INTERNATIONAL FEDERATION OF FERTILITY SOCIETIES

Fertility preservation – new frontiers?

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Disclosures

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- Congress planning, lecturing, congress participation (Gedeon-Richter, Merck, MSD, Ferring)

Indications for fertility preservation

- Cancer – treatment with alkylating agents and radiation may cause infertility
- Non-malignant disorders treated with immunosuppression and stem cell transplant
- Surgical menopause – ovarian diseases
- Sex chromosome abnormalities (45,X; 47,XXY)
- Individuals with gender diversity
- Age-related fertility preservation
- The effect of treatments and the disorder on other reproductive organs is also important (uterus, hypothalamus and pituitary)

Ovarian reserve

- population of primordial follicles
- 7 million oocytes in mid-gestation
- around 1 million remain at birth
- the decline of follicles throughout life
- serum AMH can be measured to estimate OR
- high individual variability
- gonadotoxicity is age-dependent

Effects of chemo- & radiotherapy on the ovarian cells

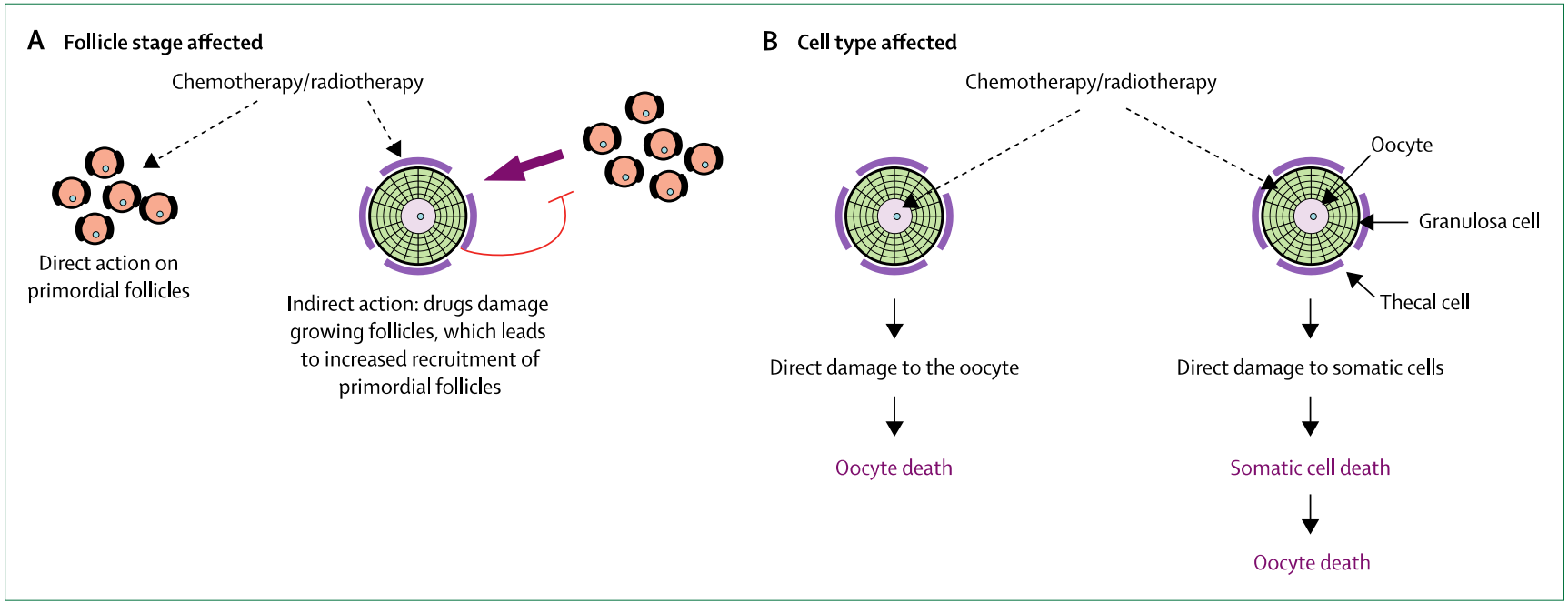

***Figure 3*: Follicle stage and cell types affected by chemotherapy and radiotherapy**
(A) Cancer treatments could directly affect the resting pool of primordial follicles or the growing follicle population. Since growing follicles inhibit recruitment of primordial follicles, loss of this growing population leads to increased activation of primordial follicles and so loss of that reserve. (B) Cancer treatments could directly target oocytes or somatic cells. Oocyte death would result from death of the follicular somatic cells, since oocytes are dependent on these for survival. Reproduced from Morgan and colleagues[10] by permission of the European Society of Human Reproduction and Embryology.

Anderson et al 2015

Effects of chemo- & radiotherapy on the testicular cells

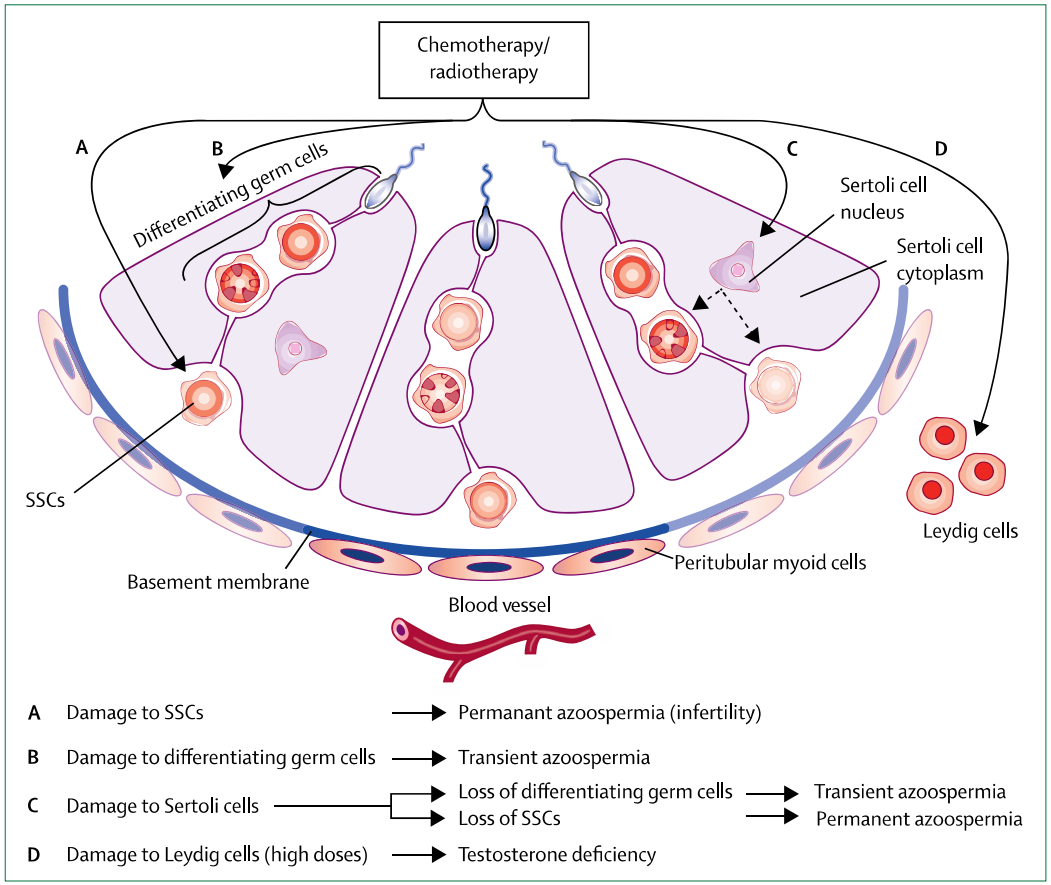

Figure 4: **Cellular targets for testicular damage after cancer treatment**
(A) Damage to the spermatogonial stem cells (SSCs) and subsequent SSC loss results in permanent azoospermia. (B) Damage to differentiating germ cells results in transient azoospermia; however, restoration of spermatogenesis can occur from the surviving SSCs. (C) Damage to Sertoli cells can result in failure of these cells to support the SSCs, differentiating germ cells, or both, resulting in permanent (A) or transient (B) loss of fertility. (D) Damage to Leydig cells after cancer treatment results in testosterone deficiency. This damage usually occurs at high doses that likewise result in germ cell loss and azoospermia.

Anderson et al 2015

Box 1 | Risk of infertility after cancer treatment

Low risk (<20%)

- Leukaemia
- Cerebral tumour <24 Gy
- Wilms' tumour
- Germinal cell tumour (no radiotherapy)

Medium risk (20–80%)

- Leukaemia
- Cerebral tumour >24 Gy
- Non-Hodgkin lymphoma
- Hodgkin lymphoma
- Ewing sarcoma, no metastases
- Osteosarcoma
- Hepatoblastoma
- Neuroblastoma

High risk (>80%)

- Total body irradiation
- Pelvic irradiation
- Bone marrow transplantation
- Hodgkin lymphoma, alkylating agent

Pelvic radiation -> POI
5 – 10 Gy is toxic to oocytes

< 2 Gy is estimated to destroy 50% of primordial follicles

Cyclophosphamide is the most harmful to oocytes of the chemotherapeutics

Risk of infertility very individual, ovarian reserve, age, treatment details

Donnez &Dolmans 2013
Nature Reviews Endocrinology

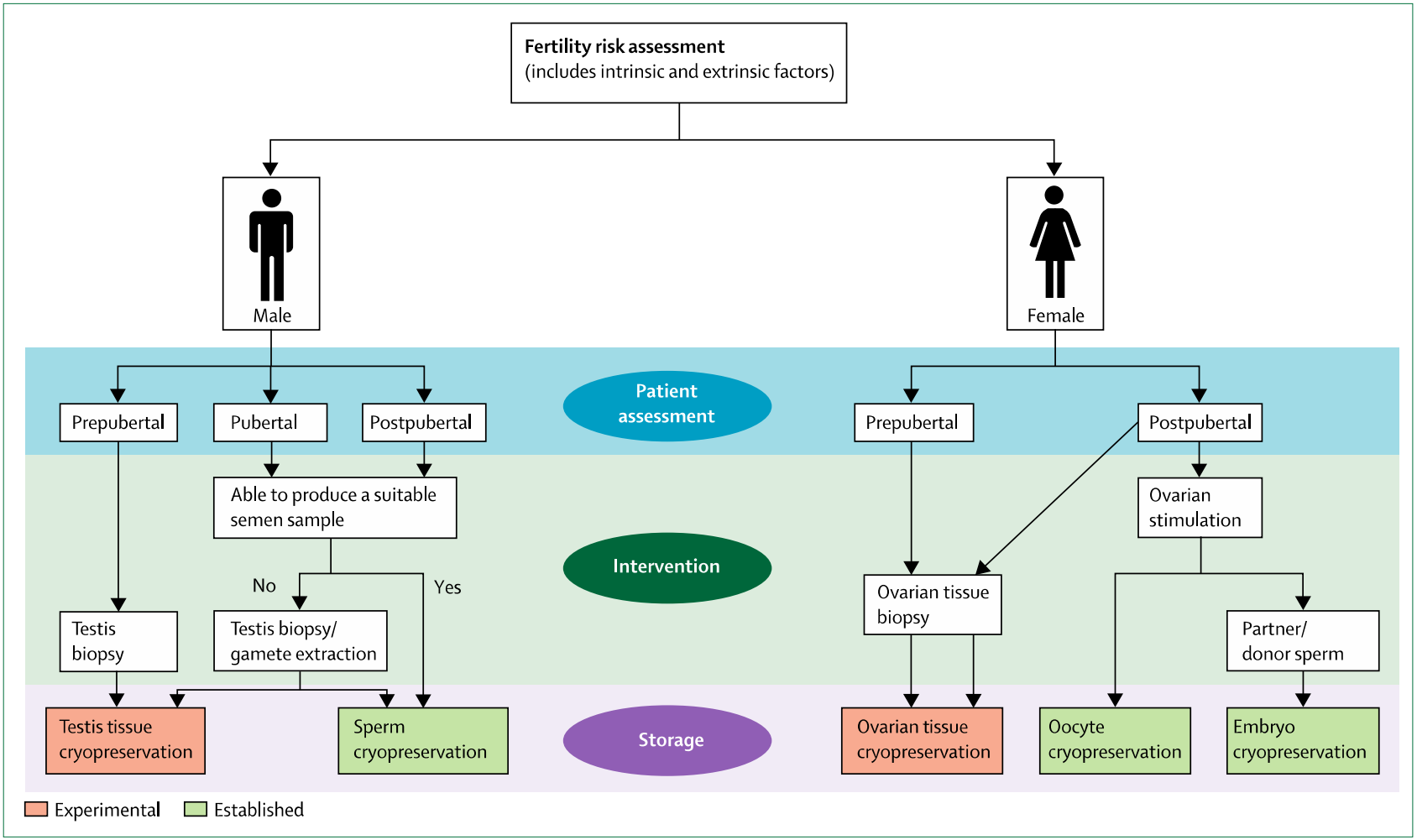

Figure 5: **Pathways to fertility preservation options for children and young adults**
In prepubertal boys, before onset of spermatogenesis, testicular biopsy and cryopreservation is an option. In pubertal and postpubertal male patients, the ability to produce a sperm-containing ejaculate enables sperm cryopreservation: if this is not possible, testicular biopsy with cryopreservation of sperm or tissue is needed. In prepubertal girls, ovarian stimulation is inappropriate, so ovarian tissue cryopreservation can be offered. After puberty, cryopreservation is an option, but ovarian stimulation enables recovery of mature oocytes for cryopreservation, or of embryos after fertilisation. Distinction is made between established and experimental options. Recovery of immature oocytes with in-vitro maturation is omitted for clarity.

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Fertility preservation - women

- Ovarian transposition (advanced cervical cancer)
  - relatively easy anterolateral fix through l-scopy
  - ovarian biopsy can be taken (cryopreserv.)
  - ovarian function retained in nearly 90% (<40y)
  - under used method

Bisharh & Tulandi 2003
Barameh et al. 2013

- GnRH agonist
  - does not reduce gonadotoxicity
  - may only be used when no other methods are available

Fertility preservation - women

- Embryo cryopreservation

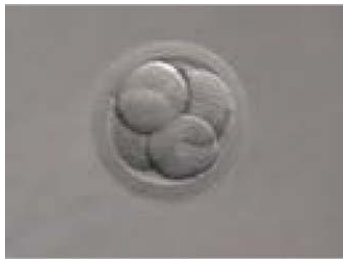
  * established methodology – good results

  * ovarian stimulation takes time and this may delay the cancer treatment (random antagonist)

  * high E2 levels may be detrimental in cases of estrogen sensitive tumors

  * spermatozoa required

  * ethical, legal and religious implication (e.g. death, divorce etc.)



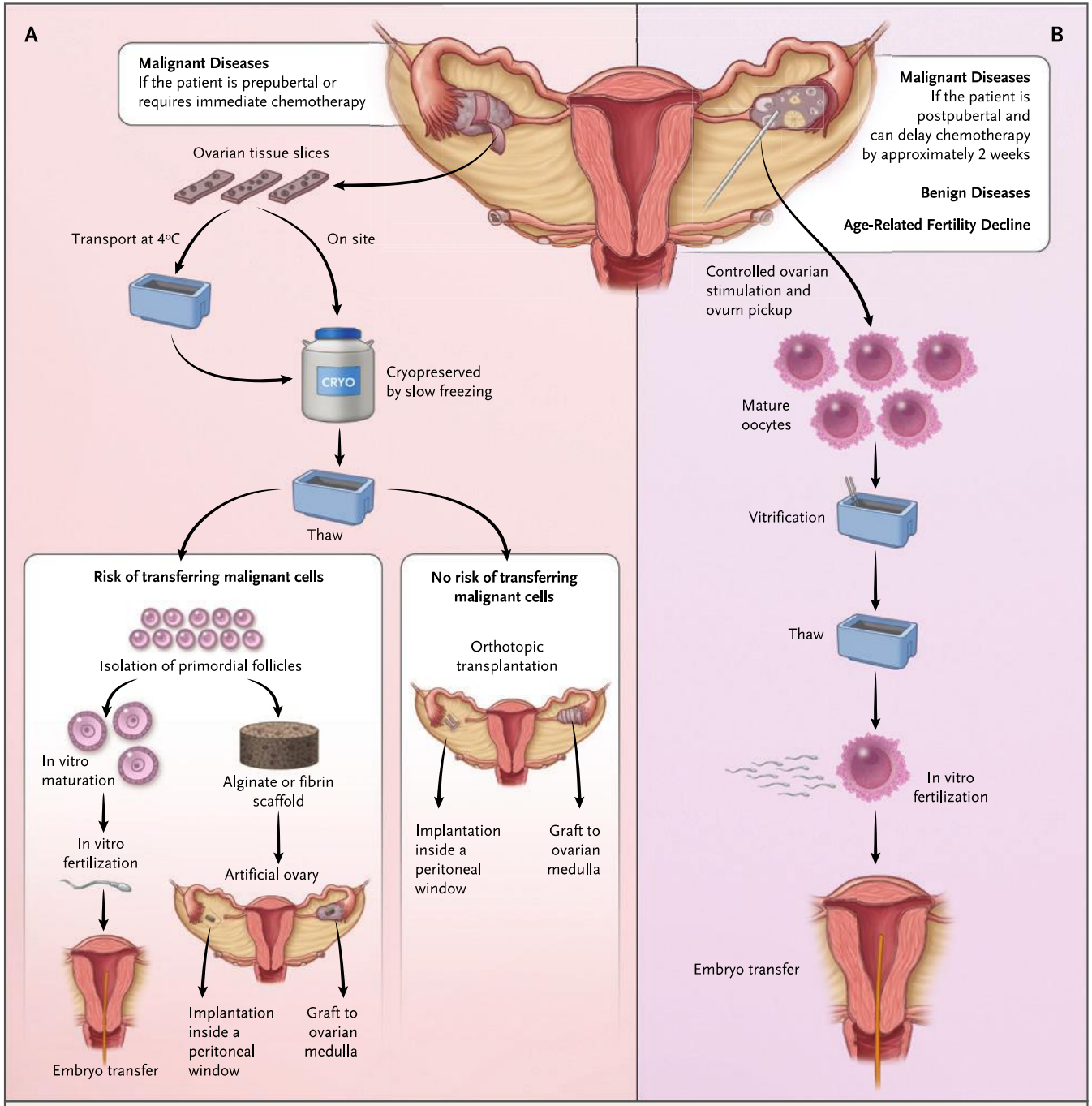


Figure 1. Options for Fertility Preservation.

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Fertility preservation - women

- Oocyte vitrification

  * comparable results to fresh oocytes

  * no sperm required - best option - all women?

  * ovarian hormone stimulation still required (delay, E2 exposure)

  * [illegible] s



Fertility preservation - women

- Non-oncological fertility preservation
- <35 years at vitrification:

  LBR: 5 oocytes ->15.4%, 8-> 40.8%, 10 ->60.5%
- >35 years at vitrification

  LBR: 5 oocytes ->5.1%, 8-> 19.9%, 10 ->29.7%

With 10 oocytes the CLBR was double in < 35 y women compared to >35 y women.

20 vitrified oocytes are required to achieve a live birth
(in egg donation programmes).

Cobo et al. 2016

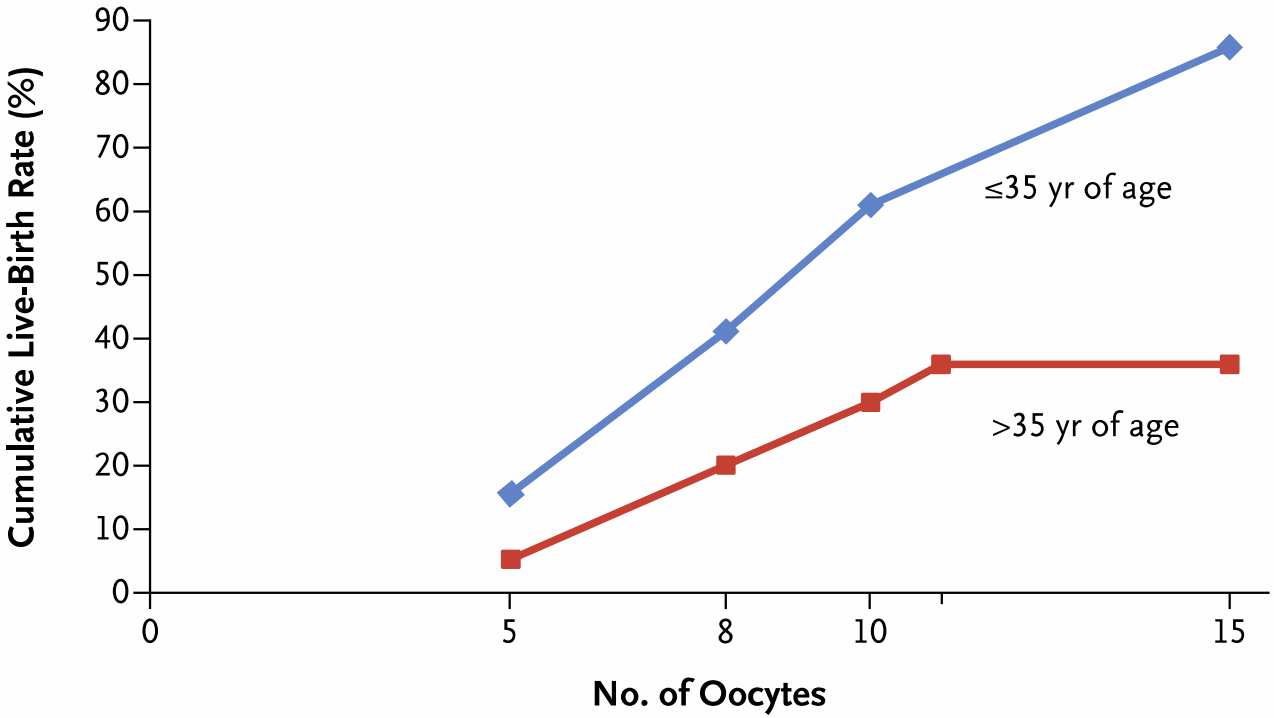

Figure 2. Cumulative Live-Birth Rates with 5 to 15 Oocytes, According to Age.

The cumulative live-birth rate increases with the number of oocytes and is higher among younger women (≤35 years of age) than among older women (>35 years of age). Data are from Cobo et al.[26]

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Fertility preservation - women

- Ovarian tissue cryopreservation

  * only available option for paediatric patients and in hormone-dependent diseases.

  * no ovarian stimulation

  * allows cancer treatment with little delay

  * ovarian cortex is rich with premordial follicles

  * reimplantation has the potential of restoring fertility and hormonal function

  * spontaneous pregnancy possible with ortotopic transplantation (>130 live births reported, June -17)

  * reseeding malignant cells remains a concern

Ovarian tissue cryopreservation - transplantation

- FertiProtekt (Germany, Austria, Switzerland)
  - Safe laparoscopic procedure – complication only **0.2%** (1302 removals and 71 transplantations)
  - 58 women received transplantation
  - Hormonal activity in 63% (E2 >100 pmol/l at 6 mos
  - Return on menses in 81 %
  - Pregnancies in 16 women (22%)
- Earlier a pregnancy rate of 14.5% has been reported

Beckmann et al 2018 RBMOnline

Indications for ovarian tissue cryopreservation

Box 2 | Indications for cryopreservation of ovarian tissue

Malignant pathology

Systemic diseases

- Hodgkin lymphoma
- Non-Hodgkin lymphoma
- Leukaemia
- Medulloblastoma

Extrapelvic diseases

- Bone cancer (osteosarcoma, Ewing sarcoma)
- Breast cancer
- Melanoma
- Neuroblastoma
- Bowel malignancy

Pelvic diseases

- Pelvic sarcoma
- Rhabdomyosarcoma
- Sacral tumour
- Rectosigmoid tumour
- Early cervical carcinoma
- Early vaginal carcinoma
- Early vulvar carcinoma
- Selected cases of ovarian carcinoma (stage IA)
- Borderline ovarian tumour

Non-malignant pathology

Unilateral or bilateral oophorectomy

- Benign ovarian tumour
- Severe and recurrent endometriosis
- *BRCA1* or *BRCA2* mutation carrier

Risk of premature menopause

- Turner syndrome
- Family history
- Benign diseases requiring chemotherapy: autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis, Behçet's disease, Wegener's granulomatosis)

Bone marrow transplantation

- Benign haematological diseases: sickle cell anaemia, thalassaemia major, aplastic anaemia
- Autoimmune diseases unresponsive to immunosuppressive therapy

Social reasons

- Age of the patient
- Childbearing postponed to later in life for social or financial reasons

Donnez &Dolmans 2013
Nature Reviews Endocrinology

Fertility preservation - women

- Ovarian tissue in vitro culture – follicle maturation
  * no risk of re-introduction of malignant cells to the individual
  * successful in mouse
  * not available for women at present

Fertility preservation - men

- Sperm cryopreservation

  * from the ejaculate or from a testicular biopsy

  * not possible for prepubertal boys – evaluation of maturity to produce a semen sample challenging.

  * spermarche occurs over a wide age range - biopsies have revealed sperm with testicular volume < 5 ml and pubic hair I (Tanner staging).

  * malignant diseases often severely impair spermatogenesis

Fertility preservation - boys

- Testicular tissue cryopreservation

  * the aim is to preserve spermatogonial stem cells

  * they may be used for transplantation (SCCs or testicular tissue) or *in vitro* culture of tissue or SCC

  * transplantation has been successfully performed in animals including non-human primates

  * in rodents tissue culture live pups have been born following in vitro culture of testicular tissue and SCC

  * no reports on humans (nor other primates).

Fertility preservation - Klinefelter

- Testicular tissue cryopreservation
- vast majority of men with Klinefelter sdr (47, XXY)
- Some authors report loss of spermatogenic sites in the testis with age – not all agree
- Testicular biopsy is typically needed to identify spermatozoa in KS, MD-TESE appears to be the most efficient method with a sperm recovery rate of 40 – 60%

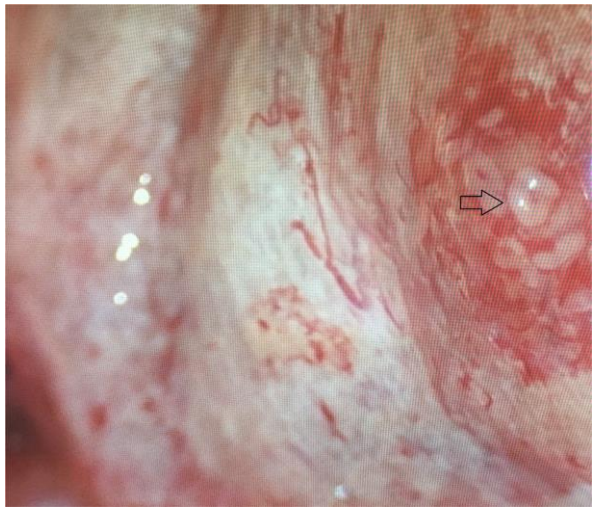

Fig. 2. Microdissection testicular sperm extraction of a man with Klinefelter

Panel 2: The Edinburgh Selection Criteria for gonadal tissue cryopreservation

These criteria were established with ethics committee review and approval because they refer to experimental procedures, and should be regarded as a starting point for future discussion, research, and refinement.

Female patients[112]

- Age younger than 35 years
- No previous chemotherapy or radiotherapy if aged 15 years or older at diagnosis, but mild, non-gonadotoxic chemotherapy is acceptable if younger than 15 years
- A realistic chance of 5-year survival
- A high risk of premature ovarian insufficiency (>50%)
- Informed consent (parent and, when possible, patient)
- Negative HIV, syphilis, and hepatitis serology
- Not pregnant and no existing children

Male patients

- Age 0–16 years
- A high risk of infertility (>80%)
- Unable to produce a semen sample by masturbation
- No clinically significant pre-existing testicular disease (eg, cryptorchidism)
- Informed consent (parent and, when possible, patient)
- Negative HIV, syphilis, and hepatitis serology

Anderson et al 2015

Risk of reimplanting malignant cells

Box 3 | Risk of ovarian metastasis according to cancer

High risk (>11%)

- Leukaemia
- Neuroblastoma
- Burkitt lymphoma

Moderate risk (0.2–11%)

- Breast cancer (stage IV infiltrating lobular subtype)
- Colon cancer
- Adenocarcinoma of the cervix
- Non-Hodgkin lymphoma
- Ewing sarcoma

Low risk (<0.2%)

- Breast cancer (stage I–II infiltrating ductal subtype)
- Squamous cell carcinoma of the cervix
- Hodgkin lymphoma
- Osteogenic carcinoma
- Non-genital rhabdomyosarcoma
- Wilms' tumour

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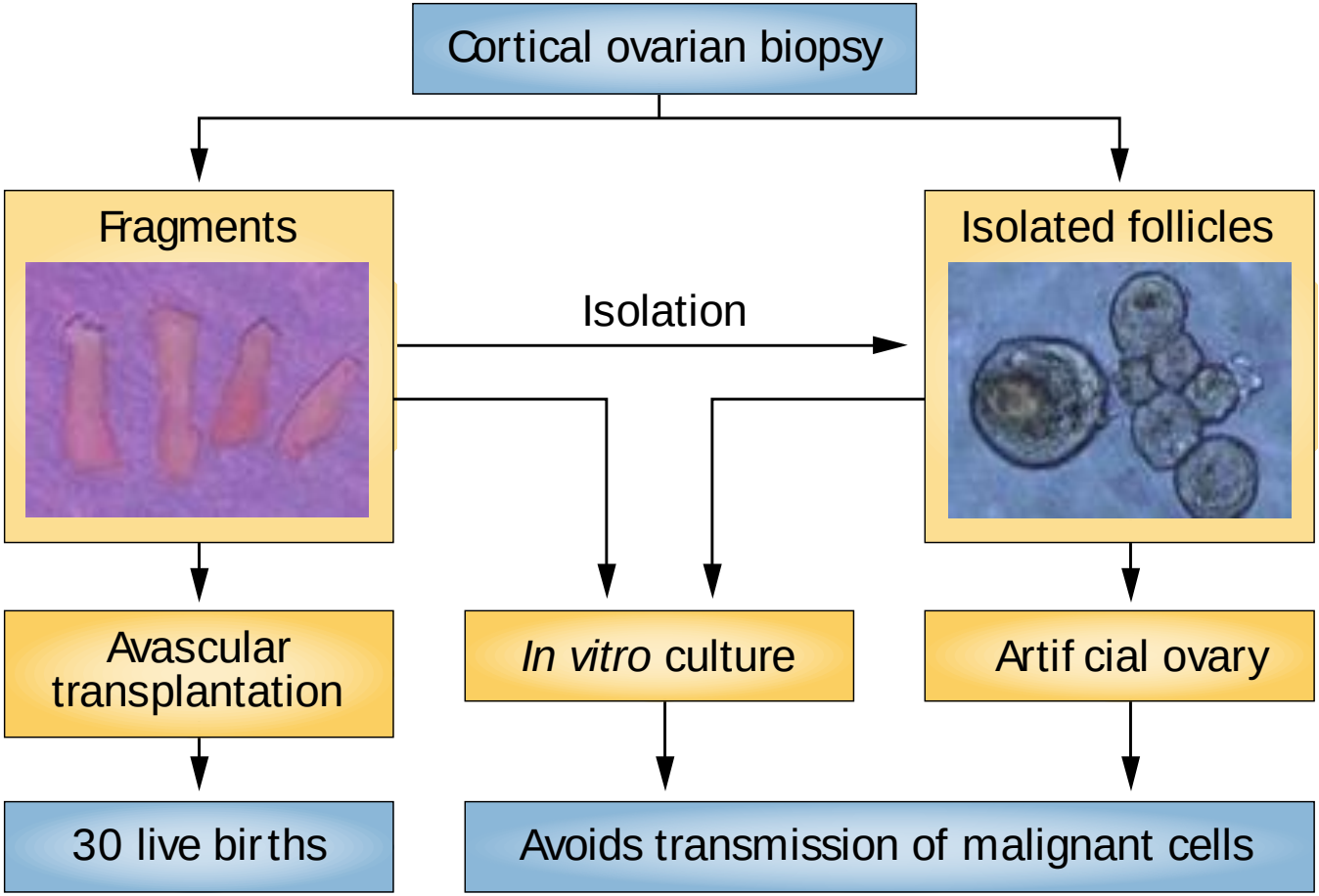
Option of ovarian tissue utilization



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The Future

- Artificial ovary

  -transferring primordial follicles onto a biocompatible and biodegradable “scaffold” (fibrin or alginate) would eliminate the risk of transmission of malignant cells.

  - survive and grow 1 wk after autotransplant

  -low recovery of follicles (prim & sec)

  -significant loss after grafting

Luyckx et al. 2014 F&S; Chiti et al. 2016 Hum Repro

The Future

- growing oocytes from the early stages of follicle

  - a dynamic multistep culture system is required to support each of the transitional stages of follicles. e.g. bidirectional communication between oocyte and the granulosa cells.

  - ovarian stem cells derivation might interfere with the complex genomic imprinting and epigenetic mechanisms required for fully competent oocytes

Telfer & Zelinski 2013 F&S

The Future

- Cyclophosphamide activates primordial follicle growth –> loss of reserve
- This “burn-out” effect was prevented by immunomodulator **AS101**
- Whether AS101 will have a role in clinical fertility preservation requires further studies
- **S1P** is a sphingolipid metabolite that inhibits radiation and chemotherapy induced cell apoptosis in mice. Possible potential in fertility preservation in human.

Kalich-Philosoph et al 2013; Soleimani et al 2011

Fertility preservation

- Several option exist for fertility preservation - individual counseling should be available prior to fertility compromising interventions.

- Despite technical challenges in certain patient groups (e.g. prepubertal boys), currently the major obstacle in ensuring the option of appropriate fertility preservation is the improvement of the service provision to match the technical and scientific advances

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Nightless night – lightless winter

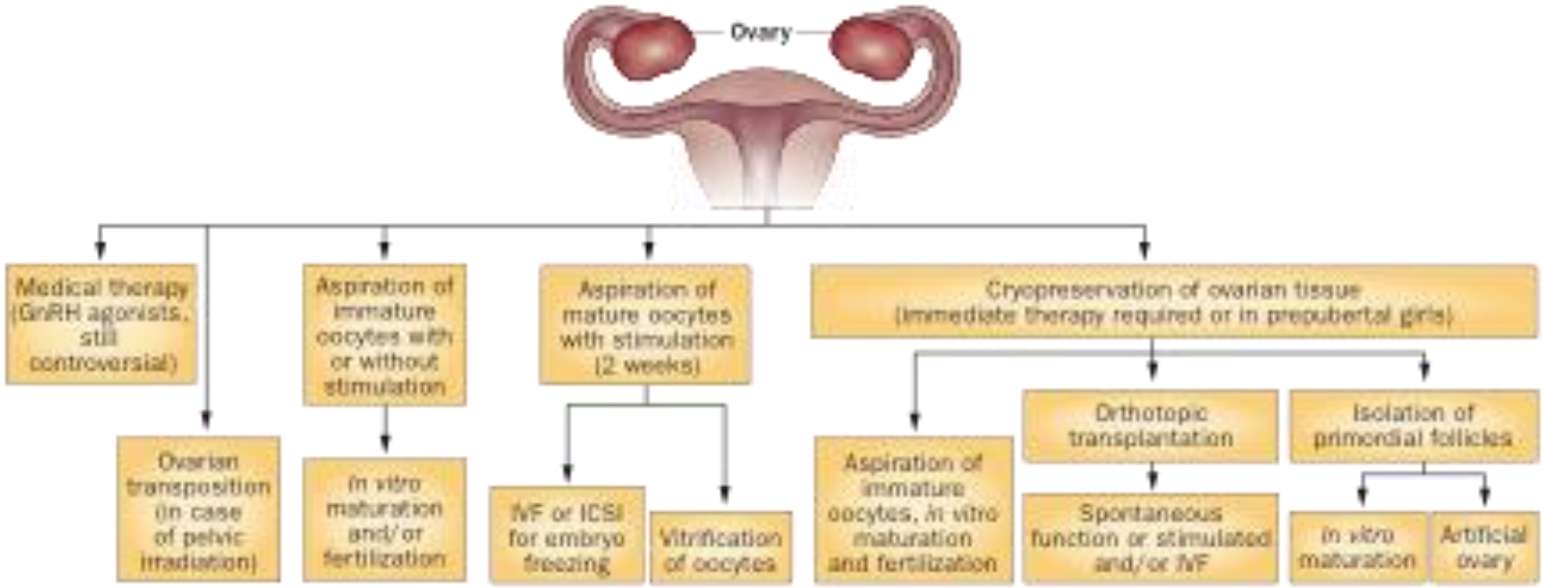

Figure 4 | Fertility preservation methods in women at risk of premature ovarian failure. Depending on the patient's age and the possible delay before starting chemotherapy, options include: medical therapy to protect the ovaries from chemotherapy (still controversial); ovarian transposition before pelvic radiotherapy; immature oocyte retrieval followed by *in vitro* maturation; mature oocyte retrieval followed by fertilization and embryo freezing or oocyte vitrification; or ovarian tissue cryopreservation. At the time of ovarian tissue cryopreservation, immature oocytes can also be retrieved. After thawing of ovarian tissue, orthotopic transplantation is currently the goal in clinical practice. Indeed, isolation of primordial follicles is still at the research stage. Abbreviations: GnRH, gonadotropin-releasing hormone; ICSI, intracytoplasmic sperm injection; IVF, *in vitro* fertilization. Permission obtained from Elsevier © Donnez, J. *et al. Fertil. Steril.* **99**, 1503–1513 (2013).

Donnez &Dolmans 2013
Nature Reviews Endocrinology

Cytotoxic treatments

- Cyclophosphamide causes most damage to oocytes (busulfan > 600 mg / m2, cyclophosphamide > 7.5 g/m2 or ifosfamide > 60 g / ms) North American Children's Oncology Group
- Pelvic radiotherapy 5 – 10 Gy toxic to oocytes, 2 Gy -> 50% of primordial follicles destroyed.

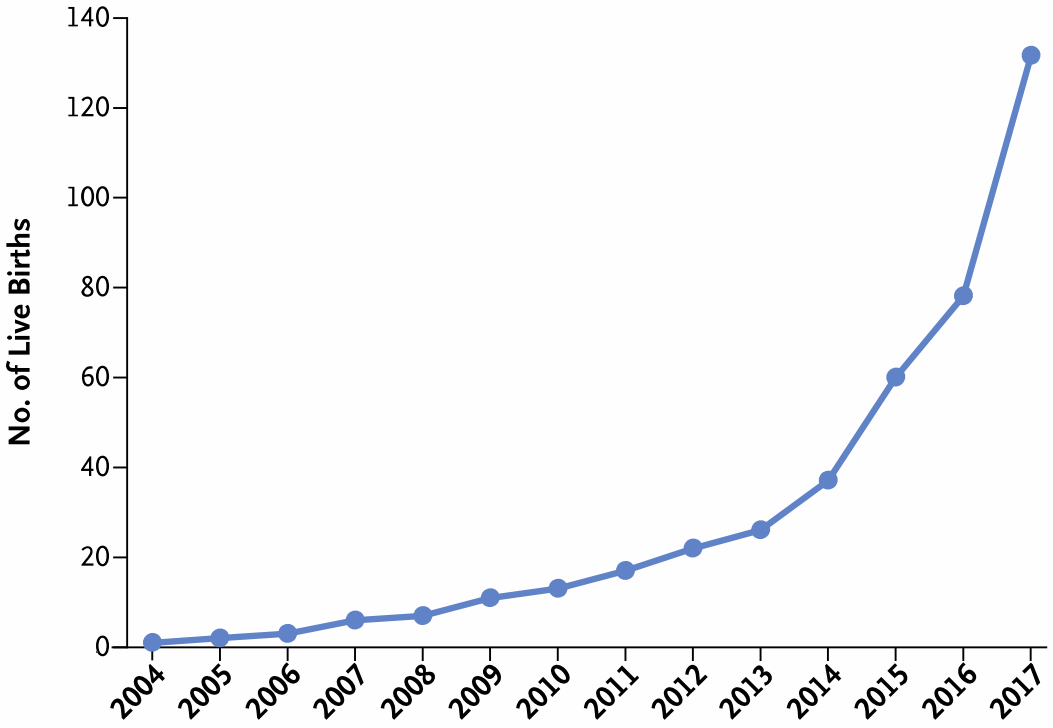

Figure 3. Reimplantation in an Orthotopic Site.

Since 2004, when the first pregnancy after reimplantation in an orthotopic site (namely, a site in the pelvic cavity) was reported, the number of live births has reached more than 130, showing a logarithmic increase during the past 2 years and highlighting the need to move from experimental studies to widespread clinical application.

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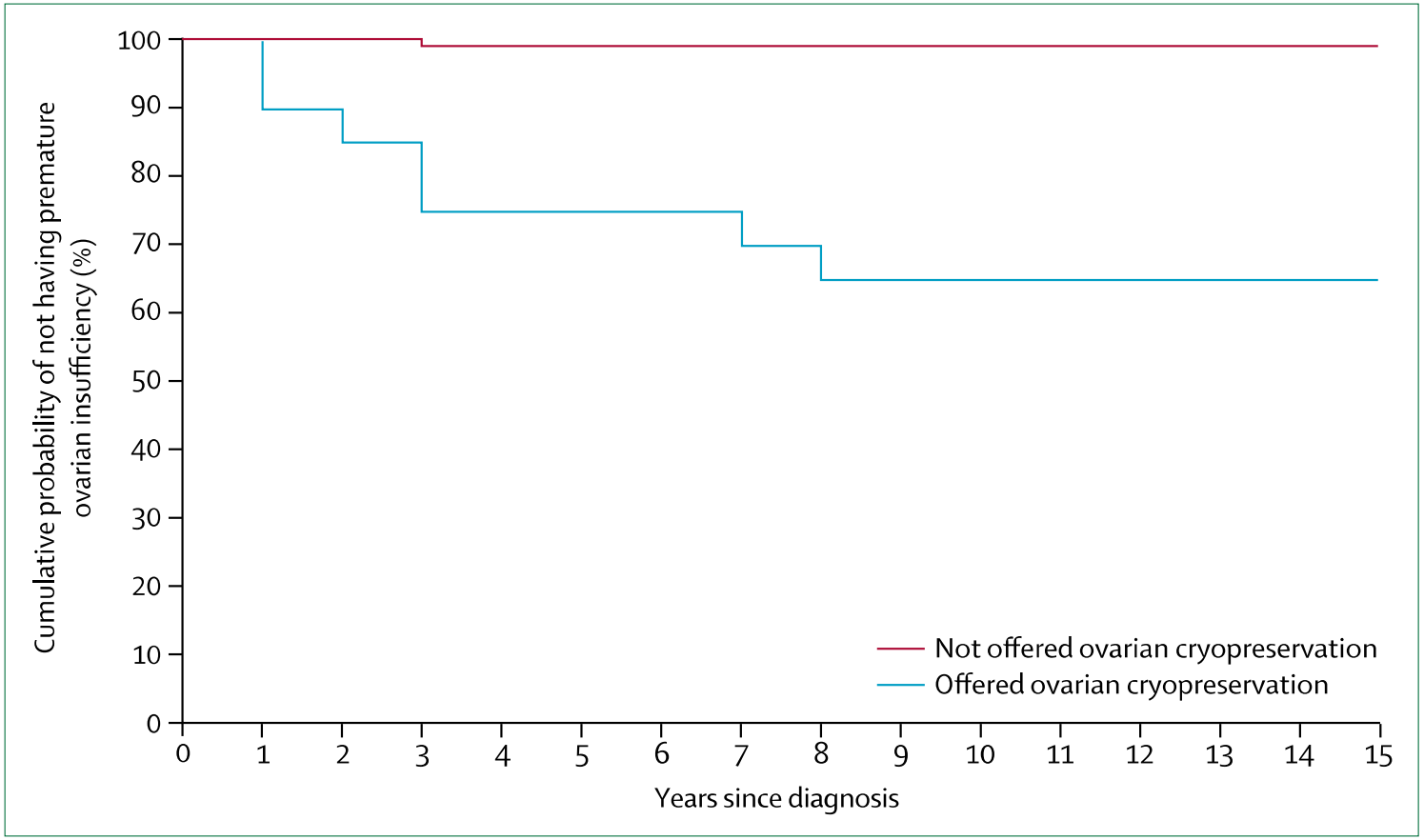
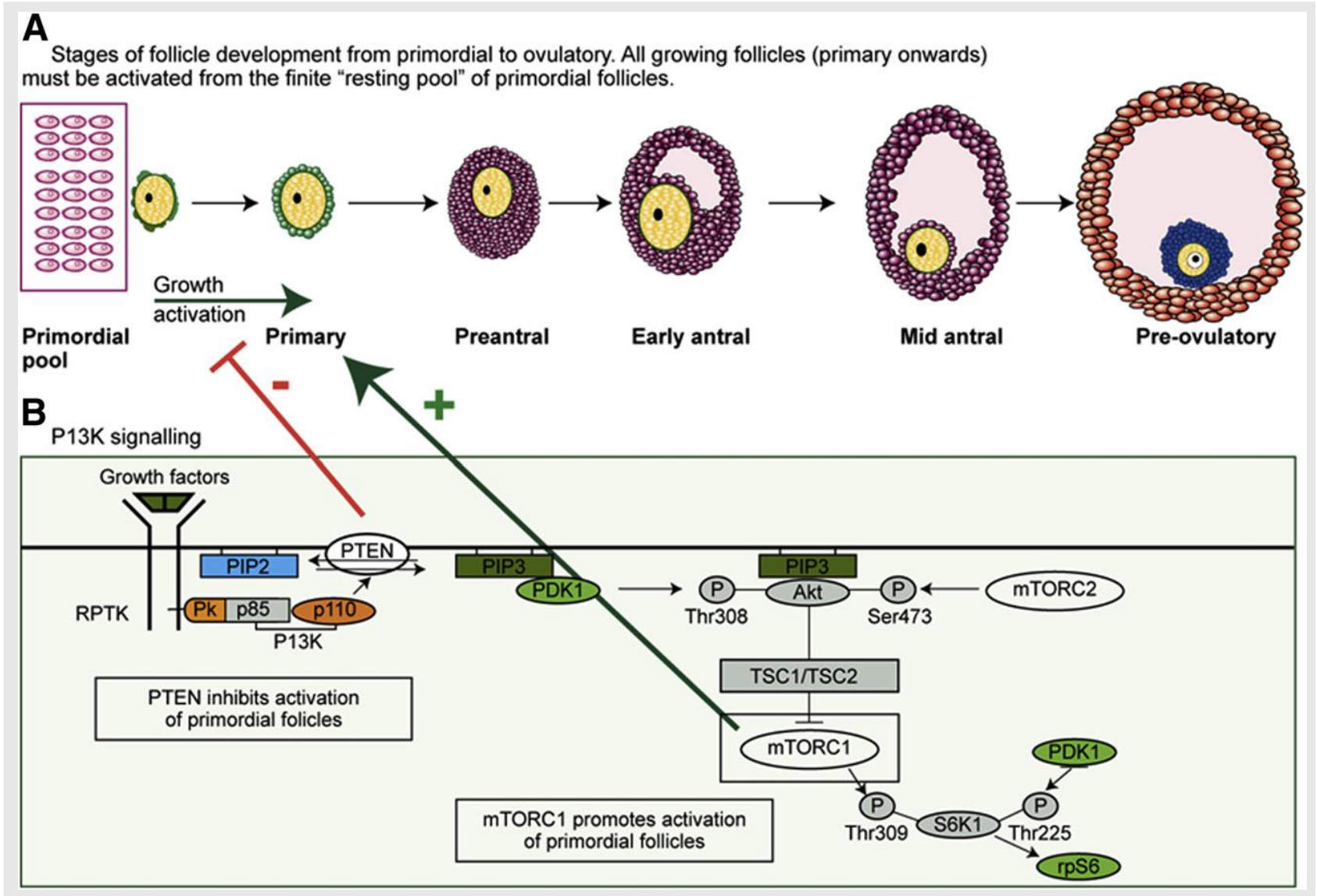


Figure 6: **The cumulative probability of not having premature ovarian insufficiency after diagnosis for patients offered ovarian cryopreservation (blue line) and those not offered ovarian cryopreservation (red line)**
The cumulative probability of developing premature ovarian insufficiency after treatment was significantly higher for the patients offered ovarian tissue cryopreservation than for those who were not offered ovarian tissue cryopreservation (15-year probability 35% [95% CI 10–53] versus 1% [0–2]; p<0·0001; hazard ratio 56·8 [95% CI 6·2–521·6]). Reproduced from Wallace and colleagues,[112] by permission of Elsevier.

Anderson et al 2015

Oocyte freezing

- < 35 y old women the cumulative LBR after oocyte vitrification was 15.4% when only 5 oocytes were used. (40.8% / 8 & 60.5% / 10)
- > 35 y old the CLBR was 5.1 % with 5 oocytes, 19.9% with 8 and 29.7% with 10 oocytes
- With 10 oocytes the CLBR was double in < 35 y women compared to >35 y women.



Telfer 2013 F&S

(A) Diagrammatic representation of follicle growth from the nonproliferating pool of primordial follicles. Primordial follicles are continuously activated into the growing population, where they become primary follicles consisting of an oocyte arrested at the dictyate stage of prophase I of meiosis (*yellow*) surrounded by granulosa cells (*green*). Primary follicles undergo oocyte growth and granulosa cell proliferation and differentiation (*purple*), when they form an antral cavity. Antral follicles continue to grow, and granulosa cells differentiate into two subpopulations of cells: 1) cumulus surrounding the oocyte (*blue*); and 2) mural lining the wall of the follicle (*orange*). Exact timings for this developmental sequence in humans are not known; estimations suggest several months, but it is not known whether the growth profile is continuous or whether there are ''resting'' phases through follicle development. (B) Simplified version of the phosphatidylinositol-3′-kinase (PI3K) pathway. The factors initiating this process are largely unknown, but a body of evidence is emerging to show that the PI3K-Akt signaling pathway is a major regulator of early follicle/oocyte development and that components of this pathway are involved in controlling the rate of activation from the nongrowing population of follicles. The phosphatase PTEN converts phosphatidylinositol phosphate (PIP) 3 to PIP2, which negatively regulates PI3K activity. Signaling mediated by PI3Ks converge at phosphatidylinositol phosphate dependent kinase-1 (PDK1). Phosphatidylinositol-dependent kinase (PDK) 1 phosphorylates Akt and activates it. Akt can phosphorylate and inactivate tuberous sclerosis complex 2 (TSC2, or tuberin), which leads to the activation of mammalian target of rapamycin complex 1 (mTORC1). mTORC1 can phosphorylate (activate) S6 kinase 1 (S6K1). S6K1 subsequently phosphorylates and activates ribosomal protein S6 (rpS6), which enhances protein translation that is needed for cell growth. mTORC1 can be inhibited pharmacologically with rapamycin and stimulated by leucine. The manipulation of this pathway could have important clinical applications in the field of fertility preservation. RPTK = receptor protein tyrosine kinase.

Telfer. Advances in ovarian follicle culture. Fertil Steril 2013.

Telfer 2013 F&S

Figure 1. Options for Fertility Preservation.

If the patient is prepubertal or requires immediate chemotherapy (Panel A), ovarian tissue is removed in the form of multiple biopsy specimens (or an entire organ) and cut into cortical strips. The tissue is then cryopreserved by slow freezing on site (or transported to a processing site at a temperature of 4°C). After thawing, if there is no risk of transmitting malignant cells, the ovarian tissue can be grafted to the ovarian medulla (if at least one ovary is still present) or reimplanted inside a specially created peritoneal window. If there is a risk of transmitting malignant cells, ovarian follicles can be isolated and grown in vitro to obtain mature eggs, which can then be fertilized and transferred to the uterine cavity. Isolated follicles may be placed inside a scaffold (alginate or fibrin), creating an artificial ovary that can be grafted to the ovarian medulla or peritoneal window. If the patient is postpubertal and chemotherapy can be delayed for approximately 2 weeks (Panel B), mature oocytes can be removed after ovarian stimulation and vitrified on site. After thawing, they can be inseminated and transferred to the uterine cavity in the form of embryos. This technique can also be used in women with benign diseases or in those with age-related fertility decline. The techniques in Panels A and B can also be combined, with ovarian-tissue cryopreservation followed by controlled ovarian stimulation and vitrification of oocytes. The combined technique theoretically yields a 50 to 60% chance of a live birth.

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Table 1. Indications for Fertility Preservation.

Malignant diseases requiring gonadotoxic chemotherapy, radiotherapy, or bone marrow transplantation
Hematologic diseases (leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma)
Breast cancer
Sarcoma
Some pelvic cancers
Nonmalignant conditions
Systemic diseases requiring chemotherapy, radiotherapy, or bone marrow transplantation
Ovarian diseases
Bilateral benign ovarian tumors
Severe and recurrent ovarian endometriosis
Possible ovarian torsion
Risk of premature ovarian insufficiency
Family history
Turner's syndrome
Personal reasons
Age
Childbearing postponed until later in life

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