PRECONGRESS COURSE 16

Addressing the broad scope of fertility preservation

Special Interest Group Fertility Preservation

Barcelona – Spain, 1 July 2018

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SCIENCE MOVING
PEOPLE
MOVING SCIENCE

Addressing the broad scope of fertility preservation

Barcelona, Spain
1 July 2018

Organised by
the Special Interest Group Fertility Preservation

Contents

Course coordination

Richard Anderson (United Kingdom), Kirsten Louise Tryde Macklon (Denmark), Michael von Wolff (Switzerland), Jan-Bernd Stukenborg (Germany), Clara Gonzalez Llagostera (Spain)

Course type

Basic

Course description

This course will cover the wide scope of current practice in FP, highlighting some of the key issues and addressing areas where there are gaps in our understanding of how best to organise and provide an effective service.

Target audience

All involved in fertility preservation: clinicians, nurses, embryologists, scientists, counsellors

Educational needs and expected outcomes

A needs assessment for this course identified that this is a rapidly changing area of medical practice with wide variation in practice between centres and countries. There is a lack of European guidelines on this subject, though this is in development, and US-based guidlelines have recently been updated. There is thus a strong need for educational events to discuss, inform and support best clinical practice and identify the main areas where further research and development is needed.

Scientific programme

Chair:	Kirsten Louise Tryde Macklon, Denmark
09:00 - 09:30	The impact of cancer treatment on female fertility Richard Anderson, United Kingdom
09:30 - 09:45	Discussion
09:45 - 10:15	Emergency ovarian stimulation for egg cryopreservation Michael von Wolff, Switzerland
10:15 - 10:30	Discussion
10:30 - 11:00	Coffee Break
Chair:	Jan-Bernd Stukenborg, Sweden
11:00 - 11:30	Spermatogenetic recovery in long term survivors of childhood cancer and hematopoietic stem cell transplantation Kirsi Jahnukainen, Finland
11:30 - 11:45	Discussion
11:45 - 12:15	How effective are we at generating pregnancies from women who have had 'fertility preservation'? Ana Cristina Cobo Cabal, Spain
12:15 - 12:30	Discussion
12:30 - 13:30	Lunch
Chair:	Clara Gonzalez Llagostera, Spain
13:30 - 14:00	In vitro gametogenesis - Status report Stefan Schlatt, Germany
14:00 - 14:15	Discussion
14:15 - 14:45	Between the patient and the cryotank: tissue transport and freezing Stine Gry Kristensen, Denmark
14:45 - 15:00	Discussion
15:00 - 15:30	Coffee break
Chair:	Richard Anderson, United Kingdom
15:30 - 16:00	FP in transgender patients Kelly Tilleman, Belgium
16:00 - 16:15	Discussion
16:15 - 16:45	Is social egg freezing a promise or a panacea? Gillian Lockwood, United Kingdom
16:45 - 17:00	Discussion
17:00 - 18:00	SIG Fertility Preservation members' meeting

The impact of cancer treatment on female fertility

Richard A Anderson
MRC Centre for Reproductive Health
University of Edinburgh

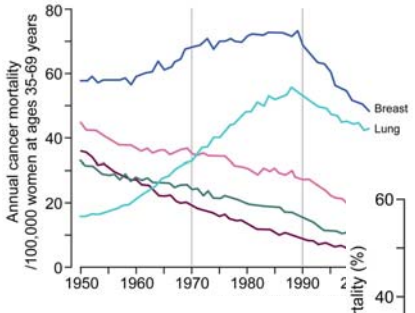
Conflicts of interest

- Research support /speaker for Roche Diagnostics and Beckman Coulter, IBSA, Merck, Ferring Pharmaceuticals
- Consultancy for Roche Diagnostics, HRA Pharma, KnDy therapeutics

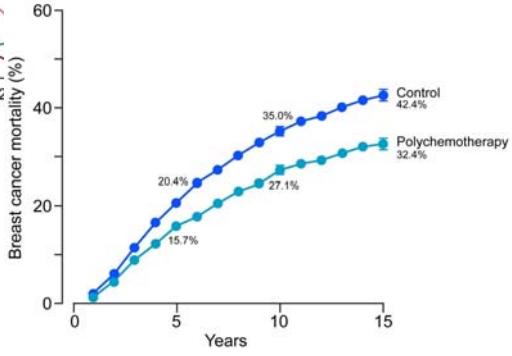
Outline of talk: key points

- Which cancer treatments can affect female fertility, and how
  - Ovary
  - Uterus
  - Hypothalamus/pituitary
- Evidence for effects on female reproductive function
  - Surrogate markers
  - Fertility
  - Reproductive lifespan

Improving survival: minimising 'late effects'

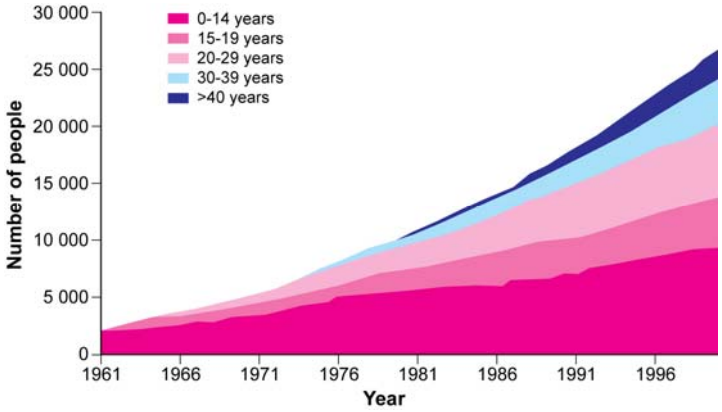


polychemotherapy reduces the annual breast cancer death rate by 38%



Early Breast Cancer Trialists' Collaborative Group. *Lancet* 2005;365:1687.

Childhood cancer survivors by current age



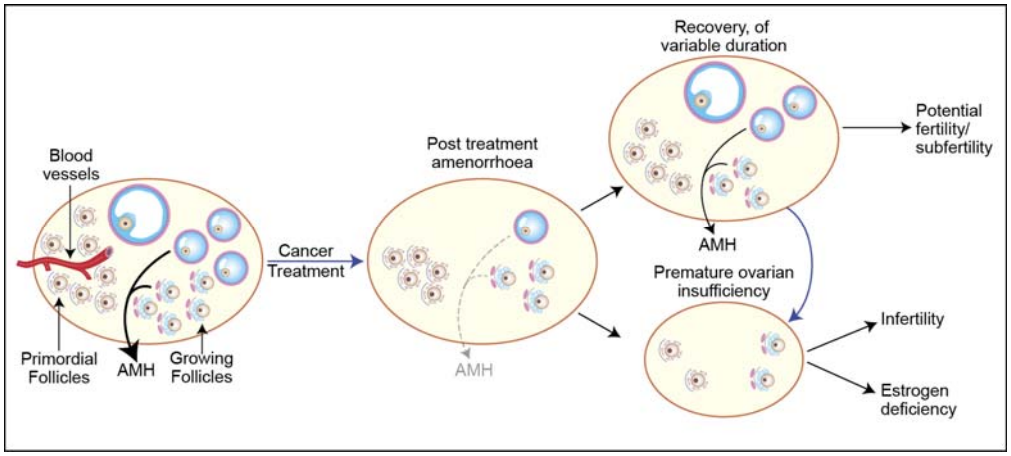
Long-term survival rate from childhood cancer is **80%**
1 in 700 adults is a childhood cancer survivor

Skinner et al 2006 Lancet Oncology 7:489

The broader 'survivorship' agenda

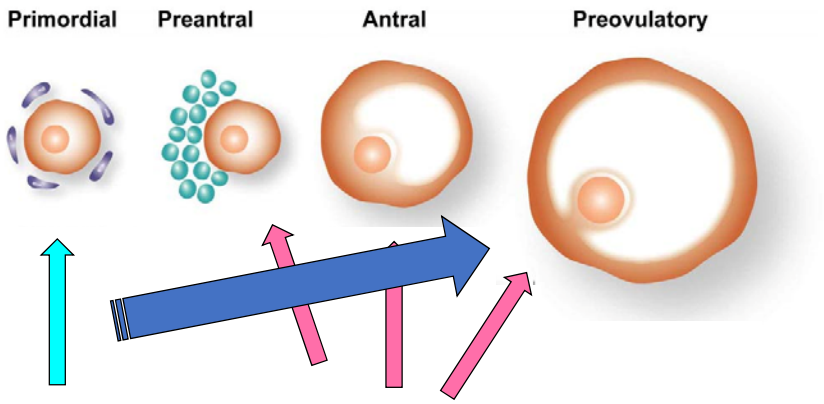
- Most cancer survivors have significant health issues
  - Oeflinger et al NEJM 2006
- Reduced chance of marriage/cohabitation with brain/CNS cancers
  - Frobisher et al Int J Cancer 2007

The variability of ovarian damage



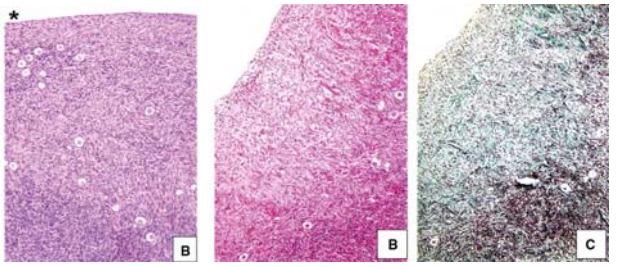
Jayasinghe, Wallace and Anderson 2018

Which stages of follicle growth are key targets of cancer therapies?



Loss of growing follicles leads to increased growth activation: 'Burn out'

The stroma and vasculature are also targets

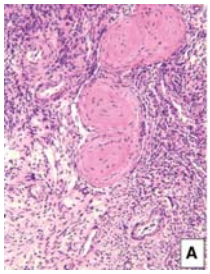


Normal control

After chemotherapy

Green: Masson stain for collagen

Focal cortical fibrosis in ovaries exposed to chemotherapy



Prominent thickening and hyalinization, with narrowing /obliteration of the lumen

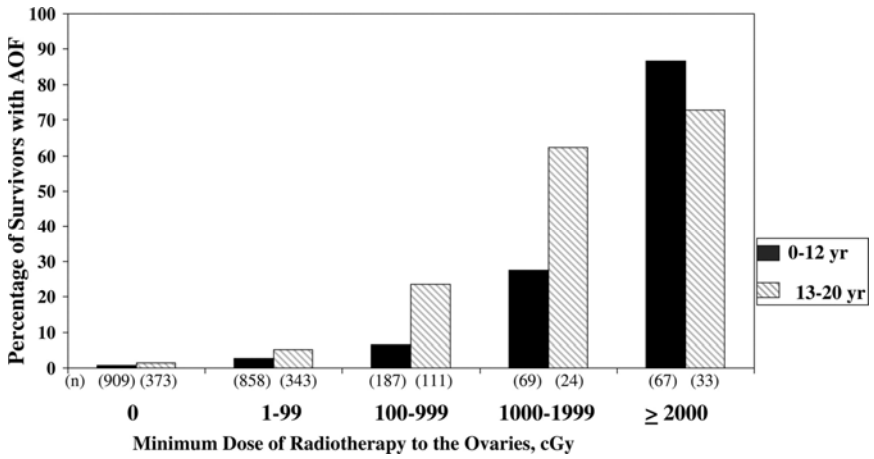
Meirow D et al. Hum. Reprod. 2007;22:1626-1633

Risks of chemo agents to fertility

High risk	Medium risk	Low risk
Cyclophosphamide	Cisplatin	Vincristine
Ifosfamide	Carboplatin	Methotrexate
Chlormethine	Doxorubicin	Dactinomycin
Busulfan	Dacarbazine	Bleomycin
Melphalan	Thiotepa	Mercaptopurine
Procarbazine	Gemcitabine	Vinblastine
Chlorambucil	Cytarabine	Azathioprine
Mechlorethamine	Daunorubicin	Fludarabine
Carmustine		Etoposide
Lomustine		

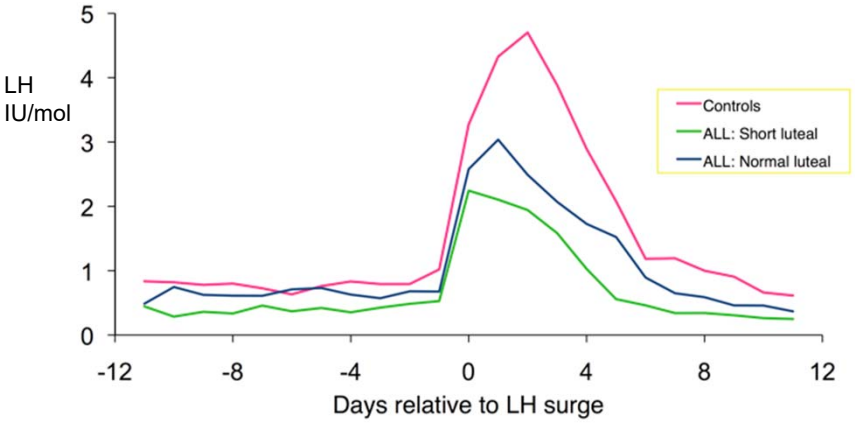
Adapted from ASCO guidelines and others

Ovarian failure and radiation to the ovary



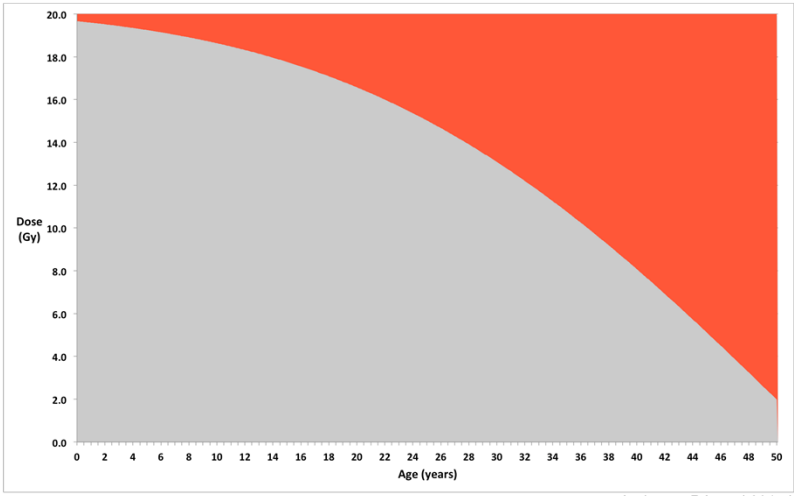
Chemaitilly, W. et al. J Clin Endocrinol Metab 2006;91:1723

Hypothalamic damage: deficient LH surge in women after craniospinal RT for childhood leukaemia



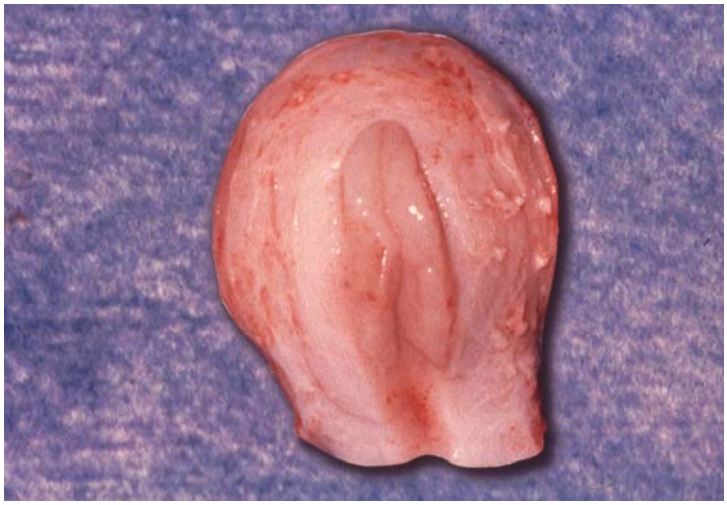
Bath et al 2001 Hum Reprod 16, 1838

Sterilising radiation dose to ovary and age

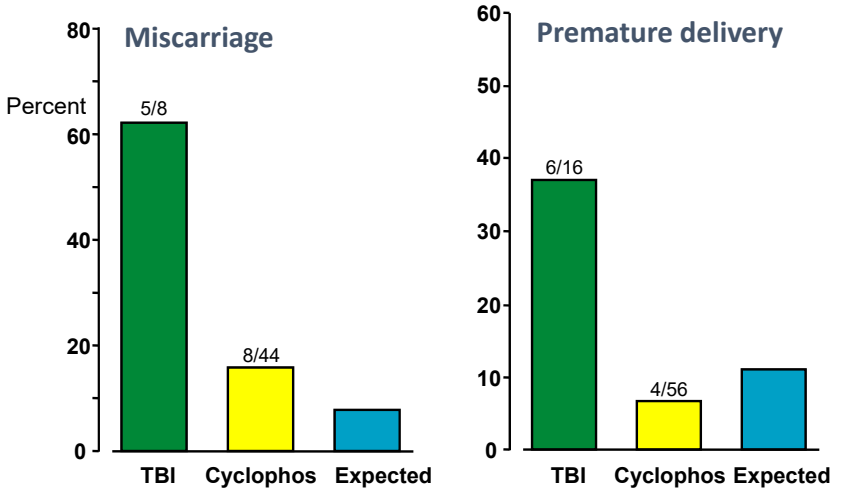


Anderson RA et al 2015 Lancet Diabetes and Endo

The uterus

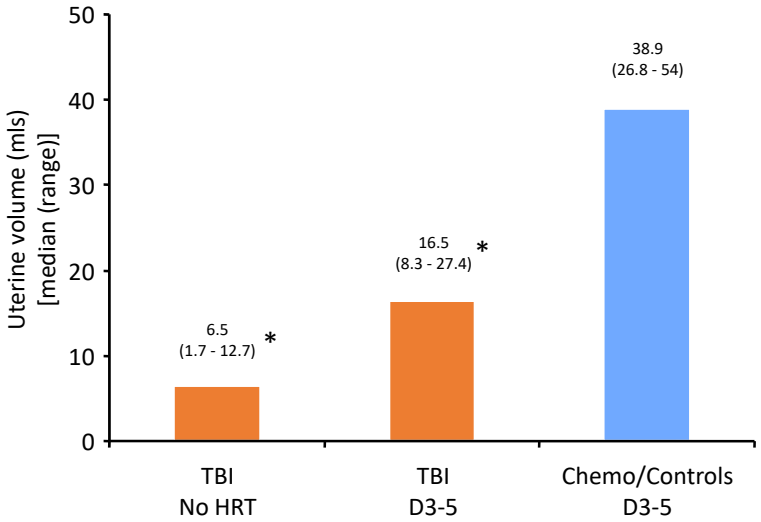


Adverse effect of radiotherapy to uterus



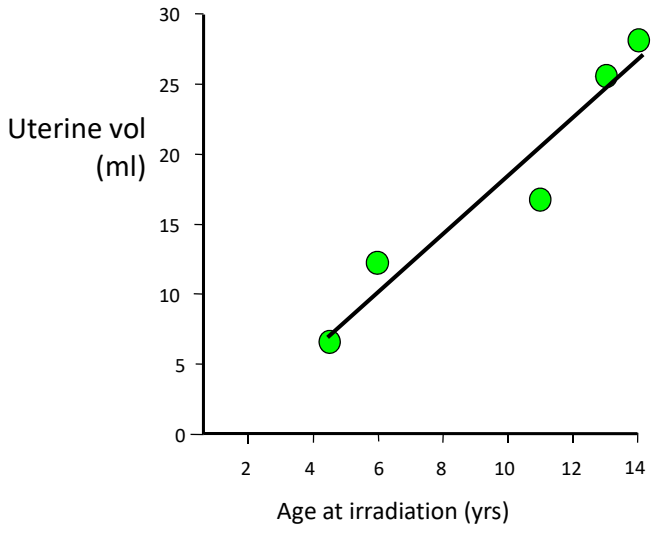
Sanders et al 1996 Blood 87, 3045

TBI and uterine volume



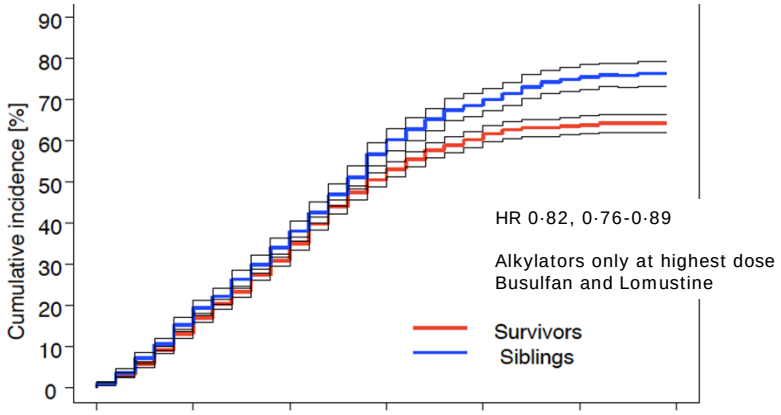
Bath LE et al BJOG 1999

Effect of age at irradiation on adult uterine volume



Bath LE et al BJOG 1999

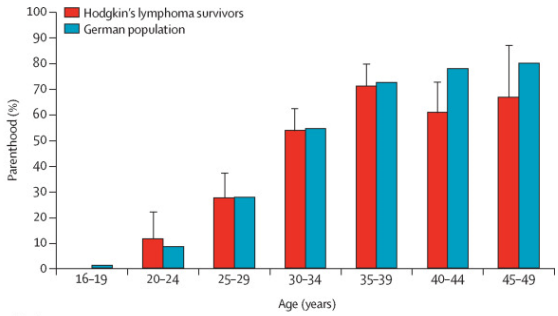
Live birth to female childhood cancer survivors: chemo only



# at risk:							
Survivor	3093	3185	2353	1076	452	197	147
Sibling	1843	1580	1056	567	268	121	75

Chow et al Lancet Oncol 2016

Parenthood in female survivors of Hodgkin lymphoma <18 at diagnosis

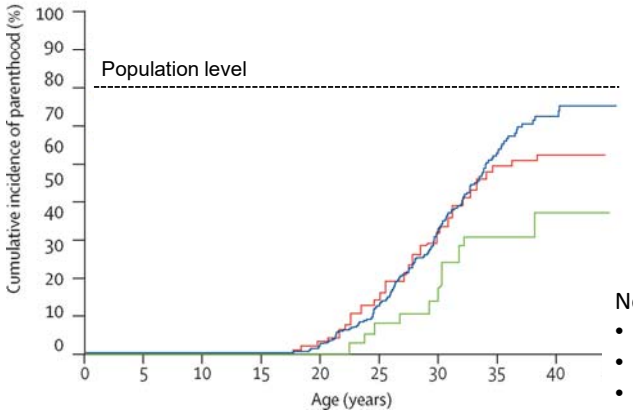


Number with first parenthood/number in age group							
Hodgkin's lymphoma survivors	0/19	4/35	23/84	69/129	78/110	40/66	14/21
German population (×1000)	15/1539	190/2246	645/2335	1284/2362	1609/2228	2208/2847	2596/3244
p value		0·53	0·96	0·84	0·76	0·001	0·13

N=590 aged <18 at diagnosis

Brämswig JH et al 2015 Lancet Oncol 16, 557-675

The impact of pelvic radiotherapy in girls with Hodgkin Lymphoma



RT above diaphragm

RT below diaphragm

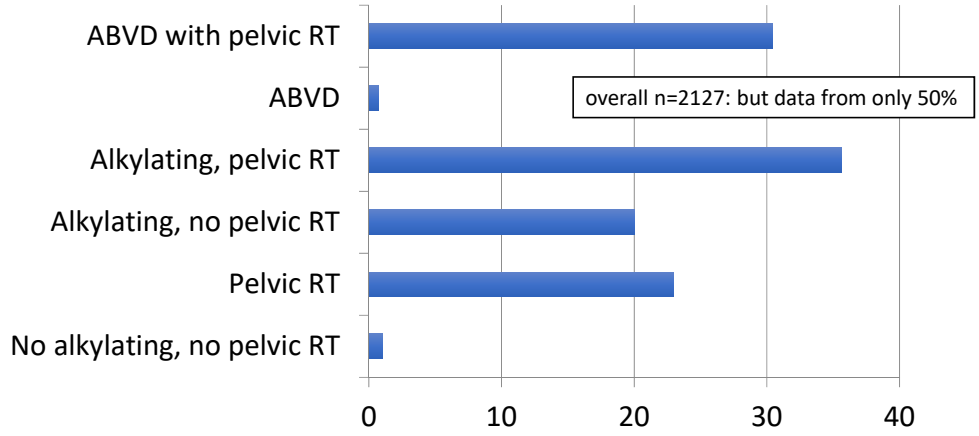
RT of pelvis

Non significant or only minor effects of:

- procarbazine (to 11 400 mg/m^2)
- cyclophosphamide (to 6000 mg/m^2)
- alkylating agent dose scores of 1–5
- treatment protocol
- age at treatment

Brämswig JH et al 2015 Lancet Oncol 16, 557-675

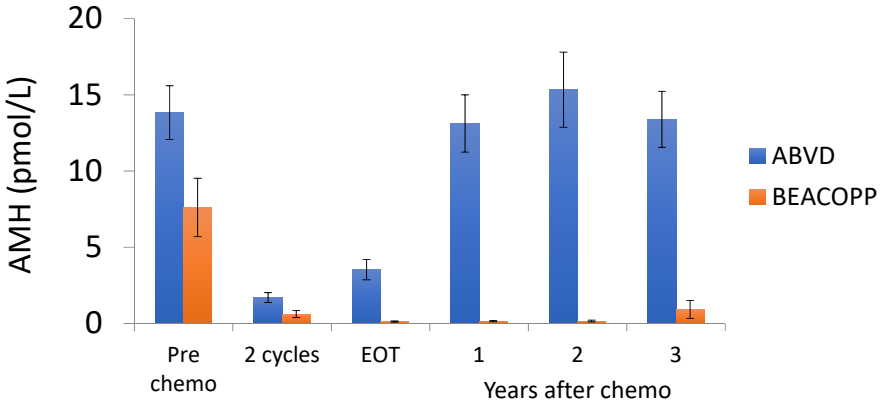
Hazard ratio for menopause <40 yrs in treatment of HL



All adjusted for age

Swerdlow AJ et al 2014, J Natl Cancer Inst

Gonadotoxicity of treatment for adult HL



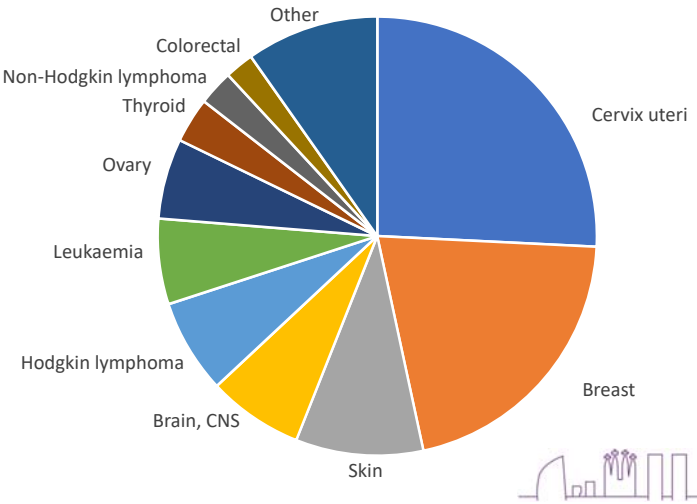
Anderson RA et al unpublished

Population-based analysis of pregnancy after cancer

1981-2012, aged 0-40
23,201 cancer survivors

38% less likely to achieve a pregnancy after diagnosis than women in the general population

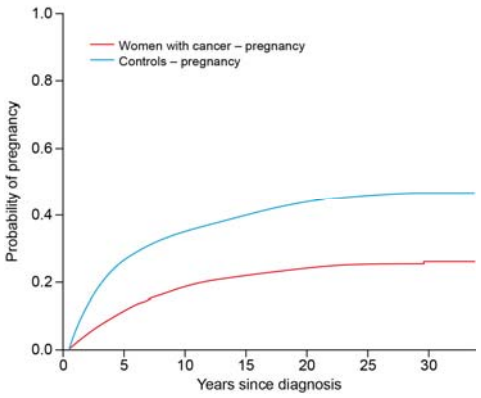
-across all diagnostic groups



RA Anderson et al unpublished

First pregnancy after cancer

- 10,271 women vs 30,881 matched controls
- Proportion achieving a first pregnancy: 20.6% vs 38.7%
- Rate ratio 0.53 (CI 0.51-0.56)

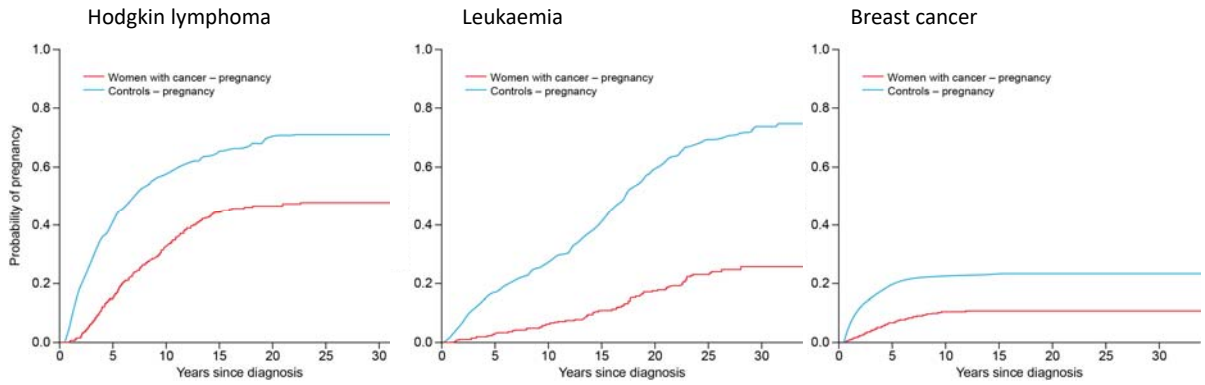


Number at risk

	0 yrs	5 yrs	10 yrs	20 yrs	30 yrs
Cancer	10271	6435	4344	2122	570
Controls	30811	20167	14294	6858	1990

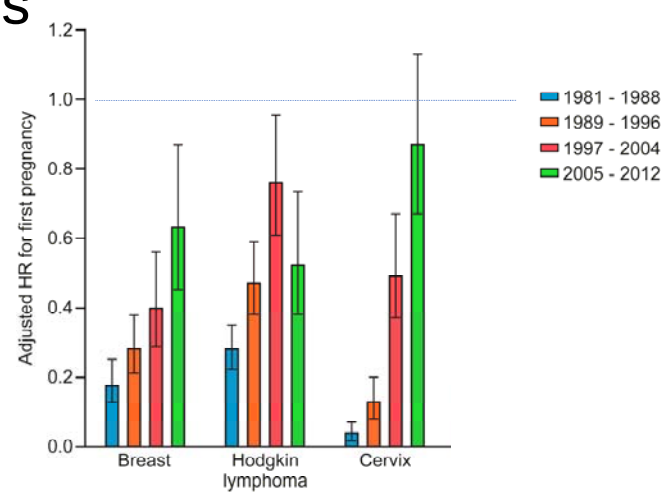
RA Anderson et al unpublished

First pregnancy after cancer



RA Anderson et al unpublished

The changing risk to fertility in some cancers

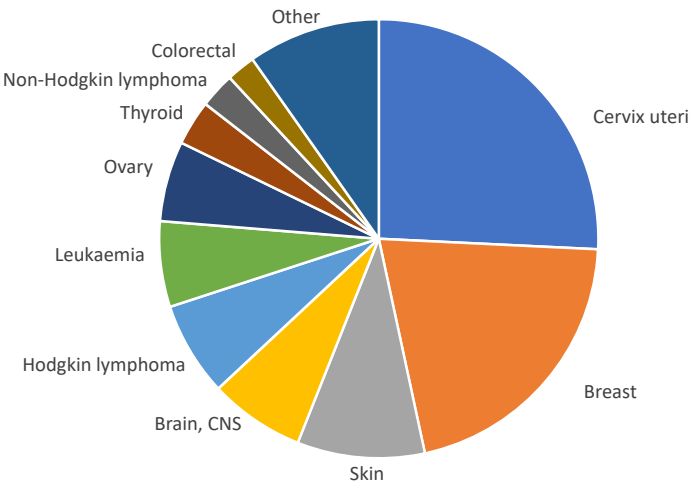


RA Anderson et al unpublished

Reducted chance of pregnancy after all diagnoses

Why is this?

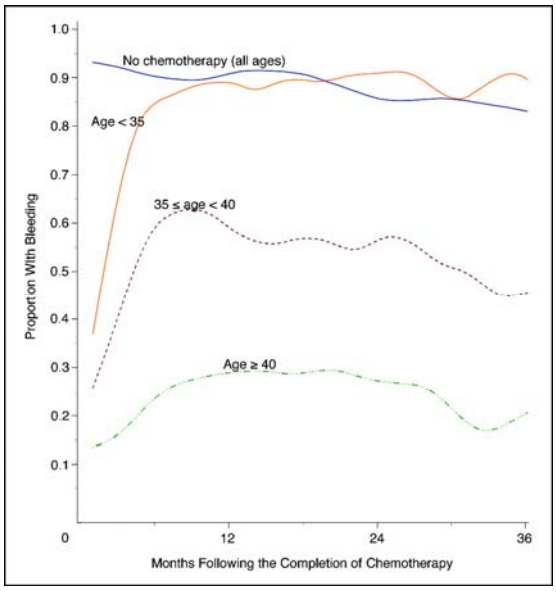
Eg skin cancer:
Unlikely to be 'biological'
Probably 'psychological'
-effect on life choices?



RA Anderson et al unpublished

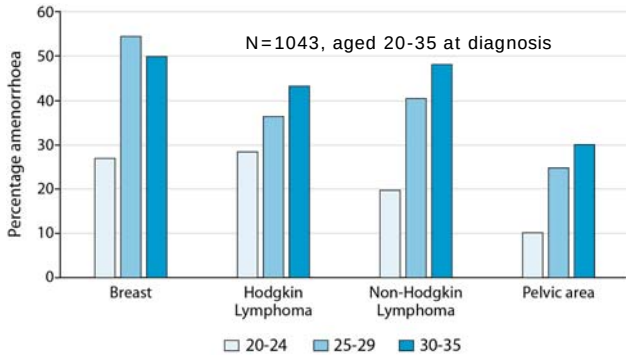
The overarching impact of age

Prevalence of ongoing menses after chemotherapy for early breast cancer



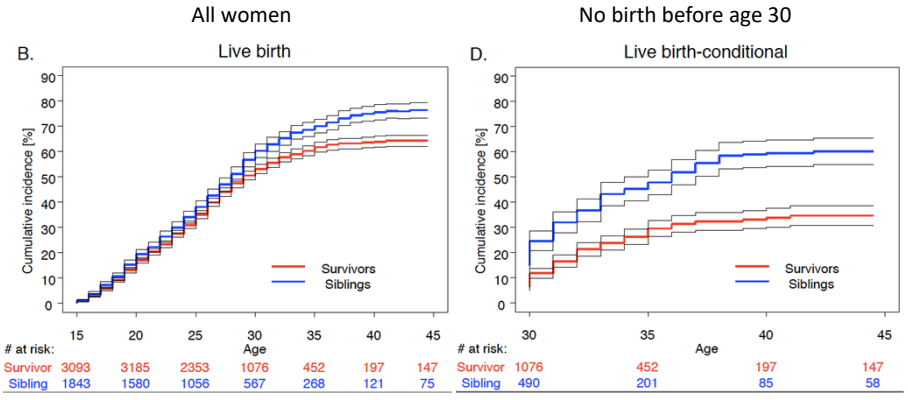
Petrek et al 2006 J Clin Oncol 24 1045

Persisting amenorrhoea by age and diagnosis



From Jacobson et al 2016 Fertil Steril 105, 765-772

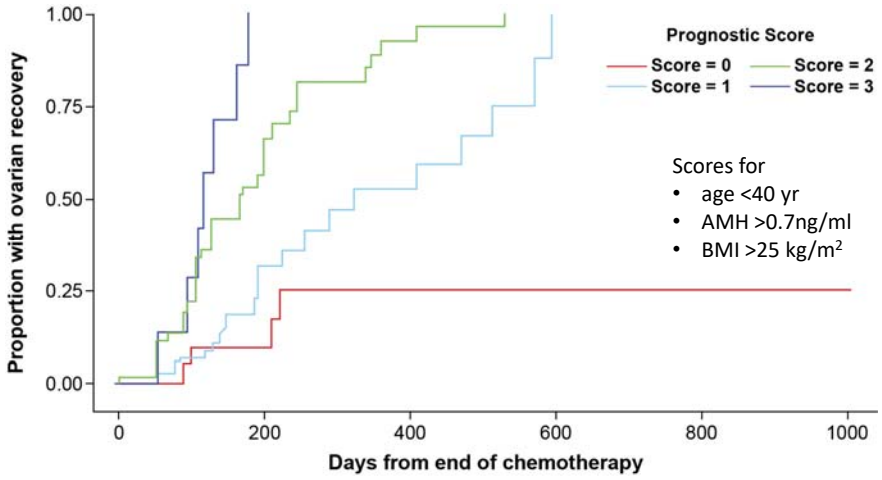
The all-importance of female age



Age <21 at diagnosis, 1970-1999

Chow EJ et al 2016 Lancet Oncol

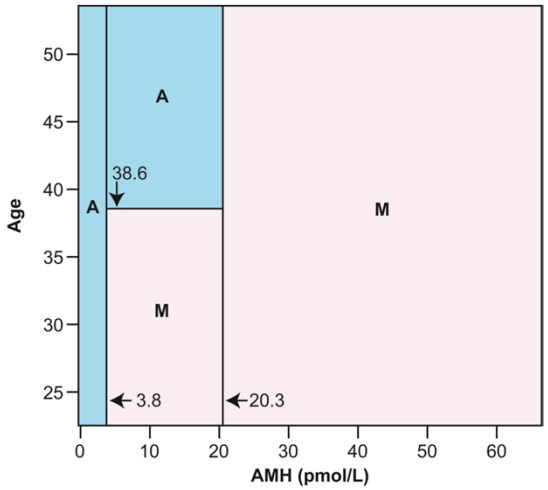
Return of ovarian function after chemo for eBC



Scores for
- age <40 yr
- AMH >0.7ng/ml
- BMI >25 kg/m^2

From Su et al 2014 Cancer

Clinical application of age and pretreatment AMH assessment: predictive mosaic chart

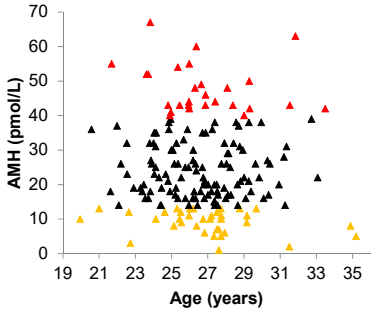


sensitivity 98.2% specificity 80.0% for correct classification of amenorrhoea vs ongoing menses

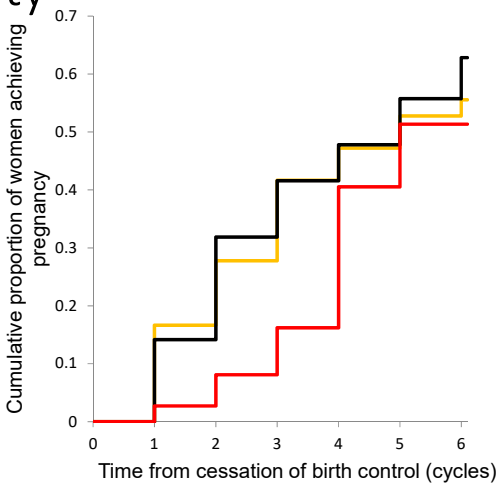
n=75

Anderson and Cameron 2011 JCE&M
Anderson et al 2013 Eur J Cancer

AMH and fecundability



AMH quintiles, middle 3 combined

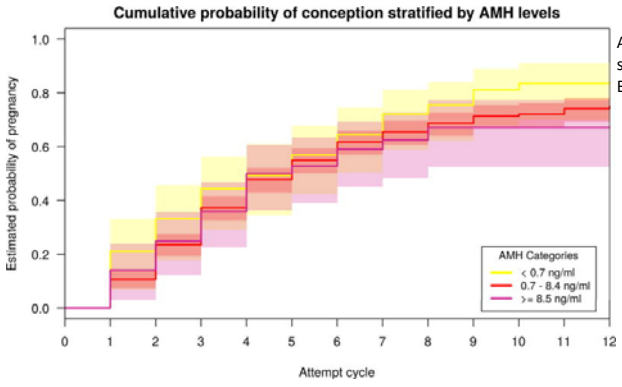


Hagen et al 2012 Fertil Steril

JAMA | Original Investigation

Association Between Biomarkers of Ovarian Reserve and Infertility Among Older Women of Reproductive Age

Anne Z. Steiner, MD, MPH; David Pritchard, MS; Frank Z. Stanczyk, PhD; James S. Kesner, PhD; Juliana W. Meadows, PhD; Amy H. Herring, ScD; Donna D. Baird, PhD, MPH



Adjusted for age, smoking, contraception, BMI, race, prev pregnancy

981 women aged 30 to 44, trying to conceived max 3 months at study entry

Steiner AZ et al, 2017, JAMA

Assessment for fertility risk

- **Intrinsic factors**
  - Health of patient, now and after treatment
  - Consent (patient/parent)
  - Age
  - Assessment of ovarian reserve
- **Extrinsic factors**
  - Nature of predicted treatment
    - high/medium/low/uncertain risk
  - Practicalities
    - Time/Expertise/funding available

Adapted from Wallace WH, Critchley HO and Anderson RA *J Clin Oncol* 2012; **30**: 3-5.

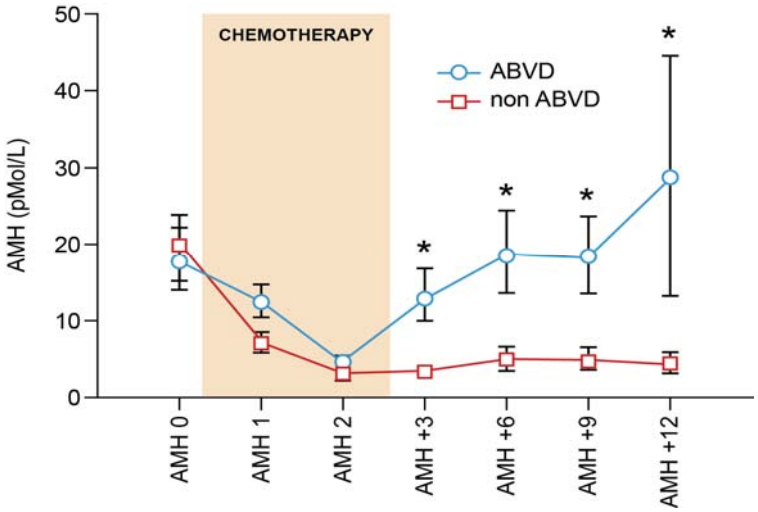
Conclusions

Cancer treatment can have a major impact on female fertility

- With treatment any age
- But many women (and most girls) will retain fertility
- Age is the final arbiter of fertility
- Remember non-ovarian effects too
- Broader issues than gonadal toxicity
  - changing priorities, postchemo endocrine treatment

- Pre-treatment assessment is valuable
- Post-treatment assessment: there will be surprises!

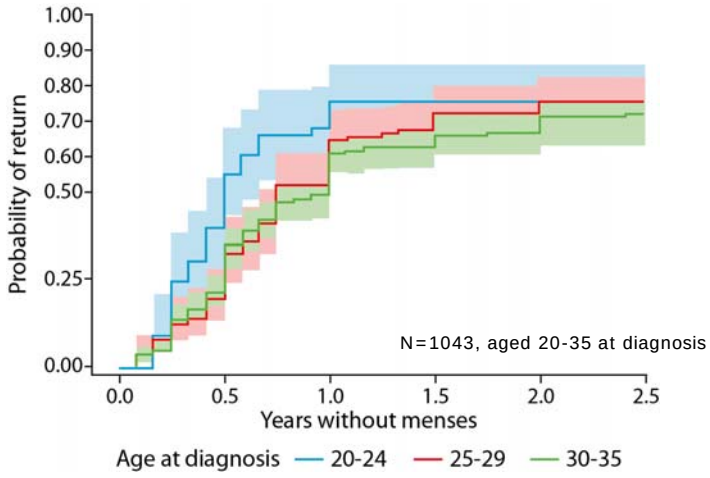
AMH differentiates high and low risk chemo



N=26, HL and NHL

Decanter C et al 2009 RBMOnline

Recovery of menses after chemo by age



Jacobson et al 2016 Fertil Steril 105, 765-772

Emergency ovarian stimulation for egg cryoconservation

INSELSPITAL
UNIVERSITÄTSSPITAL BERN
HOPITAL UNIVERSITAIRE DE BERNE
BERN UNIVERSITY HOSPITAL

Prof. Michael von Wolff

UNIVERSITÄT BERN

University Women`s hospital
Division of Gynecological Endocrinology and Reproductive Medicine

Conflicts of interest

No conflicts of interest regarding this topic

Learning objective

- to understand the efficacy of stimulations
- to understand the risks of stimulations
- to understand and to be able to perform different stimulation protocols
- to understand how to combine stimulations with other fertility preservation techniques

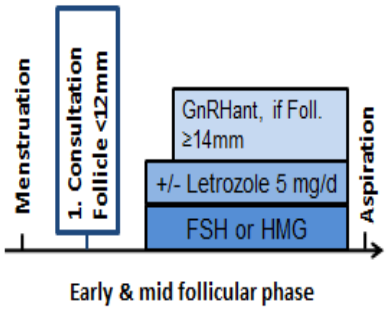
Agenda

- **Standard stimulation protocol**
- Overall efficacy
- Luteal phase stimulation
- Double stimulation
- Progestin primed ovarian stimulation
- Stimulation in breast cancer
- Stimulation in combination with GnRHa depot injections
- Stimulation in combination with ovarian tissue freezing

All stimulations will be evaluated regarding

- Rationale
- Technique
- Efficacy
- Indication

Standard stimulation protocol



- Antagonist protocol
- Gonadotropins at a dosage around 50U higher/day compared to IVF with fresh transfer
- Aromatase inhibitors etc. in breast cancer
- Ovulation induction with 0.2mg of triptoreline

von Wolff et al., Arch Gynecol Obstet, 2018

Agenda

- Standard stimulation protocol
- Overall efficacy
- Luteal phase stimulation
- Double stimulation
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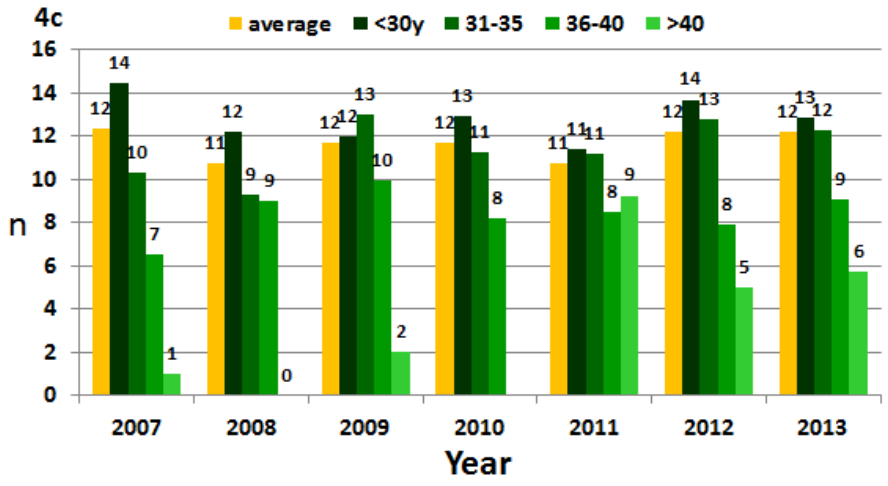
Collected oocytes per stimulation (*Ferti*PROTEKT)



von Wolff et al., RBMonline, 2015

Stimulation efficacy in breast cancer

Human Reproduction, Vol.32, No.3 pp. 568–574, 2017
Advanced Access publication on January 25, 2017 doi:10.1093/humrep/dew355

human reproduction ORIGINAL ARTICLE *Infertility*

Response to ovarian stimulation is not impacted by a breast cancer diagnosis

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Stimulation efficacy in other types of cancer

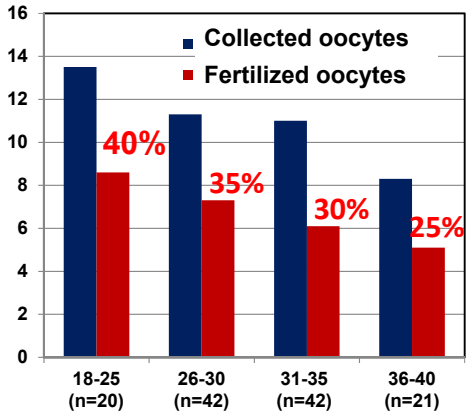
Diseases	Breast cancer n=493	Hodgkin`s lymphoma n=224	non-Hodgkin`s lymphoma n=84	Leukaemia n=25	Sarcoma n=37	Cerebral cancer n=32	Gastrointestinal cancer n=32	Ovarian cancer n=34
Age (years±SD)	32.3±4.2	25.5±4.9	28.4±5.7	27.6±6.1	26.2±5.0	28.7±4.5	30.3±4.6	25.1±5.4
Difference [95% CI]**		**-6.8 [-7.5; -6.0] ¤**	**-3.9 [-5.0; -2.8] ¤**	**-4.7 [-6.6; -2.8] ¤**	**-6.0 [-7.6; -4.5] ¤**	**-3.6 [-5.3; -1.9] ¤**	**-2.0 [-3.7; -0.3]**	**-7.2 [-8.8; -5.6] ¤**
Oocytes total (n±SD)	12.2±8.4	14.3±8.7	12.9±7.8	12.6±9.4	13.1±6.8	16.5±10.5	12.9±6.6	9.1±5.1
Oocyte total (n±SE)*	13.3±0.4	12.6±0.6	12.4±0.9	11.7±1.6	11.8±1.3	16.1±1.4	13.2±1.4	7.3±1.4
Difference [95% CI]**		-0.7 [-2.1; 0.8]	-0.9 [-2.9; 1.0]	-1.6 [-4.9; 1.7]	-1.6 [-4.4; 1.2]	2.8 [-0.1; 5.8]	-0.1 [-3.0; 2.8]	**-6.0 [-9.0; -3.1] ¤**
Days of stimulation (n±SD)	10.5±2.3	11.3±2.7	11.3±2.3	10.6±2.8	10.8±2.0	10.9±1.7	10.5±2.0	11.2±2.5
Days of stimulation (n±SE)*	10.6±0.1	11.2±0.2	11.3±0.3	10.6±0.5	10.7±0.4	10.9±0.4	10.5±0.4	11.1±0.4
Difference [95% CI]**		**0.6 [0.2;1.0] ¤**	**0.7 [0.1;1.3] ¤**	0.0 [-1.0;10]	0.1 [-0.7;1.0]	0.3 [-0.5;1.2]	-0.1 [-0.9;0.8]	0.5 [-0.4;1.3]

Compared to breast cancer, oocyte yield is not reduced in different types of cancer (only if ovarian surgery is required)

Von Wolff et al., submitted

Theoretical life birth rate - calculation based on the number of retrieved oocytes and registry data

Red numbers: Theoretical life birth rate



Lawrenz et al., 2010 Fertil Steril
v. Wolff & Dian; Dsch Aerzteblatt Int., 2011

Real Life birth rate – calculation based on observational studies using cryopreserved oocytes and embryos

8 studies:

- **1203 women cryopreserved**
- **90 women used their depot (7.5%)**
- **196 embryo transfers**
- **35 women delivered ≥1 baby**
  (Life birth rate / women: 38.9%)
- **45 children total**

Alvarez & Ramanathan, Hum Reprod, 2016

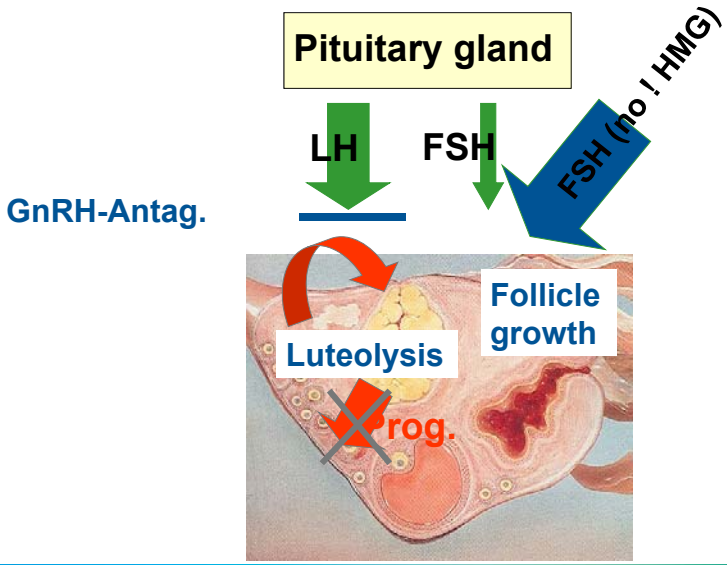
Agenda

- Standard stimulation protocol
- Overall efficacy
- Luteal phase stimulation
- Double stimulation
- Progestin primed ovarian stimulation
- Stimulation in breast cancer
- Stimulation in combination with GnRHa depot injections
- Stimulation in combination with ovarian tissue freezing

Rationale

Stimulation can be started any time during the cycle to shorten the treatment time.

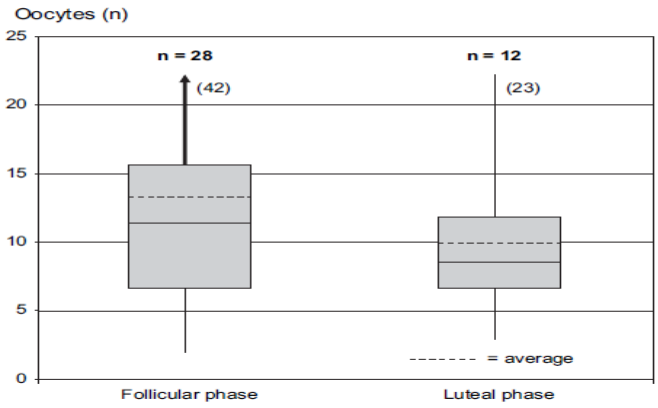
The proof of principle study



von Wolff et al., Fertil Steril, 2009

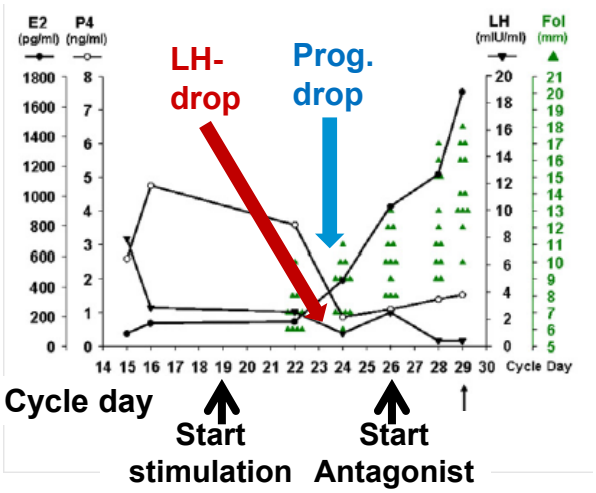
The proof of principle study

Number of oocytes collected after initiation of ovarian stimulation in the follicular (n = 28) versus the luteal (n = 12) phase.



von Wolff. Luteal phase stimulation. Fertil Steril 2009.

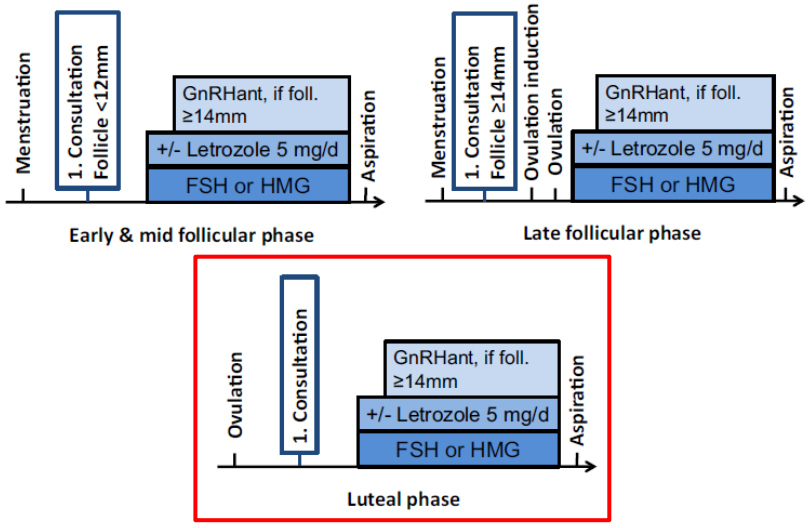
Estrogen increase in luteal phase stimulation apparently suppresses endogenous LH



…. and therefore possibly induces a luteolysis – even without GnRH antagonists

Cakmak et al., Fertil Steril 2013

Technique



von Wolff et al., Arch Gynecol Obstet, 2017

Efficacy

	von Wolff et al., 2009, Fertil Steril		Buendgen et al., 2013, Arch Gynecol Obstet		Cakmak et al. 2013 Fertil Steril		Kim et al., 2015 JKMS		von Wolff et al. Eur J Obstet Gynecol Reprod Biol 2016	
	Foll. phase	Luteal phase	Foll. phase	Luteal phase	Foll. phase	Luteal phase	Foll. phase	Luteal phase	Foll. phase	Luteal phase
Cycles (n)	28	12	30	10	103	22	6	5	472	103
Stim. (d)	10.6	11.4↑	9.1	11.7↑	9.3	11.2↑	11.8	12.3↑	10.8	11.5↑
Stim. (U)	2255	2720↑	2040	3500↑	3400	4340↑	1500	2100↑	2496	2970↑
Stim/d(U)*	172	238↑	224	299↑	365	387↑	127	170↑	231	258↑
Oocyt. (n)	13.1	10.0↓	10.0	8.8↓	14.4	15.5↑	11.5	9.0↓	11.6	13.6↑
MII (n)	11.0	8.0	7.9	7.2	9.7	10.3	4.5	6		
Fert. Oocytes	*6.7	**6.1	**4.8	**4.6	**7.0	**9.1	10	5		
Preg.Rate Cycle			20%	10%						

* calculated
** estimated

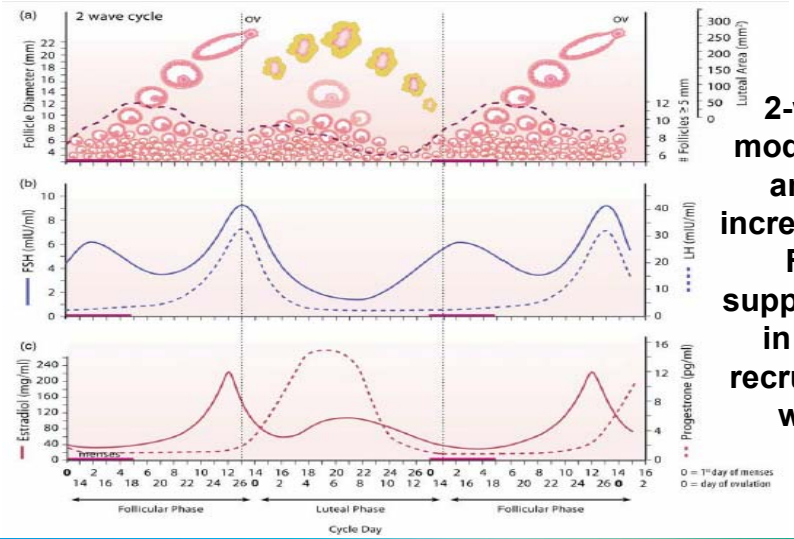
Indication

- **In women before gonadotoxic therapies.**
- **In any women to reduce treatment time and if freeze all is planned.**

Agenda

- Standard stimulation protocol
- Overall efficacy
- Luteal phase stimulation
- Double stimulation
- Progestin primed ovarian stimulation
- Stimulation in breast cancer
- Stimulation in combination with GnRHa depot injections
- Stimulation in combination with ovarian tissue freezing

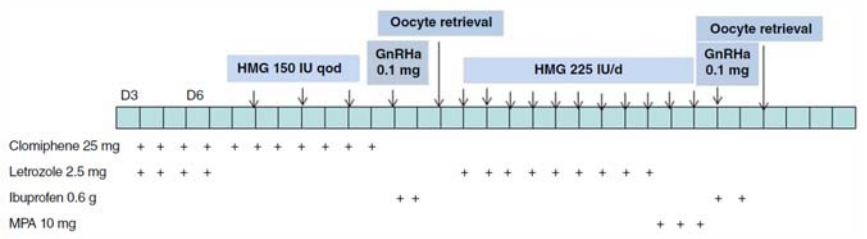
Rationale



2-wave model with an E2-increase and FSH suppression in each recruitment wave

Baerwald et al., Hum Reprod Update, 2011

Technique I & efficacy (Shanghai protocol)

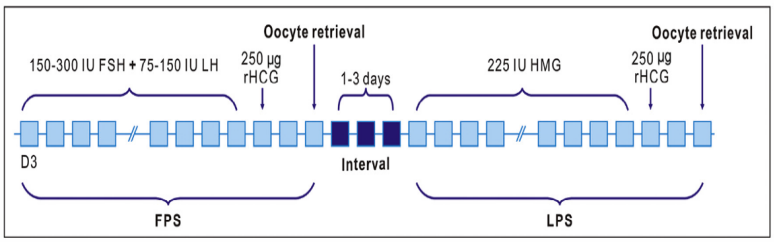


LH down regulation with MPA 10mg in long stimulations

	First oocyte retrieval (n=38)	Second oocyte retrieval (n=30)
Total dose of gonadotropins (IU)	326 ±248	1802 ±712
Oocytes retrieved	1.7 ±1.0	3.5 ±3.2
Embryos transferred	1.0 ±1.0	2.0 ±2.4

Kuang et al., RBMonline, 2014

Technique II & efficacy

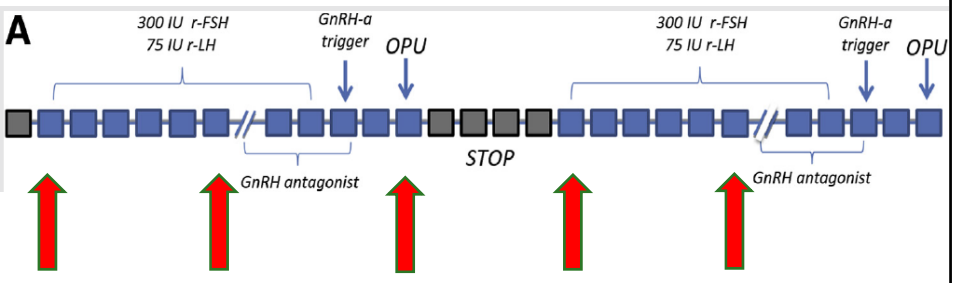


LH down regulation with MPA 10mg beginning on day 3

	First oocyte retrieval (n=116)	Second oocyte retrieval (n=116)
Total dose of gonadotropins (IU)	1882 ±958	1728 ±937
Oocytes retrieved	2.3 ±2.0	3.5 ±3.6
Embryos	1.6 ±1.5	2.4 ±2.7

Liu et al., RBMonline, 2017

Techniques II



Start Day 2

Conventional antagonist protocoll

Follicle aspiration (small follicles=2nd cohort) are not aspirated

Start 5 days after aspiration

Conventional antagonist protocoll

Ubaldi et al., Fertil Steril, 2016

Techniques II & efficacy (Results after stimulation according to Ubaldi et al., 20106)

	1st cycle	2nd cycle	P-value
Patients (n)	153	153	
Patients with aspiration OPU (n)	128	128	
MII-Oocytes (mean ±SD)	486 (3.6±2.2)	565 (4.5±2.7)	n.s
Blastocysts (% per inseminated MII-oocyte)	161 (1.3±1.1) (48.2%)	214 (1.7±1.7) (49.4.0%)	n.s
Euploid blastocyst	61 0.5±0.8) (37.9%)	81 (0.6±1.0) (37.9.0%)	n.s

Vaiarelli et al., Curr Opin Obstet Gynecol, 2017

Techniques III & efficacy (Results after stimulation according to Ubaldi et al., 20106)

	1st cycle	2nd cycle
Number of single embryo transfers (n)	27	30
Miscarriage rate (n, %)	2 (14.3%)	2 (10.0%)
Ongoing pregnancies (n, %)	12 (44.4%)	18 (60.0%)

Vaiarelli et al., Curr Opin Obstet Gynecol, 2017

Indication

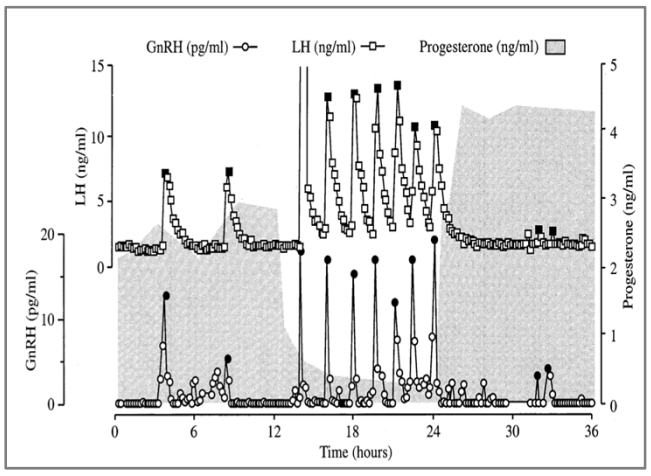
- **In women before gonadotoxic therapies.**
- **In poor responders and if freeze all is planned.**

Agenda

- Standard stimulation protocol
- Overall efficacy
- Luteal phase stimulation
- Double stimulation
- Progestin primed ovarian stimulation
- Stimulation in breast cancer
- Stimulation in combination with GnRHa depot injections
- Stimulation in combination with ovarian tissue freezing

Rationale

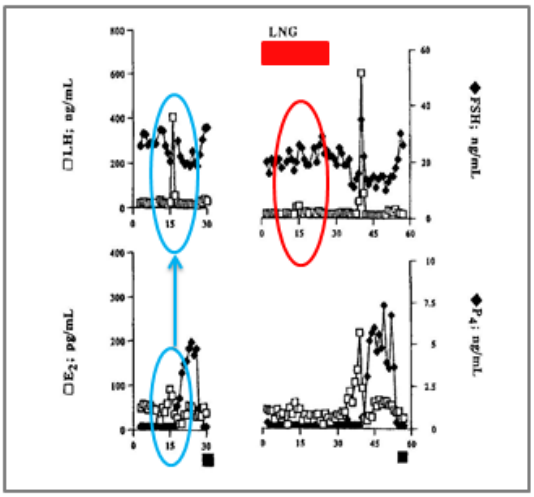
Effect of progesterone on GnRH and LH secretion in ewes



Progesterone modulates GnRH secretion by decreasing GnRH pulse frequency (hypothalamic action)

Chabbert-Buffet et al. Steroid 2000; Skinner et al. PNAS 1998

Rationale



Levonorgestrel (LNG) inhibits physiologic estradiol induced LH surge
It's action is reversible

Heikinheimo et al. Contraception 1996

Technique & Efficacy

day 2 ↓

Kuang et al., Fertil Steril 2015

	Patients	Duration of FSH d	Total FSH	Oocytes	Implantation rate
HMG 150-225/d + MPA 10mg/d	150	9.3*	2014*	7.0	31.9
HMG 150-225/d + Short agonist	150	8.4*	1636*	6.4	27.7

Zhu et al.,Medicine 2015

	Patients	Duration of FSH d	Total FSH	Oocytes	Implantation rate
HMG 150-225/d + oral Utrogestan 2x100/d	187	8.9*	1844*	7.6	33.6
HMG 150-225/d + Short agonist	187	8.3*	1446*	7.1	34.0

* p<0.001

Indication

- If GnRH agonists/antagonists should be avoided.
- If freeze all is planned.

Agenda

- Standard stimulation protocol
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Stimulations in combination with TAM or letrozole

TABLE 2. Comparison of various characteristics between letrozole+FSH and control groups

	Letrozole+FSH[a]	Control[b]	*P* value
Age at IVF (yr)	36.4 ± 3.6	36.9 ± 3.9	0.44
Baseline FSH	7.1 ± 3.1	4.2 ± 2.0	<0.001
E_2 at hCG	483.4 ± 278.9	1464 ± 644.9	<0.001
Endometrial thickness	8.7 ± 2.8	10.9 ± 2.5	<0.001
Follicle no. > 17	4.0 ± 1.7	2.7 ± 1.2	<0.001
Peak follicle size (mm)	21.3 ± 2.6	18.7 ± 1.5	<0.001
Total oocytes (n)	12.4 ± 7.0	11.1 ± 5.5	0.43
Mature oocytes (n)	8.7 ± 4.8	9.7 ± 5.1	0.43
Mature oocytes (%)	73.2 ± 22.9	86.3 ± 12.7	0.003
No. of 2 pn zygotes	6.6 ± 4.0	6.9 ± 4.1	0.73
Fertilization rate	74.1 ± 24.0	73.2 ± 21.5	0.71
No. of days stimulated	11.7 ± 2.3	12.2 ± 1.5	0.09
Total FSH dose	1317.8 ± 578.2	2382.5 ± 1062.8	<0.001

Oktay et al., J Clin Endocrinol Metab, 2006

Stimulations in combination with TAM or letrozole

Human Reproduction, Vol.32, No.5 pp. 1033–1045, 2017
Advanced Access publication on February 27, 2017 doi:10.1093/humrep/dex027

human reproduction REVIEW *Infertility*

The safety and efficacy of controlled ovarian hyperstimulation for fertility preservation in women with early breast cancer: a systematic review

Rachael J. Rodgers[1,2,*], Geoffrey D. Reid[3], Juliette Koch[1,2], Rebecca Deans[1,2], William L. Ledger[1,2], Michael Friedlander[4,5], Robert B. Gilchrist[2], Kirsty A. Walters[2], and Jason A. Abbott[2]

WIDER IMPLICATIONS OF THE FINDINGS: The co-administration of 5 mg of letrozole daily commencing on Day 2 and continuing throughout COH is recommended as it reduces peak oestradiol concentrations without significantly decreasing oocyte yield. The use of a GnRH agonist trigger is beneficial as oestradiol concentrations rapidly decrease post-administration and rates of ovarian hyperstimulation are lower than with an hCG trigger, without a corresponding reduction in clinical pregnancy or live birth rates in cryopreservation cycles. The protective effect of tamoxifen has not been evaluated although theoretically may be of benefit due to its action on the oestrogen receptor.

Safety of letrozole

OPEN ACCESS Freely available online 2014 PLOS ONE

Congenital Malformations among Babies Born Following Letrozole or Clomiphene for Infertility Treatment

Sunita Sharma[1]*, Sanghamitra Ghosh[1], Soma Singh[1], Astha Chakravarty[1], Ashalatha Ganesh[2], Shweta Rajani[1], B. N. Chakravarty[1]

Methods and Material: A total of 623 children born to infertile women who conceived naturally or following clomiphene citrate or letrozole treatment were included in this study. Subjects were sorted out from medical files of both mother and newborn and follow up study was done based on the information provided by parents through telephonic conversations. Babies with suspected anomaly were called and examined by specialists for the presence of major and minor congenital malformations. Other outcomes like multiple pregnancy rate and birth weight were also studied.

Results: Overall, congenital malformations, chromosomal abnormalities were found in 5 out of 171 (2.9%) babies in natural conception group and 5 out of 201 babies in the letrozole group (2.5%) and in 10 of 251 babies in the CC group (3.9%).

Conclusions: There was no significant difference in the overall rate of congenital malformations among children born to mothers who conceived naturally or after letrozole or CC treatment.

Agenda

- Standard stimulation protocol
- Overall efficacy
- Luteal phase stimulation
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- Stimulation in combination with ovarian tissue freezing

The efficacy of GnRH agonists

Breast cancer: Odds ratios for POI/amenorrhoea with and without GnRHagonists

Study or Subgroup	CT plus GnRHa Events	Total	CT alone Events	Total	Weight	Risk Ratio M-H, Random, 95% CI
1.1.1 Breast Cancer						
Badawy 2009	4	39	21	39	6.2%	0.19 [0.07, 0.50]
Elgindy 2013	9	50	10	50	7.9%	0.90 [0.40, 2.02]
Gerber 2011	5	30	6	30	5.4%	0.83 [0.28, 2.44]
Karimi-Zarchi 2014	2	21	14	21	3.7%	0.14 [0.04, 0.55]
Lambertini 2015	40	148	48	133	16.4%	0.75 [0.53, 1.06]
Leonard 2017	12	65	23	66	11.0%	0.53 [0.29, 0.97]
Moore 2015	5	66	15	69	6.4%	0.35 [0.13, 0.90]
Munster 2012	3	26	2	21	2.5%	1.21 [0.22, 6.59]
Song 2013	15	89	27	94	11.8%	0.59 [0.33, 1.03]
Sverrisdottir 2009	14	22	18	20	16.4%	0.71 [0.50, 1.00]
Subtotal (95% CI)		**556**		**543**	**87.6%**	**0.57 [0.43, 0.77]**
Total events	109		184			

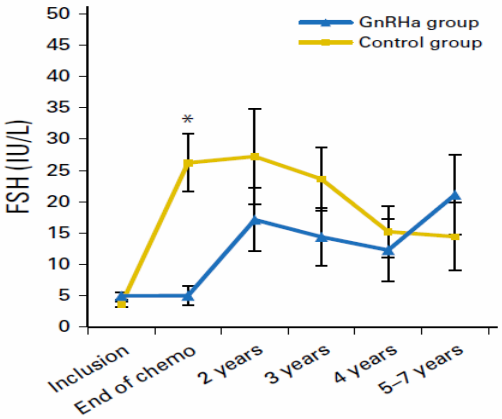
Heterogeneity: $Tau^2 = 0.09$; $Chi^2 = 16.23$, df = 9 (P = 0.06); $I^2 = 45\%$

Test for overall effect: Z = 3.69 (P = 0.0002)

Senra et al., Ultrasound Obst Gynecol, 2017

Long term efficacy of GnRH agonists

Analysis of the ovarian reserve after a chemotherapy in hodgkins lymphoma patient with (n=32) and without (n=35) GnRH-agonists.



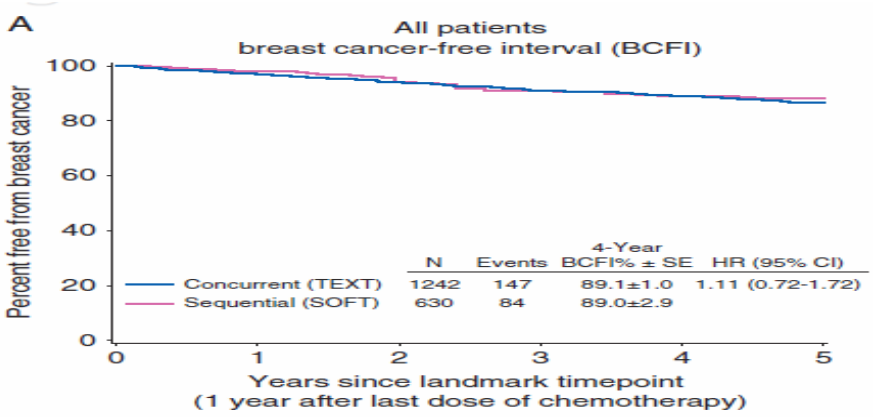
Conclusion:

GnRHa do have a short term but possibly not a long term effect, requiring an individual decision concerning its use.

Demeestere et al.,
J Clin Oncol, 08 2016

Risk of GnRH agonists in hormone receptor positive breast cancer?

TEXT: GnRHa with chemotherapy,
SOFT: GnRHa after chemotherapy



Regan et al., Ann Oncol, 2017

GnRH depot agonists – when to start?

	Advantages	Disadvantages
At the time of ovulation trigger	Early application	Risk of OHSS increased? Intervention with ovulation trigger?
At the time of aspiration	Timing of GnRH application can adapted in relation to the individual risk of OHSS	Risk of OHSS increased (personal communication)
A few days after aspiration	No risk of OHSS	Late application

It is unknown when GnRH agonists should be applied. According to experiences of many centers, application at the time of oocyte triggering seem to be a good option.

Agenda

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- Stimulation in combination with ovarian tissue freezing

3 Studies concerning the combination of ovarian stimulation and tissue freezing

Fertil Steril. 2011 Jan;95(1):342-4. doi: 10.1016/j.fertnstert.2010.07.1074. Epub 2010 Aug 24.

Improving fertility preservation in cancer: ovarian tissue cryobanking followed by ovarian stimulation can be efficiently combined.

Huober-Zeeb C[1], Lawrenz B, Popovici RM, Strowitzki T, Germeyer A, Stute P, von Wolff M.

J Ovarian Res. 2014 Aug 26;7:80. doi: 10.1186/s13048-014-0080-8.

Ovarian tissue cryopreservation followed by controlled ovarian stimulation and pick-up of mature oocytes does not impair the number or quality of retrieved oocytes.

Dolmans MM, Marotta ML, Pirard C, Donnez J, Donnez O.

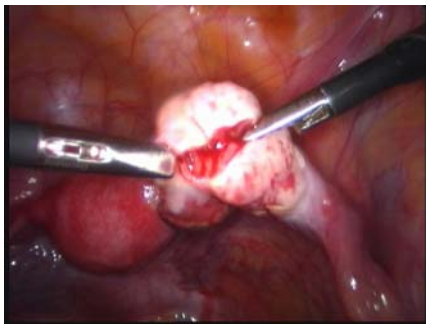
Reprod Biol Endocrinol. 2013 Mar 5;11:19. doi: 10.1186/1477-7827-11-19.

Oncofertility: combination of ovarian stimulation with subsequent ovarian tissue extraction on the day of oocyte retrieval.

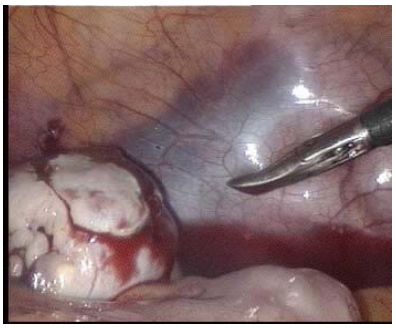
Dittrich R[1], Lotz L, Mueller A, Hoffmann I, Wachter DL, Amann KU, Beckmann MW, Hildebrandt T.

Removal of tissue before or after stimulation?

Before stimulation

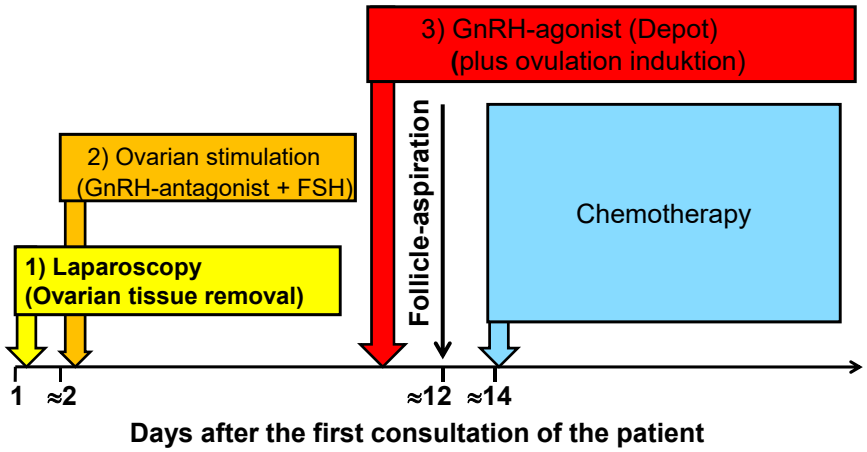


After stimulation



Conclusion: Removal seem to be better before ovarian stimulation

Combination of several fertility preservation techniques



von Wolff et al., Arch Gynecol Obstet, 2018

Summary

- Standard: Antagonist protocol and GnRHa für ovulation trigger.
- The average «Baby take home rate» after one stimulation in women <35y is around 40% and around 20% at the age of 40y.
- Luteal phase stimulation requires around 50U higher stimulation dosage and one more stimulation day.
- Double stimulation doubles the outcome.
- Progestogens can be used instead of GnRH antagonists.
- In breast cancer E2 lowering co-medications such as letrozole can be used.
- GnRHa depots can be applied at the same time as ovulation trigger.
- Ovarian tissue should be removed before ovarian stimulation.

Spermatogenetic recovery in long term survivors of childhood cancer and hematopoietic stem cell transplantation

Kirsi Jahnukainen, professor, MD

Children´s Hospital, Helsinki

Karolinska Institutet, Stockholm

Disclosure statement

"I declare that I have no commercial or financial interests pertaining to the subject of this presentation or its content"

Learning Objectives

- What factors contribute to impaired spermatogenesis?
- How does spermatogenetic recovery occur after low and high dose exposures?
- What is the effect of follow-up time for spermatogenetic recovery?
- What are the future challenges?

International Harmonisation Group / PCSF-WP6, Male Gonadotoxicity Guidelines Group

NORDFERIL Nordic centre for fertility preservation

HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI

Pancare Childhood and Adolescent Cancer Survivor Care and Follow-up Studies
PanCareSurFup

International Guideline Harmonization Group
for Late Effects of Childhood Cancer

NORDFERTIL
nordic centre for fertility preservation

What factors contribute to impaired spermatogenesis?

Risk of long term/permanent infertility is associated with treatment with **Alkylating agents**

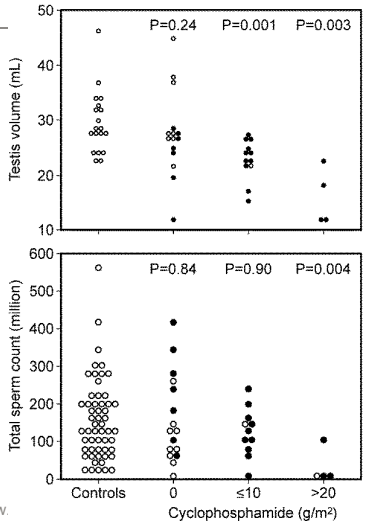
- Cyclophosphamide
- Nitrogen mustard
- Procarbazine

Lopez Andreu et al. Pediatr Hematol Oncol. 2000;16:21-30.
van Beek et al. Hum Reprod. 2007;22:3215-3222

→ Sperm concentration decreases with increasing dose

→ No threshold dose for azoospermia can be identified

genetic susceptibility to subfertility? Green et al. Lancet Oncol 2014;15:1215-1223



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International Guideline Harmonization Group
for Late Effects of Childhood Cancer

Skinner et al. Lancet Oncol 2017; 18: e75–90

What factors contribute to impaired spermatogenesis?

Risk for permanent infertility is associated with **radiotherapy**

- Exposing testes at any dose
- Especially doses >2-3 Gy
- Especially TBI (level C)

There is no evidence of

- Safe irradiation dose

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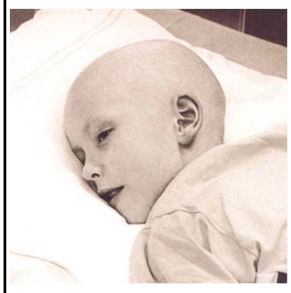
Skinner et al. Lancet Oncol 2017; 18: e75–90

Who are at risk of subfertility?



- Spinal tumors, CNS leukemia, pelvic sarcomas, testicular leukemia
  - Localized pelvic, spinal or testicular radiotherapy
- Relapsed and resistant hematological malignancies
  - Total body irradiation before HSCT
- Relapsed and resistant hematological malignancies, myelodysplastic syndrome, bone marrow failure, and hemoglobinopathies
  - Preconditioning chemotherapy before HSCT with alkylating agents
- Hodgkin disease not responding standard therapy, metastatic sarcoma
  - High dose alkylating agent based therapies

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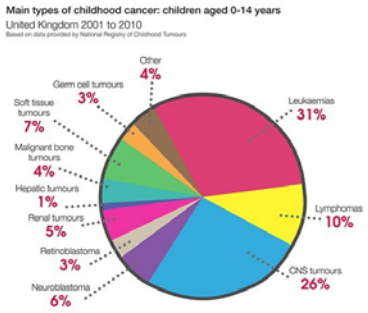
Who will recover spermatogenesis?

>70% of all childhood cancer cases

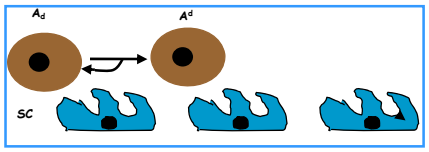
Low risk of subfertility

- AML and ALL
- Low risk NHL
- Renal tumors, no radiotherapy
- Soft tissue sarcoma, low stage
- Germ cell tumors, no radiotherapy
- Brain tumors
  - surgery only
  - cranial irradiation <24 Gy dose

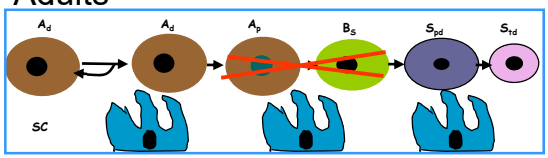


Effects of low dose cancer therapy in children and adult testis

Children



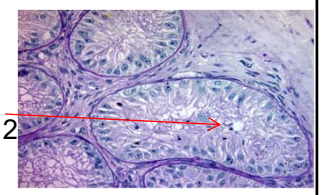
Adults



Adult men

- Low doses kill differentiating spermatogonia
- Surviving spermatocytes and spermatids, continue maturation into sperm

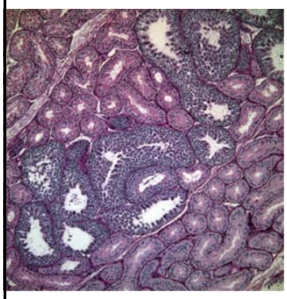
→ some sperm production 4-10w (do not cryopreserve!)
→ short term loss of sperm production and recovery at w12



Children

- No morphological effects

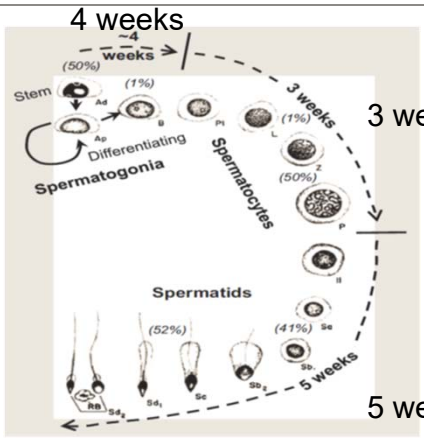
Effects of high dose cancer therapy in testis



- Germ cell toxicity shows no age-dependency
- Toxic insult with HIGH INTENSITY
  - Depletes spermatogonial stem cell pool
    → Permanent spermatogenetic failure = azoospermia
- Toxic insult with NEARLY STERILIZING INTENSITY
  - Surviving spermatogonial stem cells begin to proliferate and repopulate the tubules
    → Recovery follows, but takes time!
- Androgen production - less sensitive → OFTEN REMAINS.

Recovery at different stages of spermatogenesis

- Even moderate gonadotoxic doses produce azoospermia that lasts longer than the 12 weeks
- Recovery is delayed for months or decades if gonadotoxic therapy kills stem cells



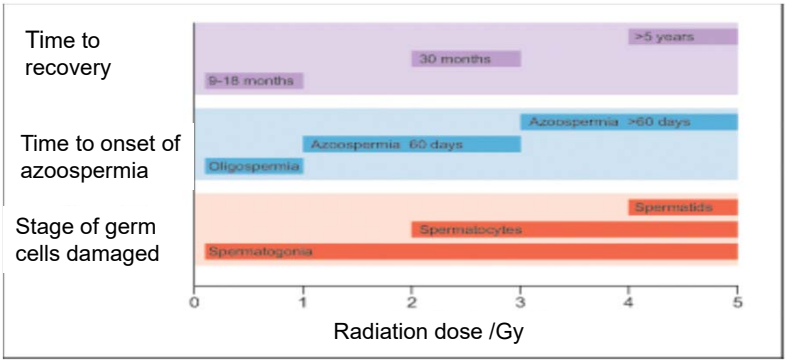
Adapted from Rowley et al. Radiat Res 1974;59:665–78

Recovery of spermatogenesis depends on the dose of irradiation

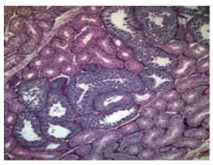
≥0,1 Gy
The differentiating spermatogonia are killed → short term cessation of spermatogenesis

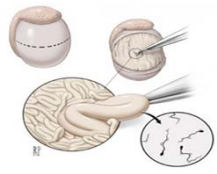
2–3 Gy
Kills also SSCs → long term azoospermia.

>6 Gy
Able to deplete the SSCs pool →permanent/long term infertility



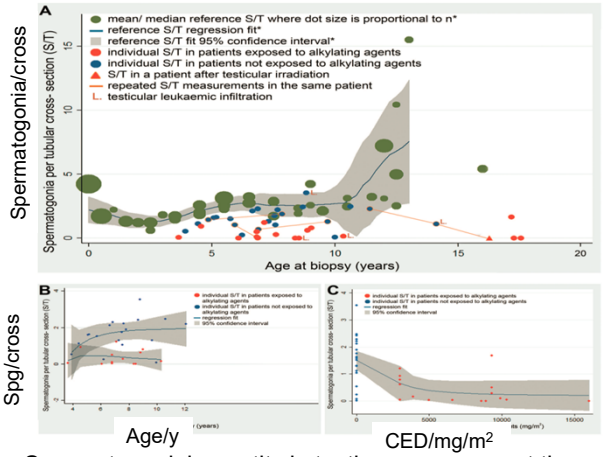
Sperm quality after spermatogenetic recovery



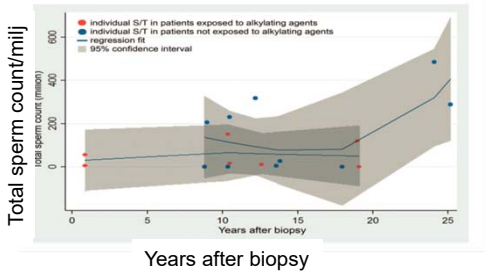


- The recovery is almost always progressive, and significant declines in sperm counts are rare
- Many recover to normospermic levels
- When the human testis contains <3–4 million sperm, sperm do not survive epididymal transit and do not reach the ejaculate
- It is possible that some sperm are produced in the testis.
- Recovery may be patchy.
- Spermatozoa can be retrieved from the testes by microdissection testicular sperm extraction (TESE)

Acute depletion of spermatogonia and recovery after leukemia therapy



Spermatogonial quantity in testicular samples at the end of leukemia therapy



No difference in total sperm counts after a follow-up period of 9-19 years between non-alkylating vs alkylating therapy

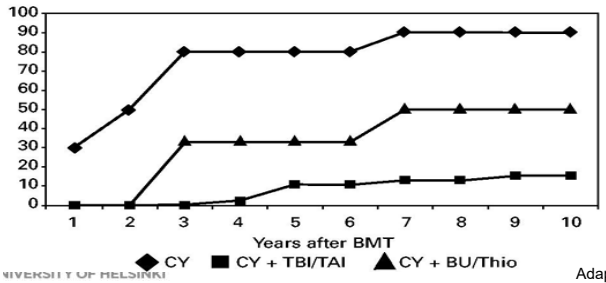
Poganitsch-Korhonen et al Leukemia 2017;31:1460-1463.

Spermatogenetic recovery after HSCT in adults

Recovery of spermatogenesis after allogeneic HSCT is predicted by

- younger age (<25 years)
- a non–total body irradiation (TBI) -based conditioning regimen
- no cancer therapy before HSCT
- no chronic graft-versus-host disease

Rovó et al. Blood 2006:108:1100-1105



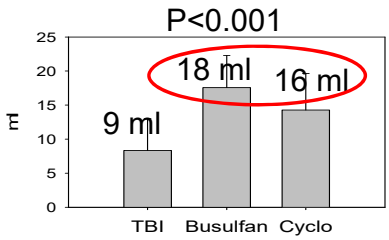
Cyclophosphamide 90%

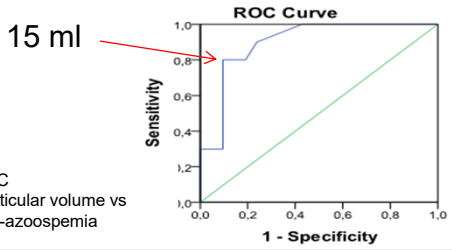
Cyclophosphamide 50%
+ busulphan or thiotepa

Cyclophosphamide 17%
+TBI

Adapted from Anserini et al. Bone Marrow Transplantation 2002;30:447-451

Testicular growth in puberty reflects spermatogenetic recovery after pediatric HSCT





ROC
Testicular volume vs non-azoospemia

Follow up 13 ± 4.8 y, median age at study 22 ± 6.0 years, n=106

Mean adult testicular size is >15 ml after

- busulfan-based regimes
- cyclophosphamide alone as conditioning

→ suggest very long-term recovery of spermatogenesis after chemotherapy-based HSCT regimens in children

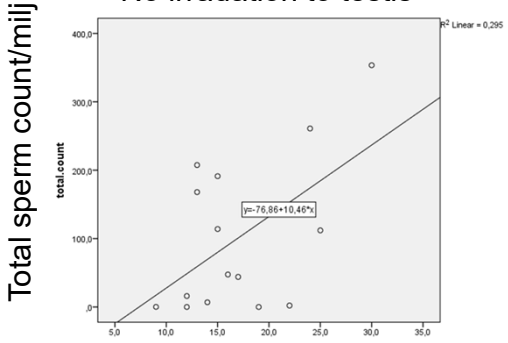
Wilhelmsson et al PBC 2014;61:1094-100.

Spermatogenetic recovery after pediatric HSCT is progressive

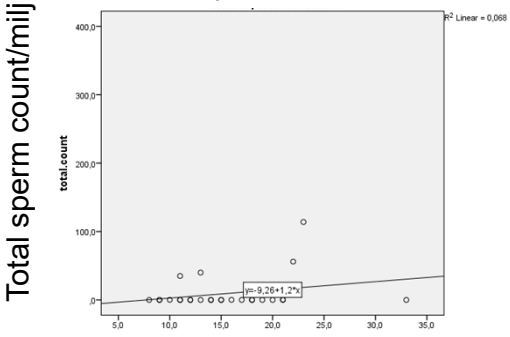
Follow up 16.1 ± 5.7 y, median age at study 26.1 ± 6.3 years

Preliminary HSCT data Helsinki-cohort, n=41 with no testosterone replacement

No irradation to testis



Testis exposed to irradiation



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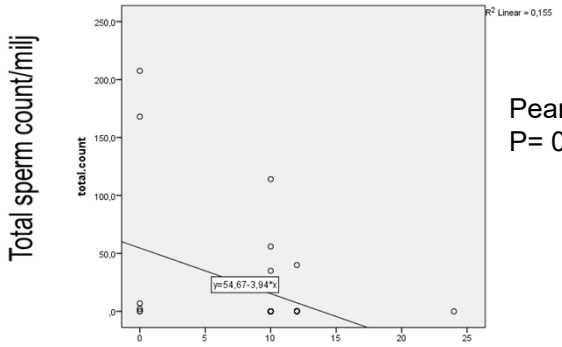
Spermatogenetic recovery associates to the testicular irradiation dose

Preliminary HSCT data Helsinki-cohort, n=41 with no testosterone replacement



Pearson correlation -0.510
P= 0.001

Cumulative testicular irradiation dose/Gy

Predictors of spermatogenetic recovery after pediatric HSCT

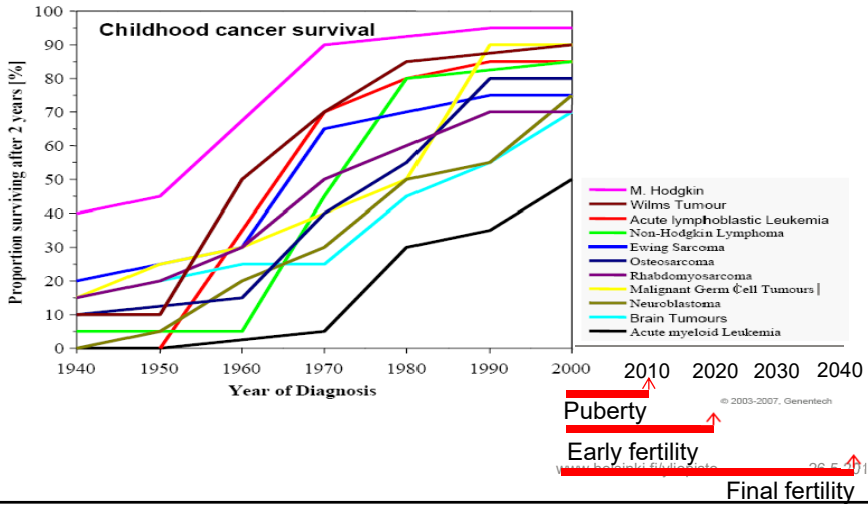
Preliminary HSCT data Helsinki-cohort, n=41 with no testosterone replacement

Follow up 16.1 ± 5.7 y, median age at study 26.1 ± 6.3 years

Total sperm count	beta	P<
Cum testicular irradiation dose	**-0.364**	**0.013**
Time from HSCT	**0.346**	**0.012**
Testicular volume	**0.344**	**0.018**

Gaps in knowledge 1

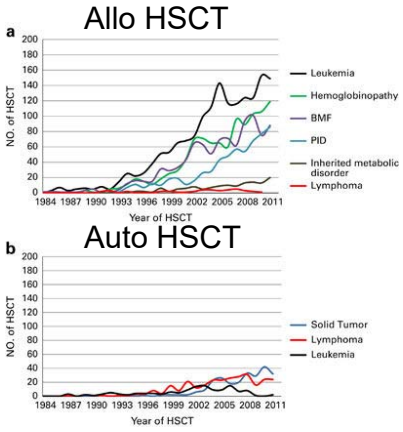
Fertility data matures slowly and reflects the past therapies



Gaps in knowledge
Impaired spermatogenesis

- Risks of, and dose thresholds, for impaired spermatogenesis of
  - Busulfan, chlorambucil, ifosfamide, melphalan and thiotepa
  - Dacarbazine, temozolomide
  - Carboplatin, cisplatin
  - Carmustine, lomustine
- No data on treosulfan (widely used to replace busulfan)
- No data on modern targeted therapy

Gaps in knowledge 2
Indications for HSCT are changing



Non-malignat conditions will dominate in future

Increase of allogeneic HSCT

- Hemoglobinopathy
- Primary immunodeficiences
- Bone marrow failure

Only smaller increase in autologous HSCT

- Solid tumors (high risk neuroblastoma and brain tumors)
- Non-Hodgkin's lymphoma

Adapted from Hussein et al. Bone Marrow Transplantation 2017; 52:120–125

Hematological malignancy is no more the major indication for pediatric HSCT

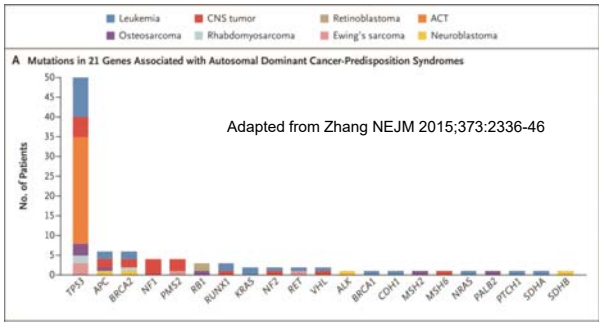
Gaps in knowledge 3
There may be genetic susceptibility to treatment related subfertility

>10% of pediatric cancer patients will have germ line mutation in cancer predisposition gene, RASopathy or telomeropathy

Germ line mutations are frequent in primary immunodeficiency and bone marrow failure

- Increased risk to treatment related toxicity
- Increased risk to subsequent cancer
- Increased risk to subfertility?

WGS (whole genome sequencing) broadens our knowlege about genetic variation



Adapted from Zhang NEJM 2015;373:2336-46

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Future challenges

- More accurate information is needed about spermatogenetic recovery after modern therapies
- Understanding about genetic susceptibility to subfertility needs to be increased
- Only minority of young men after childhood cancer or modern HSCT protocols will be infertile

  → The challenge is individualized counselling and optimized patient selection for fertility preservation

Multi-disciplinary teams are required

NORDFERTIL nordic centre for fertility preservation

GROW SPERM

Children´s Hospital Helsinki
- Anu Vatanen
- Melanie Poganitsch

University of Amsterdam Center for Reproductive Medicine
- Ans van Pelt
- Ieva Masliukaite

University of Münster
- Stefan Schlatt
- Jens Ehmcke

NORDFERTIL Research Lab Stockholm Karolinska institutet
- Olle Söder
- Jan-Bernd Stukenborg
- Mari Wilhelmsson
- Valentina Pampanin
- Lena sahlin
- Halima Al-Balushi
- Magdalena Kurek
- Joao Lopes

Rigshospitalet, Copenhagen
- Sidsel Mathiesen
- Malene Mejdahl
- Klaus Müller

Acknowledgements

A bibliography

- Skinner et al. Lancet Oncol 2017;18:e75–90
- Rowley et al. Radiat Res 1974;59:665–78
- Poganitsch-Korhonen et al. Leukemia 2017;31:1460-1463
- Rovó et al. Blood 2006:108:1100-1105
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- Wilhelmsson et al. Pediatr Blood Cancer 2014;61:1094-100.
- Hussein et al. Bone Marrow Transplantation 2017; 52:120–125
- Zhang et al. NEJM 2015;373:2336-46

Precongres course 16.
Addressing the broad Scope of Fertility Preservation

Ana Cobo

IVIRMA Global

Barcelona, 1 July 2018

I have nothing to disclose

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How effective are we at generating pregnancies from women who have had fertility preservation?

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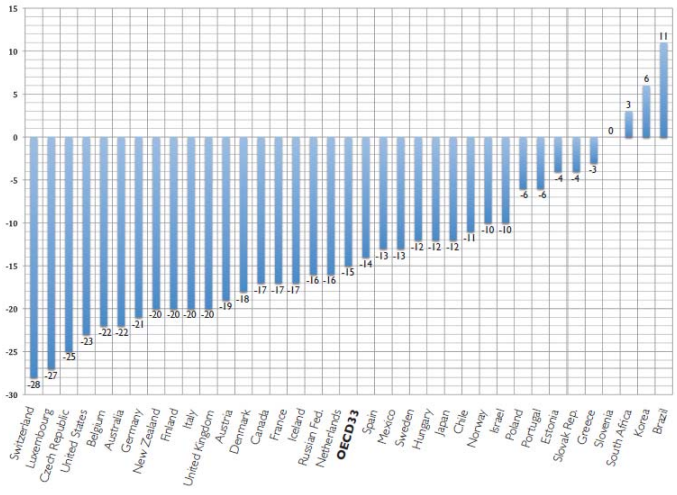
Cancer mortality rates in OECD countries(1099-2011)



Fuente: OECD (Organisation for Economic Co-operation and Development) (http://dx.doi.org/10.1787/health_glance-2013-en).

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Today's woman…

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Today's Society

Mean age in 1st birth from 2000 to 2014 - USA

24.9 **26.3 y**

28% rise in first births to women 30 to 34

21% rise in first births to women 35 and over

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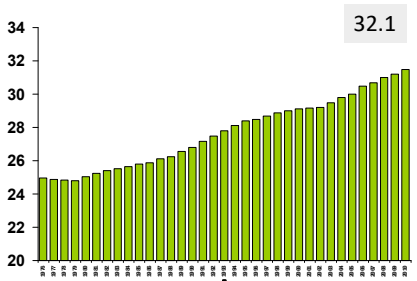
NCHS data - 2016

Our reality

Delayed maternity….and more:



each decade ↑2 years

51 y. in 22nd century?

Best embryos in the 20's

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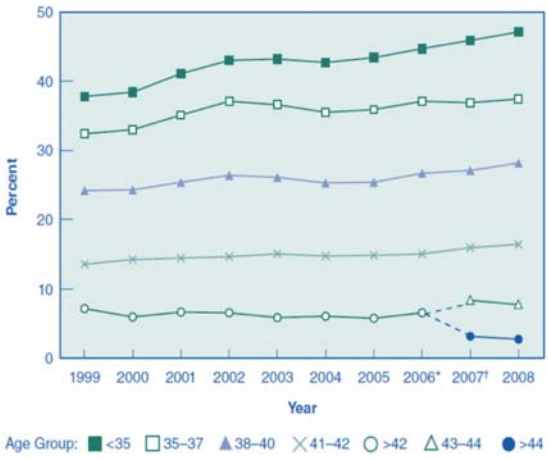
Live birth according to age



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CDC Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

Who can benefit from FP?

Medical Reasons

- Cancer
- Other diseases requiring chemo- or immunotherapy:
  - Rheumatoid arthritis etc.
- Other medical reasons:
  - Bilateral ovariectomy etc.
- Associated with a medical condition:
  - Endometriosis
  - Low ovarian reserve
  - Unilateral ovariectomy
  - Others

"Social" Reasons

- Age related fertility decline

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Options for FP

GnRHa + chemotherapy

Ovarian tissue cryopreservation

Embryo Criopreservation

Oocytes cryopreservation

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Onco and Elective FP

Oocytes Cryopreservation

Update on fertility preservation from the Barcelona International Society for Fertility Preservation–ESHRE–ASRM 2015 expert meeting: indications, results and future perspectives

Francisca Martinez, on behalf of the International Society for Fertility Preservation–ESHRE–ASRM Expert Working Group

VOL. 108 NO. 3 / SEPTEMBER 2017

Embryo and oocyte cryopreservation are first-line FP methods in post-pubertal women. Metaphase II oocyte cryopreservation (vitrification) is the preferred option.

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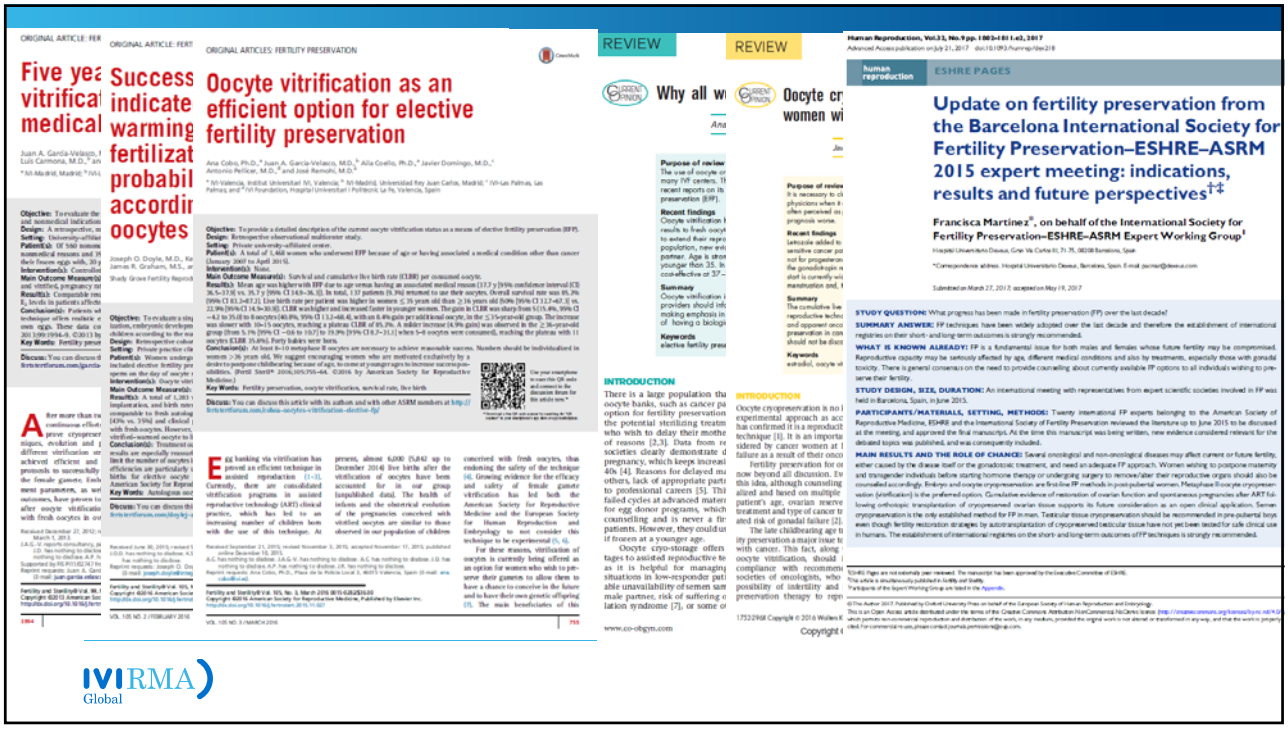

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Limitations of oocytes cyopreservation for Onco-FP

- ✓ Need for IVF (limited attempts).
- ✓ It may delay chemo therapy.
- ✓ Hormone-sensitivity of the tumor.
- ✓ Not for prepubertal patients.
- ✓ Oocytes survival may be compromised.
- ✓ No guarantee of success.
- ✓ Age and ovarian reserve.

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FP in oncological patients

REVIEW

CURRENT OPINION

Oocyte cryopreservation for fertility preservation in women with cancer

Javier Domingo[a] *and Juan A. Garcia-Velasco*[b,c]

Curr Opin Endocrinol Diabetes Obes 2016, 23:465–469

Some resolved issues…

- Use of letrozole to avoid high estradiol levels after COS.
- Use of GnRH antagonists to minimize the effect of progesterone.
- Not delaying chemotherapy by the random randomly obtaining of oocytes irrespectively of the menstrual cycle phase.

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Social Egg Freezing And The Modern Woman

Medical / oncological vs social reasons

If technically possible. why don't do it?

- Career aspiration
- Relationship instability and breakdown
- Not finding the right partner
- Late marriage
- Financial barriers

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Human Reproduction, Vol.26, No.3 pp. 655–661, 2011
Advanced Access publication on January 5, 2011 doi:10.1093/humrep/deq367

human reproduction ORIGINAL ARTICLE *Psychology and counselling*

A survey on the intentions and attitudes towards oocyte cryopreservation for non-medical reasons among women of reproductive age

D. Stoop*, J. Nekkebroeck, and P. Devroey

Centre for Reproductive Medicine, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel, Laarbeeklaan 101, B-1090 Brussels, Belgium

*Correspondence address. E-mail: dominic.stoop@uzbrussel.be

Submitted on September 14, 2010; resubmitted on November 19, 2010; accepted on November 30, 2010

BACKGROUND: Although cryopreservation of semen is a routine procedure for preserving male gametes, an efficient method of preserving fertility through oocyte freezing has only recently become available for women. In view of the limited female reproductive lifespan, oocyte freezing can now offer women some protection against the decline in fertility with aging.

METHODS: A survey was performed in Belgium among 1914 women of reproductive age (21–40 years) to assess public attitudes towards the phenomenon called 'social oocyte freezing'. Women were questioned on their awareness of the age-related fertility decline and their views and intentions towards considering undergoing oocyte cryopreservation.

RESULTS: The electronic questionnaire was completed by 1049 women, giving a response rate of 55%, and 25 were excluded as they were incomplete/inconsistent. Our results demonstrate that 31.5% of respondents consider themselves as potential social oocyte freezers, of which 3.1% would definitely consider the procedure. Just over half of the women (51.8%) would not consider the procedure while 16.7% indicated they had no opinion. Potential oocyte freezers are characterized by a higher number of desired children and more openness to oocyte donation. The decision to actually embark on such treatment would primarily depend on conditions, such as the procedure not affecting their natural fertility and the health of future children.

CONCLUSIONS: We conclude that a significant proportion of young women would consider safeguarding their reproductive potential or are at least open to the idea of social oocyte freezing.

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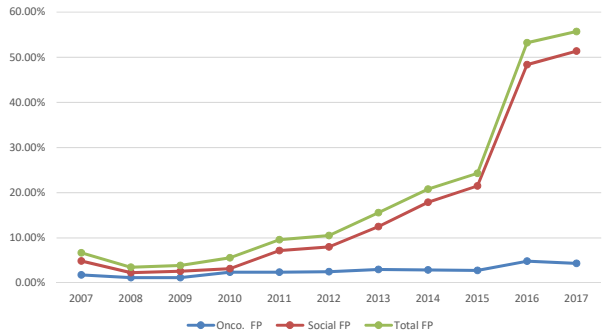
Oocytes Vitrification for FP

% FP procedures with respect of total own oocytes vitrification procedures



Year	Onco. FP	Social FP	Total FP
2007	1,80%	4,90%	6,70%
2008	1,20%	2,30%	3,50%
2009	1,20%	2,60%	3,90%
2010	2,40%	3,20%	5,60%
2011	2,40%	7,20%	9,60%
2012	2,50%	8,00%	10,50%
2013	3,00%	12,50%	15,60%
2014	2,90%	17,90%	20,80%
2015	2,80%	21,50%	24,30%
2016	4,8%	48,4%	**53,2%**
2017	4,3%	51,4%	**55,7%**
Total	3,7%	21,1%	24,8%

Nº Total Vit proc. = 30606
NºTotal Social FP= 6465
NºTotal Onco FP= 1139

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IVI unpublished data

Freezing Your Eggs—Is This What Everyone Is Doing

Is this really effective?

Analysis of actual FP population

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TABLE 2

	Nononcological	Oncological
No. of patients/warming cycles	26	4
"Fresh" ETs (%)	24 (92.3)	4
No. of embryos transferred	37 (1.5 ± 0.6)	8 (2)
CPR/patient (%)	11 (42.3)	1 (25)
OPR/patient (%)	8 (30.7)	1 (25)
No. of patients with surplus embryos	17 (65.3)	2 (50)
No. of surplus embryos vitrified	49 (2.8 ± 4.2)	4 (2)
No. of cryotransfers	15 (88.2)	1
No. of embryos transferred per cryotransfer	2.3 ± 0.7	2
CPR/patient (%)	7 (46.6)	1 (100)
OPR/patient (%)	5 (33.3)	0
Total live birth	5	1
Mean birth weight (g)	3,150 ± 0.3	3,440
Sex of the baby		
Female (%)	3 (60)	0
Male (%)	2 (40)	1

Note: Unless otherwise indicated, values are mean ± SD. CPR = clinical pregnancy rate; FP = fertility preservation; OPR = ongoing pregnancy rate.

Garcia-Velasco. 5-year experience with oocyte vitrification. Fertil Steril 2013.

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Garcia-Velasco, et al. (2013). Fertility and sterility **99**(7): 1994-1999.

SUPPLEMENTAL TABLE 3

Clinical outcomes according to the reason for EFP.

Outcome	EFP due to age	95% CI	EFP due to nononcologic medical reason	95% CI
No. of patients	1,382	NA	86	NA
No. of cycles	2,009	NA	128	NA
Mean age at vitrification, y	37.7	37.5–37.9	35.7[a]	34.9–36.3
No. of retrieved oocytes (per patient)	17,665 (12.7)	12–12.2	1,250 (14.5)[a]	12.9–13.7
No. of retrieved oocytes (per cycle)	17,665 (8.8)	8.98–9.03	1,250 (9.7)	8.9–9.5
No. of MII oocytes vitrified (per patient)	13,444 (9.7)	9.6–9.9	971 (11.2)[a]	10.2–11.9
No. of MII oocytes vitrified (per cycle)	13,444 (6.7)	6.6–6.9	971 (7.6)	6.4–7.9
No. of patients returning	120	NA	17	NA
Return rate (%)	8.7	7.2–10.2	19.7[a]	11.3–28.1
Survival rate	870/1,080 (80.5)	78.1–82.9	139/153 (90.9)[a]	86.3–95.5
Total no. of ETs/patient	102	NA	15	NA
Total no. of embryos transferred	154 (1.5)	1.4–1.6	27 (1.8)	1.5–2.1
Implantation rate, %	30.5	23.2–37.8	66.7[a]	48.9–84.5
CPR/transfer (%)	42/102 (41.2)	31.6–50.7	12/15 (80)[a]	59.8–100
CPR/patient (%)	42/120 (35)	25.7–44.3	12/17 (70.6)[a]	48.9–92.3
OPR/transfer (%)	26/102 (25.5)	17.0–33.9	11/15 (73.3)[a]	50.9–95.7
OPR/patient (%)	26/120 (21.6)	14.2–28.9	11/17 (64.7)[a]	42.0–87.4
No. of deliveries	21	NA	5	NA
No. of live births	24	NA	7	NA

Note: ET = embryo transfer; MII = metaphase II; other abbreviations as in Supplemental Tables 1 and 2.
[a] $P<.05$.

Cobo. Oocyte vitrification for elective FP. Fertil Steril 2016.

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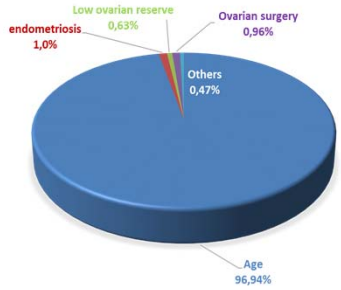
Cobo, A. et al. (2016). Fertil Steril **105**(3): 755-764 e758.

Ten years of elective FP (2007 -2018).

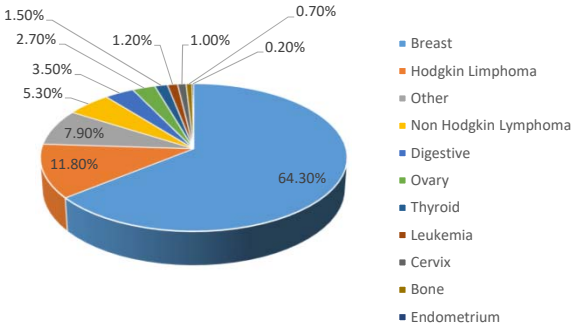
Who's doing it?

Elective FP



N= 4821 patients

Onco-FP



N= 1041 patients

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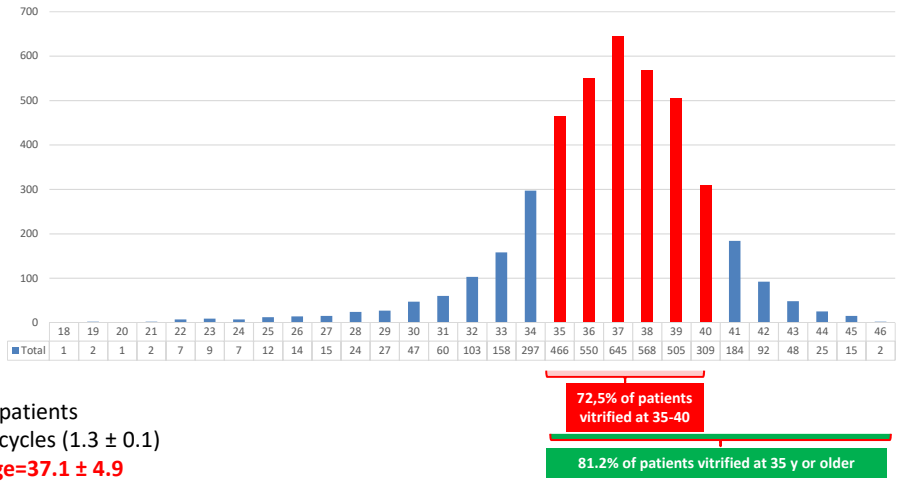
IVI unpublished data

Elective Fertility Preservation (2007-1017)

Age at vitrification



N=4198 patients
N=6467 cycles (1.3 ± 0.1)
Mean age=37.1 ± 4.9

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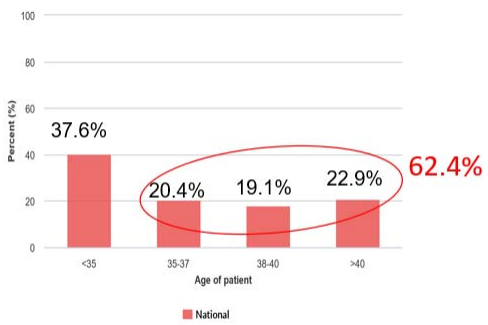
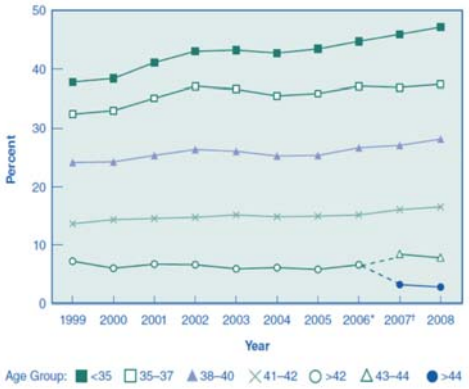
Age of women using ART and Live birth according to age



National information based on 169,602 ART cycles in 2014.



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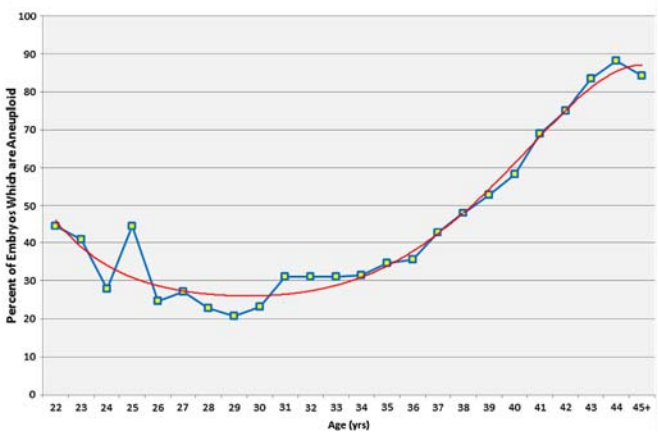
CDC Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

Aneuploidies and Age



Franasiak et al. Fertil Steril 2016

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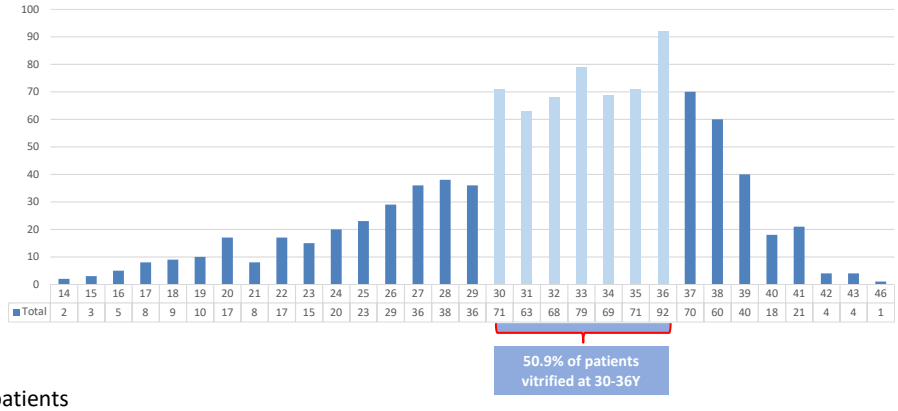
Onco- Fertility Preservation

Age at vitrification



N=1008 patients
N=1105 cycles (1.1 ± 1.1)
Mean age=32.3 ± 2.2

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IVI unpublished data

Baseline characteristics COS parameters and IVF data

	EFP	Onco-FP
Baseline Characteristics		
Nº Patients	4821	1041
Nº Cycles (mean)	6467 (1.3 ± 1.9)	1132 (1.1 ± 1.1)
Age at FP (mean)	37.2 ± 4.9*	32.3 ± 3.5*
Nº patients with previous children (%)	40	3
Protocol for Controlled Ovarian Stimulation		
GnRH Antagonist (%)	73.9*	22.2*
GnRH Agonist (%)	8.9	3.1
Clomiphene (%)	15.6	-
Letrozole (%)	1.4*	77.7*
Controlled Ovarian Stimulation Parameters		
Length of stimulation	10.7 ± 7.1	10.6 ± 21.2
Mean FSH dose (IU)	1780.1 ± 972.3	1652.5 ± 2121.3
Mean hMG dose (IU)	1160.1 ± 1484.9	1077.6 ± 2916.8
Mean LH dose (IU)	658.5 ± 159.1	689.6 ± 848.5
E2 on day of hCG (pg/ml)	1750.1 ± 38.9*	714.8 ± 310.4 *
IVF data		
Nº retrieved oocyte /patient (mean)	61380 (12.7 ± 7.4)	12991 (12.4 ± 3.2)
Nº retrieved oocyte /cycle (mean)	61380 (9.7 ± 8.4)*	12991 (11.4 ± 3.5)*
Nº MII vitrified /patient (mean)	47210 (9.8 ± 6.4)	9931 (9.5 ± 2.6)
Nº MII vitrified/cycle (mean)	47210 (7.3 ± 11.3)*	9931 (8.8 ± 2.1)*

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*P<0.05

IVI unpublished data

When they come back…

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Results of ten years' of Elective FP

Elective Fertility Preservation	
Patients returning (%)	622 (12.9)
Mean age at vitrification	37.6 ±3.5
Mean age at return	39.9 ± 0.7
Mean storage time (years)	2.1 ± 1.6
Warming Cycles/patient	657 (1.1 ± 0.05)
Mean warmed oocytes/patient	5623 (9.0 ± 3.8)
Survival rate	4718 (83.9)
Nº of transfers/warming cycle	330 (50.2)
Mean embryos Transferred	469 (1.4 ± 0.8)
IR	41.7
CPR/transfer	167 (50.6)
OPR/transfer	128 (38.7)
Nº Live Birth	107/456* (23.5)
Cryotransfers of surplus embryos	
Nº patients	147 (24.5%)
NºET/patient	247 (1.7 ± 0.5)
Nºwarming cycles/patient	203 (1.4 ± 0.9)
NºCryo transfers/warming cycle	196 (0.9 ±1.2)
IR	44.8
CPR/transfer	110 (56.1)
OPR/transfer	91 (46.4)
Nº Live Birth	53/112* (47.3)
C Live birth rate/Patient	160/456* (35.1)

IVI unpublished data

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Results of ten years' of Onco-FP

Elective Fertility Preservation	
Patients returning (%)	78 (7.4)
Mean age at vitrification	34.5 ± 2.1
Mean age at return	38.3 ± 3.5
Mean storage time (years)	4.0 ± 0.9
Warming Cycles/patient	79 (1.0 ± 0.01)
Mean warmed oocytes/patient	592 (7.6 ± 2.8)
Survival rate	81.8
Number of transfers	56 (71.8)
Mean embryos Transferred	1.4 ± 0.1
IR	33.0
CPR/transfer	23 (41.8)
OPR/transfer	18 (32.1)
Nº Live Birth/patient	18/69* (26.0)
Cryotransfers of surplus embryos	
Nº patients	20 (25.6%)
Nº warming cycles/patient	28/20 (1.4 ± 1.7)
NºET/patient	36 (1.3 ± 1.4)
NºCryo transfers/warming cycle	27 (0.96 ± 0.1)
IR	31.3
CPR/transfer	9 (37.5)
OPR/transfer	7 (25.9)
Nº Live Birth	7 (35.0)
C Live birth rate/Patient	25/69* (36.2)

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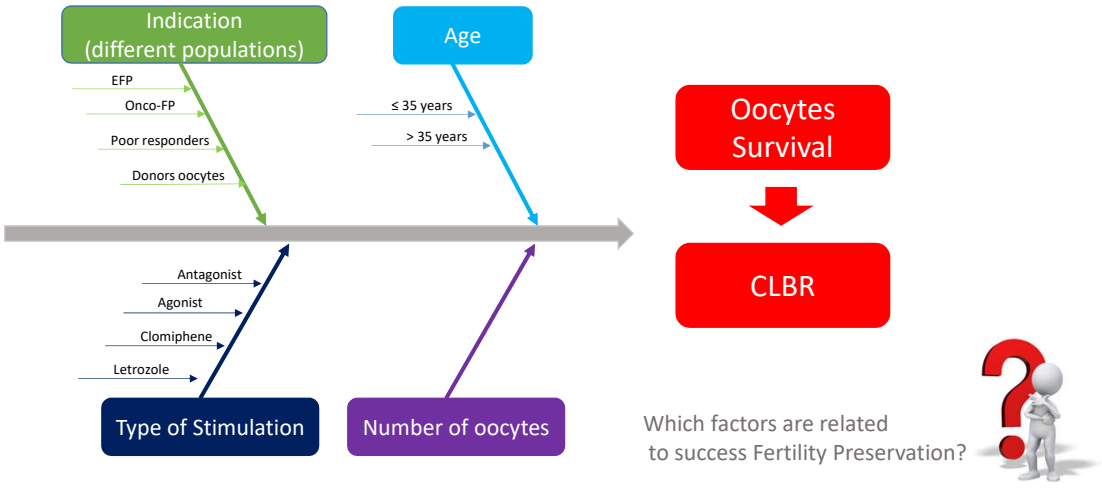
IVI unpublished data



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1. Effect of Age, Indication and Stimulation on Oocytes Survival and CLBR

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Results in different populations using vitrified oocytes.

	Oocyte Donation	Poor Responder ≤35	Poor Responder ≥36	EFP ≤35	EFP ≥36	Onco-FP ≤35	Onco-FP ≥36
N patients	15899*	316	648	119	503	42	36
N Cycles	18579	332	680	125	532	42	37
Mean age ± SD	25.3 ± 2.2 [a **]	33.3 ± 1.4 [b]	38.6 ± 1.5 [c]	32.5 ± 2.8 [b]	38.7 ± 1.0 [c]	31.6 ± 2.1 [b]	38.0 ± 2.1 [c]
Nº oocytes inseminated	11.4 ± 2.1 [a]	7.5 ± 4.2 [b]	6.8 ± 1.5 [c]	10.3 ± 3.7 [d]	8.6 ± 1.4 [e]	6.4 ± 3.7 [c,f]	5.9 ± 2.1 [f]
Survival rate (%)	92.3 [a]	83.6 [b, d]	84.9 [b]	91.4 [a]	82.1 [c,d]	81.2 [c]	82.7 [c,d]
Clinical pregnancy rate (%)	59.3 [a]	38.7 [b]	30.6 [c]	47.2 [a]	20.3 [c]	30.9 [c]	10 (27.0) [b,c]
CLBR/patient (%)	11445 (71.9) [a]	164 (51.8) [b]	208 (32.1) [c]	61/89 (68.5) [a]	99/367 (26.8) [c]	16/38 (42.1) [b,c]	9/31 (29.0) [c]

*Recipients; **Donors age. Different superscripts= P<0.05

Young social freezers achieve similar outcomes as donors.
Results are lower in young poor responders, onco FP and in older patients.
Young onco FP achieve low outcomes

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IVI unpublished data

Fertility Preservation Clinical Outcome According to Age.

OR for SV≥90% for EFP vs. ONCO-FP= 1.484 (CI95%= 0.876-2.252); P=0.202

LR Model- SV≥90%. Categorized by age			
	Adj. OR	CI95%	P value
EFP vs ONCO	1.968	(1.121-3.445)	0.018
≤35 y. vs ≥36y.	1.922	(1.274-2.900)	0.025
≤30 y. vs >40y.	4.116	(1.566-10.820)	0.004
31-35y. vs >40y.	2.746	(1.474-5.115)	0.001
36-40y. vs >40y.	1.658	(1.259-2.778)	0.045

LR- SV≥90%. Categorized by Age and Type of Stimulation			
	OR	CI95%	P value
EFP vs ONCO	1.396	(0.563-3.460)	0.472
≤30 y. vs >40y.	3.942	(1.423-10.918)	0.008
31-35y. vs >40y.	2.603	(1.368-4.953)	0.004
36-40y. vs >40y.	1.696	(1.099-2.906)	0.045
Agonist vs Antagonist	1.506	(0.582-3.895)	0.398
Letrozole vs Antagonist	2.546	(0.840-7.718)	0.099
Clomiphene vs Antagonist	1.455	(0.508-4.169)	0.485

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IVI unpublished data

Effect of Age and Indication on CLBR

OR for CLBR for EFP vs. ONCO-FP= 1.275 (CI95%= 0.711-2.284); P=0.414

LR Model- CLBR. Categorized by age			
	Adj. OR	CI95%	P value
EFP vs ONCO	2.204	(1.162-4.183)	0.016
≤35 y. vs ≥36y.	3.106	(2.039-4.733)	<0.0001
≤30 y. vs >40y.	36.477	(9.847-135.132)	<0.0001
31-35y. vs >40y.	16.514	(5.581-48.863)	<0.0001
36-40y. vs >40y.	7.411	(2.640-20.804)	<0.0001

LR- CLBR Categorized by Age and Type of Stimulation			
	OR	CI95%	P value
EFP vs ONCO	2.648	(0.949-7.390)	0.063
≤30 y. vs >40y.	37.261	(8.790-157.941)	<0.0001
31-35y. vs >40y.	19.010	(5.564-64.957)	<0.0001
36-40y. vs >40y.	9.252	(2.838-30.159)	<0.0001
Agonist vs Antagonist	1.109	(0.576-2.134)	0.757
Letrozole vs Antagonist	0.524	(0.277-1.001	0.051
Clomiphene vs Antagonist	1.120	(0.383-3.273)	0.836

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IVI unpublished data

2. Effect of the number of Oocytes on Survival and CLBR

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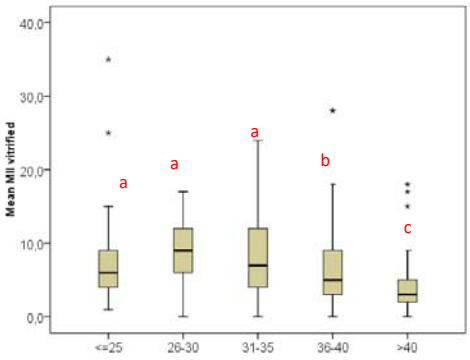
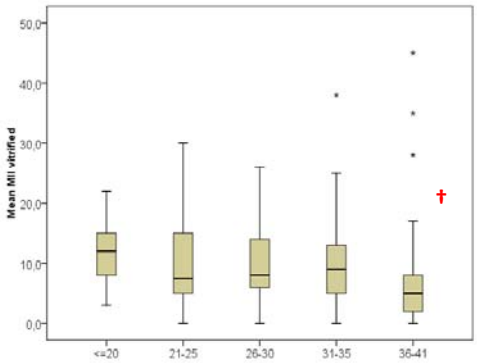
FP. Number of vitrified oocytes according to age

EFP. Vitrified oocytes



Mean	8.8	9.6	8.6	6.6	3.9

Onco-FP Vitrified oocytes



Mean	13.6	10.1	10.8	9.8	6.3

Effect of age on the ovarian reserve

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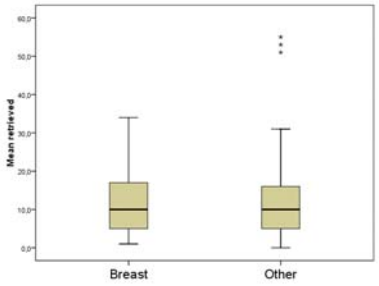
Cobo, A. et al. (2016). Fertil Steril **105**(3): 755-764 e758.

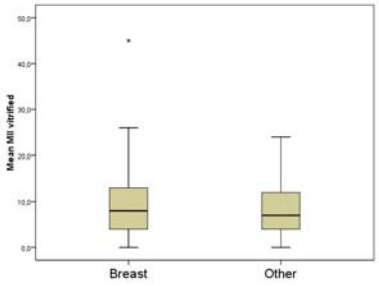
Vitrified and retrieved oocytes in oncological patients

Retrieved oocytes



Mean 11.9 11.8

Vitrified oocytes



Mean 9.3 9.1

NS

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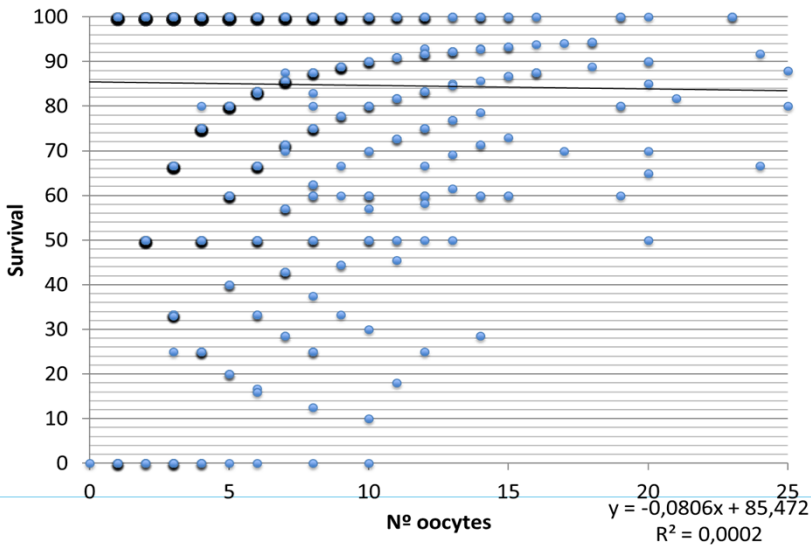
Effect of the Nº of Oocytes on the Survival



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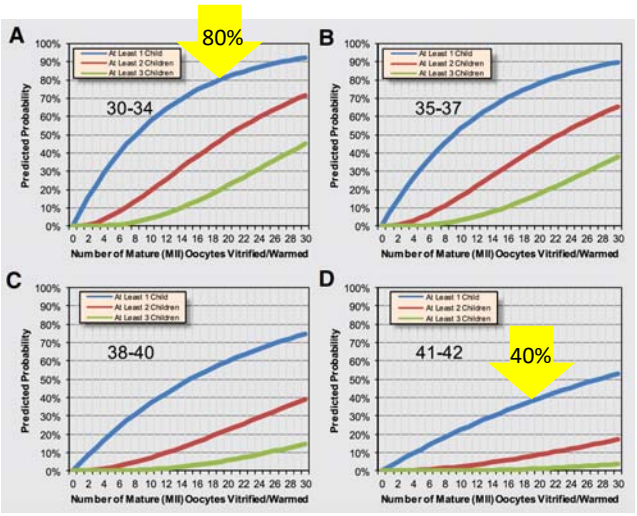
Success rates and expectations



1283 vitrified oocytes
128 IVF cycles

oocyte to baby rate

6.4%

IVI. 6215 oocytes
736 cycles

≤35 y. 6.5%

>35y. 2.9%

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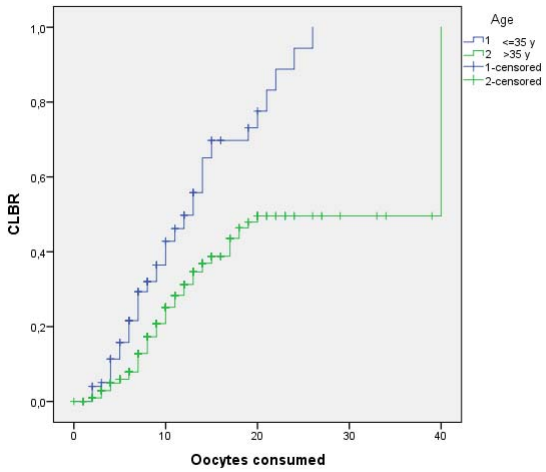
Doyle, J. et al. (2016). Fertil Steril 105(2): 459-466 e452.

EFP: Effect of the Nº of Oocytes on CLBR According to Age



≤35 (N=119)		≥36 (N=503)	
Nº oocytes	CLBR (95%CI)	Nºoocytes	CLBR (95%CI)
5	15.8 (8.4-23.1)	5	5.9 (3.6-8.3)
8	32.0 (22.1-41.9)	8	17.3 (13.3-21.3)
10	42.8 (31.7-53.90)	10	25.2 (20.2-30.1)
15	69.8 (57.4-82.2)	15	38.8 (32.0-45.6
20	77.6 (64.4-90.9)	20	49.6 (40.7-58.4)
24	94.4 (84.3-100.4)		

Log Rank (Mantel-Cox). P<0.0001
Breslow (generalized Wilcoxon. P>0.0001
Tarone-Ware. P<0.0001

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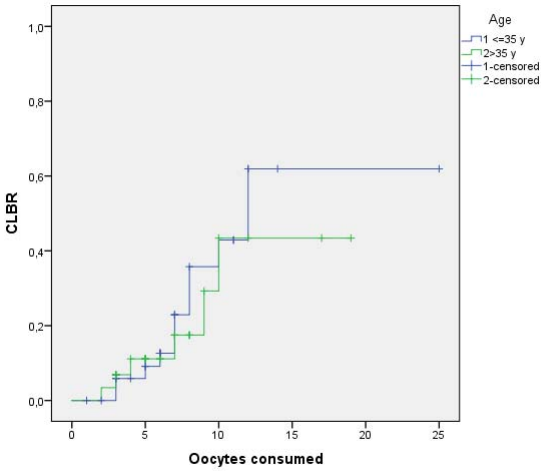
IVI unpublished data

Onco-FP: Effect of the Nº of Oocytes on CLBR According to Age



≤35 (N=42)		≥36 (N=36)	
Nºoocytes	CLBR(95%CI)	Nºoocytes	CLBR(95%CI)
5	9.1 (-0.7-19)	4	11.1 (-0.8-23.1)
8	35.8 (14.3-57.2)	9	29.3 (3.7-54.8)
10	42.9 (19.7-66.1)	10	43.4 (11.3-75.3)
12	61.9 (35.4-88.5)		

Log Rank (Mantel-Cox). P=0.577
Breslow (Generalized Wilcoxon. P=0.833
Tarone-Ware. P=0.703

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Yes, we can be effective in producing pregnancies with FP.

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Conclusions

1. Age and number of oocytes are the most powerful factors that affect success.

2. Differential survival and CLBR are observed in different indications:
   - Young social freezers achieve similar outcomes as donors.
   - Results are lower in young poor responders and onco FP patients.
   - Cancer patients achieve lower survival and CLBR but this effect remains to be clarified.

3. Type of stimulation has no clear impact on final outcomes.

4. Older women need more oocytes to equate the outcomes of younger patients. Although the gain per oocyte is lower for older women.

5. 36 years could be considered as a limit between better and worse results: with 8 oocytes the CLBR rate is ~ 30% in patients ≤35; while in patients ≥ 36% the probability is ~ 15%

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Closing Remarks

Women who consider elective oocyte cryopreservation should be encouraged to do so before the age of 35, although this could be less cost-effective.

It remains important to counsel women that elective oocyte cryopreservation can increase future reproductive chances but cannot guarantee reproductive success.

We freeze GAMETES not fertility.

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ESHRE Annual Meeting 2018
CCIB Barcelona, Spain
Pre-congress Course 16
July 1-4, 2018

In vitro gametogenesis: Status report

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Disclosure

I have no conflict of interest

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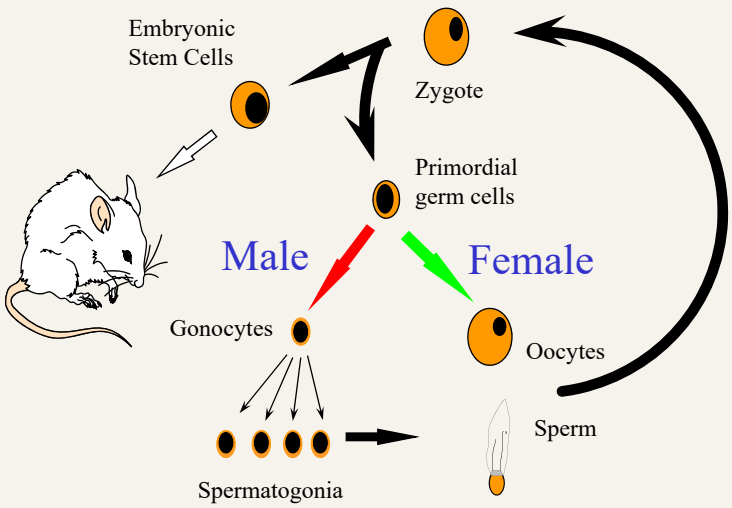
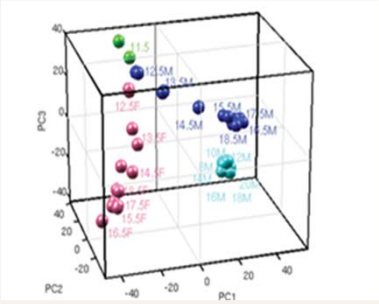
Learning Objectives

- Update knowledge on the germ line and the nature and origin of germ cells
- Understanding the gonadal niche and mechanisms controlling sexual differentiation and sex-specific germ cell differentiation
- Discussing options for *in vitro* systems to generate germ cells and mimic the gonadal microenvironment
- Exploring future options for *ex vivo* generation of eggs and sperm

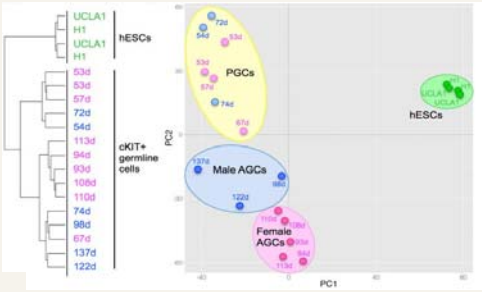
The Biology of Germ Cells



The Biology of Germ Cells

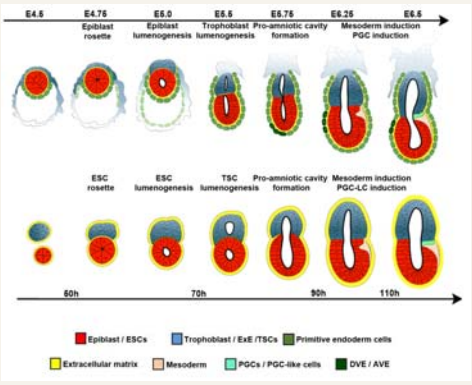


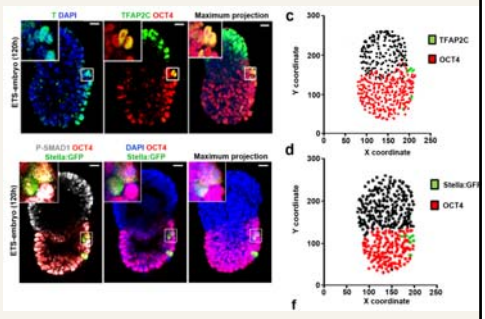
Sabour et al., **Identification of genes specific to mouse primordial germ cells through dynamic global gene expression**.
Hum Mol Genet. 20:115-25 (2011)



Gkountela et a. **DNA Demethylation Dynamics in the Human Prenatal Germline**.
Cell 161:1425-36 (2015)

The Biology of Germ Cells





Assembly of embryonic and extra-embryonic stem cells to mimic embryogenesis in vitro. SE Harrison, B Sozen, N Christodoulou, C Kyprianou, M Zernicka-Goetz . *Science* 10.1126/science.aal1810 (2017)

Plasticity of germ cells

Diploid premeiotic cells
Potentially totipotent
Often act as stem cells (self renewing population dynamics)

Located in somatic (stem cell) niches
Highly selected for DNA integrity

Limits for expansion?

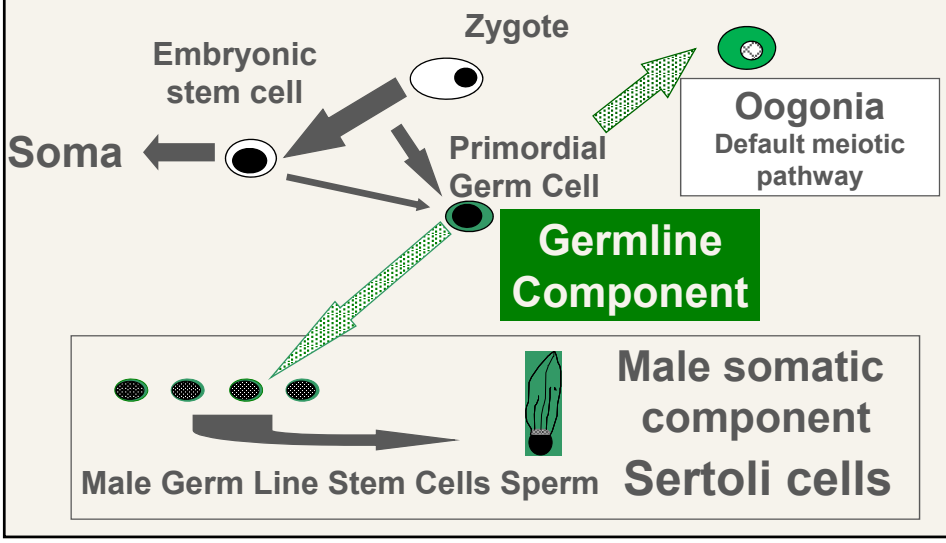
In vitro: Unrestricted developmental potential
In vivo: Often restricted developmental potential (Spermatogonia generate only sperm as unique endpoint)

ES ⟷ EG ⟷ PGC ⇒ Gonocyte ⇒ Spermatogonium

Toti/Pluripotency

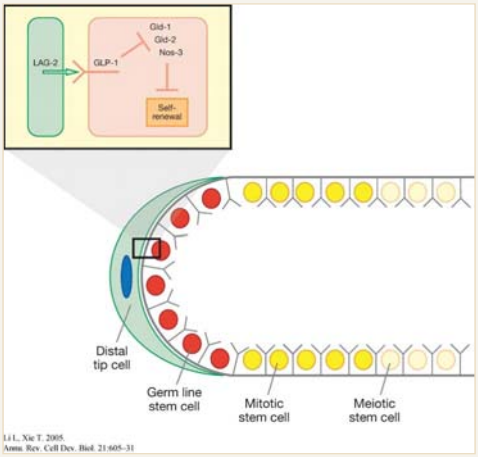
Germline sexual differentiation

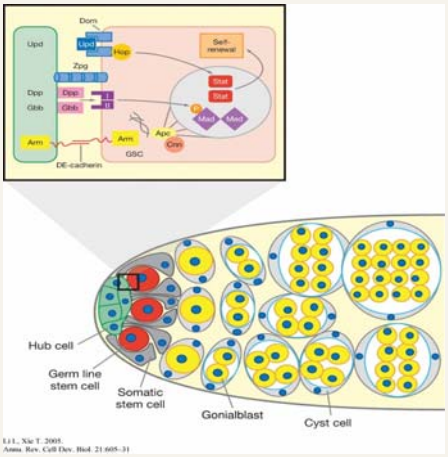
Soma acts as carrier of germ cells and guides its differentiation



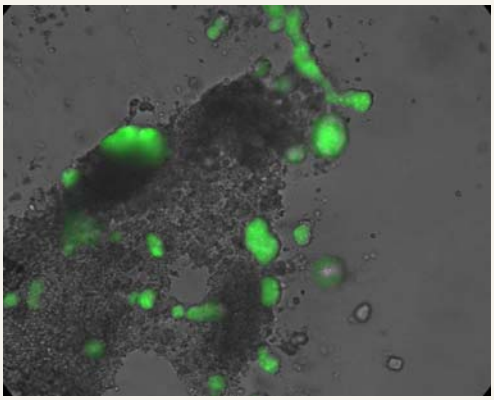
Gonadal stem cell niches: *Worm and Fly*

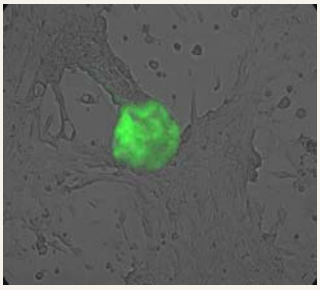
Phenotype, number and function of germline stem cells depends on the gonadal niche

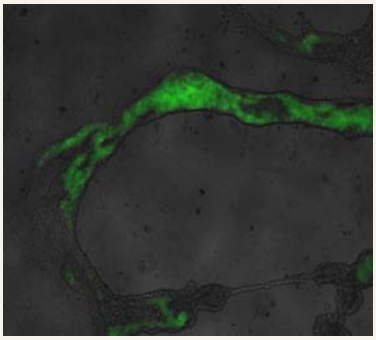




Embryonic Germ Cells in Testicular Cell Cultures







GFP-expression in mouse embryonic germ cells

Mouse testis (day 7) cultured in DMEM
No matrix substance

Klein AM, Nakagawa T, Ichikawa R, Yoshida S, Simons BD.

Mouse germ line stem cells undergo rapid and stochastic turnover.

Cell Stem Cell. 2010 7:214-24.
doi: 10.1016/j.stem.2010.05.017

Klein AM, Simons BD.

Universal patterns of stem cell fate in cycling adult tissues.

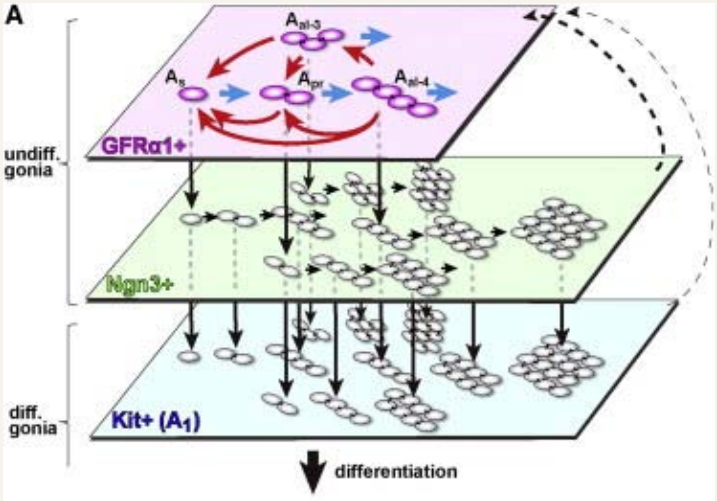
Development. 2011 138:3103-11.
doi: 10.1242/dev.060103.

Krieger T, Simons BD.

Dynamic stem cell heterogeneity.

Development. 2015 142: 1396-406.
doi: 10.1242/dev.101063. Review

Model for SSC dynamics



Hara et al. **Mouse spermatogenic stem cells continually interconvert between equipotent singly isolated and syncytial states.** Cell Stem Cell 14: 658-72 (2014)

Scenarios for *in vitro gametogenesis*

(PubMed: in vitro spermatogenesis: > 3000 hits)

Some experiments on spermatogenesis in vitro.
Goldschmidt. PNAS 1: 220-222 (1915)

Initiation of spermatogenesis in vitro.
Steinberger et al. Endocrinol 74: 788-792 (1964)

In vitro differentiation of germ cells from nonobstructive azospermic patients using three-dimensional culture in a collagen matrix.
Lee et al., Fertil Steril 87: 824-833 (2007)

In vitro post-meiotic germ cell development from human embryonic stem cells.
Aflatoonian et al., Hum Reprod 24: 3150-3159 (2009)

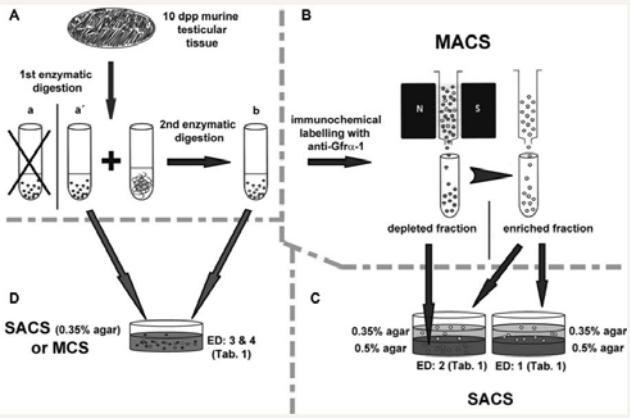
PSCDGs of mouse multipotent adult germine stem cells can enter and progress through meiosis to form haploid germ cells in vitro.
Nolte et al., Differentiation 80:184-94 (2010)

In vitro production of functional sperm in cultured neonatal mouse testes.
Sato et al. Nature 471: 504-507 (2011)

New horizons for in vitro spermatogenesis?

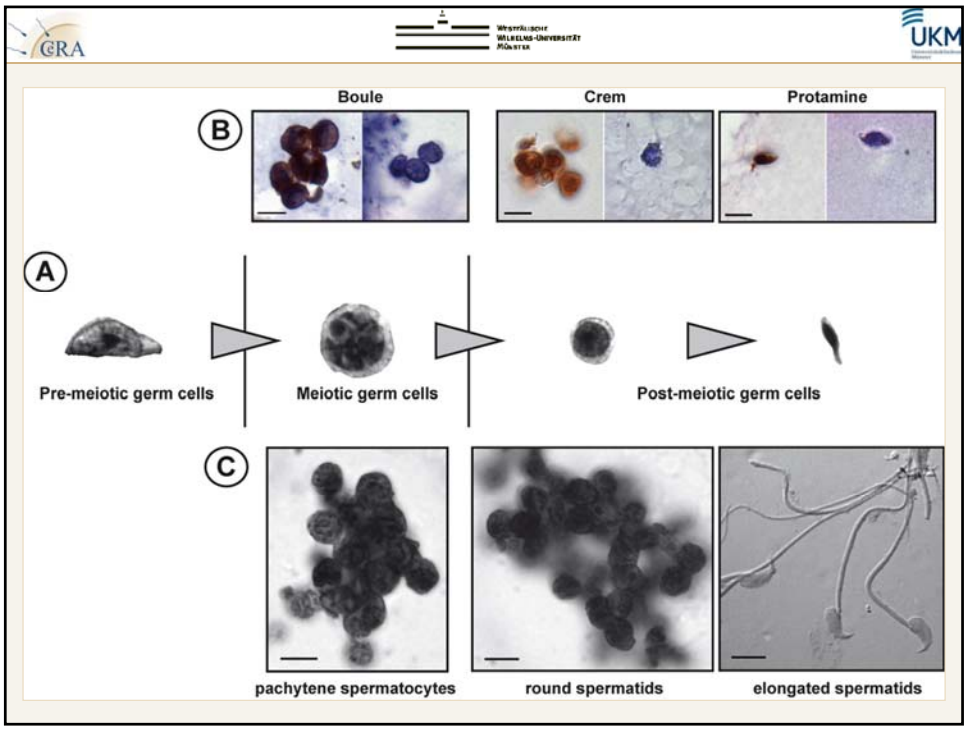
An update on novel three-dimensional culture systems as tools for meiotic and post-meiotic differentiation of testicular germ cells

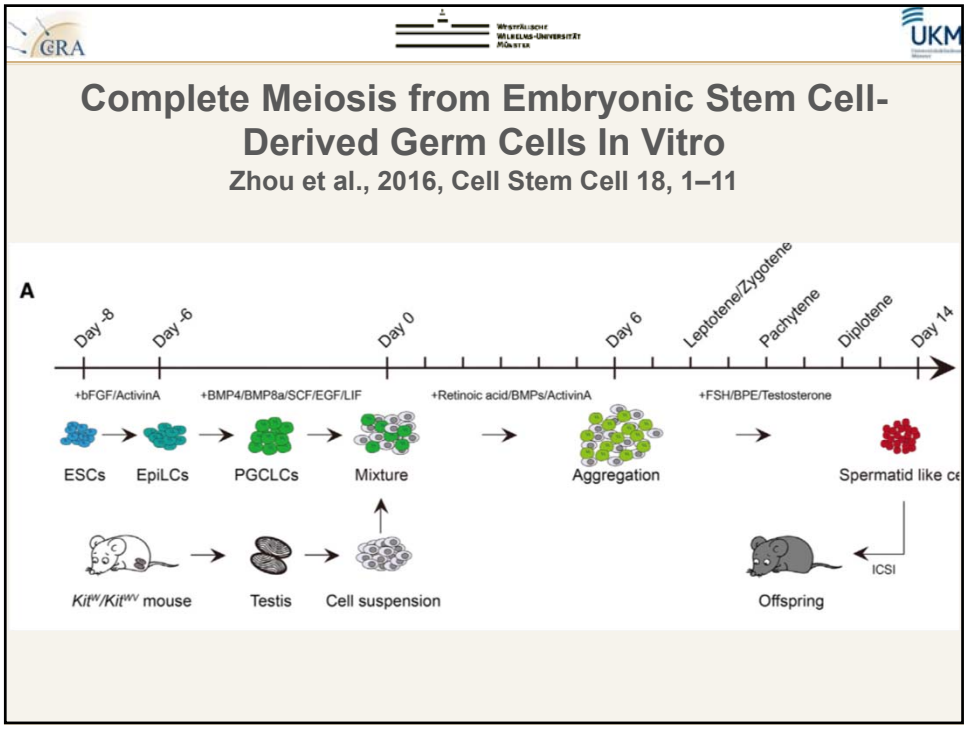
Stukenborg et al. Mol Hum Reprod 15: 521–529, 2009

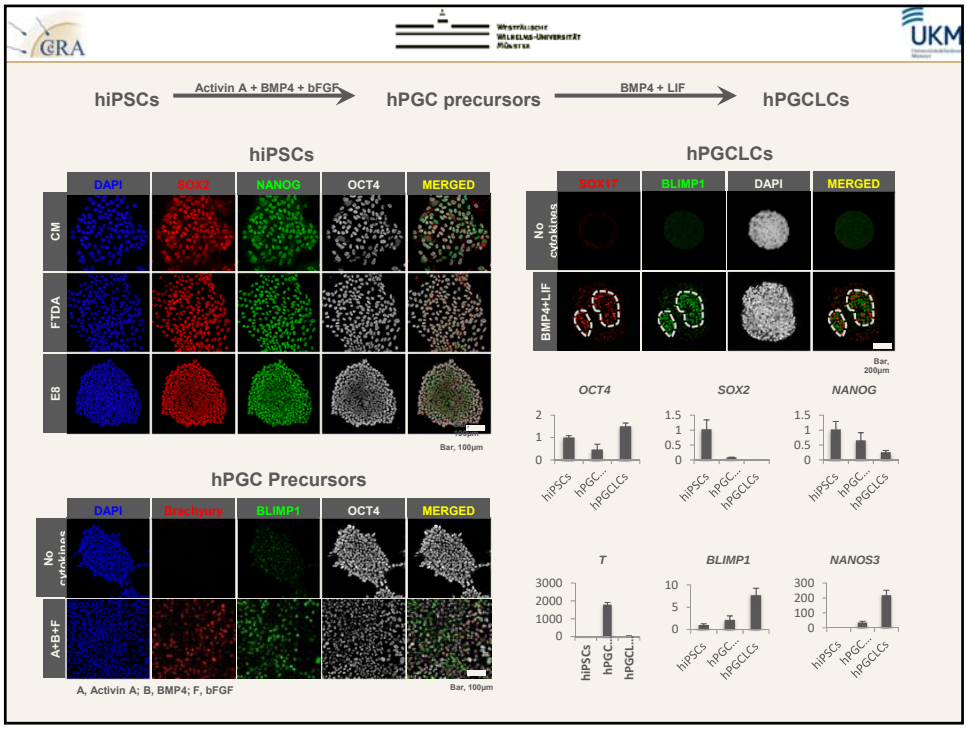


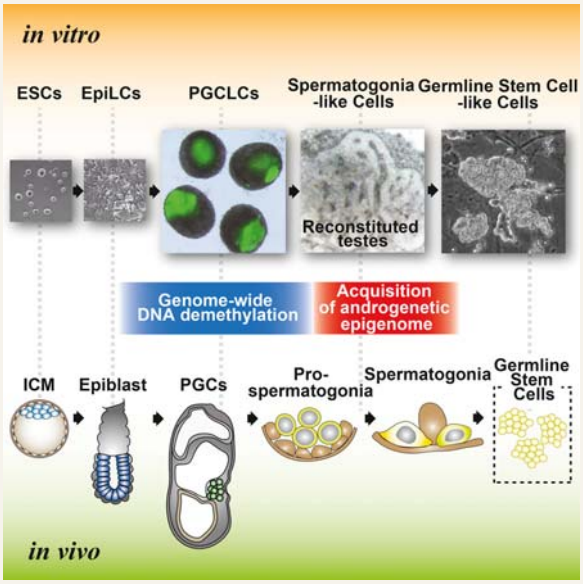
Abu Elhija M, Lunenfeld E, Schlatt S, Huleihel M. **Differentiation of murine male germ cells to spermatozoa in a soft agar culture system.** Asian J Androl. 14:285-93 (2012)

Stukenborg JB, Wistuba J, Luetjens CM, Elhija MA, Huleihel M, Lunenfeld E, Gromoll J, Nieschlag E, Schlatt S. **Coculture of spermatogonia with somatic cells in a novel three-dimensional soft-agar-culture-system.** J Androl 29: 312-29 (2008)


Boule
Crem
Protamine
B
A
Pre-meiotic germ cells
Meiotic germ cells
Post-meiotic germ cells
C
pachytene spermatocytes
round spermatids
elongated spermatids


Complete Meiosis from Embryonic Stem Cell-Derived Germ Cells In Vitro
Zhou et al., 2016, Cell Stem Cell 18, 1–11
A
Day -8
Day -6
Day 0
Day 6
Leptotene/Zygotene
Pachytene
Diplotene
Day 14
+bFGF/ActivinA
+BMP4/BMP8a/SCF/EGF/LIF
+Retinoic acid/BMPs/ActivinA
+FSH/BPE/Testosterone
ESCs
EpiLCs
PGCLCs
Mixture
Aggregation
Spermatid like ce
Kit^W/Kit^WV mouse
Testis
Cell suspension
ICSI
Offspring





Zhou et al.
Complete Meiosis from Embryonic Stem Cell-Derived Germ Cells In Vitro.
Cell Stem Cell 18, 1–11 (2016)

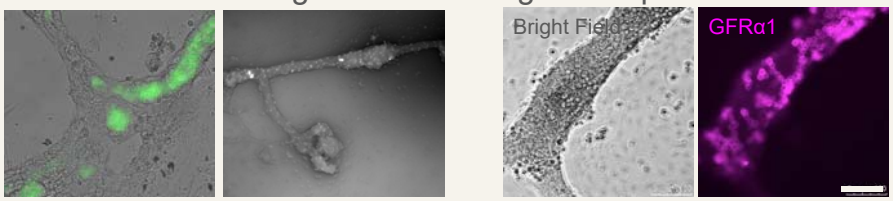
Ishikura et al.
In Vitro Derivation and Propagation of Spermatogonial Stem Cell Activity from Mouse Pluripotent Stem Cells.
Cell Rep. 17:2789-2804 (2016)

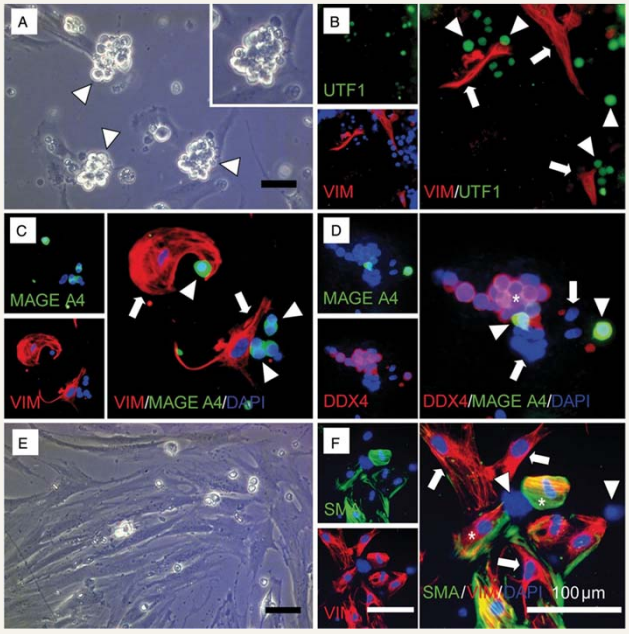
How to create a testis in vitro?

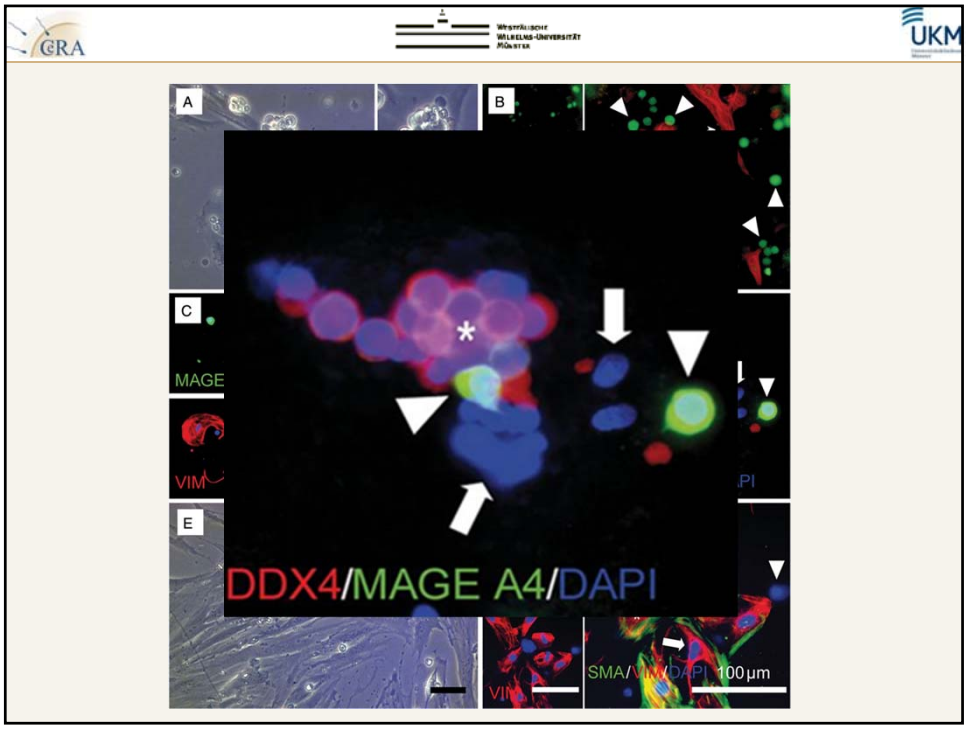
In vitro cord formation: Sertoli cell recapitulate organogenesis

Pan F, Chi L, Schlatt S. **Effects of nanostructures and mouse embryonic stem cells on *in vitro* morphogenesis of rat testicular cords.** PLoS One 8:e60054. (2013)

Observe germ cell homing and expansion







Hypothesis on in vitro gametogenesis

Will rely on better understanding of

- **germ cell segregation/establishment**
- **germ cell plasticity**
- **soma/germline interactions and communication**
- **stem cell niche (clonal expansion)**

Gamete production can be achieved by *in vitro* recapitulation of the gonadal microenvironment

- **Female: embryonic/fetal stages of ovarian development**
- **Male: Adult seminiferous epithelium (Sertoli cells)**

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Hum Reprod. 2015; 30: 2463-75

Schlatt S, Ehmcke J. Regulation of spermatogenesis: an evolutionary biologist's perspective. Semin Cell Dev Biol. 2014; 29: 2-16.

Reuter K, Schlatt S, Ehmcke J, Wistuba J. Fact or fiction: In vitro spermatogenesis. Spermatogenesis. 2012; 2: 245-252

BETWEEN THE PATIENT AND THE CRYOTANK:
TISSUE TRANSPORT AND FREEZING

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NO CONFLICT OF INTEREST

- No conflict of interest

OUTLINE OF TALK

- ESTABLISHING A CENTRALISED SERVICE FOR OVARIAN TISSUE CRYOPRESERVATION (OTC)
  - OVERNIGHT TRANSPORT
- FREEZING OVARIAN TISSUE:
  - SLOW FREEZING VERSUS VITRIFICATION
  - VALIDATION OF FREEZING

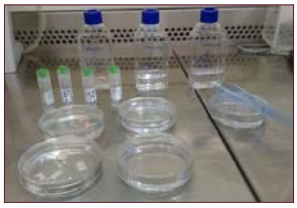
3

CRYOPRESERVATION OF HUMAN OVARIAN TISSUE AND RESTORATION OF OVARIAN FUNCTION

Retrival of one ovary

Preparation of cortical tissue

Freezing



Thawing

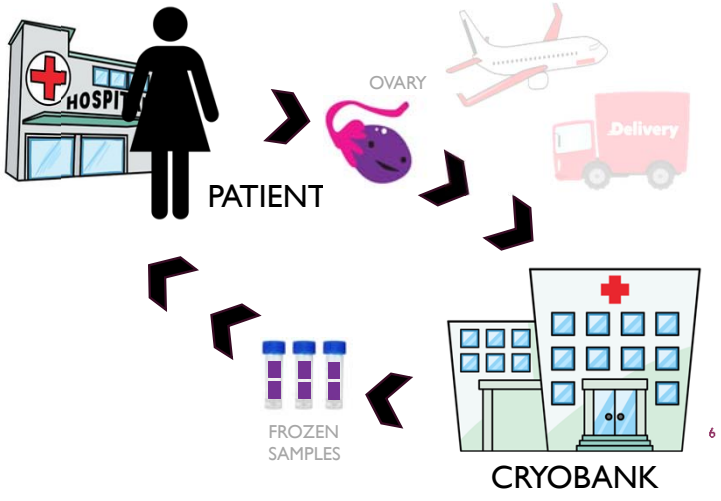
Transplantation

4

ESTABLISHING A CENTRALISED SERVICE FOR OVARIAN TISSUE CRYOPRESERVATION

5

THE DANISH MODEL
”THE WOMAN STAYS – THE TISSUE MOVES”



6

NATIONAL PROGRAMMES WITH CENTRALIZED CRYOBANKS PROVIDE SPECIALIZED SERVICE

- Ovarian tissue is removed at the local hospital and transported to a central laboratory where cryopreservation and storage is performed.
- The advantages of either centralized or network-organized cryopreservation are;
  - It keeps the expertise of both consulting doctors and technical performance concentrated and updated in a few centers.
  - It secures a high quality service of this relative seldomly performed procedure.
  - It provide patients who are very ill with an option to have their fertility preserved.

7

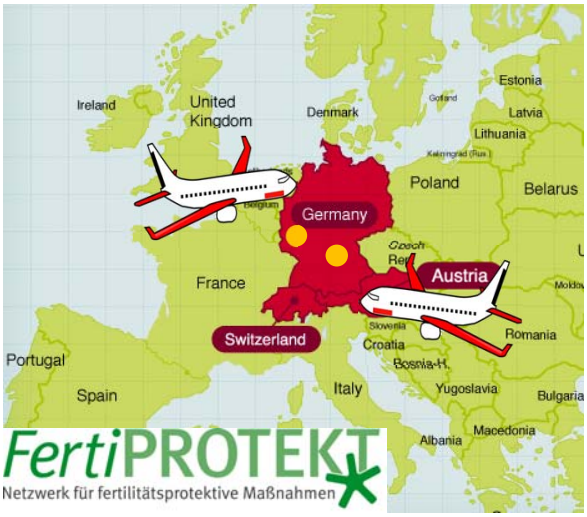
TRANSPORT OF OVARIAN TISSUE FOR UP TO 4-5 HOURS ON ICE

- Tissue is transported on crushed ice.
- Direct contact between the tissue and ice should be avoided (boble-plast or 'fruit bags' can be used for insulation)
- The metabolic rate of the cells is limited when the tissue is cooled around zero degrees.
- Therefore, the transportation media only need to comprise a basic medium with salts and small amount of nutrients is sufficient.

8

FERTIPROTEKT: A THREE-COUNTRY NETWORK

- In the FertiPROTEKTnetwork, cryopreservation of tissue is performed more often than ovarian stimulation and cryopreservation of oocytes (vonWolff et al., 2015).
- The ovarian tissue is mainly stored in two central cryobanks (Bonn and Erlangen) and the majority of the transplantations are carried out in specialized centres.
- All centres of the network have to meet strict constantly controlled and optimised standards of consultancy and therapy.



TRANSPORTATION OF OVARIAN TISSUE WITHIN FERTIPROTEKT

- The ovarian tissue is transported in a tube filled with an organ perfusion solution (Custodiol) or a phosphate-buffered saline (PBS)solution.
- Overnight transportation are carried out in special isolated transportation containers with precise temperature documentation.
- The mean period between harvesting and final cryopreservation is 18 hours to a max. of 24 hours (Van der Ven H. et al., Human Reproduction, 2016).
- FertiPROTEKT has so far published 17 live births.

11

FOLLICLE VIABILITY FOLLOWING OVERNIGHT/PROLONGED TRANSPORT OF OVARIAN TISSUE

- First case of prolonged transport was reported by Rosendahl M. et al. 2011. Viable follicles were present after 20 hours of transport on ice.
- Isachenko et al., showed that exposing human cortical tissue for suprazero temperatures for 0–26 hours did not inhibit the development of follicles during subsequent in vitro culture (Isachenko E. et al., Fertil Steril 2009).
- Effect of cooling on bovine ovarian tissue (Lucci CM et al., Theriogenology, 2004).
  - Storage of ovarian tissue at 4 °C for up to 18 hours kept the percentage of normal follicles similar to controls.
  - Storage of ovarian tissue at 20 °C for 18 hours significantly reduced the percentage of morphologically normal follicles compared to controls.
  - The type of solution (saline or coconut water) that the ovaries were immersed in had little effect on the results.

12

CURRENT ACTIVITY OF OVARIAN TISSUE CRYOPRESERVATION SUPPORTS A CENTRALIZED SERVICE

- The impact of transportation and prolonged cooling remains an area which is poorly explored, as the final endpoints with pregnancies and live births are decades ahead of us.
- However, so far the clinical outcomes support transportation, as live births have been reported in both the Danish and German cohorts.
- In Germany, 400 ovarian tissue cryopreservation procedures are carried out each year, representing a total figure of more than 2500 cryopreservations to date (2016).
- Approximately 5 cryopreservations per million population per year in Germany and 13 per million in Denmark.
- Currently, the indications for ovarian tissue cryopreservation and the level of activity support the centralization of the technique, which is very different from normal IVF and is a tremendous work for clinics to implement on their own.

13

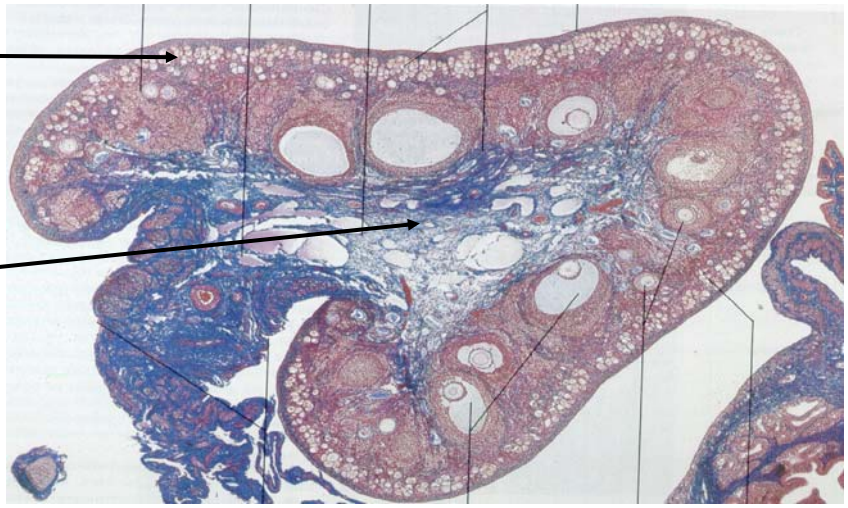
THE OVARY HAS ARRIVED SAFELY TO THE CRYOBANK – THEN WHAT?

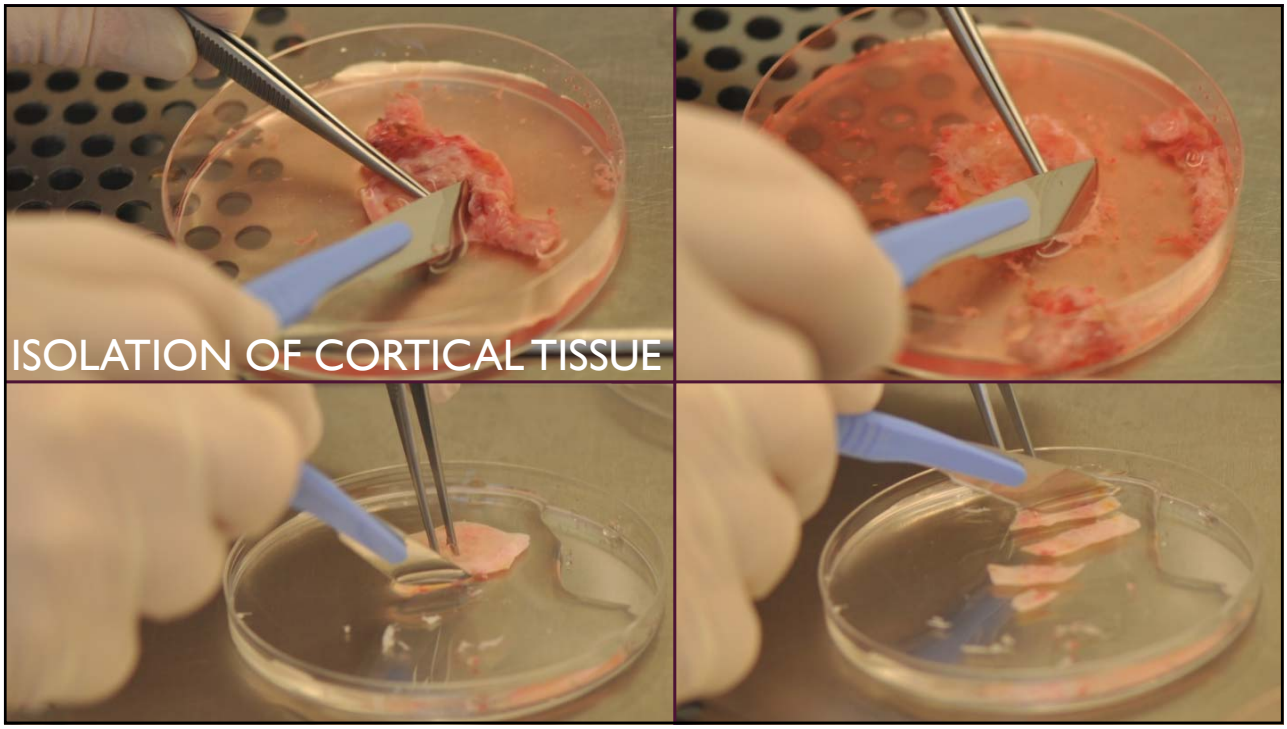
14

ONLY THE CORTICAL TISSUE IS CRYOPRESERVED

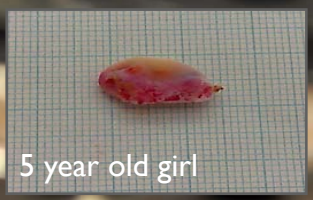
Cortex
Contains 90% of the follicular reserve (primordial follicles)

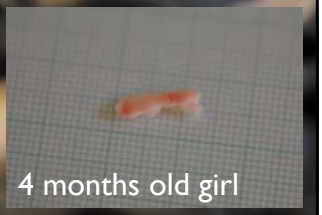
Medulla
Contains the growing follicles



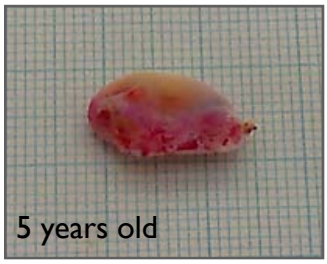


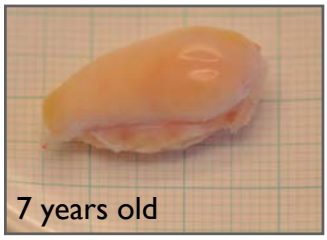
OVARIAN TISSUE PREPARED FOR CRYOPRESERVATION

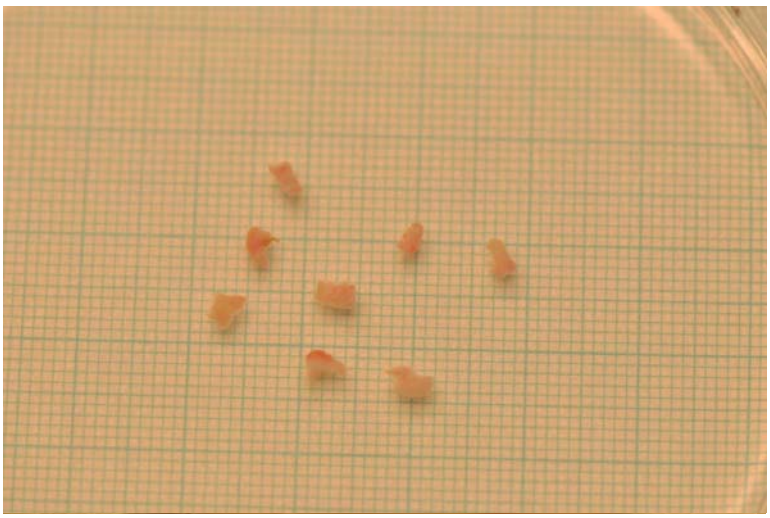




OVARIES FROM LITTLE GIRLS ARE PREPARED A LITTLE DIFFERENT FROM ADULT OVARIES







FREEZING OVARIAN TISSUE

19

IN DENMARK THE OVARIAN TISSUE IS FROZEN BY CONTROLLED SLOW-FREEZING

Freezing solution:
1.5 M Ethyleneglycol; 0.1 M Sucrose;
10 mg/ml HSA

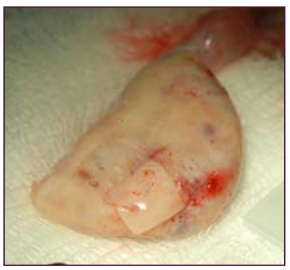
Temperature profile:
1. Equilibration – rotation (1-2 ^{0}C for 30 min)
2. – 2 ^{0}C/min until – 9 ^{0}C
3. Manuel seeding
4. – 0,3 ^{0}C/min until – 40 ^{0}C
5. – 10 ^{0}C/min until – 140 ^{0}C
6. Liquid nitrogen (– 196 ^{0}C)

SLOW FREEZING PROTOCOLS – USE OF CRYOPROTECTIVE AGENTS

- Denmark: 1.5 mol/l ethylene glycol + 0.1 mol/l sucrose + 10 mg/ml HSA in PBS, tissue equilibrated for 30 minutes.
- Belgium: 1.5 mmol/L DMSO + 4 mg/mL of human serum albumin in Leibovitz medium, tissue equilibrated for 30 minutes
- Israel: 1.5 M DMSO + 15% synthetic serum substitute supplement + 0.1 M sucrose, tissue equilibrated for 30 minutes
- Germany: 10% CryoSure-DMSO + 10% serum substitute supplement in Leibovitz's L-15 GlutaMAX medium, tissue equilibrated for 30 minutes
- Australia: 1.5 mol/l propanediol with 0.1 mol/l sucrose in base medium at room temperature for 90 min

21

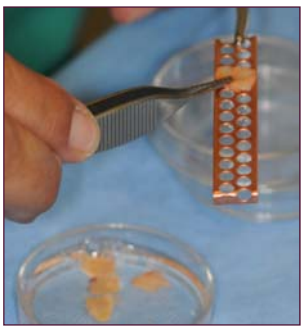
VITRIFICATION OF OVARIAN TISSUE
(PROTOCOL: KAGAWA N, et al., 2009; SILBER S, et al., 2010)



Cortical tissue prepared
10mm x10mm
Thickness: 1-1.5mm

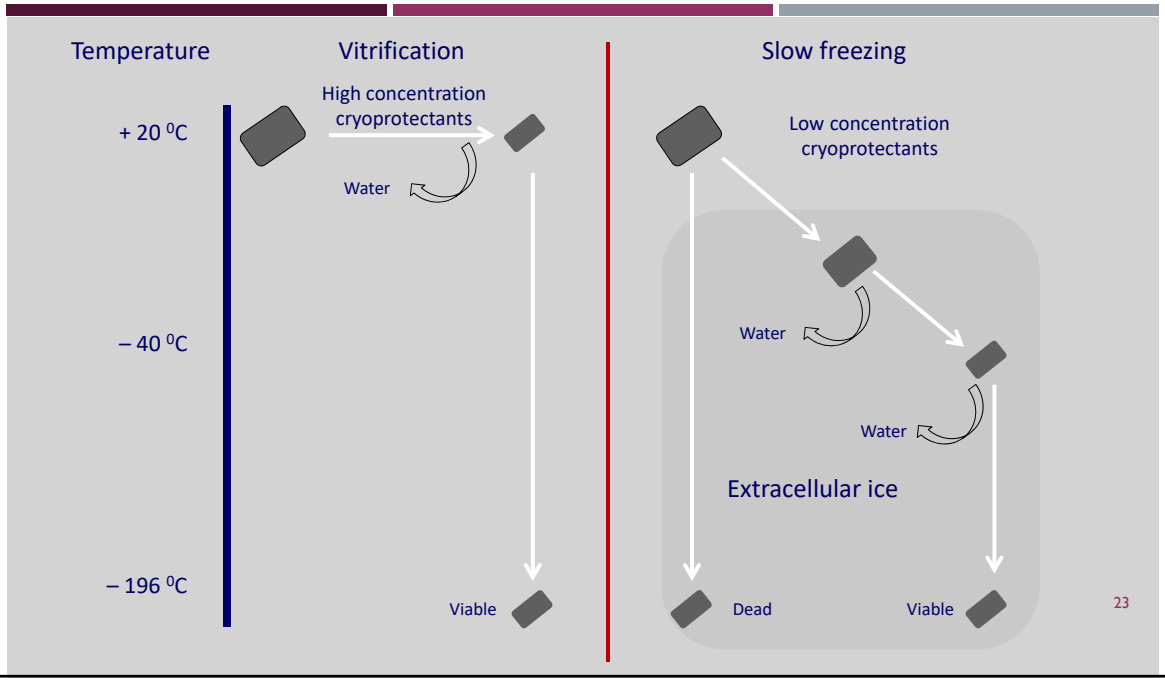
Media 1:
7.5% EG + 7.5% DMSO+ 20% SSM for 25 min

Media 2:
20% EG + 20% DMSO + 0.5M Sucrose for 15 min



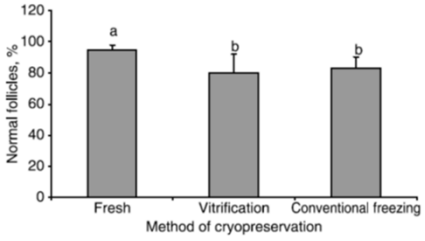
Tissue placed on metal strip and plunged directly into liquid N_2

22



SLOW-FREEZING VERSUS VITRIFICATION OF OVARIAN TISSUE: WHICH IS THE BETTER?

- Isachenko V et al., 2009:
  - During a 16 day culture period there were no difference in oestradiol, progesterone secretion or follicle quality.
  - The percentage of follicles with normal morphologically was similar in both groups after thawing.
- Keros V et al., 2009:
  - Based on tissue from 20 women the study concluded that vitrification was comparable to slow freezing in terms of preserving follicles in human ovarian tissue
  - However, ovarian stroma retained a better morphological integrity after vitrification.

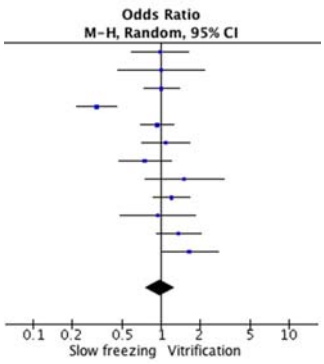


Isachenko V *et al., Reproduction*, 2009

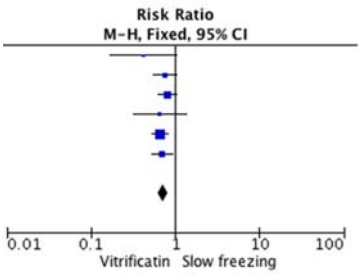
24

VITRIFICATION VERSUS SLOW FREEZING: A SYSTEMATIC REVIEW AND META-ANLAYSIS

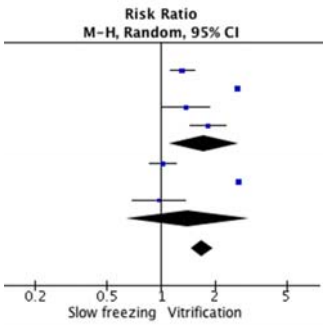
Proportion of intact primordial follicles



DNA fragmentation in primordial follicles



Proportion of normal stromal cells



- Meta-analysis of 14 studies comparing vitrification with slow freezing for cryopreservation of human ovarian tissue (Shi Q et al., Sci Rep. 2017)

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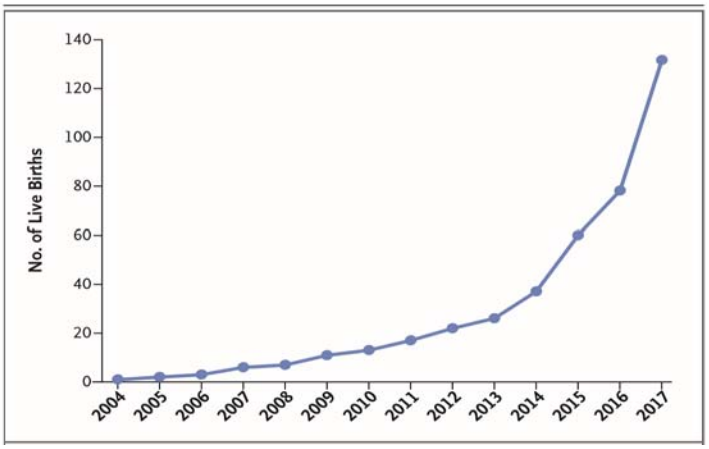
VITRIFICATION APPEARS TO BE A LITTLE BETTER, HOWEVER, CLINICAL RESULTS ARE AWAITED

- Meta-analysis (Shi Q et a., 2017) conclude that vitrification may be more effective than slow freezing, with less primordial follicular DNA strand breaks and better preservation of stromal cells. These advantages could lead to improved ovarian function after transplantation.
- **However**, the primordial follicle is pretty resistant to freezing and well-preserved with both methods, and in the end it is the clinical data which will finally decide which technique will be superior – but this will take years, if not decades…
- Basically slow-freezing and vitrification do the same thing but at a different speed and at different temperatures - they can probably be used with almost equal efficacy.

26

THE VAST MAJORITY OF BIRTHS HAVE BEEN ACHIEVED AFTER SLOW FREEZING



Donnez J & Dolmans MM, N Engl J Med. 2017

- So far, only a handful of babies have been born after vitrification of the ovarian tissue (Sherman Silber, US, and Kawamura, Japan/China).
- The efficacy of vitrification cannot be evaluated – too few clinical results.

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LONG TERM STORAGE OF FROZEN OVARIAN TISSUE

HOW DO WE SECURE SURVIVING FOLLICLES?

29

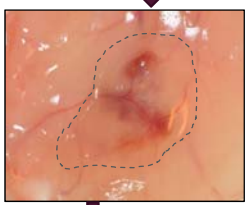
GOLDEN STANDARD FOR VALIDATION OF FOLLICULAR SURVIVAL; XENOTRANSPLANTATION TO NUDE-MICE

NUDE-MICE
(Immunodeficient)

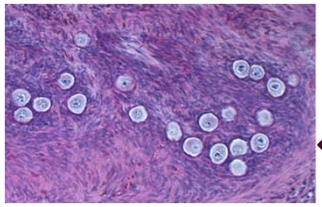
The deficiency in T cell function allows athymic mice to accept and grow xenografts of normal and malignant tissues.

Xeno-grafting

Graft retrival
(4 weeks)



Histology

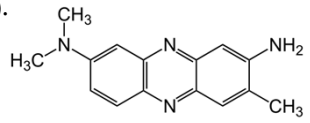


A <u>FUNCTIONAL</u> TEST WITH A <u>QUALITATIVE</u> OUTCOME

30

FOLLICULAR VIABILITY AND DENSITY ASSESSMENT USING NEUTRAL RED STAINING

- **Neutral Red** is a weak cation that passes through the intact plasma membrane and becomes concentrated in lysosomes of viable cells (Triglia et al., 1991).



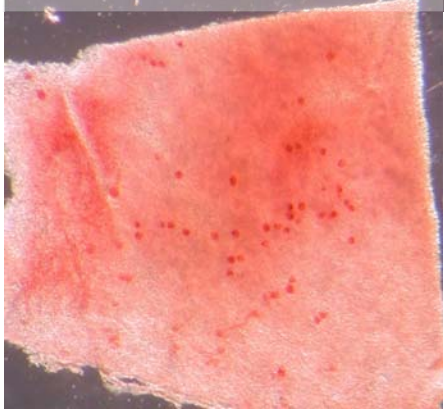
(Chambers et al., 2010, Hum Reprod)


human reproduction ORIGINAL ARTICLE *Reproductive biology*

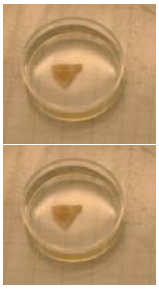
***In situ* identification of follicles in ovarian cortex as a tool for quantifying follicle density, viability and developmental potential in strategies to preserve female fertility**

E.L. Chambers[1], R.G. Gosden[2], C. Yap[3], and H.M. Picton[1,*]

Human ovarian cortex stained with Neutral Red

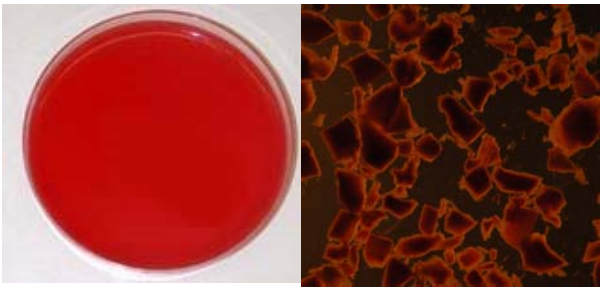


SIMPLE PROTOCOL FOR NEUTRAL RED STAINING *IN SITU*



1-2 pieces of thawed ovarian cortex

Ovarian cortex chopped into smal pieces (<100µm) with the McIlwain Tissue Chopper

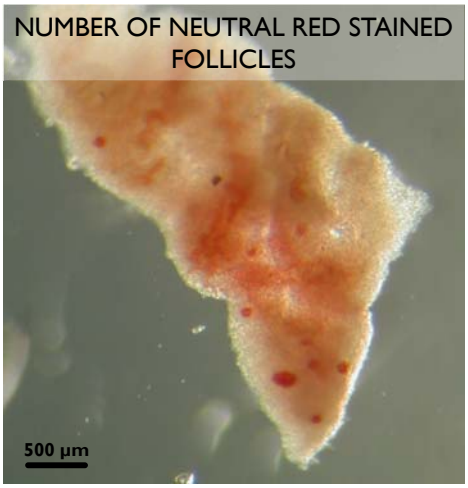


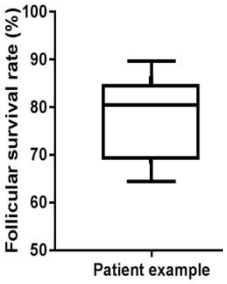
Incubated for 3-4 hours in Neutral Red (15µl NR per 1 ml of prewarmed and CO_2 equillibrated culture media)

32

(Kristensen SG et al., Hum Reprod, 2011)

A NEW TOOL TO QUANTIFY FOLLICULAR SURVIVAL





Piece no.	NR stained follicles	Total no. of follicles	Survival Rate (%)
1	21	28	75.0
2	14	18	77.8
3	13	20	65.0
4	10	12	83.3
5	15	18	83.3
6	26	29	89.7
7	24	30	80.0

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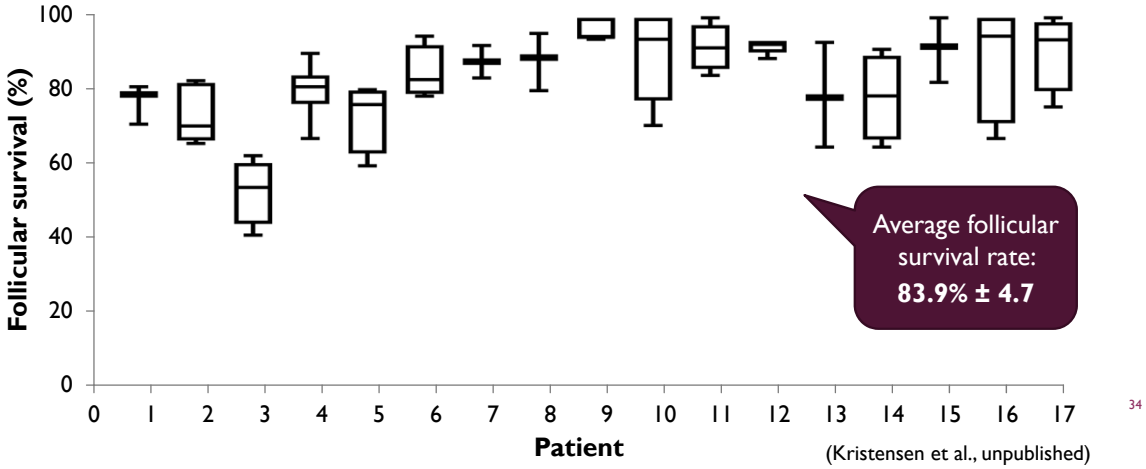
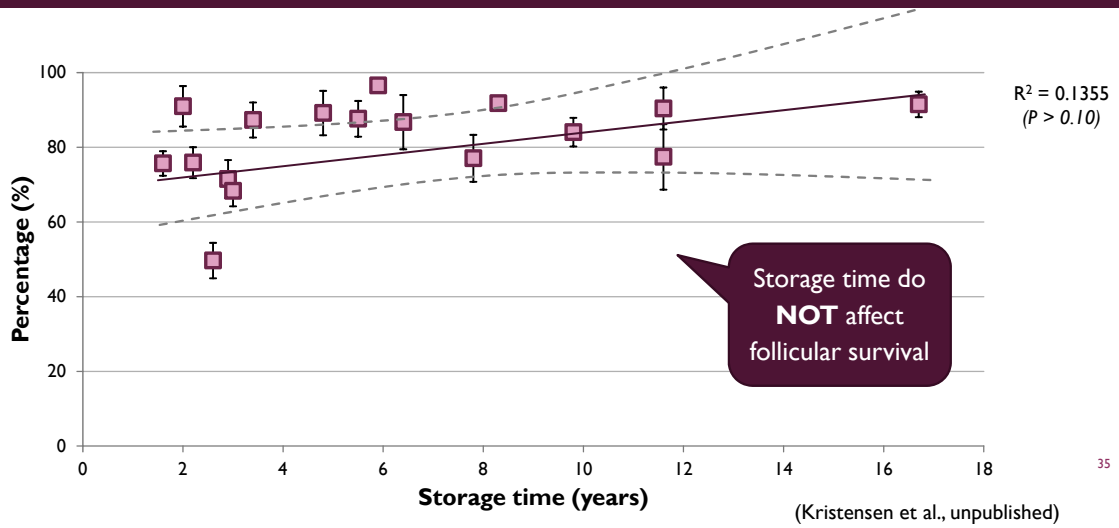
FOLLICULAR SURVIVAL RATE IN OVARIAN TISSUE FROM 17 PATIENTS



(Kristensen et al., unpublished)

34

FOLLICULAR SURVIVAL RATE IN RELATION TO STORAGE TIME OF THE OVARIAN TISSUE



(Kristensen et al., unpublished)

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NEUTRAL RED: A NEW AND SIMPLE TEST TO QUANTIFY FOLLICULAR SURVIVAL

- NEUTRAL RED STAINING COMBINED WITH HISTOLOGICAL EVALUATION IS A QUANTITATIVE METHOD TO PROVIDE AN EXACT SURVIVAL RATE FOR FOLLICLES IN FROZEN/THAWED HUMAN OVARIAN TISSUE.

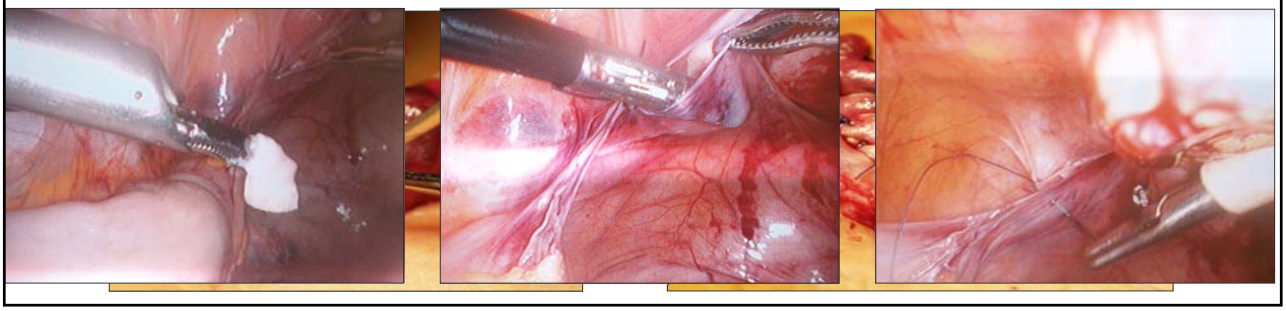
- THIS NEW AND SIMPLE METHOD COULD BE APPLIED IN MOST CENTERS TO VALIDATE THE CRYOPRESERVATION PROCEDURE.

AN IN VITRO TEST WITH A QUANTITATIVE OUTCOME

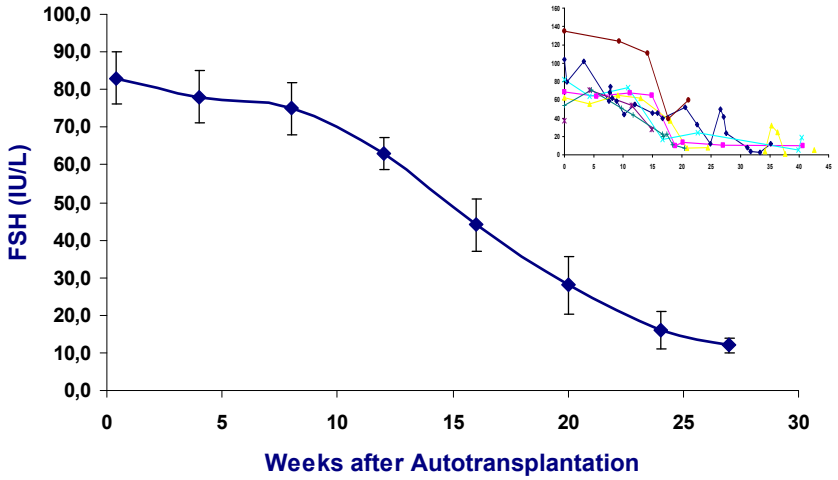
36

ONCE FOLLICULAR SURVIVAL HAS BEEN SECURED IN THE CRYOSTORED TISSUE, TRANSPLANTATION CAN BE PERFORMED

- Laparoscopy / mini-laparotomy
- **Orthotopic** (ovary)
- **Heterotopic** (sub-peritoneal on anterior abdominal wall and lateral pelvic wall)



RESTORATION OF ENDOCRINE FUNCTION



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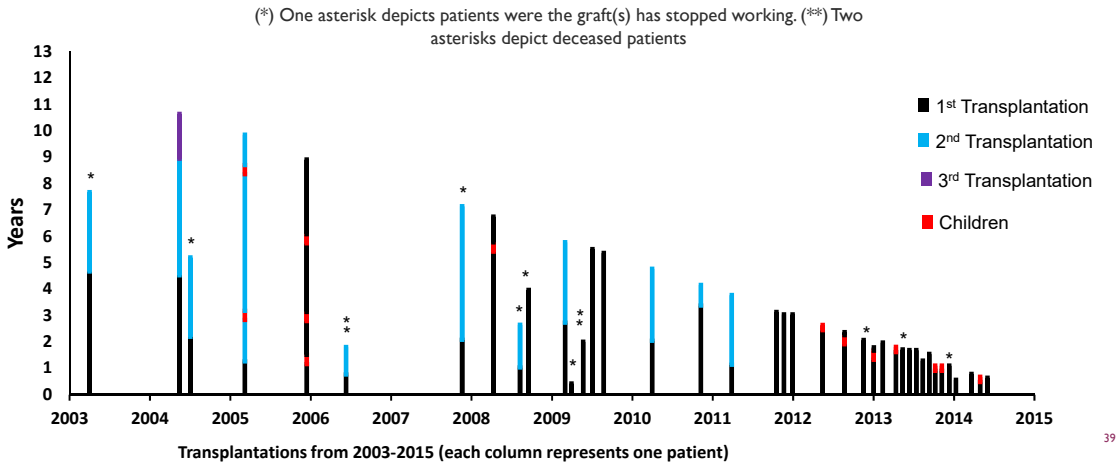
LONGEVITY OF TRANSPLANTED OVARIAN TISSUE IS SURPRISINGLY LONG



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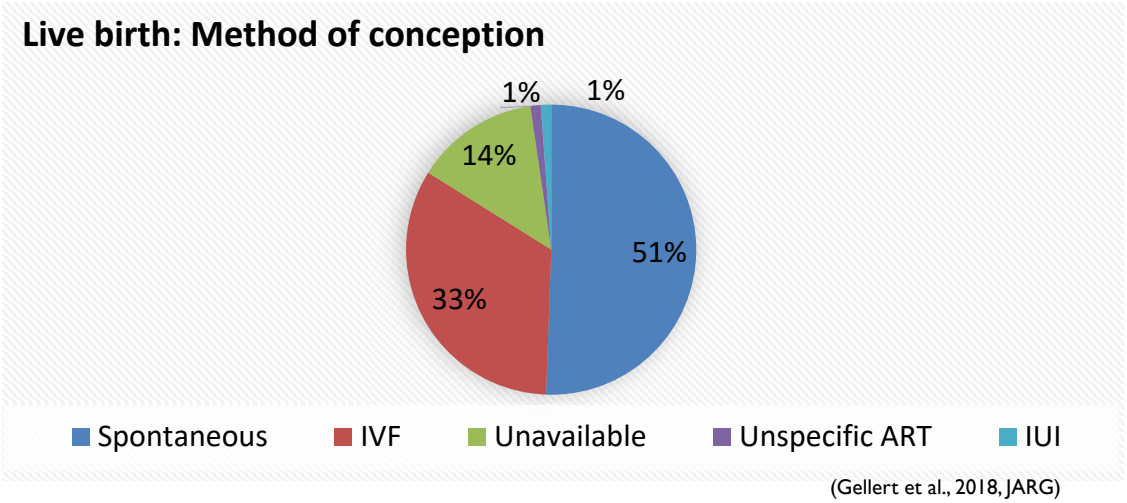
Jensen *AK et al. Hum Reprod.* 2015

HALF OF THE CHILDREN BORN ARE FROM NATURAL CONCEPTION



40

(Gellert et al., 2018, JARG)

PERINATAL OUTCOME OF 40 CHILDREN BORN WORLDWIDE AFTER TRANSPLANTATION OF FROZEN/THAWED OVARIAN TISSUE

	NC/IVF/ IUI	Delivery mode (CS/VD/NS)	GA (weeks) Median	GA (weeks) (Range)	Birth weight (g) Median	Birth weight (g)(Range)	Girls N	Boys N
Singletons	17/16/1	15/12/7	38	39 ±0.2 (36-41)	3168	3217 ±82 (2370-4230)	17	17
Twins	3 sets IVF	2 sets/1 set	37	36 ±1 (33-38)	2650	2560 ±286 (1650-3320)	2	4

Jensen AK *et al.*, *JARG* 2017;**34**:325

The majority of women who have given birth were under the age of 30 years when they had their ovarian tissue cryopreserved.

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OTC: A VALID AND EFFECTIVE TECHNIQUE, BUT…

42

A HUGE LOSS OF FOLLICLES FOLLOWING GRAFTING

Transplantation

TABLE 2. Survival of primordial follicles after grafting fresh or frozen-thawed cortical tissue from sheep ovaries to SCID mice

Graft type (group no.)	Primordial follicles	
	No. ± SEM	% of control
1) Control	192 ± 47	
2) Fresh	68 ± 11	35
3) Frozen-thawed	54 ± 12	28

(Baird DT et al., Endocrinology,1999)

- Transplanted tissue undergoes massive follicle loss in the early post-grafting period due to hypoxia and ischemia ⇨ Up to **2/3** of the follicles are lost

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ADVANCE OTC BY FOCUSING ON THE TRANSPLANTATION PROCESS

Human Reproduction, pp. 1–10, 2018
doi:10.1093/humrep/dey080

human reproduction ORIGINAL ARTICLE *Reproductive Biology*

Two-step transplantation with adipose tissue-derived stem cells increases follicle survival by enhancing vascularization in xenografted frozen–thawed human ovarian tissue

D.D. Manavella[1], L. Cacciottola[1,2], S. Pommé[1], C.M. Desmet[3], B.F. Jordan[3], J. Donnez[4], C.A. Amorim[1], and M.M. Dolmans[1,5,*]

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WE NEED TO IMPROVE THE TRANSPLANTATION PROCESS!

Table I Follicle outcomes: follicle analyses performed on HE-stained sections.

Group	Primordial follicles		Total follicles	
	No. of follicles	% of controls ± SEM	No. of follicles	% of controls ± SEM
Non-grafted controls	166 ± 63.2[ab]		217 ± 84.67[ef]	
OT	42.4 ± 16.96[a]	24.6 ± 4.80[c]	64.6 ± 25.51[e]	30.26 ± 7.52[g]
Fi+OT	45.4 ± 16.55[b]	33.24 ± 6.87[d]	64.8 ± 24.53[f]	36.39 ± 6.97
Fi/ASCs+OT	93.4 ± 36.45	61.7 ± 14.12[cd]	146.2 ± 59.62	69.46 ± 12.84[g]

One-way ANOVA and post-hoc tests. Values are means ± SEM.

Manavella DD et al., Hum Reprod. 2018

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TAKE HOME MESSAGES

- National programmes with centralized cryobanks have been established in several countries (Denmark and FertiPROTEKT) in order to concentrate cryopreservation in centers with a high quality service and substantial expertise.
- Current activity of OTC and clinical data on prolonged transportation support a centralized service for the procedure.
- Freezing of ovarian tissue can be performed equally good with both slow freezing and vitrification, even though vitrification appears to perform a little better, clinical data are too sparse to validate the superiority of the procedure.
- Finally, the transplantation procedure is where we could really advance OTC by improving follicular survival…

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FP in transgender patients

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PCC 17, The broad scope of fertility preservation, Barcelona 2018

FP in transgender patients

Conflicts of interest:

None

FP in transgender patients

Learning objectives:

1) Gender dysphoria and its social context
2) Transgenderism and reproduction
3) FP option for transgender patients
4) Maximising FP options in FtM transgenders: IViMOVA

Gender dysphoria and its social context

Trans = persons whose gender identity and/or gender expression differs from the sex assigned at birth.
The term covers many gender identities.

The concept of gender identity and gender expression are based upon:

International human rights law to sexual orientation and gender identity issues

Yogyakarta Principles (UN Human rights system)

Gender dysphoria and its social context

Gender identity:

deep and individual experience of gender
(may or may not correspond to sex assigned at birth)

personal sense of the body (*may involve if freely chosen modification of bodily appearance)*

other expressions of gender *(including dress, speech, mannerims)*

Gender expression:

a person's manifestation of gender identity
(masculine, feminine, gender variant, behaviour, clothing, hair, voice, body characteristics)

Trans persons may choose to express their gender identity in different ways.

Gender dysphoria and its social context

Between 1.9% and 3.2% natal female report ambivalent gender identity
Between 2,2% and 4,2% natal men report ambivalent gender identity

Equal identification with the other sex as with the sex assigned at birth

Between 0.6% and 0.8% natal female report incongruent gender identity
Between 0.7% and 1,1% natal men report incongruent gender identity

Stronger identification with the other sex than with the sex assigned at birth

Being trans in the EU, comparative analysis of EU LGBT survey data by the European Union Agency of Fundamental Rights (2014)

Gender dysphoria and its social context

GID = Gender identity disorder = mental disorder

GID = strong and persistent cross-gender identification, involving persistent discomfort with one's sex or sense of its inappropriateness and the experience of significant distress or impairment in social interactions, occupations or other important areas of function so long as these disturbances are not concurrent with a physical intersex condition

(American Psychiatric Association (APA, 1994)

eshre

Gender dysphoria and its social context

For years advocates have lobbied the American Psychiatric Association to change or remove categories labeling transgender people in a psychiatric manual, arguing that terms like "Gender Identity Disorder" characterize all trans people as mentally ill.
Based on the standards to be set by the DSM-V, individuals will be diagnosed with **Gender Dysphoria** for displaying

"a marked incongruence between one's experienced/expressed gender and assigned gender."

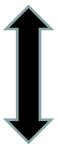
-> shift from treatment and fixing a disorder to resolving distress

Remember: Homosexuality -> pathological DSM 1973

eshre

Gender dysphoria and its social context

a trans woman risked losing the children she fathered before her transition. Because she is trans, a lawyer has argued that her GID is a "severe, chronic **mental illness** that might be harmful to the child."



But in other cases, a GID **diagnosis** justifies insurance coverage for gender reassignment surgery and other medical procedures that sometimes accompany a transition. Having a diagnosis is the difference between a necessary medical procedure and something that can be perceived as cosmetic surgery that insurance won't cover

Gender dysphoria and its social context

POLICY & ETHICS

Where Transgender Is No Longer a Diagnosis

Denmark becomes the first country to declassify it as a mental disorder

By Francine Russo on January 6, 2017

Danish politicians -> WHO to remove transgender from a category of mental illness in the ICD-10

If not by 01/01/2017 -> they would act unilaterally and they did!

Transgender and reproduction

Life satisfaction in trans persons ~ positively having children
Parenting children ~ suicide protective factor
Loss of fertility ~ perceived as problematic

70% of those who enter the clinic trans request **full treatment**
(in total 50% of all trans people)

→ Release tremendous burden of having a price to pay for going through life in a gender they identify themselves with

Being trans in the EU, comparative analysis of EU LGBT survey data by the European Union Agency of Fundamental Rights (2014)
T'sjoen G. et al. J.Sex Med. 2017 Dec 14(12): 1494-1495
Dierckx M. et al. Int. Rev. Psychiatry 2016 28(1): 36-43

Transgender and reproduction

Full treatment:

9 months – 1 year	1 year		6 to 9 months after SRS
Diagnostic phase	Hormonal phase	Sex reassignment surgery	Genital reconstruction
Mental health professional (1/6 weeks) DSM criteria (DSM-5) ICD-10	Psychosocial evaluation (1/2 months) Real life experience -> real life therapy		

Transgender and reproduction

Transition -> reproductive age

Relationships

Desire to have children

The sex-change sweethearts: How a pageant princess and colonel's son fell in love after BOTH had transgender treatment

- Katie Hill and her boyfriend Arin Andrews were both born the opposite sex
- Katie, 18, and Arin, 16, met at a support group in Tulsa, Oklahoma
- Both have undergone hormone therapy and Katie had gender reassignment surgery shortly after her 18th birthday

Transgender and reproduction

Transgender persons should be encouraged to consider fertility issues **before** starting cross-gender hormonal treatment

(World Professional Association for Transgender Health WPATH, 2011, sect. IX)
(Clinical practice guidelines of the Endocrine Society, 2009)

→ Loosing fertility ≠ the price to pay for transitioning

Transgender and reproduction

How? → **Legislation**

Hormone treatment

Body modifications

Sex reassignment surgery

Masculizing hormonal therapy:
- amenorrhea
- no depletion of follicle pool
- no affect on developmental capacity after stop

Feminizing hormonal therapy:
- hypospermatogenesis
- azoospermia

Removal of gonads = sterility

Transgender and reproduction

Thomas Beattie lives in Oregon and is married to a woman named Nancy. He's pregnant.

To our neighbors, my wife, Nancy, and I don't appear in the least unusual. To those in the quiet Oregon community where we live, we are viewed just as we are -- a happy couple deeply in love. Our desire to work hard, buy our first home, and start a family was nothing out of the ordinary. That is, until we decided that I would carry our child.

I am transgender, legally male, and legally married to Nancy. Unlike those in same-sex marriages, domestic partnerships, or civil unions, Nancy and I are afforded the more than 1,100 federal rights of marriage. Sterilization is not a requirement for sex reassignment, so I decided to have chest reconstruction and testosterone therapy but kept my reproductive rights. Wanting to have a biological child is neither a male nor female desire, but a human desire.

MaCDonald T., et al. BMC Pregn. Childbirth 2016 May 16(16): 106

Transgender and reproduction

Transgender and reproduction

How! → **Fertility preservation**

- The World Professional Association for Transgender Health Standards of Care and the Clinical practice guidelines of the Endocrine Society clearly state that transsexual persons should be encouraged to consider fertility issues before starting cross-sex hormonal treatment.
- The majority of transsexual men and women are of reproductive age at the moment of transition and have relationships following transition.
- Reproductive options for all trans persons are not equal because not only the gametes are of importance, but also the sex of the (future) partner.
- In trans men, use of donor sperm is most common, but in theory, there are three options available to preserve fertility: oocyte banking, embryo banking and banking of ovarian tissue
- In trans women, sperm cryopreservation is advised before starting hormonal therapy.

FP option for transgender patients

Male to Female transgender (MtF)

FP option for transgender patients

Male to Female transgender (MtF)

Table 2. Fertility preservation options in transgender women.

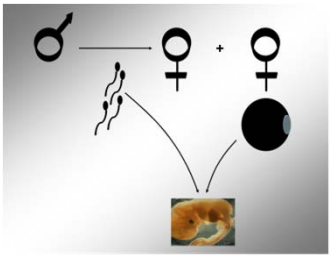
Technique	Description	Considerations	Future use
Sperm cryopreservation	Cryopreservation of ejaculated sperm through masturbation or vibratory stimulation	Established technique Masturbation Post-pubertal	Male partner: Needs a donor oocyte and surrogate mother Female partner: Intra-uterine insemination or IVF/ICSI depending on sperm quality followed by embryo transfer in partner
Surgical sperm extraction	Percutaneous aspiration of sperm from testis or epididymis	Established technique No masturbation Surgical procedure Post-pubertal	Male partner: Needs a donor oocyte and surrogate mother Female partner: IVF/ICSI treatment followed by embryo transfer in partner
Immature testicular tissue cryopreservation	Surgical biopsy of testicular tissue	Experimental Pre- or post-pubertal Possible at moment of genital reconstructive surgery	Male partner: *In vitro* maturation and need of a donor oocyte and surrogate mother (not possible at this stage) Female partner: *In vitro* maturation and IVF/ICSI followed by embryo transfer in partner (not possible at this stage)

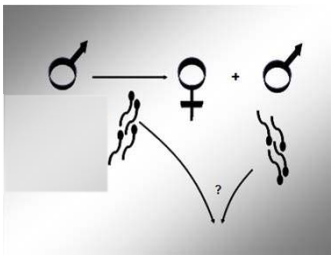
IVF, *in vitro* fertilization; ICSI, intracytoplasmic sperm injection.

De Roo et al. Int. Rev. Psy 2016: 28;1, 112-119

FP option for transgender patients

Male to Female transgender (MtF)





Oocyte donor
+
Surrogate mother

FP option for transgender patients

Male to Female transgender (MtF)

University of Gothenburg

Seven Swedish women have had embryos reintroduced after receiving wombs from living donors. Now the first transplanted woman has delivered a baby – a healthy and normally developed boy.

texasnews

Trans woman seeks uterine transplant

Sarah Luiz of The Colony, who's no stranger to the media spotlight, aims to be world's 1st trans mom

File photo: Surgeon specialists, L-R: Andreas G Tzakis, Pernilla Dahm-Kähler, Mats Brannstrom, Michael Olausson and Liza Johannesson after the world's first mother-to-daughter uterine transplants in 2012. The same team performed the womb transplant birth in Sep 2014. (AFP/Adam Ihse)

FP option for transgender patients

Female to Male transgender (FTM)

FP option for transgender patients

Female to Male transgender (FtM)

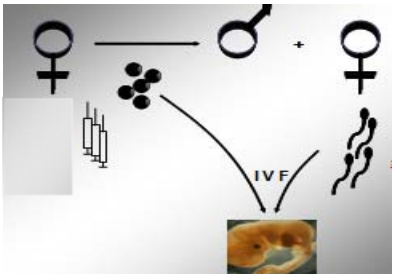
Table 1. Fertility preservation options in transgender men prior to a hysterectomy and bilateral oophorectomy procedure.

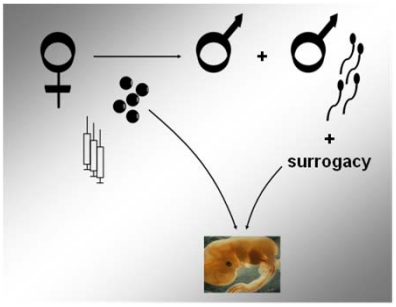
Technique	Description	Considerations	Future use
Embryo cryopreservation	Controlled ovarian stimulation for oocyte retrieval and fertilization to obtain embryos for cryopreservation	Established method Controlled ovarian stimulation Vaginal procedure Post-pubertal Partner or donor sperm	Male partner: Use of partner's sperm prior to cryopreservation, needs a surrogate mother Female partner: Fertilization by donor sperm prior to cryopreservation, implantation into the partner's uterus
Oocyte cryopreservation	Controlled ovarian stimulation to obtain oocytes for cryopreservation	Established method Controlled ovarian stimulation Vaginal procedure Post-pubertal No partner required	Male partner: Use of partner's sperm, needs a recipient uterus (surrogate mother) Female partner: Fertilization by donor sperm, implantation into the partner's uterus
Ovarian tissue cryopreservation	Surgical excision of ovarian tissue for cryopreservation	Experimental Pre- or post-pubertal No controlled ovarian stimulation Possible at moment of genital reconstructive surgery No partner required	Male partner: *In vitro* maturation and use of partner's sperm, need of a recipient uterus (surrogate mother) (not possible at this stage) Female partner: *In vitro* maturation, fertilization by donor sperm, implantation into the partner's uterus (not possible at this stage)

De Roo et al. Int. Rev. Psy 2016: 28;1, 112-119

FP option for transgender patients

Female to Male transgender (FtM)





Maximising FP options in FtM transgenders: IViMOVA

Transgender men's experiences of fertility preservation: a qualitative study

G. Armuand[1,*], C. Dhejne[2,3], J.I. Olofsson[4,5], and K.A. Rodriguez-Wallberg[5,6,*]

Discontinouing testosterone: **challenging** and a mental strain
(feelings tiredness and exhaustion) – smelling different, feminizing voice

Resumption of menstruation: **psychiologically stressfull**, relapse of self-harming behaviour

Stimulation treatment: **huge impact** on mood swings, body changes

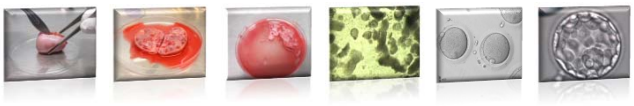
Pelvic examinations and being seen by others: **uncomfortable**, **humiliated**,
they felt exposed, the penetration involving TVS triggered negative feelings

Using of pronouns: egg, vagina, uterus: reminding them of their **gender incongruence**
confirming others saw them as a women

Armuad G. et al. Hum. Reprod. 2107; Feb 32(2): 383-390

Maximising FP options in FtM transgenders: IViMOVA

'ex-vivo' method for oncology patients -> 1st EU child (2015)



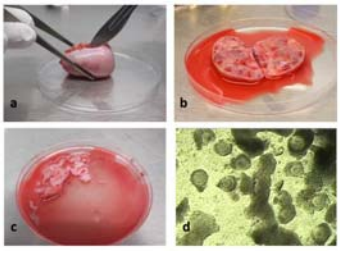
Presence of immature oocytes during processing of trans ovaries

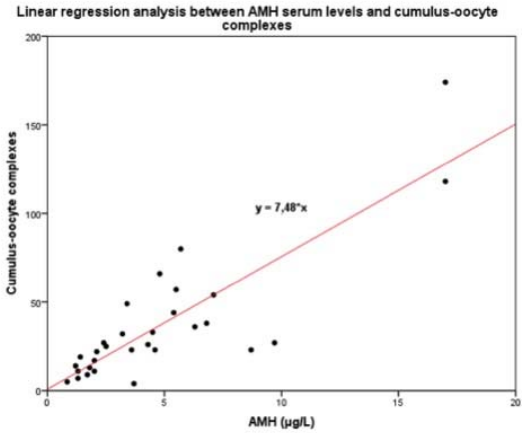
In **Vi**tro **M**aturation and vitrification of oocytes collected during **OVA**rian cortex cryopreservation: a realistic fertility preservation therapy for trans men

-> IViMOVA a trans men tailored FP possibility?

Maximising FP options in FtM transgenders: IViMOVA

Fertility preservation for transsexual persons: COCs collected from ovaries after prolonged testosterone therapy can be in vitro matured $\sim AMH$





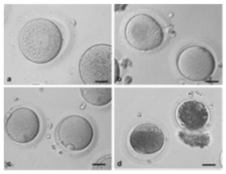
De Roo C, et al. Reprod Biomed Online. 2017 Jun;34(6):557-566

Maximising FP options in FtM transgenders: IViMOVA

COCs collected from ovaries after prolonged testosterone therapy can be in vitro matured:

Maturation rate: 38,1%
Normal spindles after maturation: 85,7%

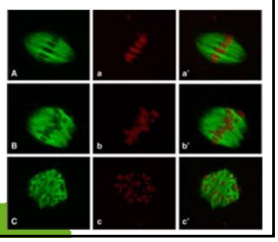
Oocyte survival rate: 67,7%
Normal spindles after warming: 92,2%



Age	Patients (*n*)	Age mean ± SD	AMH mean (range)	Oocytes collected (*n*) (range)	Oocytes collected/ patient mean ± SD	IVM rate (%) (range)
<20 years	4	18.3 ± 0.5	8.4 (1.3–17.0)	245 (7–174)	61.3 ± 76.2	31.7 (14.3–46.2)
20–30 years	9	23.0 ± 2.9	5.5 (0.8–17.0)	369 (5–118)	41.0 ± 35.7	39.0 (20.5–63.6)
>30 years	3	35.0 ± 1.0	2.2 (2.0–2.4)	66 (17–27)	22.0 ± 5.0	30.2 (22.7–44.4)

No statistical significant differences were observed in the IVM rate after 44–48 h IVM culture, among the three groups.

-> morphologically : IViMOVA oocytes seem okay



Lierman S, et al. JARG. 2017 Nov.34(11):1445-1456

Maximising FP options in FtM transgenders: IViMOVA

IViMOVA oocytes – fertilization rate

Time lapse shows aberrant and irregular cleavage patterns

Comprehensive genetic screening: normal patterns

IViMOVA embryo's: how do they look like?

D2016.11.22 2pn_embryo 45

D2016.11.22 2pn_embryo 33_blastocyst

Almost 100 patients included in study
Over 1500 COCs collected
Over 200 oocytes frozen for research

-> developmental capacity?
-> study results will be complete by mid 2019
Unpublished results

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to the research project

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Is social egg freezing a promise or a panacea?

Gillian Lockwood, United Kingdom

Contribution not submitted by the speaker