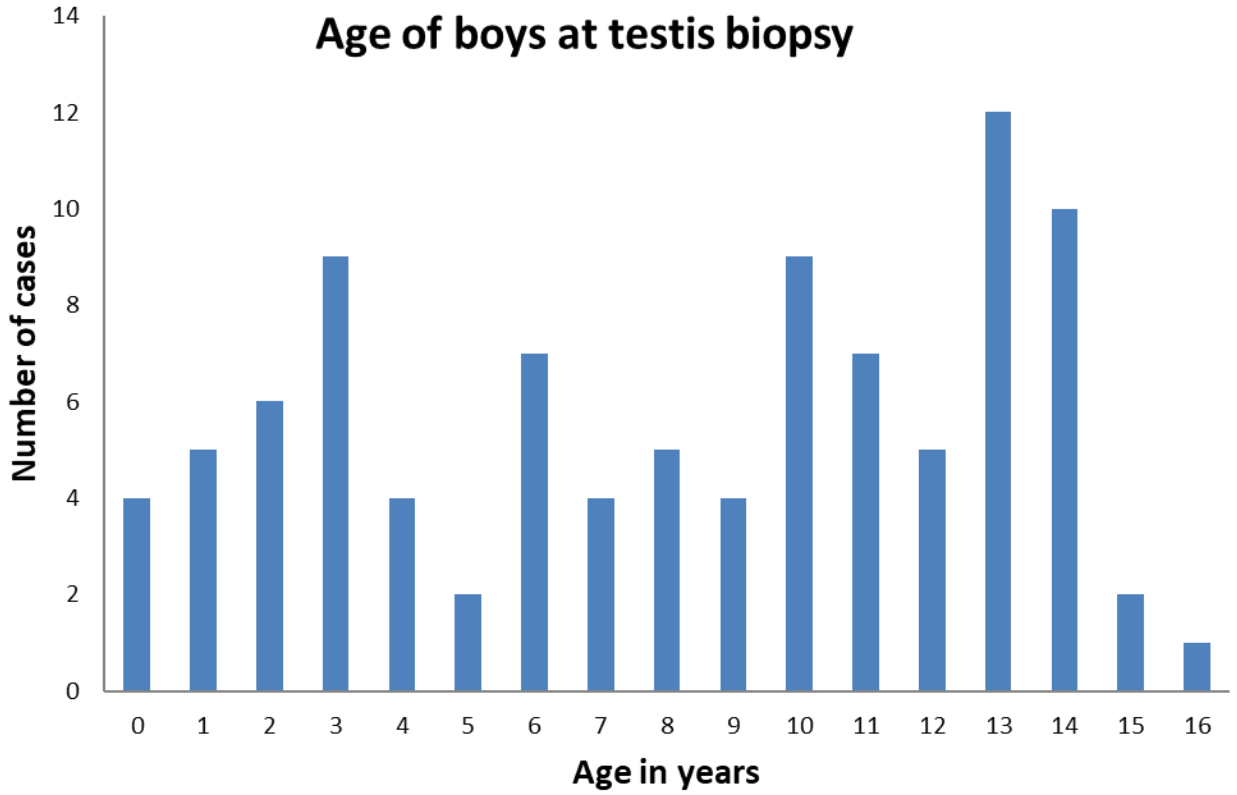
Preparation and cryopreservation



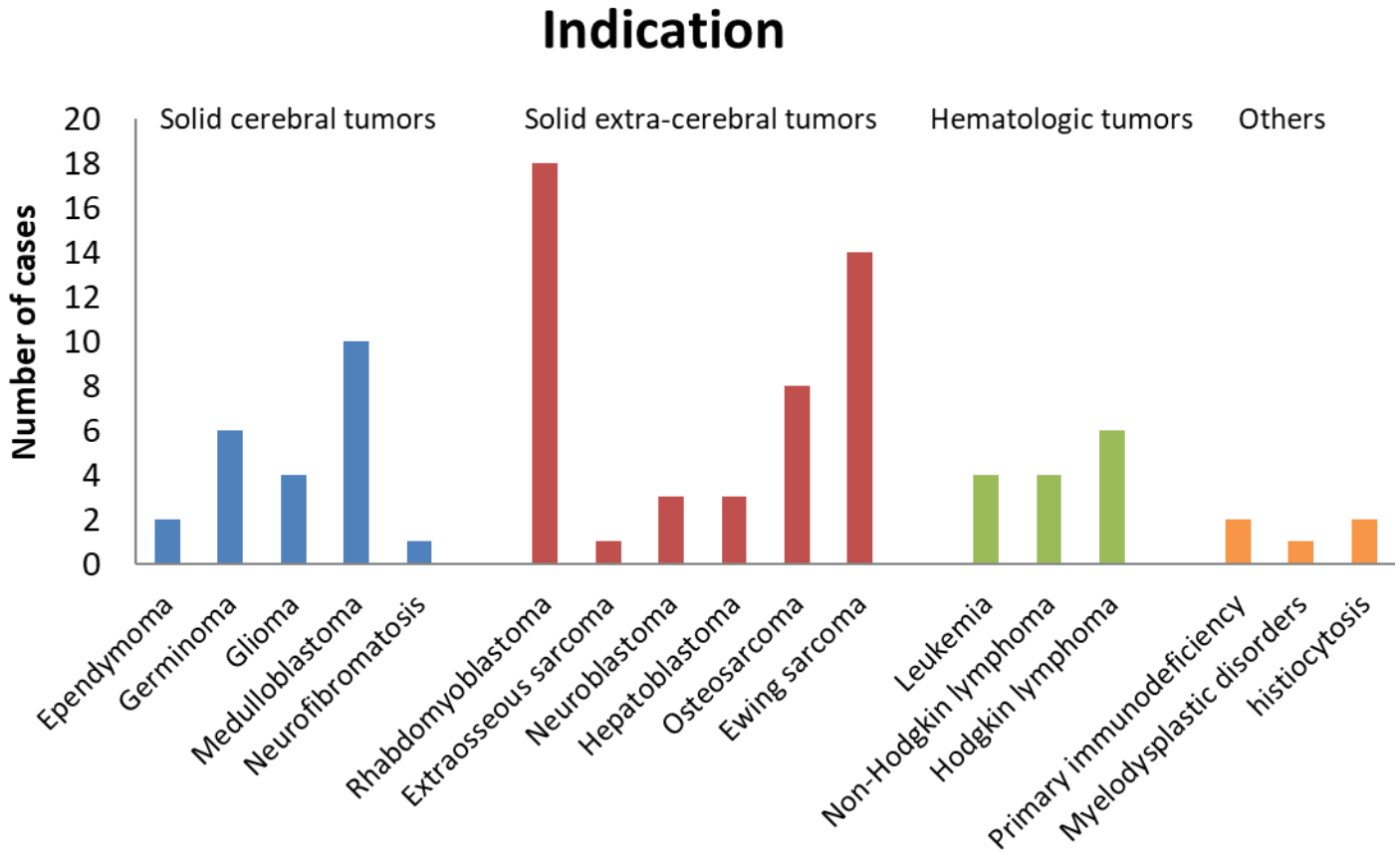
Cryopreservation of testis biopsies



Age range of patients



Indications of patients



Handling of sperm and testicular tissue for cryopreservation

- Each step in handling of samples is approved by 2 persons based on name and birthday (or patients MDN) unique for that day
- Yearly registration of newly stored and distributed cells and tissue according to European standard.

Summary

- Ejaculated, MESA and TESE sperm is cryopreserved in glyserol-based cryoprotectant directly in N2 container and used for IUI/IVF/ICSI
  - Donors, patients diagnosed with azoospermic or cancer (all post puberty)

- Testicular biopsies are cryopreserved in DMSO-based cryoprotectant by controlled slow freezing.
  - Prepubertal boys diagnosed with disease that need gonadotoxic treatment or risk for loss of germ cells

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