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
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
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

**Female**

- PGC
- Oogonia
- Primary oocyte
- Secondary oocyte

**Male**

- PGC
- Gonocyte
- Spermatogonia
- Primary spermatocyte
- Secondary spermatocyte
- Spermatid

<table>
<thead>
<tr>
<th>Female</th>
<th>Male</th>
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<tbody>
<tr>
<td><strong>PGC</strong></td>
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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 treatment, 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 intruterin 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

Cryopreservation of sperm before onset gonadotoxic treatment

Sperm for fertility preservation obtained from:

- Ejaculate
- Epididymis
- Testis

Cryopreserved similar as for infertile men or donors
For prepubertal boys with cancer there is no means to preserve fertility with sperm.

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

- Testis tissue transplantation and spermatogenesis in vivo for TESE
- Spermatogonial stem cell propagation and transplantation
- Spermatogenesis in vitro in testis organ culture
- Testis tissue transplantation and spermatogenesis in vivo for TESE
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 3mm³
- 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
Development of testis is not hampered after biopsy for fertility preservation in prepubertal boys

Table II Secondary outcomes.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Biopsied tests</th>
<th>Contra lateral tests</th>
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<tbody>
<tr>
<td>Acute adverse effects (n = 78)</td>
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<tr>
<td>Post-operative bleeding, n (%)</td>
<td>1 (1.3%)</td>
<td>2 (2.6%)</td>
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<tr>
<td>Wound infection, n (%)</td>
<td>2 (2.6%)</td>
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Uijldert et al., Hum Rep 2017
Preparation and cryopreservation
Cryopreservation of testis biopsies
Age range of patients

Age of boys at testis biopsy

Number of cases

Age in years
Indications of patients

- Solid cerebral tumors
- Solid extra-cerebral tumors
- Hematologic tumors
- Others

Number of cases:

- Ependymoma
- Germinoma
- Glioma
- Medulloblastoma
- Neurofibromatosis
- Rhabdomyosarcoma
- Extraskeletal sarcoma
- Neuroblastoma
- Hepatoblastoma
- Osteosarcoma
- Ewing sarcoma
- Leukemia
- Non-Hodgkin lymphoma
- Hodgkin lymphoma
- Primary immunodeficiency
- Myelodysplastic disorders
- Histiocytosis
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 glycerol-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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