OVARIAN CRYOPRESERVATION: BACKGROUND, FERTILITY PREDICTION AND THE EDINBURGH EXPERIENCE

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Is there a fertility deficit for females?

and if so..

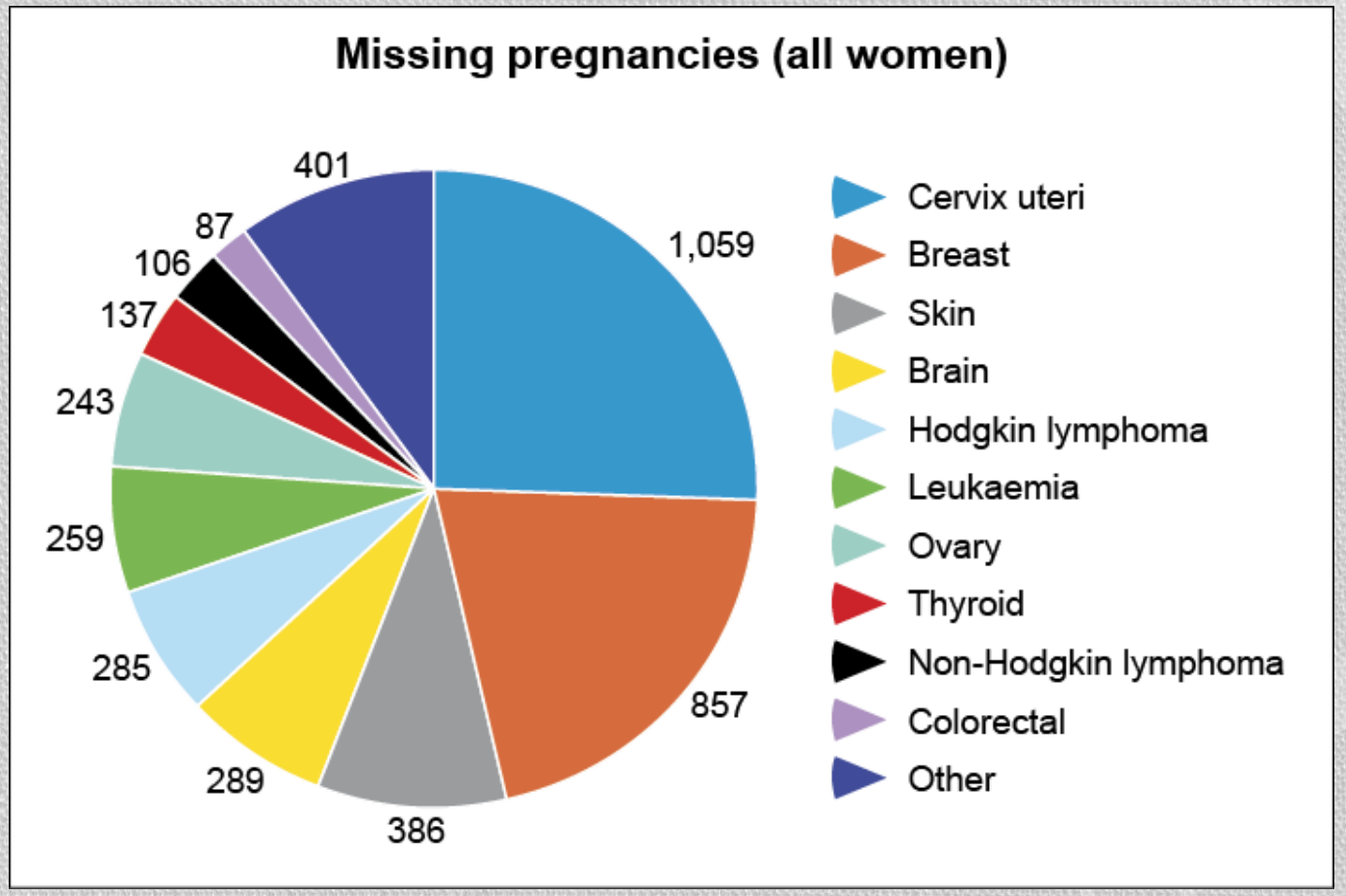
who is at risk?

Pregnancy after cancer in girls and women in Scotland: a population-based analysis

- All female patients aged < 39 years at the incidence date of first cancer diagnosed 1981-2012 on Scottish Cancer Registry records.
- These were then linked to general and maternity hospital discharge records to ascertain subsequent pregnancies up until the end of 2014.
- 23,201 women aged < 39 at time of cancer diagnosis.
- Overall the cancer survivors achieved a lower than expected number of pregnancies compared to the general population of women, SIR 0.62 (95% CI 0.60-0.63)
- Cancer survivors were approximately 38% less likely to achieve pregnancy after diagnosis compared to women in the general population

Anderson RA et al. unpublished

Pregnancy after cancer in girls and women in Scotland: a population-based analysis



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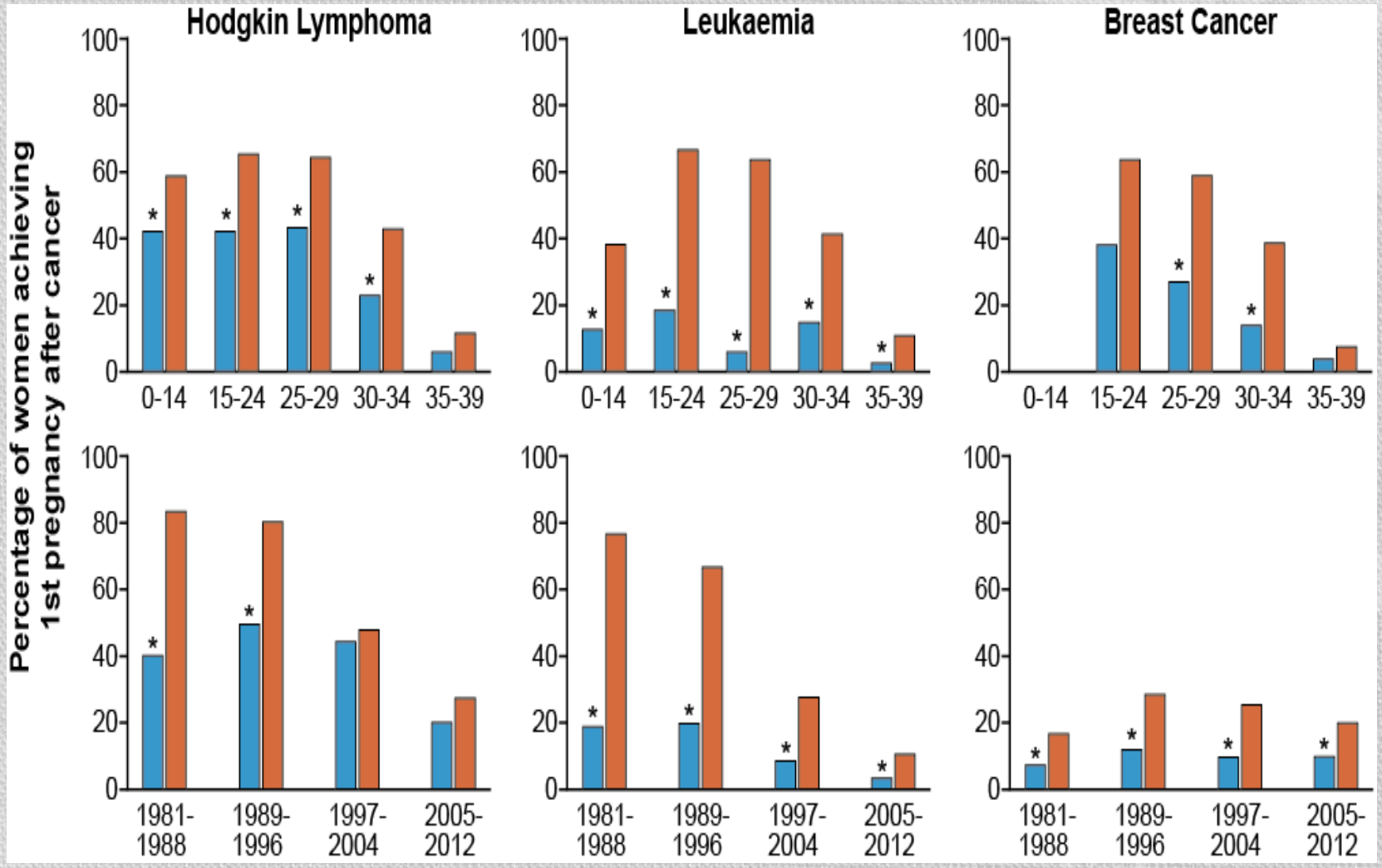


Anderson RA et al. unpublished

Risk assessment for fertility preservation

* Intrinsic factors
  * Health status of patient
  * Consent (Patient/Parent)
  * Assessment of ovarian reserve
* Extrinsic factors
  * Nature of predicted treatment
    * High/Medium/Low/Uncertain Risk
  * Time available
  * Expertise available

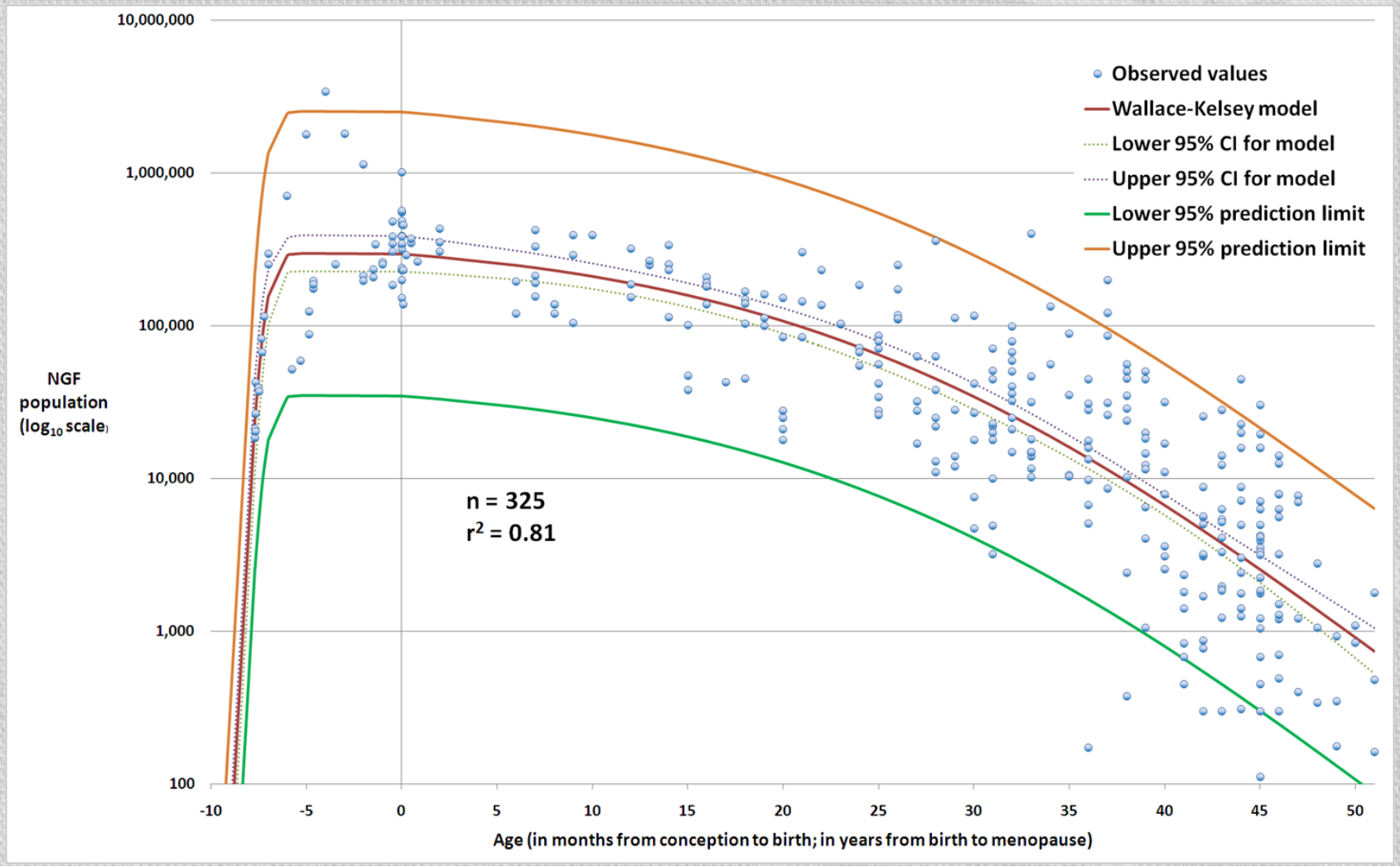
Wallace WH et al. JCO, 2012

Risk of infertility

Low risk (<20%)	Medium risk	High risk (>80%)
ALL Wilms' tumour Brain tumour Sx, RT < 24Gy Soft tissue sarcoma (stage1) Hodgkin's Lymphoma HL(Low stage)	AML Osteosarcoma Ewing's sarcoma STS: stage II/III Neuroblastoma NHL Brain tumour RT>24Gy HL (High Stage)	Total Body Irradiation Pelvic/testes RT Chemo pre BMT Metastatic Ewing's HL (Pelvic RT)

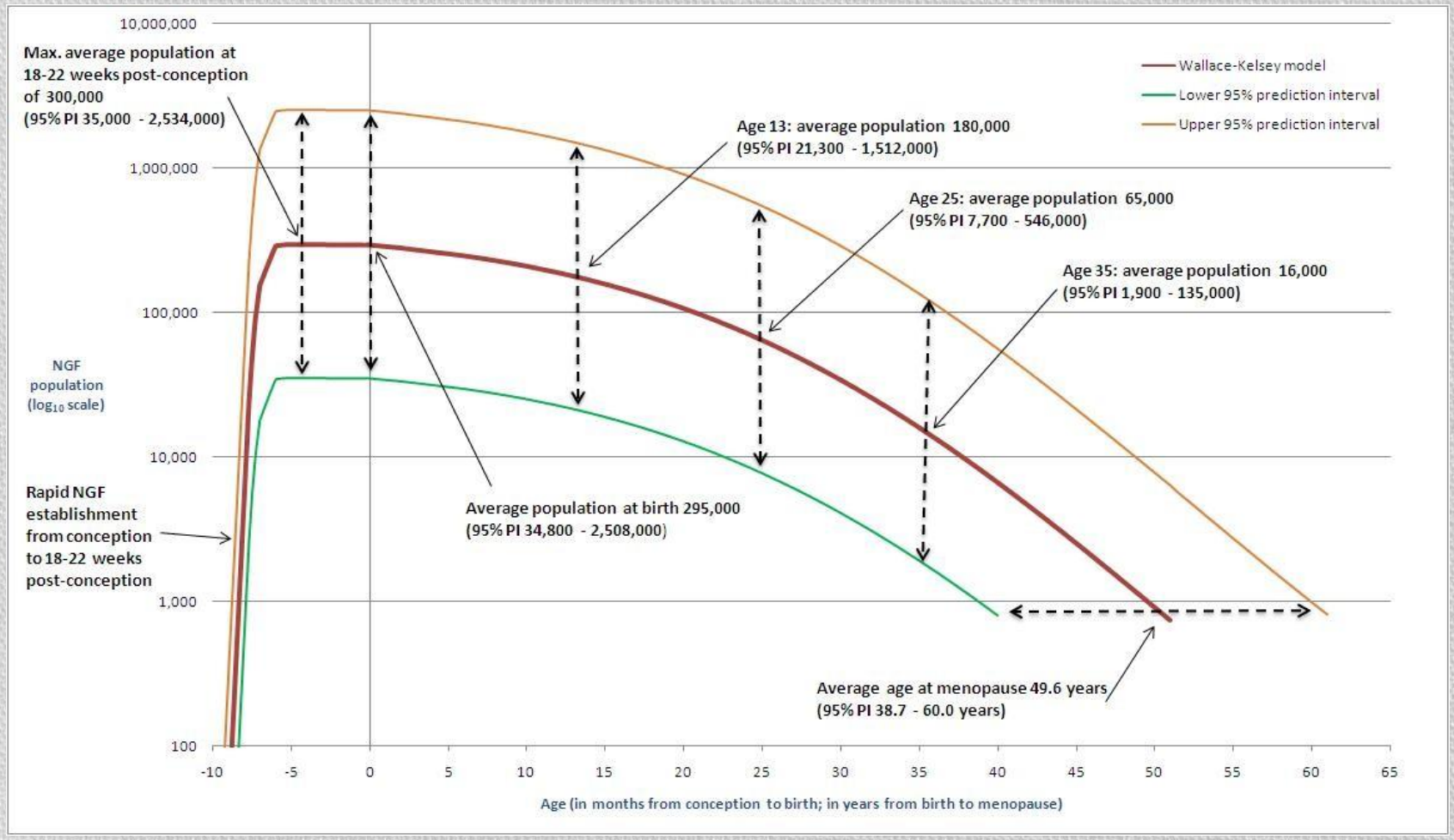
Ovarian Reserve

The Wallace-Kelsey NGF Model



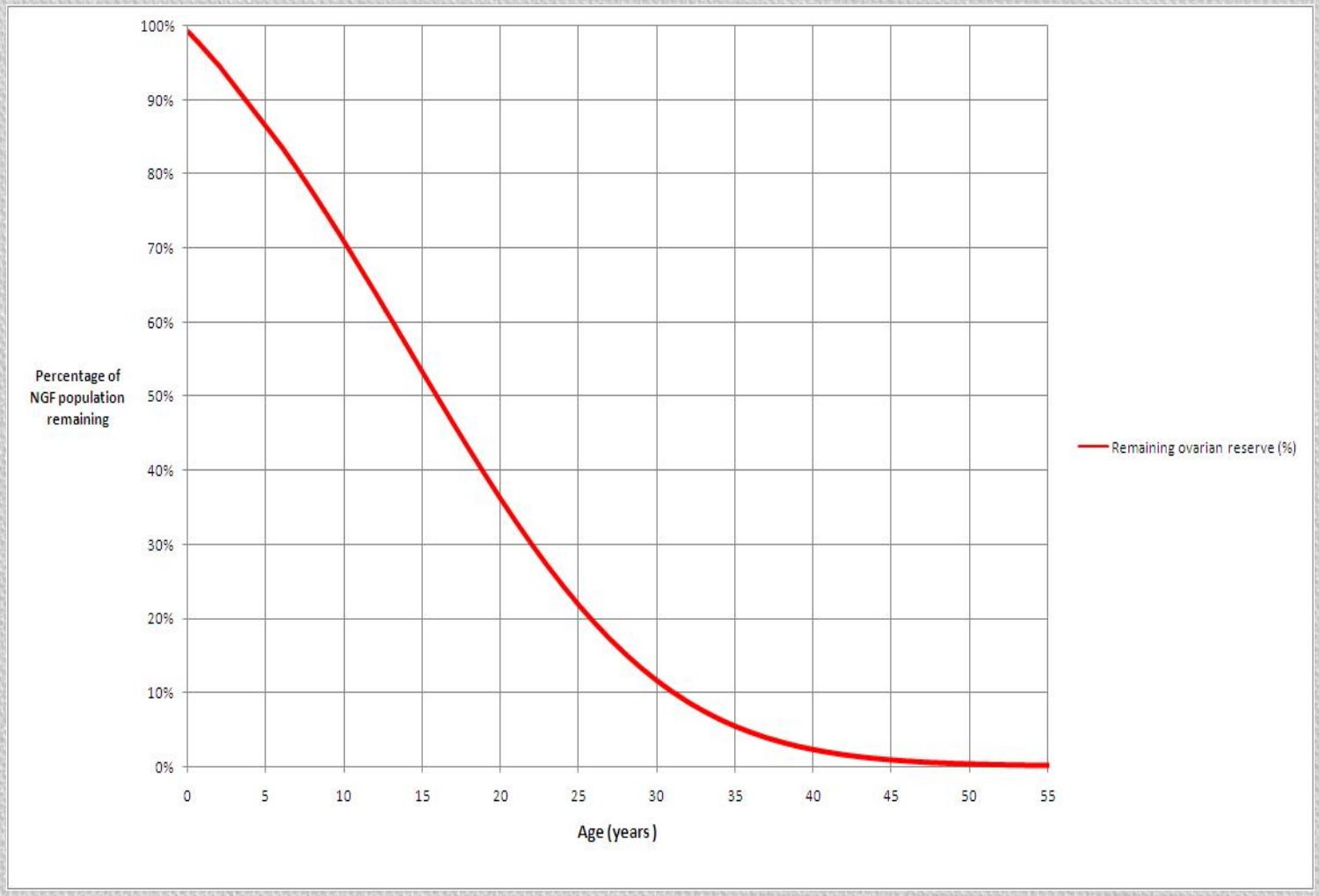
Wallace & Kelsey (2010) PloS ONE

Ovarian reserve: Conception to Menopause



Wallace & Kelsey (2010) PloS ONE

Percentage of NGF population remaining with increasing age



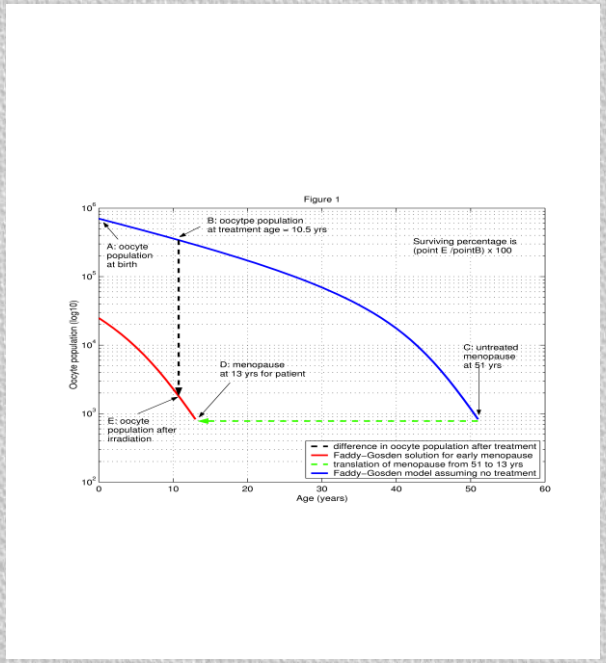
Wallace & Kelsey (2010) PloS ONE

Radiation-induced ovarian damage

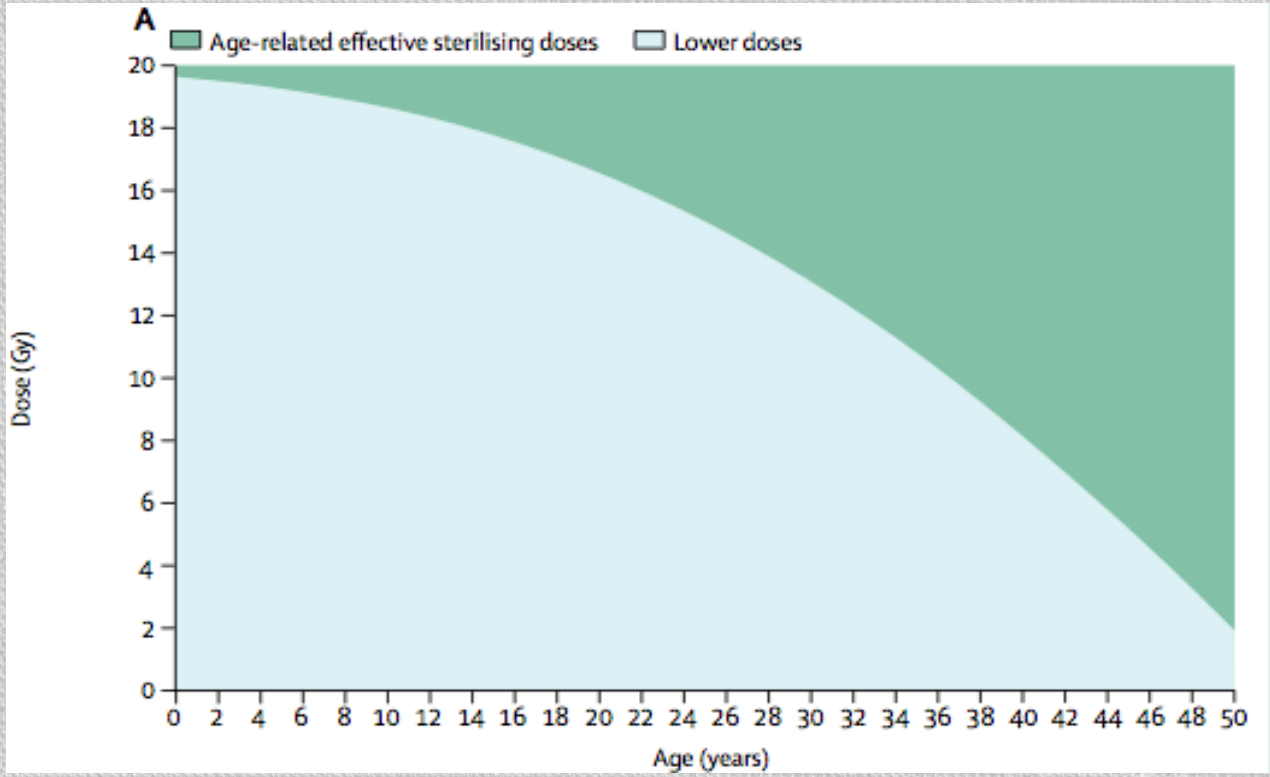
Human oocyte
(Primordial follicle)

* $LD_{50} < 2$ Gy

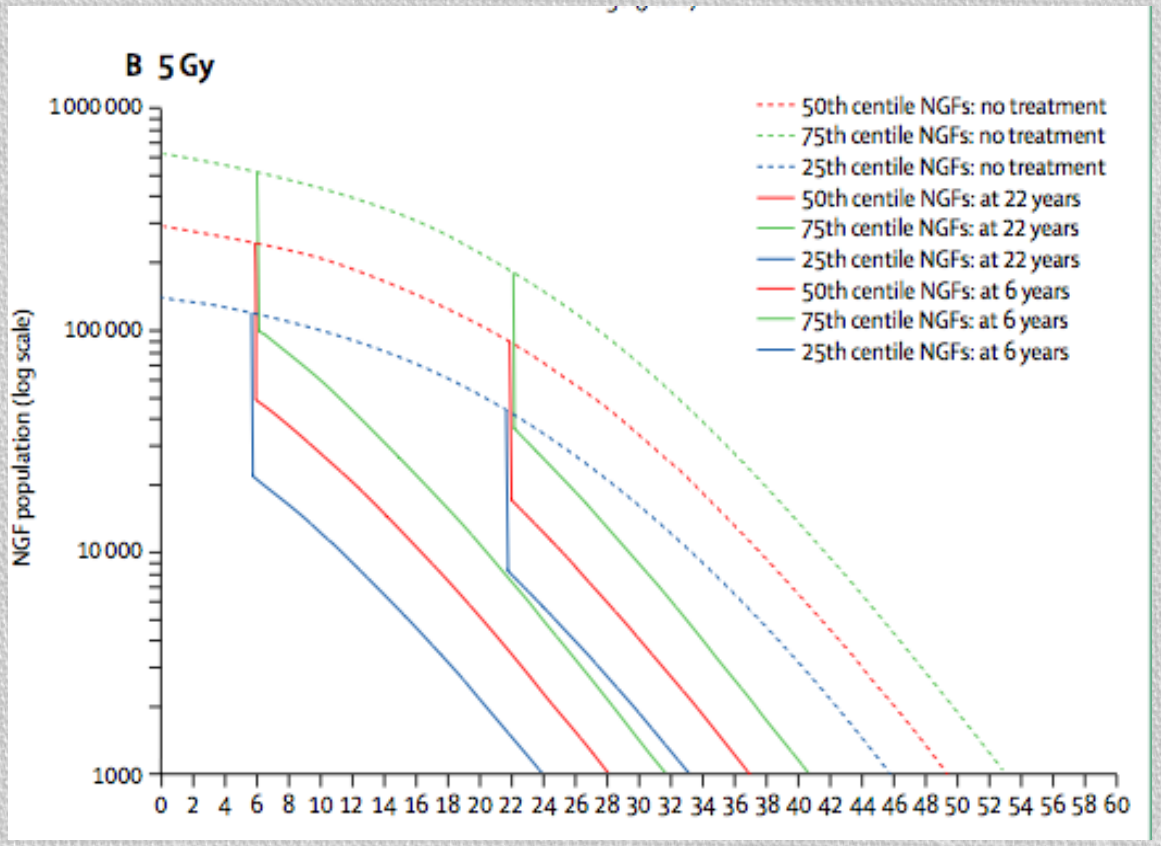
Wallace, Thomson, Kelsey. (2003)
Hum Reprod.



Effective ovarian sterilizing doses of radiotherapy with increasing age



Anderson RA…Wallace WH. Lancet Diabetes Endocrinol. 2015



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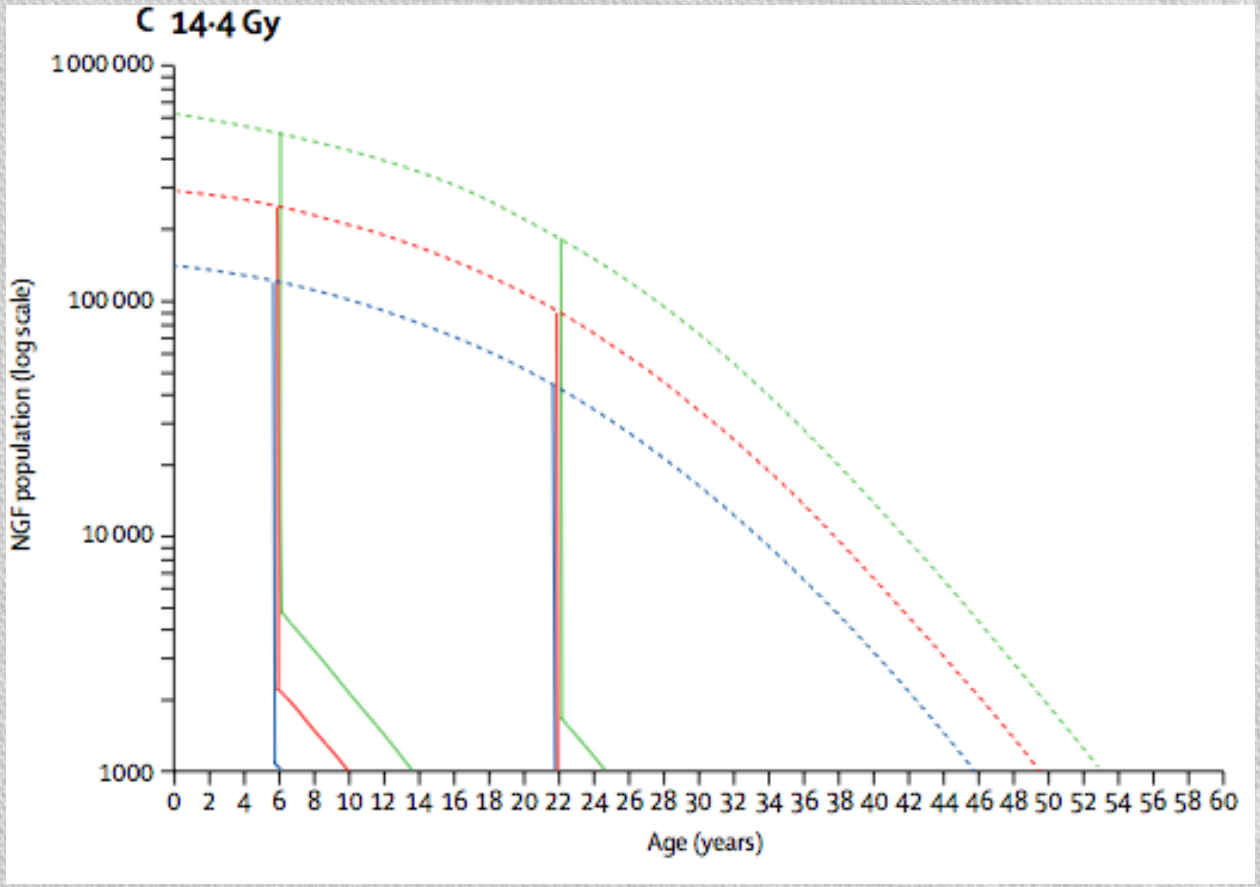
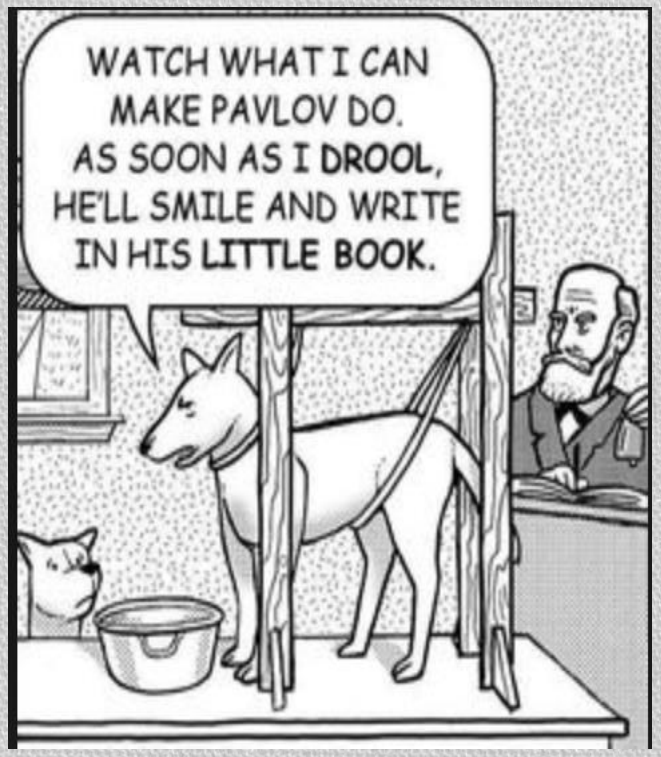
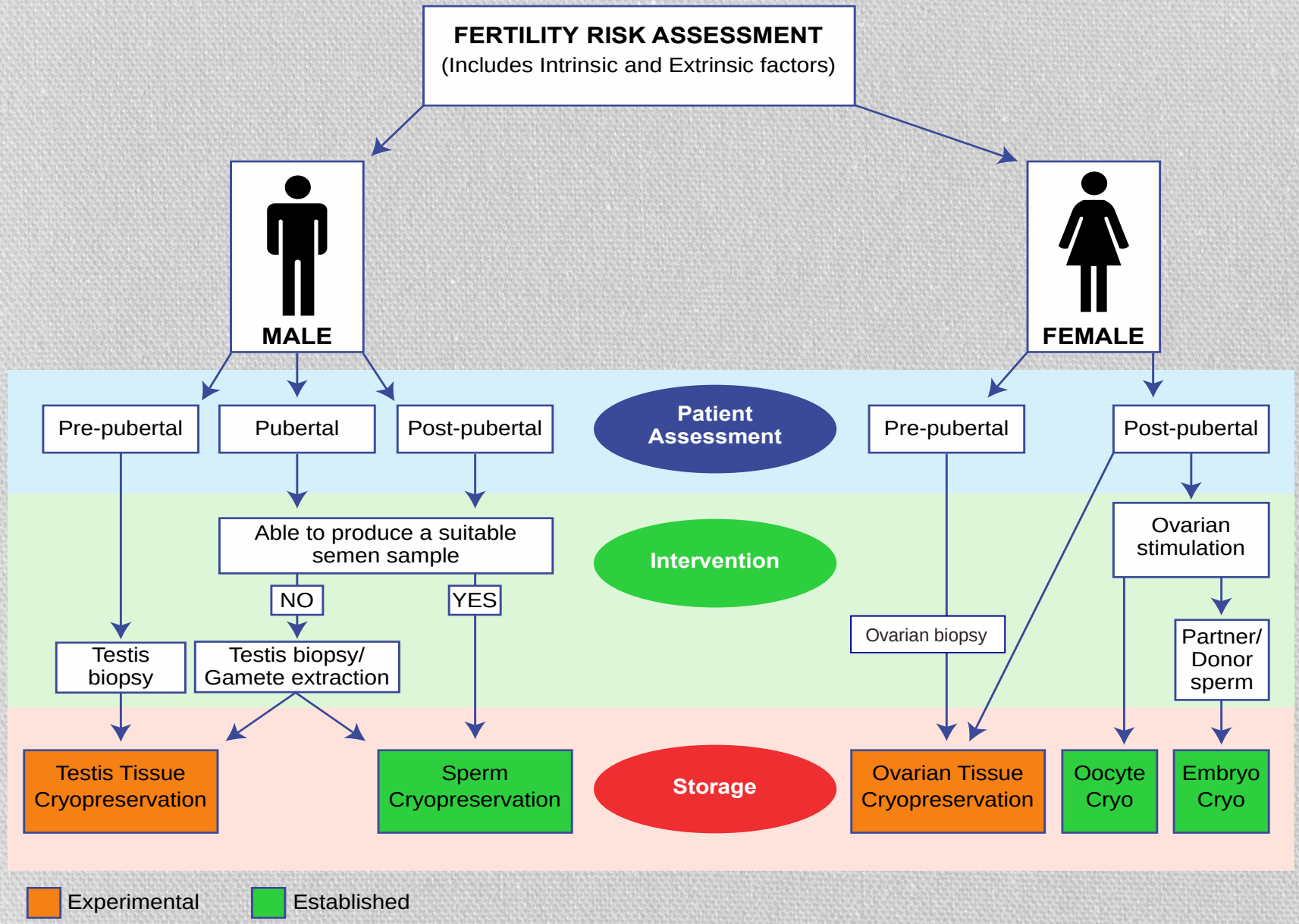


Anderson RA…Wallace WH. Lancet Diabetes Endocrinol. 2015

Fertility preservation options: established and experimental

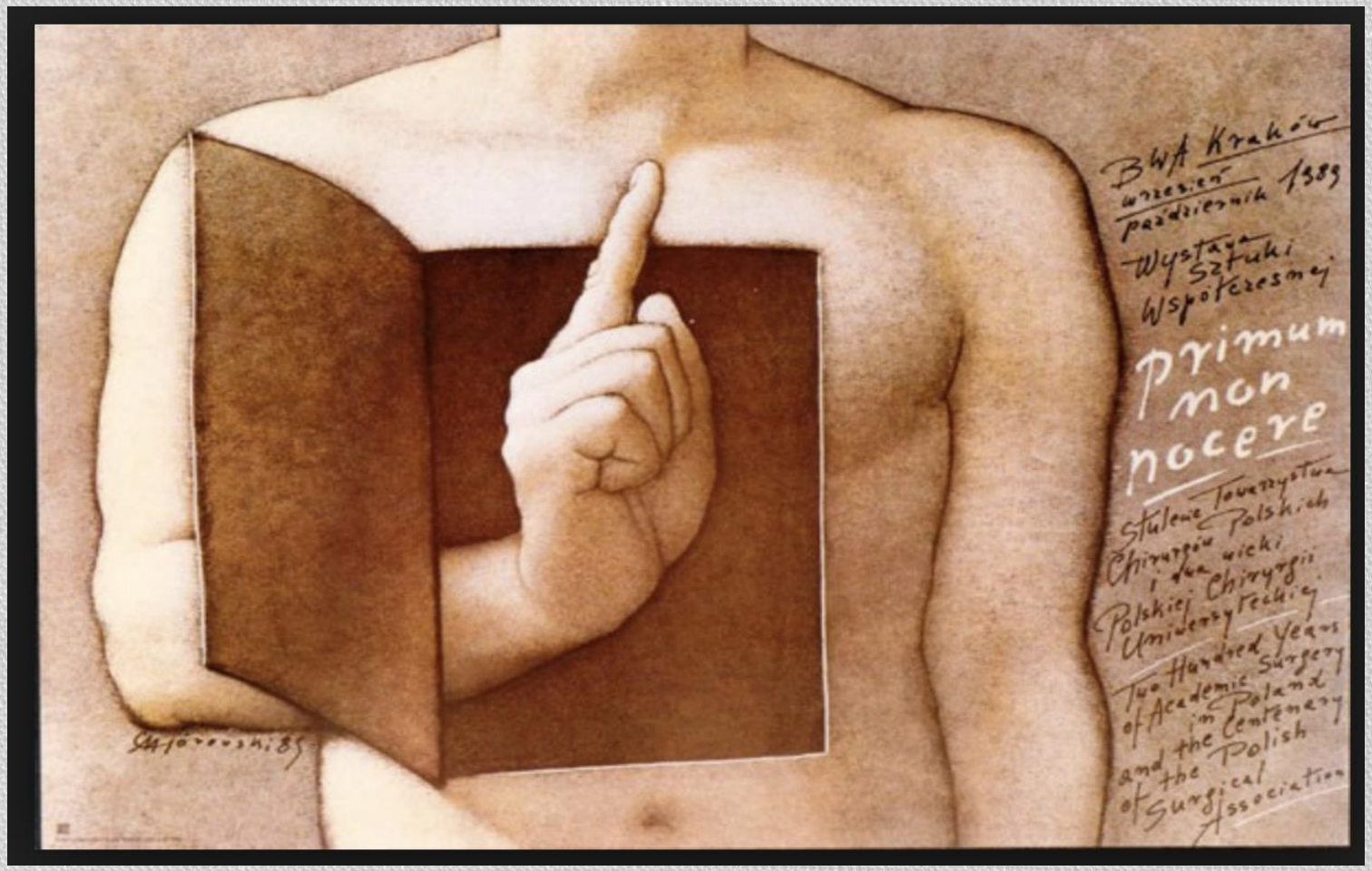



FERTILITY RISK ASSESSMENT
(Includes Intrinsic and Extrinsic factors)
MALE
FEMALE
Pre-pubertal
Pubertal
Post-pubertal
Patient Assessment
Pre-pubertal
Post-pubertal
Able to produce a suitable semen sample
Intervention
Ovarian stimulation
NO
YES
Ovarian biopsy
Partner/ Donor sperm
Testis biopsy
Testis biopsy/ Gamete extraction
Testis Tissue Cryopreservation
Sperm Cryopreservation
Storage
Ovarian Tissue Cryopreservation
Oocyte Cryo
Embryo Cryo
Experimental
Established

Key features of the 3 options for fertility preservation for women

- Embryo cryopreservation
  - Established but require time and a partner
- Oocyte cryopreservation
  - Established but require time and hormone stimulation (success rate per oocyte low)
- Ovarian tissue cryopreservation
  - Minimal delay
  - No lower age limit
  - Surgical procedure
  - Allows for future developments

Primum non nocere



Cryopreservation: European experience

- Three centres (Denmark, Spain and Belgium)
- 60 cases of orthotopic reimplantation.
- Of these women, 11 (21%) became pregnant
- Six have delivered 12 healthy babies.
- Restoration of ovarian activity was observed in 93% of the patients between 3.5 months and 6.5 months after grafting
- The mean duration of ovarian function after trans-plantation is ~4–5 years but can persist for up to 7 years.

Donnez, J. *et al.* *Fertil. Steril.* 99, 1503–1513 (2013).

Efficacy of Ovarian Tissue Cryopreservation

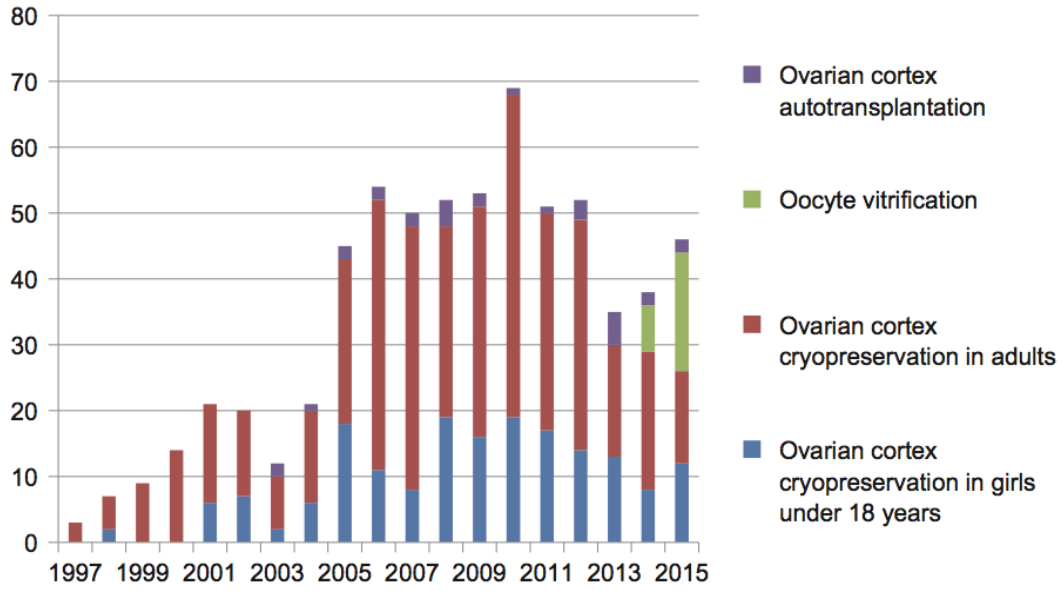


Figure 1 Numbers of patients undergoing ovarian tissue cryopreservation (OTC), oocyte vitrification and ovarian tissue transplantation between 1997 and 2015.

Jadoul P et al., Human Reproduction (2017) 32:1046-54

Efficacy of Ovarian Tissue Cryopreservation

- N=545 (22.3+/-8.8 yrs); (29% <18 yrs and 15% pre-pubertal)
- 79% for cancer
- 10% died
- 21 (3.9%) autotransplantation
- 7 delivered a heathy baby (Post transplantation live birth rate 33%)
- POI in 31.5% of 92 evaluable pts by questionnaire.
- Of 140 pts evaluable by questionnaire 96% satisfied, five minor and one major complication (intra-abdominal haemorrhage)

Jadoul P et al., Human Reproduction (2017) 32:1046-54

Efficacy of Ovarian Tissue Cryopreservation

- Concluded

- Four reasons OTC established for women at risk of POI:

- Risk of POI can change from low to high
- Real risks of POI increase with longer FU
- High patient satisfaction (96%)
- Complication rates low (Five minor, one major in 140 of 545)

Jadoul P et al., Human Reproduction (2017) 32:1046-54

Live birth after autograft of ovarian tissue cryopreserved during childhood

Sickle cell disease Aged 5 from Rep of Congo

Onset of puberty Aged 10, No menstruation

BU/CY HSCT from matched sibling for severe disease

Lap collection of whole ovary Aged 13 and 11 months, October 2000 before HSCT

Developed POI, started on HRT aged 15

Aged 25 ovarian tissue replaced. After five months menstruation, continued for two years. Assisted conception due to male factor. No pregnancy

Aged 27 spontaneous conception with new partner. Healthy male 3.14 Kg.

Demeestere I et al Hum Rep 2015

Ovarian tissue cryopreservation: World-wide experience

* At least 44-100 ? pregnancies worldwide after othotopic reimplantation of frozen–thawed ovarian cortex
* Success rate is unclear as the denominator is unknown
* No pregnancies reported following the reimplantation of ovarian tissue harvested pre-pubertally
* Young children are potentially ideal candidates

Donnez, J. & Dolmans, M.-M. *Nat. Rev. Endocrinol.* 9, 735–749 (2013)

Ovarian Cryopreservation & Ovarian Function

Edinburgh experience in children (< 18 yrs) 1996-2012

Cryopreservation of ovarian cortical tissue – Edinburgh criteria

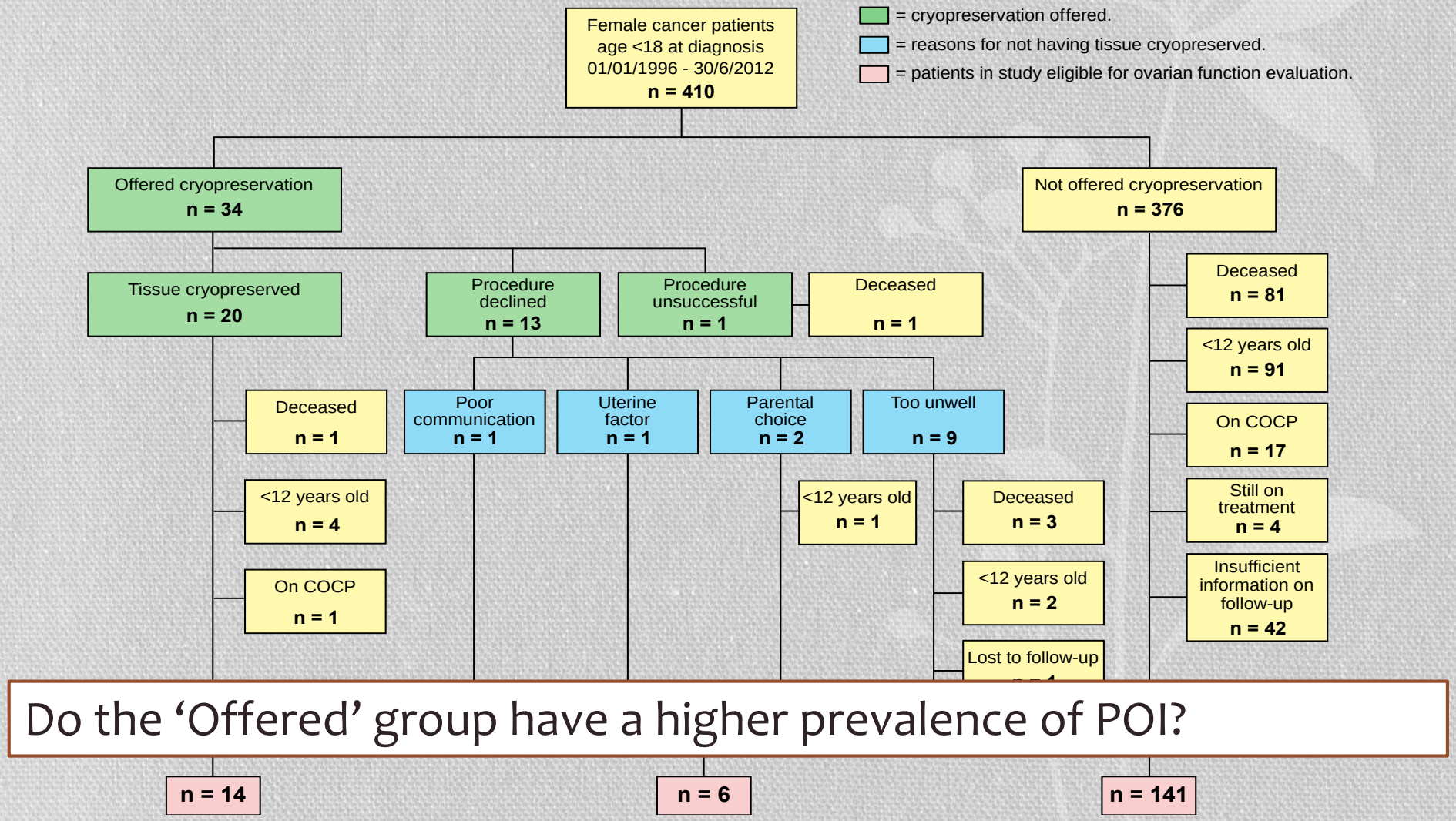
Selection criteria (1995, modified 2000)

- Age < 35 years
- No previous chemotherapy/radiotherapy if age >15 years
- Mild, non gonadotoxic chemotherapy if < 15 years
- A realistic chance of surviving five years
- A high risk of ovarian failure
- Informed consent (parent and where possible patient)
- Negative HIV and Hepatitis serology
- No existing children

Revised Indications ?

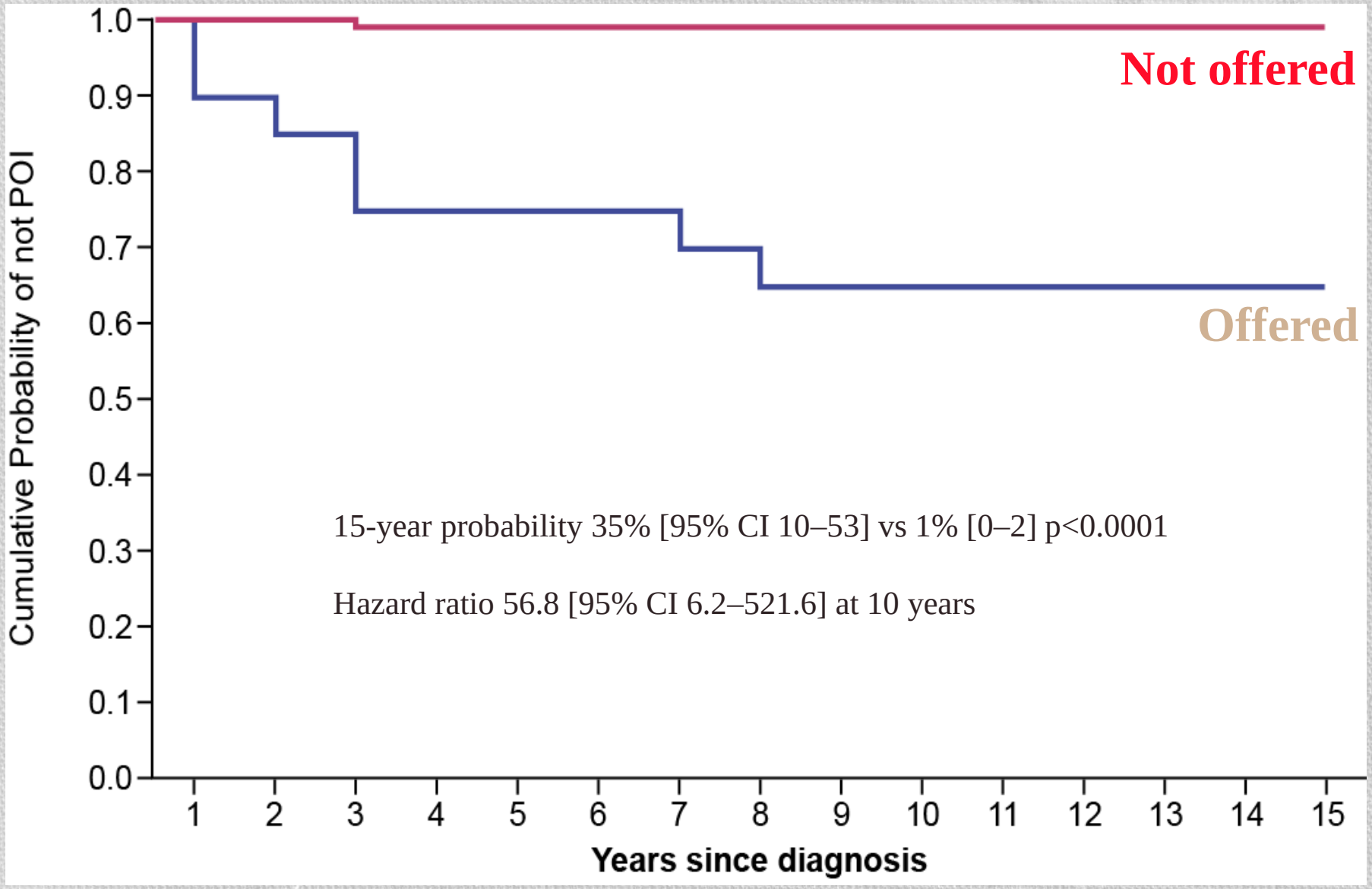
- Neutropenia?
- Thrombocytopenia
- On chemotherapy?
- Within three months of receiving chemotherapy?

15 year, population-based analysis of criteria for ovarian cryopreservation



Walllace WH et al. 2014 Lancet Oncology

Cumulative incidence of POI



Walllace…..and Anderson 2014 Lancet Oncology

Conclusion

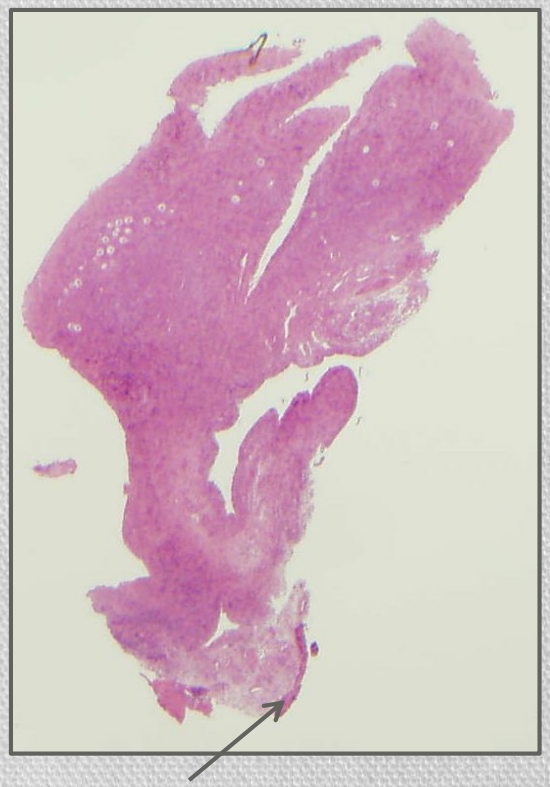
- Ovarian cryopreservation was offered to 9% of our patients, and performed in 5%
- The procedure was safe and without complications
- No patients have asked for re-implantation of their tissue – to date
- All patients who have thus far developed premature ovarian insufficiency were identified except one patient
- The Edinburgh Selection Criteria have proved to be helpful in selecting those patients at highest risk of POI

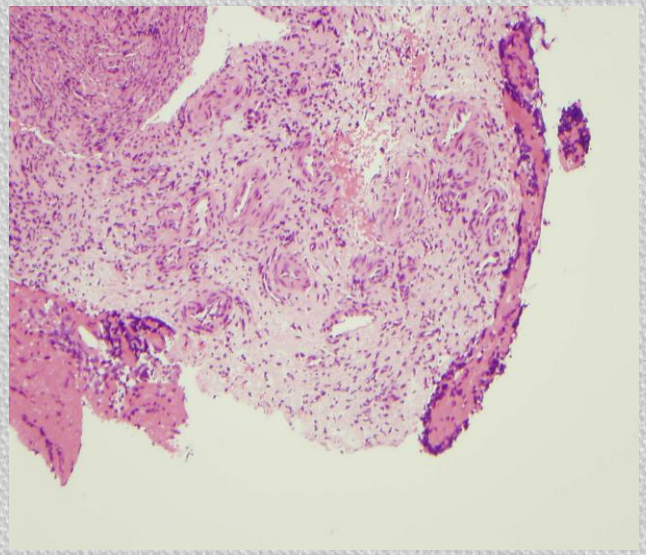
Wallace WH…..and Anderson 2014 Lancet Oncology

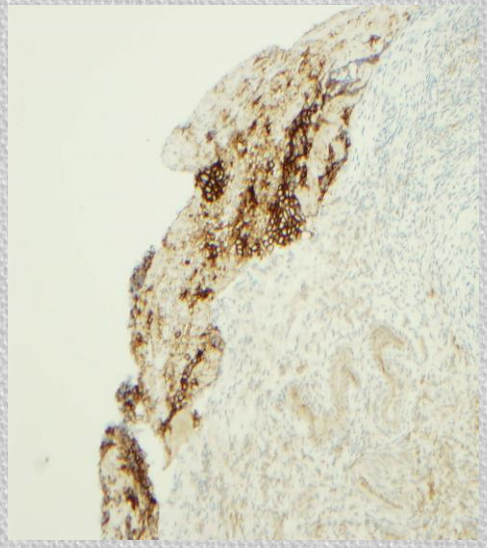
Reimplantation?

- It is important to be aware that reimplantation of ovarian cortical tissue is a separate procedure at a time distant from the treatment of the original cancer
- Consent for harvesting ovarian tissue from children often will have been obtained from their parents
- Informed consent for reimplantation can be obtained from the patients at a much later date when they are competent to assess the complex issues themselves.

Ewings sarcoma localised T 7 Vertebrae (Age 12) – unexpected contamination of ovarian biopsy

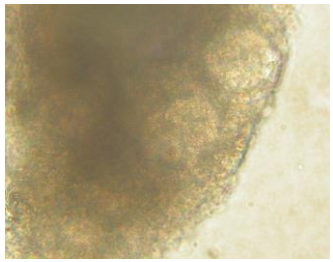


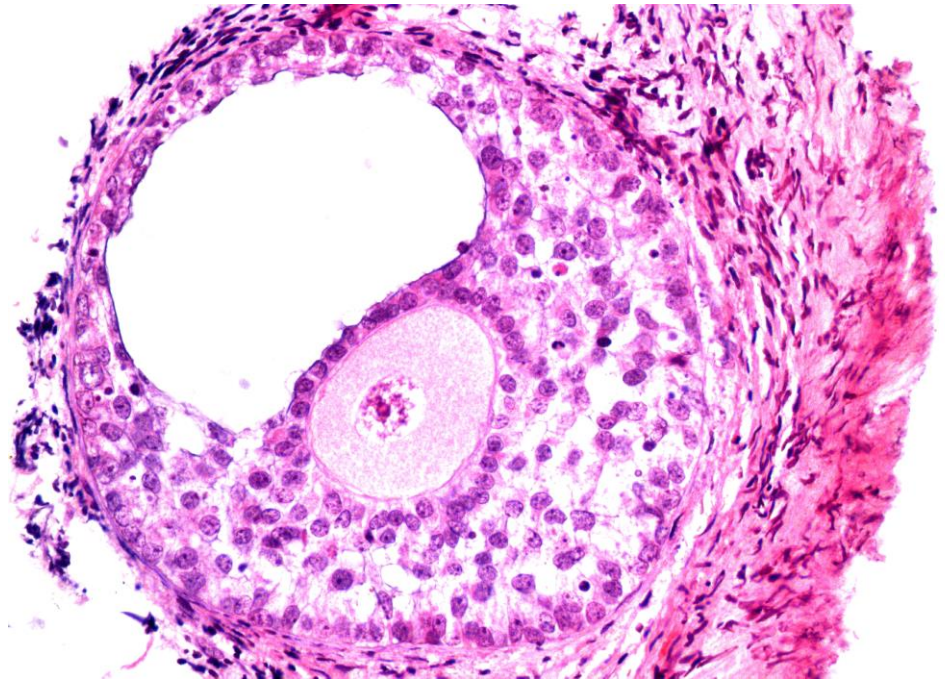




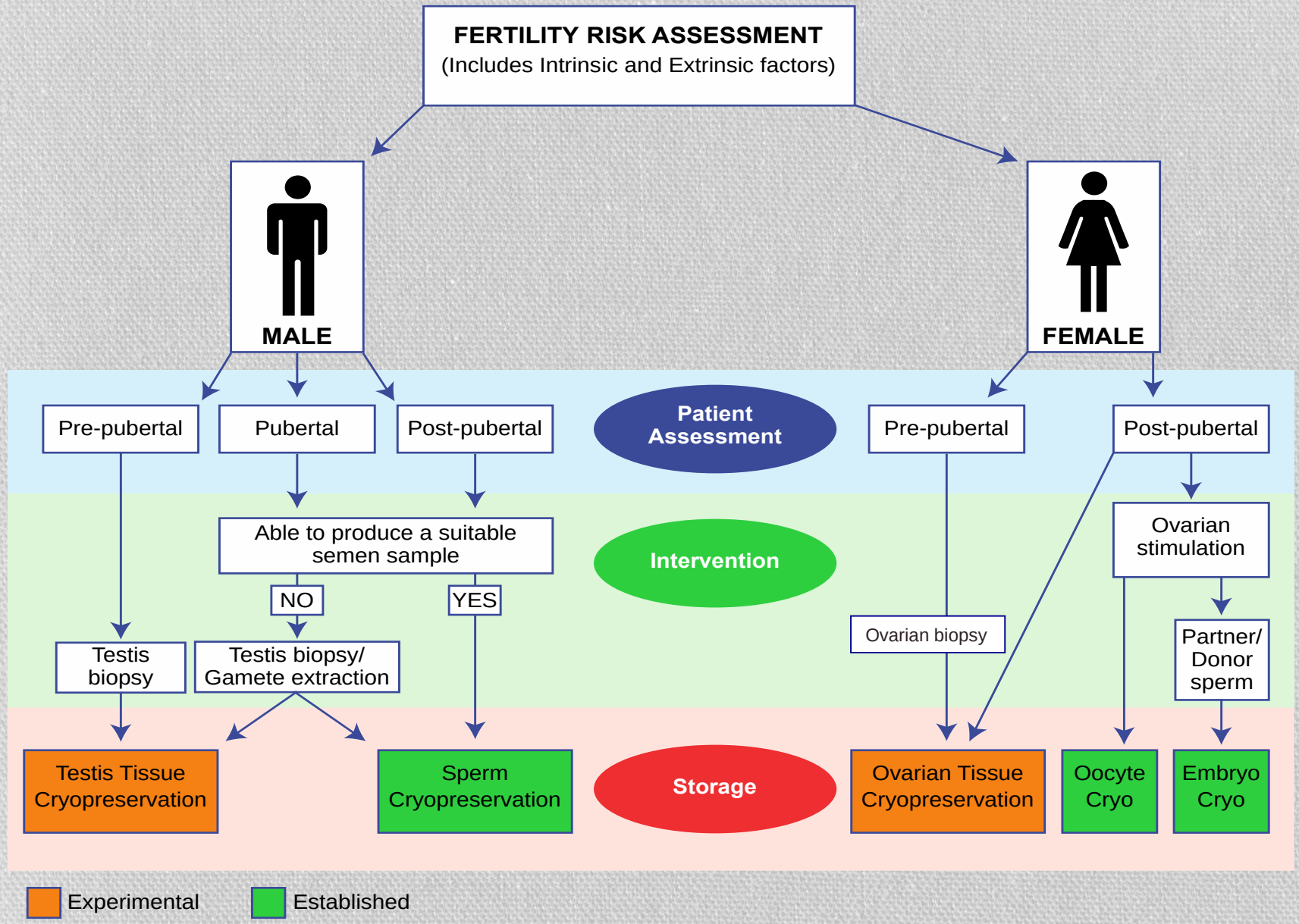
CD99

Antral development from *in vitro* grown human primordial follicles within 10 days





Telfer et al., 2008: A two step serum free culture system supports development of human oocytes from primordial follicles in the presence of activin. **Human Reproduction** 23: 1151-1158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Oocyte Cryo
Embryo Cryo
Experimental
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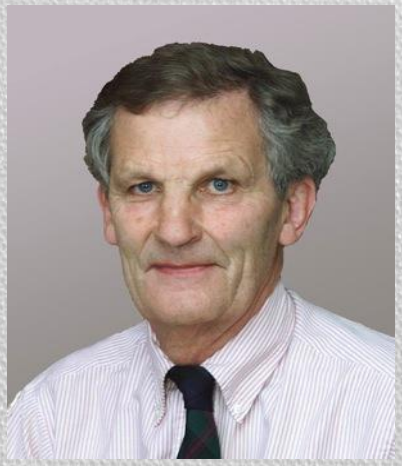
Challenges

- Provide fertility counseling to all young patients with cancer
- Cryopreserve ovarian tissue from the right (high risk) patients
- Define the success rate of the procedures
- Develop IVG/M as a safe alternative to re-implantation through basic research

owledgements



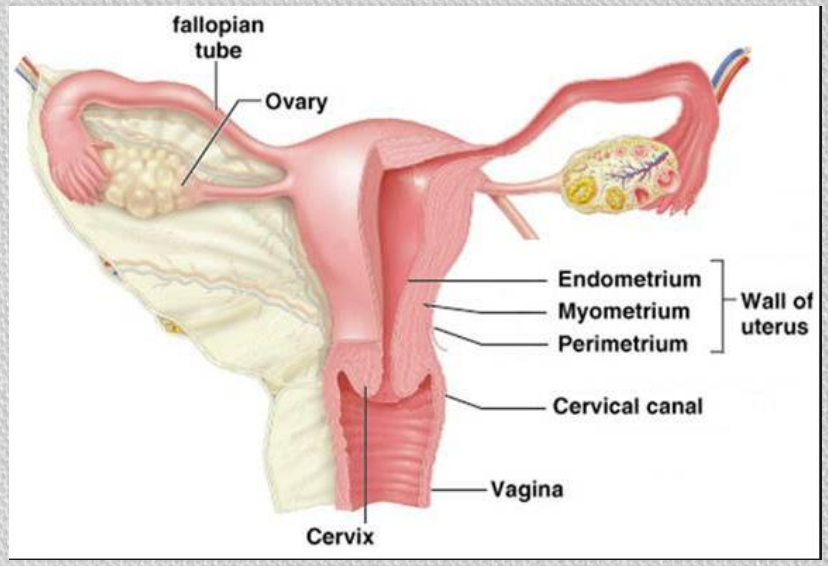
- Richard Anderson
- David T Baird
- Tom Kelsey
- Evelyn Telfer
- Marie McLaughlan
- Alice Grove Smith
- George Galea
- Rod Mitchell
- Louise Bath
- Chris Kelnar
- Angela Edgar
- Mark Brougham
- Fraser Munro

THANK YOU

Key features of the 3 options for fertility preservation for women

Technique	Main advantages	Main disadvantages
Embryo cryopreservation	Established technique	May incur delay Sperm required: partner or donor Fixed potential for future fertility
Oocyte cryopreservation	Does not require sperm	May incur delay Not appropriate for pre-pubertal child Limited numbers of eggs can be stored in time available
Ovarian tissue cryopreservation	Minimal delay No lower age limit Allows for spontaneous and repeated conception Greater allowance for future developments	Requires surgical procedure Malignant contamination in some conditions precludes reimplantation In vitro follicle growth unlikely to be available for several years.

The Uterus


fallopian tube
Ovary
Endometrium
Myometrium
Perimetrium
Wall of uterus
Cervical canal
Vagina
Cervix


A

Re-implantation or IVG and maturation?

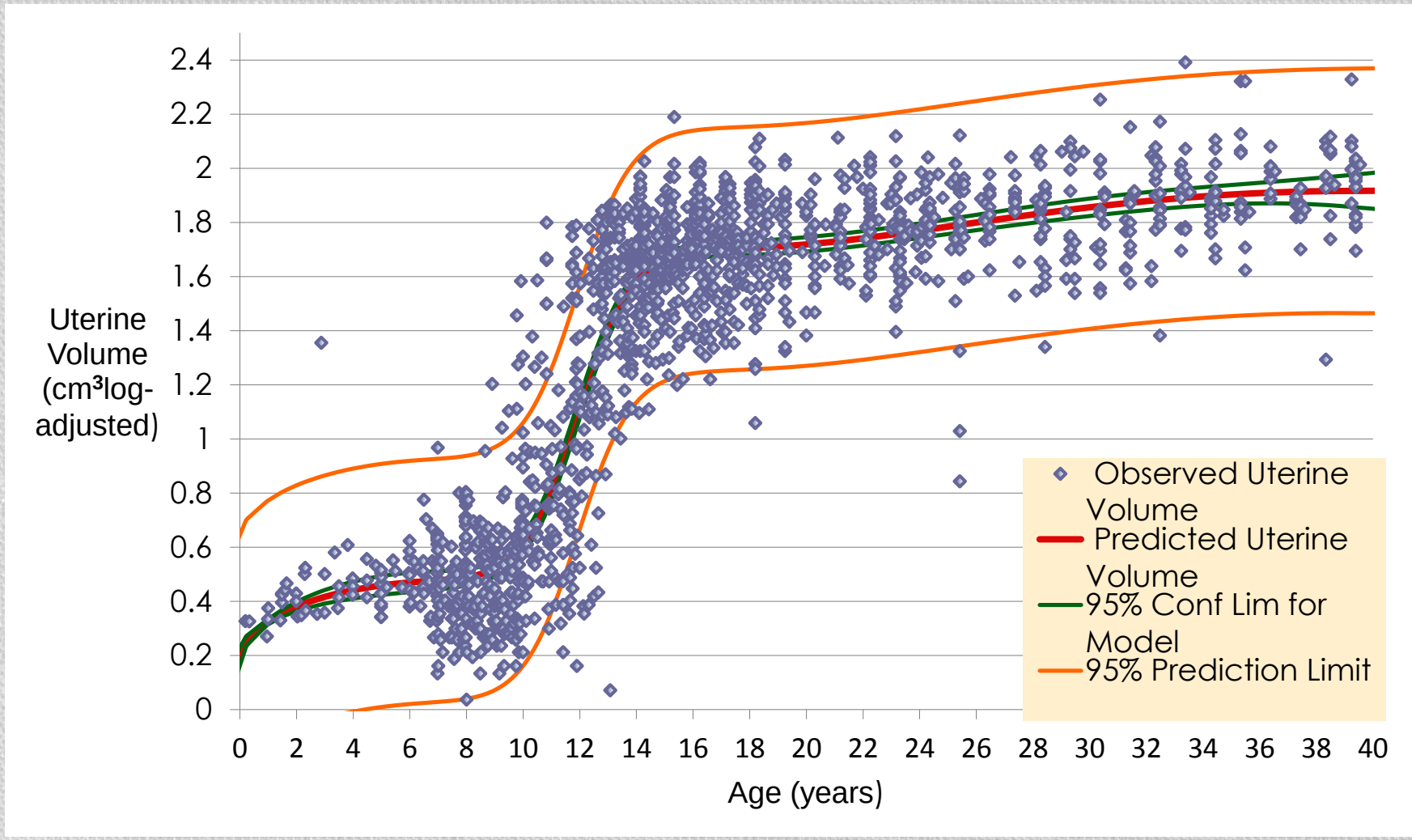
- Contamination of the cryopreserved tissue with malignant cells, particularly in haematological malignant disease – shown in a rodent lymphoma model – to cause recrudescence of the original disease
- Oocyte maturation in vitro, followed by IVF, would eliminate this risk

Uterine function after cancer treatment

- No reports of uterine damage due to chemotherapy

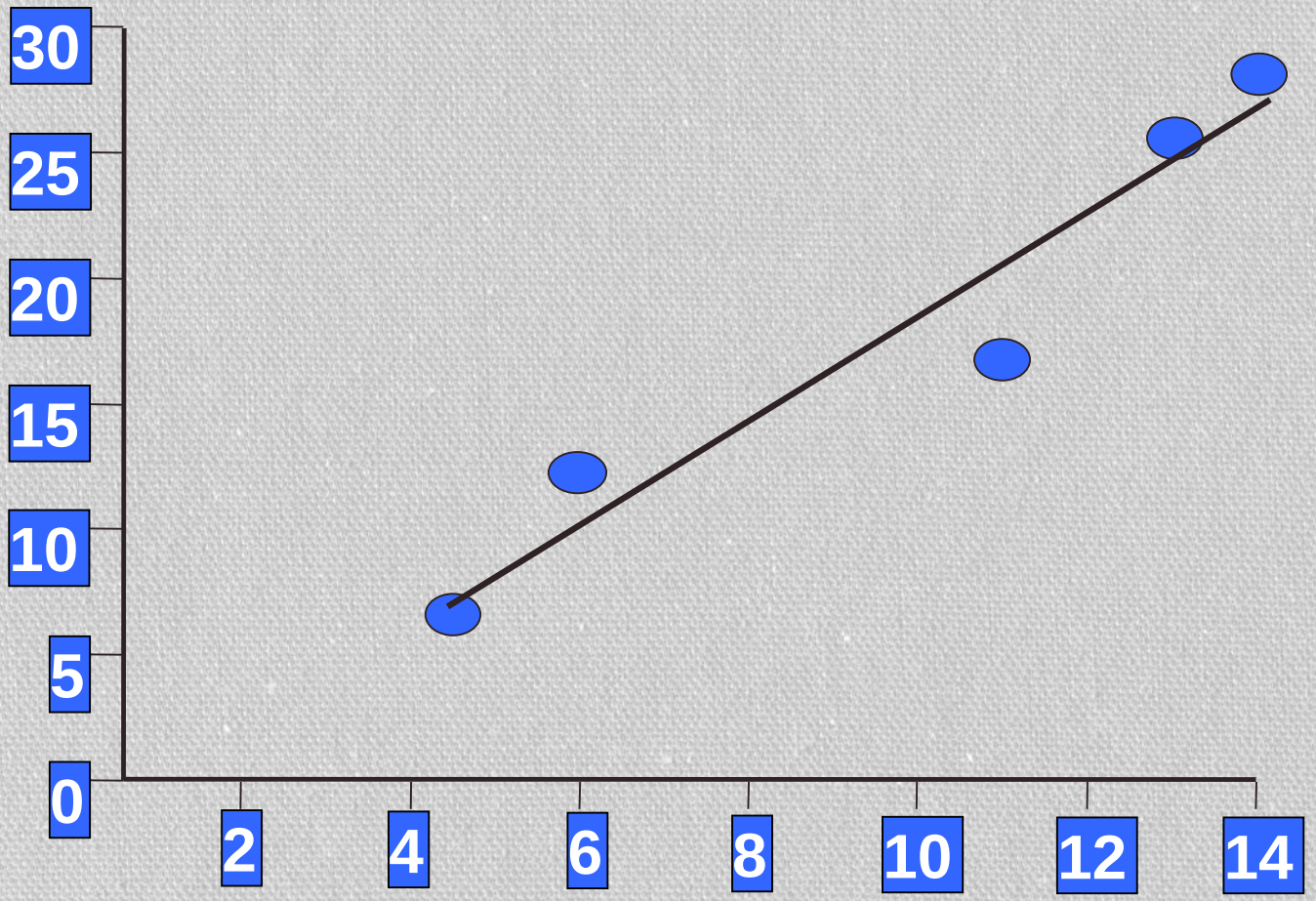
- Radiotherapy:
- Uterine damage, manifest by impaired growth and blood flow.
- Uterine volume correlates with age at irradiation.
- Exposure of the pelvis to radiation is associated with an increased risk of miscarriage, mid-trimester pregnancy loss, PPH, pre-term birth and low birth weight.

Normative model for uterine volume from birth to 40 years. The r^2 is 0.859.



Kelsey et al. 2016

Uterine volume and age at irradiation (TBI)



Bath et al. BJOG (1999)

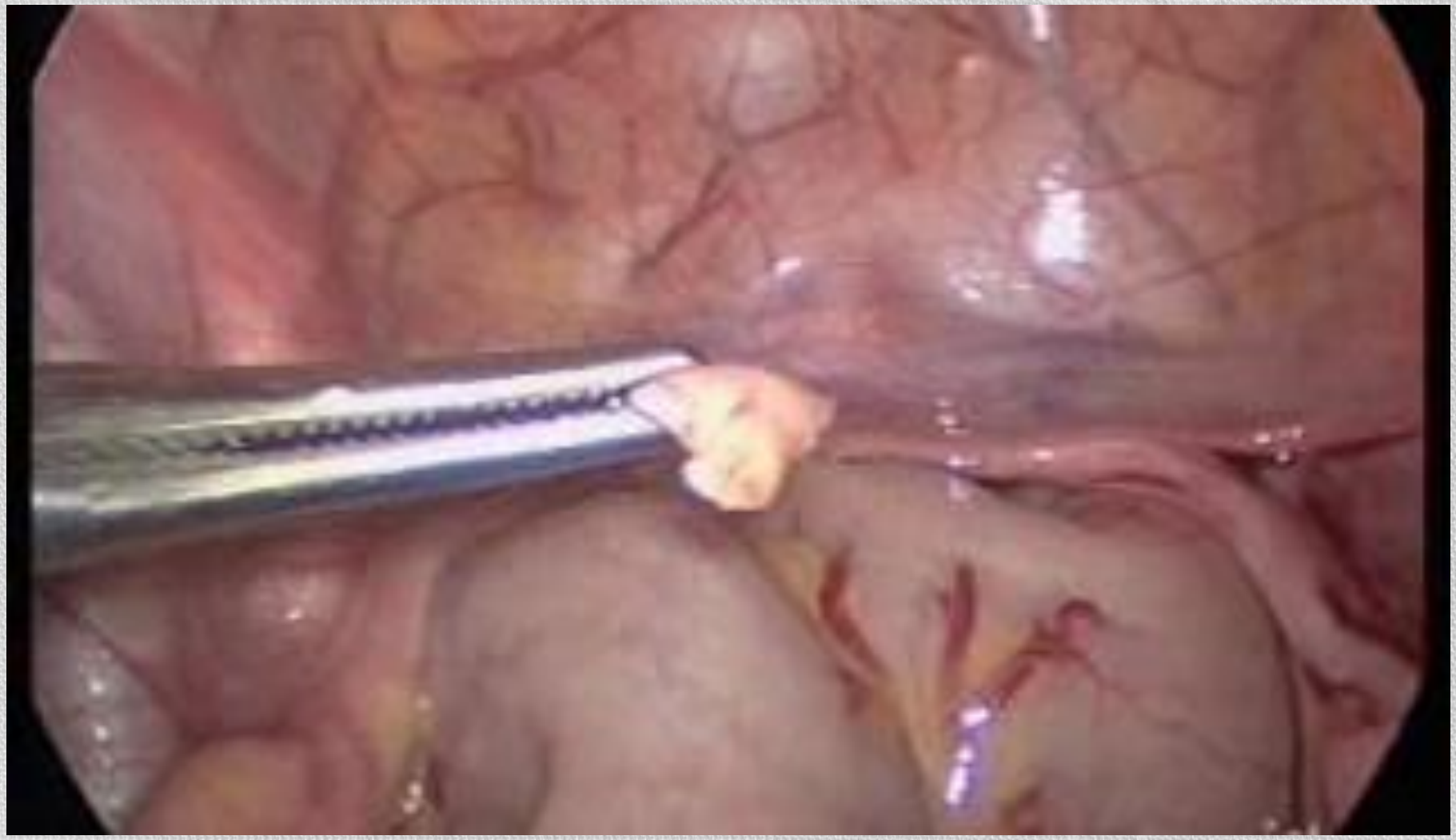
Ovarian cortical strips

- rich in primordial follicles
- survive cryopreservation
- technique validated in sheep

Live births following cryopreservation of ovarian tissue and transplantation

Diagnosis	Age (yrs)	Surgical method	Reimplantation	Pregnancy	Reference
Hodgkin's Lymphoma	25	Unilateral ovarian biopsy	Orthotopic	Spontaneous, live birth	Donnez, 2004
Non-Hodgkin's Lymphoma	28	Unilateral ovarian biopsy (after 1st course chemo)	Orthotopic (Both ovaries)	IVF, live birth	Meirow 2005; 2007
Hodgkin's Lymphoma	31	Unilateral ovarian biopsy (after 1st course chemo)	Ortho and heterotopic	Spontaneous, miscarriage then livebirth	Demeestere 2007
Hodgkin's lymphoma	27	Whole ovary	Orthotopic	Livebirth male Week 37 B.Wt 2.6 Kg	Andersen et al 2008
Ewings Sarcoma	36	Whole ovary	Orthotopic	Livebirth Female Term B Wt 3.2 Kg	Andersen et al 2008

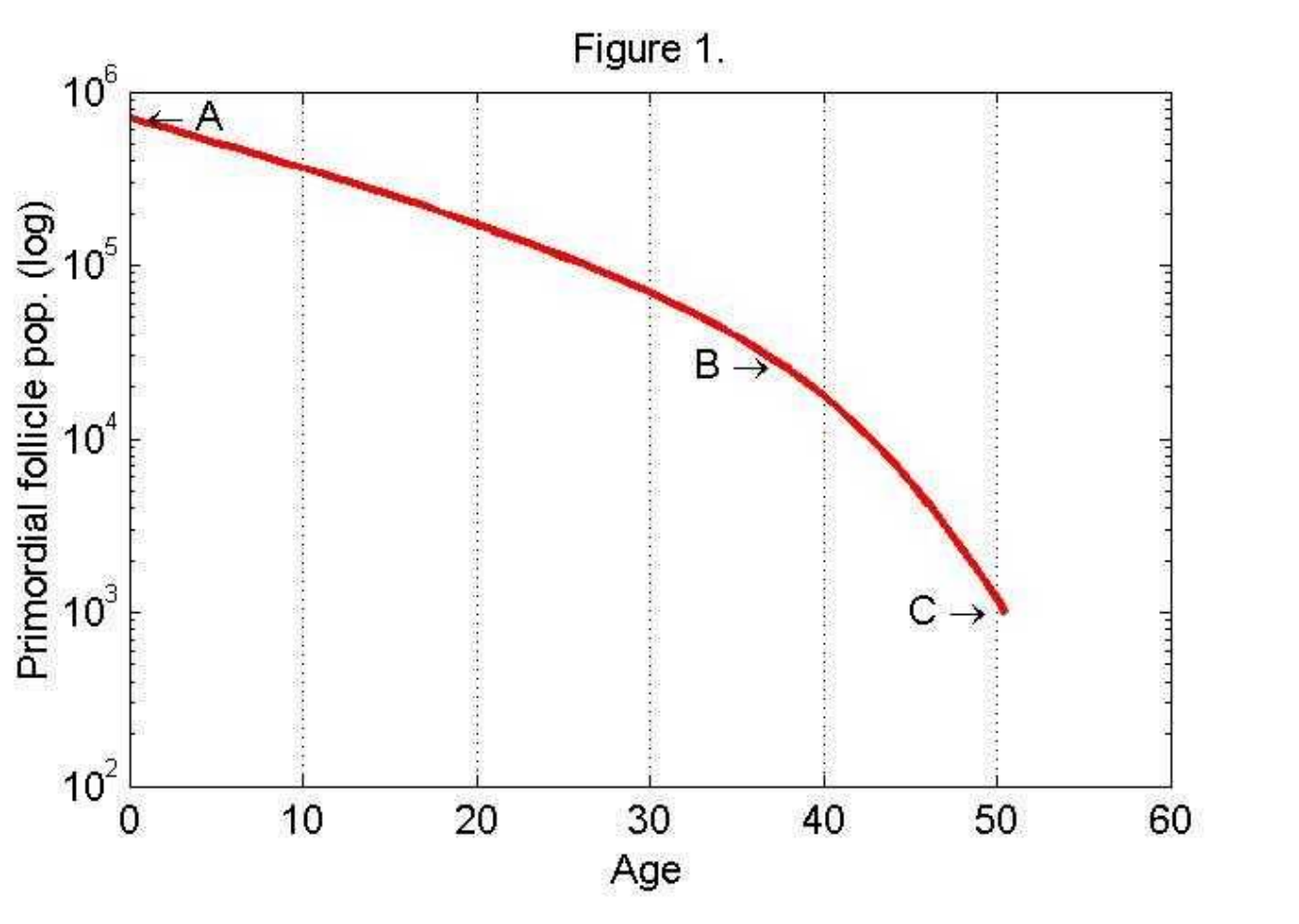
Ovarian biopsy at laparoscopy



Cryopreservation: World-wide experience

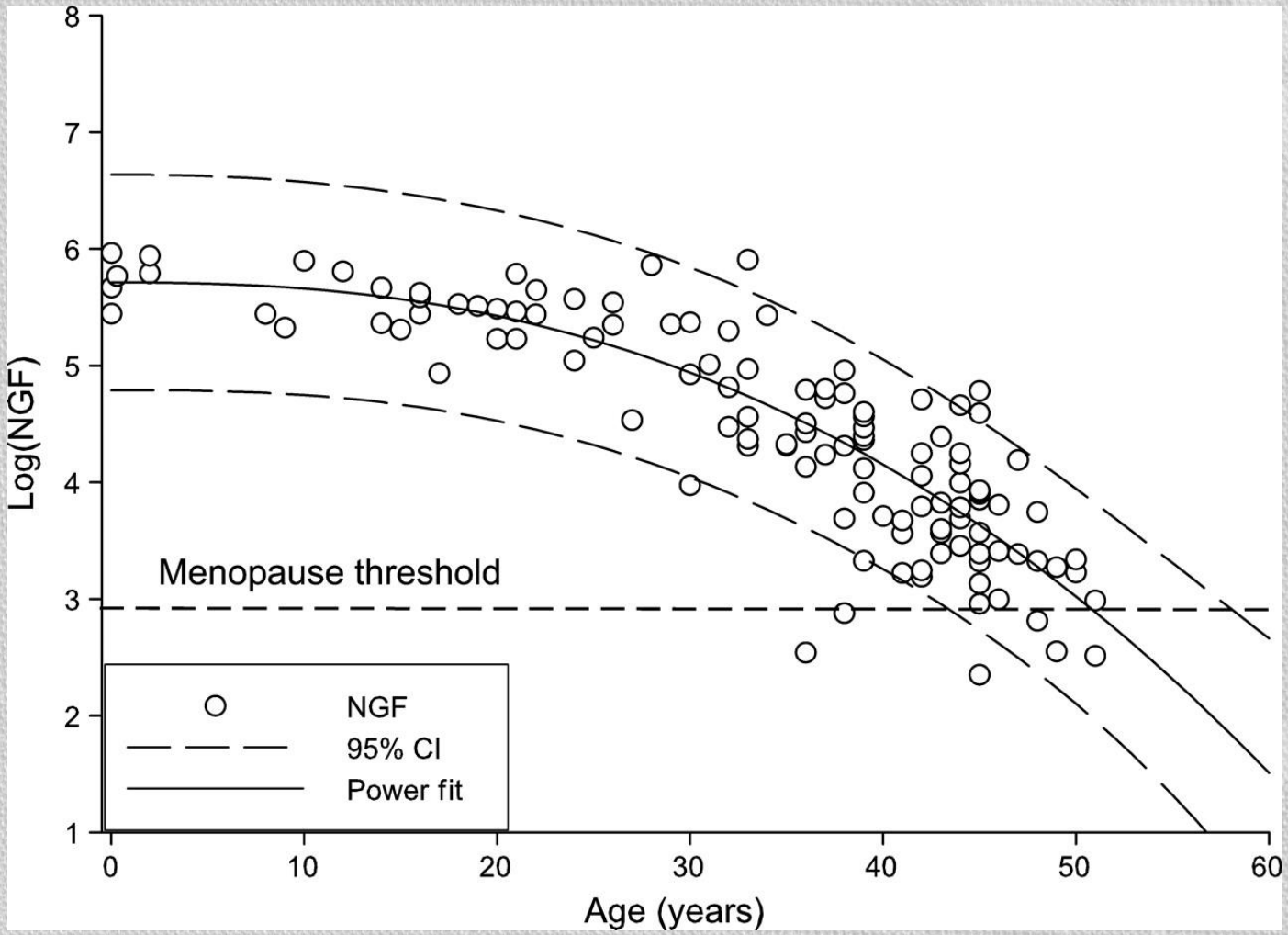
* At least 20 pregnancies worldwide after othotopic reimplantation of frozen–thawed ovarian cortex
* Success rate is unclear as the denominator is unknown
* No pregnancies reported following the reimplantation of ovarian tissue harvested pre-pubertally
* Young children are potentially ideal candidates

The Faddy-Gosden model of primordial follicle decline (birth-menopause)



Faddy MJ, Gosden RG (1996) A model conforming the decline in follicle numbers to the age of menopause in women. Human Reproduction 11: 1484–1486.

Power-model of human ovarian NGF decay



Hansen, K. R. et al. Hum. Reprod. 2008 23:699-708

Data set:
Eight quantitative histological studies

Study			Statistics			
Number	First author	Year	No. ovaries	Min. age	Max. age	Median age
1	Bendsen	2006	11	−0.6	−0.6	−0.6
2	Baker	1963	11	−0.6	7.0	−0.2
3	Forabosco	2007	15	−0.5	0.5	−0.3
4	Block	1953	19	−0.2	0.0	0.0
5	Hansen	2008	122	0.1	51.0	38.0
6	Block	1951	86	6.0	44.0	28.0
7	Gougeon	1987	52	25.0	46.0	39.5
8	Richardson	1987	9	45.0	51.0	46.0
Overall			325	−0.6	51.0	32.0

Ovarian transplantation: World-wide experience

* Silber et al. have also extensively reported their experience of successful fresh ovarian transplantation in identical twins discordant for premature ovarian failure
* 12 pregnancies and eight healthy babies have been reported from nine homozygotic transplants

Silber et al. MHR 2012

Cryopreservation: World-wide experience

- Recent report of three women who have experienced long-term (> 7 years) duration of function of ovarian cortical tissue grafts.
- Birth of eight healthy babies in total following a single graft per patient.

Andersen et al. 2012 RBMonline

Ethical issues

- Ethical considerations for children are different and more challenging from those involving adults
  - who are assumed to be competent
- interventions in children can only be ethical if they can be considered to be therapeutic and in the best interests of the minor

HRT and pubertal induction

- An intriguing question remains: Should ovarian tissue that has been harvested and frozen be reimplanted to provide HRT?
- or even pubertal induction in the young patient with premature ovarian failure?
  - Poirot et al., Lancet 2012
- Ovarian grafts will survive for up to 7 years
  - Andersen et al ., 2012
- several groups have reimplanted ovarian tissue once the initial graft has failed
  - Silber et al., 2008
- Our view is that this precious tissue should only be reimplanted if fertility is requested

Technology or evidence led?

- In the field of fertility preservation there is a dearth of well-designed studies to fully evaluate exciting new techniques
- Unlikely to be feasible or ethical to perform an RCT in a well characterized group of young women to test laparoscopic collection of ovarian cortex versus either dummy laparoscopy or no intervention
- It is highly unlikely that IRBs would pass such a study, or that such a randomized study would be able to recruit sufficient patients

Technology or evidence led?

- When there is uncertainty about a new experimental procedure, it is important for it to be evaluated in IRB-approved clinical trial
- the ASCO guideline recommends that ovarian cryopreservation and transplantation procedures should only be performed in centres with the necessary expertise under IRB-approved protocols that include follow-up for recurrent cancer

Lee et al. JCO 2006, 24(18):2917-31

Ovarian cryopreservation & ovarian function

Edinburgh experience in children (< 18 yrs) 1996-2012

Cryopreservation of ovarian cortical tissue – Edinburgh criteria

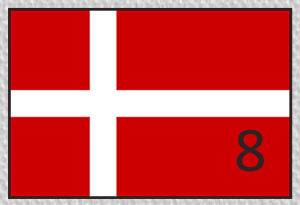
Selection criteria (1995,modified 2000)

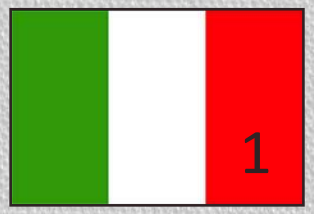
- Age < 30 years
- No previous chemotherapy/radiotherapy if age >15 years
- Mild, non gonadotoxic chemotherapy if < 15 years
- A realistic chance of surviving five years
- A high risk of ovarian failure
- Informed consent (Parent and where possible Patient)
- Negative HIV and Hepatitis serology
- No existing children

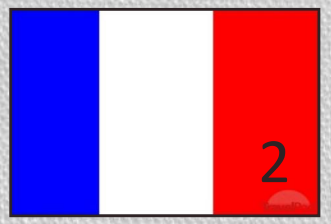
Consent

- We emphasize in the information sheet that the procedure is voluntary and experimental, and not part of routine practice
- We obtain informed consent for disposal of ovarian tissue if it is no longer required or the patient dies
- If consent has been obtained, it may be used for ethically approved research studies
- Separately, we ask if an additional small amount can be taken at the time of collection for research studies
- Our practice constitutes research and has been approved by the local institutional review board (IRB)

Children born from transplantation of frozen/thawed ovarian tissue







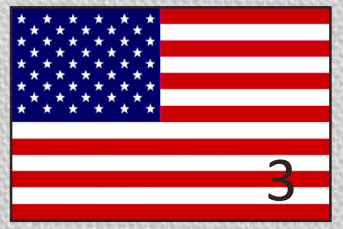
All Normal Babies

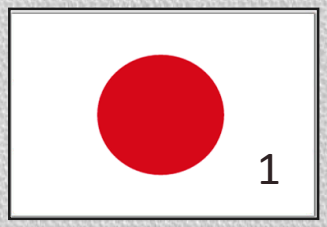
weight and duration

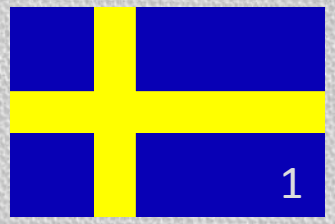
Orthotopic >> heterotopic

All except for one is a result of a slow-freezing protocol

An estimated excess of 150 transplantations have been performed







Induction of puberty by autograft of cryopreserved ovarian tissue

- 10 year old with Sickle cell disease 2003 before HSCT Rt Oophorectomy and cryopreservation
- Aged 13 , developed POI, and requested return for pubertal induction
- B2, 4 months; Menstruation, 8 months
- Regular menstruation for two years post graft, Normal breast development
- This case shows the first restoration of endocrine ovarian function from tissue harvested before puberty.

Poirot et al.Lancet, 2012

Induction of puberty by autograft of cryopreserved ovarian tissue

9 year old with Ewing, intensively treated with CT and RT

OTC before treatment commenced

Developed POI . No pubertal development. In remission

4.5 years later (13.5years) ovarian tissue returned for pubertal induction. Tanner B4 and menstruation within one year.

Graft ceased to function after 19 months

Several years later she relapsed and died from recurrent Ewing sarcoma

No evidence of EWS FLI1 in remaining stored ovarian tissue.

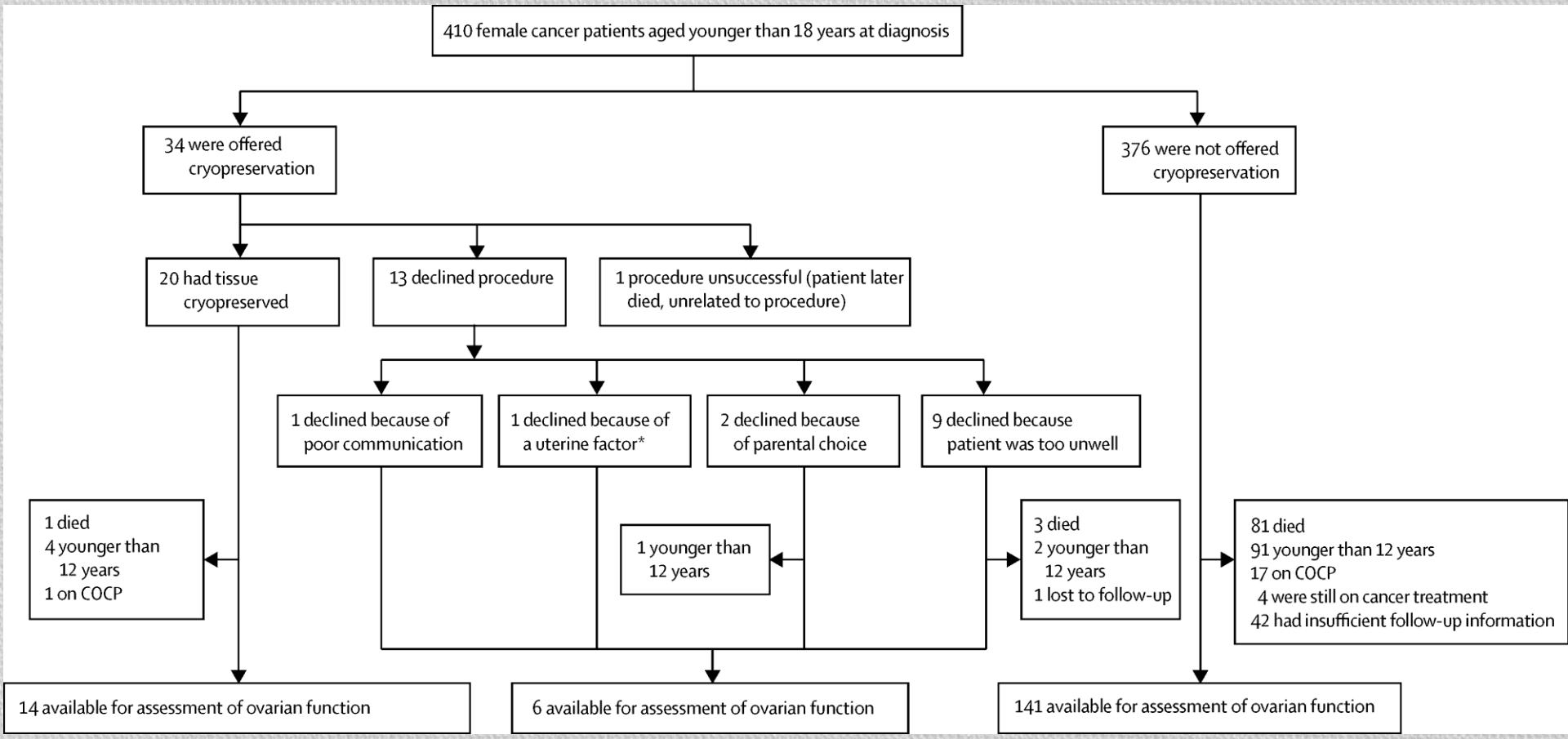
Ernst et al EJC, 2013

Edinburgh Paediatric Experience

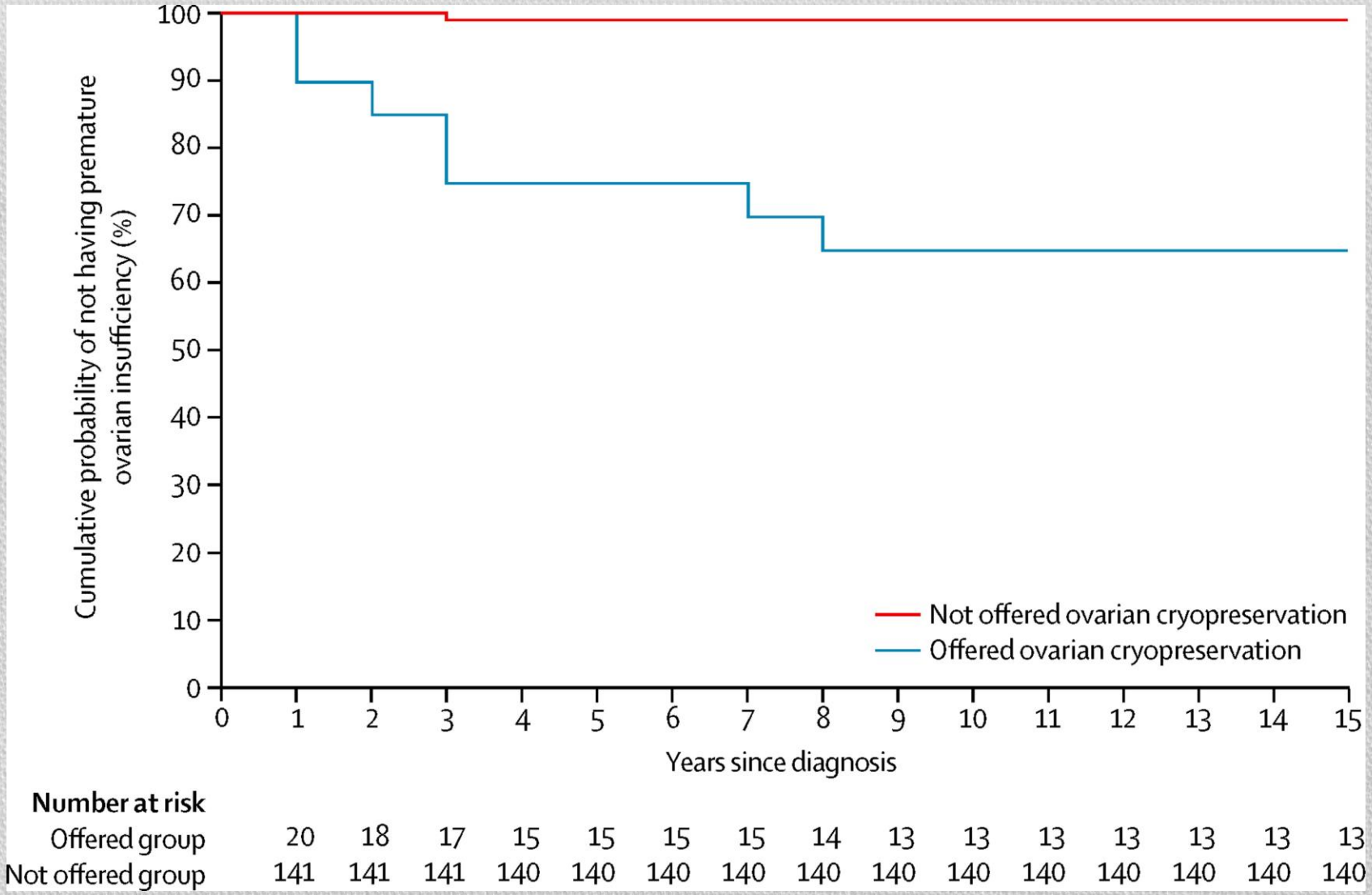
Table 3: Patients that had ovarian tissue cryopreserved

Patient No.	Diagnosis	Age at procedure	Method	Complications
1	Hodgkin's lymphoma¤	14.9	Laproscopic Cortical Strip	None
2	Ewing's sarcoma of pubic bone	14.9	Laproscopic Cortical Strip	None
3	Sacral ependymoma	11.3	Laproscopic Cortical Strip	None
4	Hodgkin's lymphoma	13.7	Laproscopic Cortical Strip	None
5	Hodgkin's lymphoma	11.0	Laproscopic Cortical Strip	None
6	Chronic granulocytic leukaemia	9.9	Laproscopic Cortical Strip	None
7	Rhabdomyosarcoma	5.3	Laproscopic Cortical Strip	None
8	Ewing's sarcoma (pelvic)	9.8	Laproscopic Cortical Strip	None
9	Uterine Cervix Rhabdomyosarcoma*	16.5	Laproscopic Cortical Strip	None
10	Hodgkin's lymphoma°	14.1	Laproscopic Cortical Strip	None
11	Abdominal embryonal Rhabdomyosarcoma	7.9	Laproscopic Cortical Strip	None
12	Ewing's sarcoma	12.1	Laproscopic Cortical Strip†	None
13	Hodgkin's lymphoma	12.7	Laproscopic Cortical Strip	None
14	Metastatic Medulloblastoma	8.1	Laproscopic Cortical Strip	None
15	Hodgkin's lymphoma	15.2	Laproscopic Cortical Strip	None
16	Alveolar Rhabdomyosarcoma	10.5	Laproscopic Cortical Strip	None
17	Embryonal Rhabdomyosarcoma	3.0	Oophorectomy	None
18	Ewing's Sarcoma	12.0	Laproscopic Cortical Strip	None
19	Undifferentiated Sarcoma	12.3	Laproscopic Cortical Strip†	None
20	Wilm's Tumour	1.2	Oophorectomy	None

Cohort Summary



Wallace et al. *The Lancet Oncology* 2014 15, 1129-1136



The cumulative probability of developing premature ovarian insufficiency after treatment was completed was significantly higher for patients who met the criteria for ovarian tissue cryopreservation than for those who did not (15-year probability 35% *vs* 1%; p<0·0001; hazard ratio 56·8 at 10 years).

Wallace et al. *The Lancet Oncology* 2014 15, 1129-1136

Conclusion

- Ovarian cryopreservation was offered to 9% of our patients, and performed in 5%
- The procedure was safe and without complications
- No patients have asked for re-implantation of their tissue – to date (15.7 [1.3-30.9] yrs)
- All patients who have thus far developed ovarian failure were identified
- The Edinburgh Selection Criteria have proved to be helpful (only one patient not offered cryopreservation who has uncertain ovarian function)

Outcomes of transplantations of cryopreserved ovarian tissue to 41 women in Denmark

- 41 women who had thawed ovarian tissue transplanted 53 times over a period of 10 years
- Majority had breast cancer or lymphoma, all <39 years at ovarian tissue cryopreservtion
- Among 32 women with a pregnancy-wish, 10(31%) had a child/children
- The transplanted ovarian tissue can last up to 10 year
- Three relapses occurred (2 Breast Ca, 1 Ewings)

Jensen AK…Andersen CY Hum Rep 2015

Transplantation of Ovarian Tissue - The Israeli experience

- N= 20 cancer survivors
- Ovarian Tissue harvested 14-39 years
- N=15 haematological malignancies
- N=10 exposed to pre-harvest chemotherapy
- 93% reported endocrine recovery
- N=16 pregnancies(10: IVF, 6 spontaneous)
- 32% had at least one live birth and 53% had a pregnancy
- No cancer relapses
- **Safe and no longer experimental!**

Meirow et al., Fertility and Sterility 2016

Summary

Females

- It remains difficult to predict which patients are at high risk of a premature menopause
- Cryopreservation of ovarian tissue before treatment is the best option for girls and young women
- Orthotopic reimplantation works but so far there have been very few live births.
- Accelerated IVG of human oocytes is likely to become a realistic possibility.

Anderson, Mitchell et al. The Lancet Diabetes & Endocrinology 2015

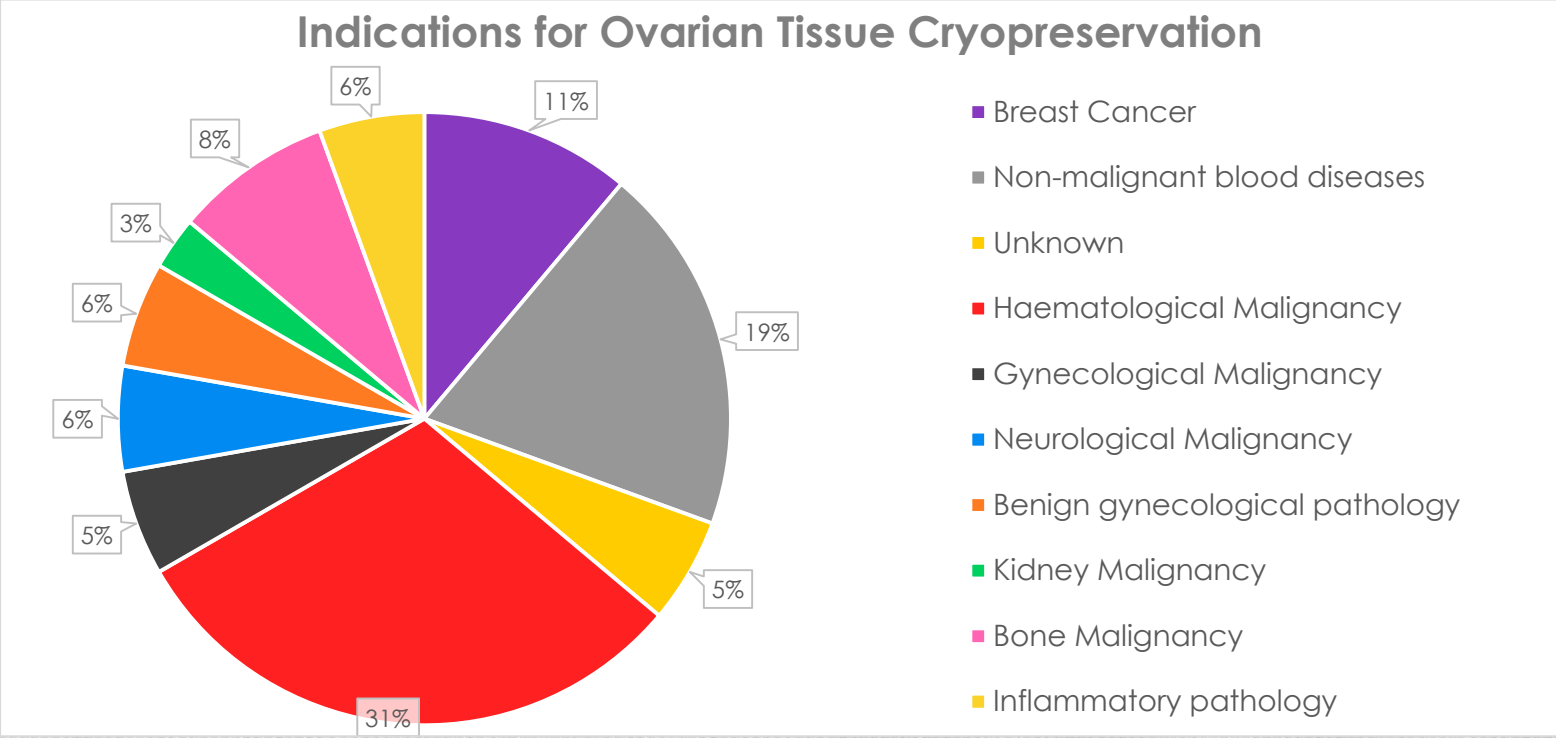
Challenges

- Provide fertility counseling to all young patients with cancer
- Cryopreserve ovarian tissue from the right patients
- Define the success rate of the procedure
- Develop IVG/M as a safe alternative to reimplantation

Induction of puberty by autograft of cryopreserved ovarian tissue

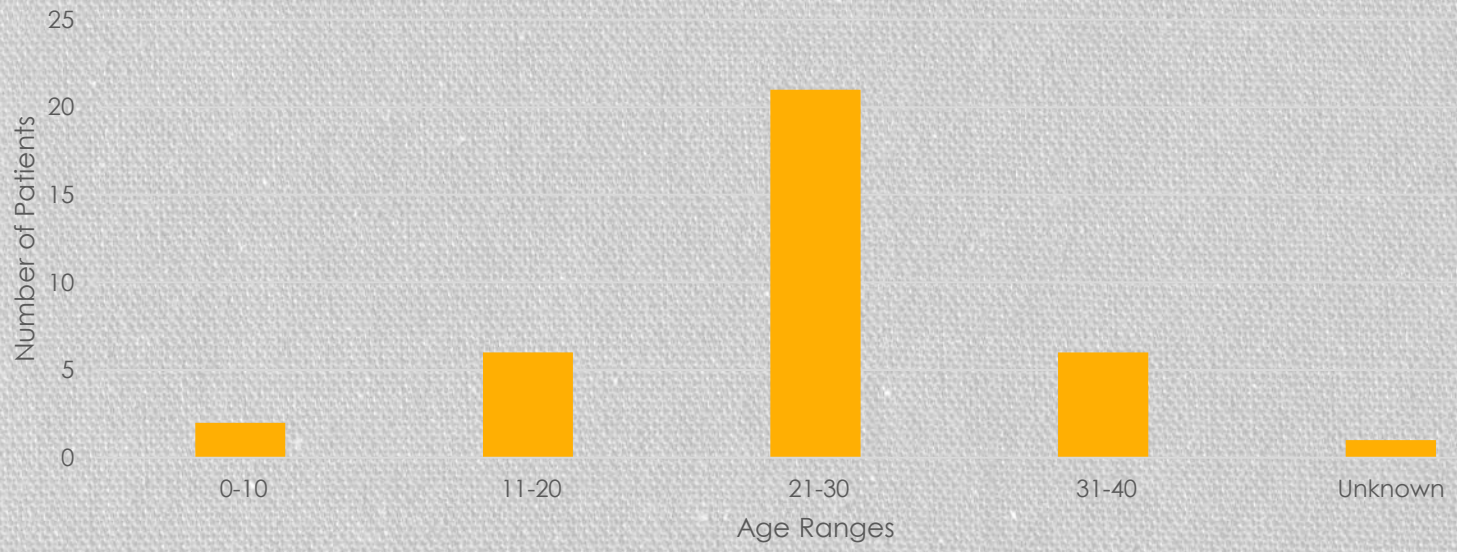
- Induction of puberty with exogenous steroid hormones either orally or trans-dermally is well established
- The re-implantation of ovarian tissue in a hypergonadotrophic environment not ideal
- Potential waste of a finite number of germ cells
- Risk of relapse ..particularly in haematological malignancies

Indications for ovarian tissue cryopreservation (n=36).



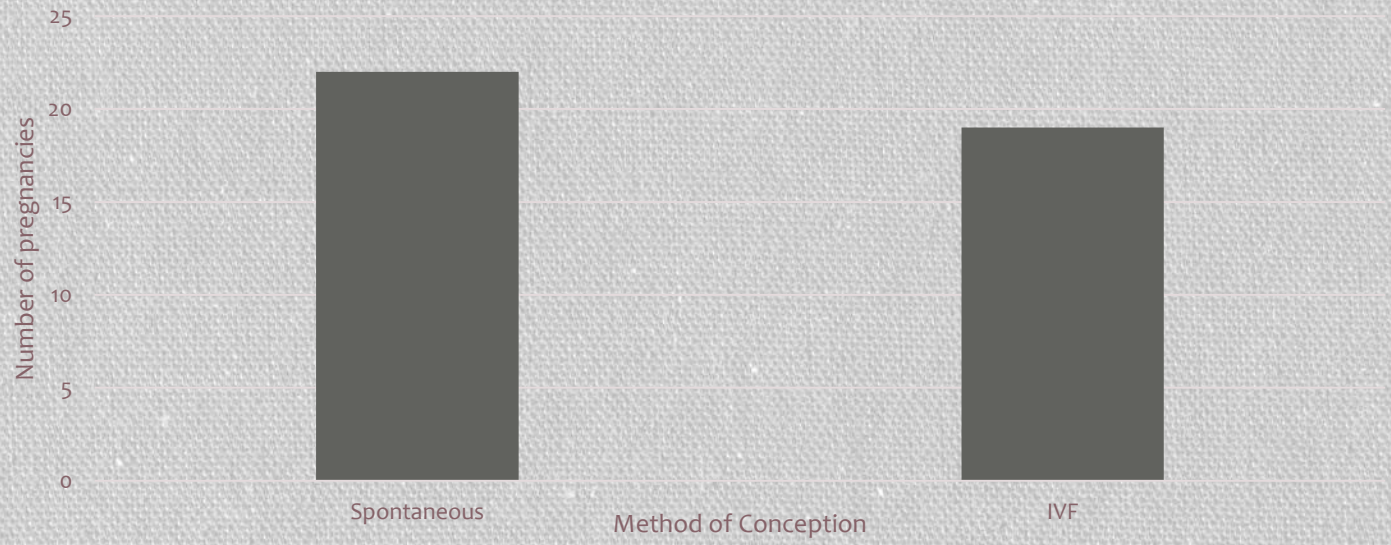
Chalk K & Wallace WH (unpublished)

Age ranges of patients from published data who underwent ovarian tissue cryopreservation (n=36)



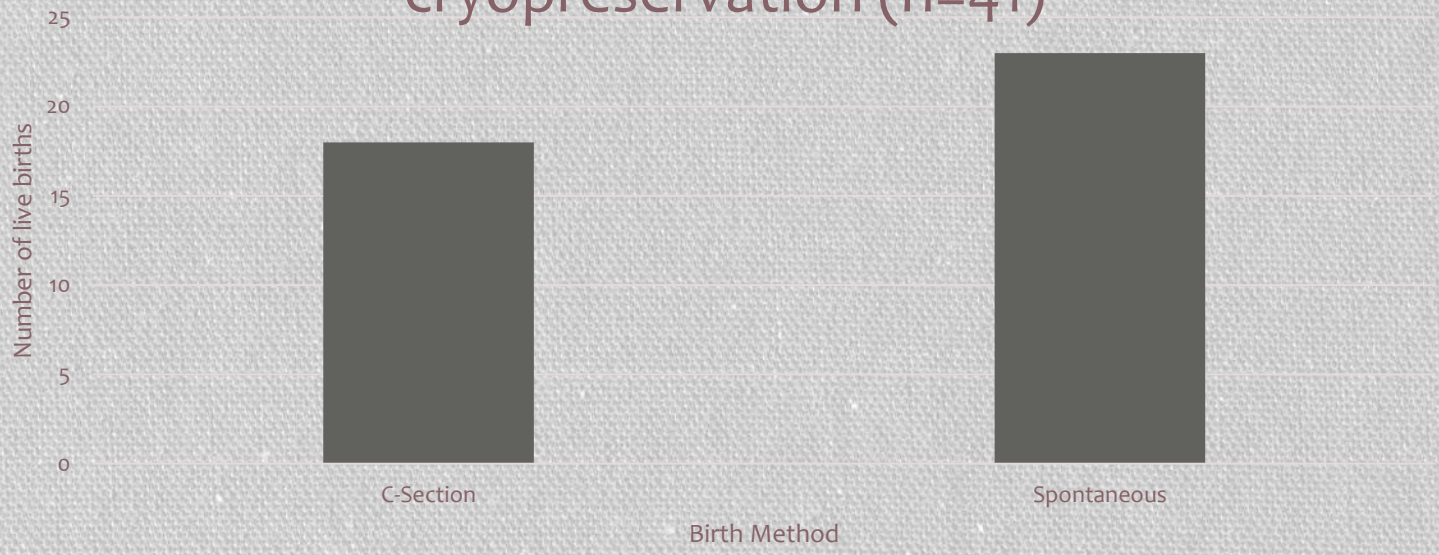
Chalk K & Wallace WH (unpublished)

Method of conception for successful live births after ovarian tissue cryopreservation based on published data (n=41)



Chalk K & Wallace WH (unpublished)

Birth method for published live births after the mother had undergone ovarian tissue cryopreservation (n=41)

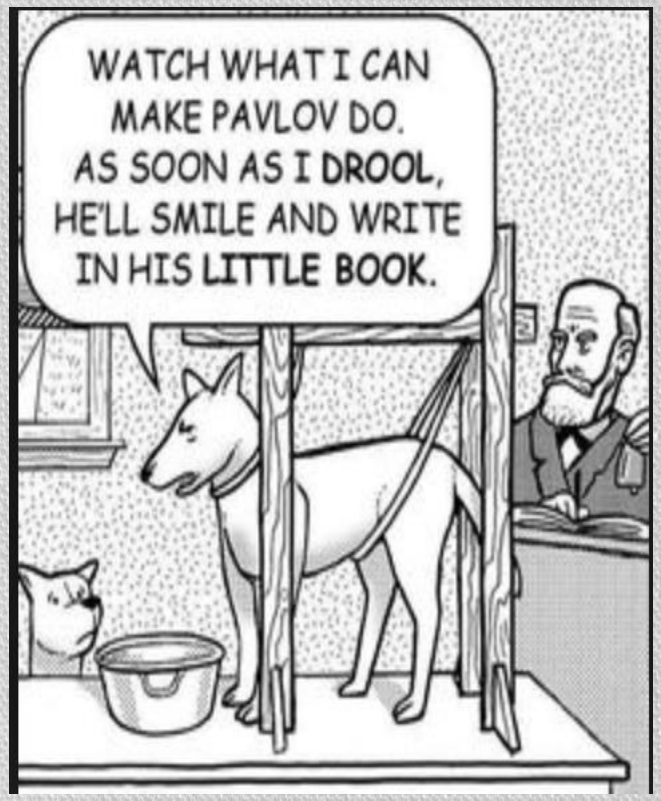


Chalk K & Wallace WH (unpublished)

Number of patients who underwent chemotherapy before the procedure (n=34)

Chalk K & Wallace WH (unpublished)

Experimental or Established



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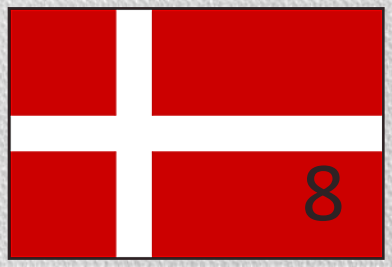

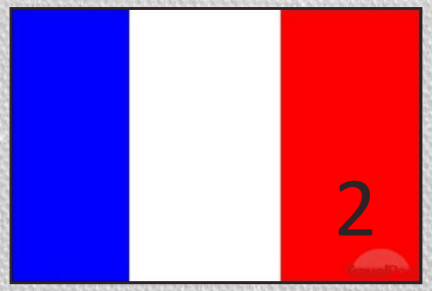

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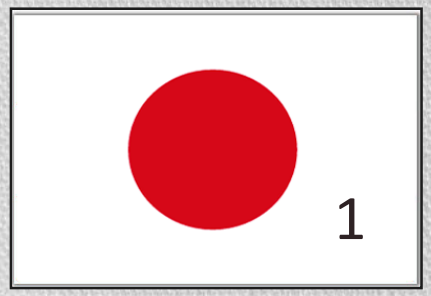

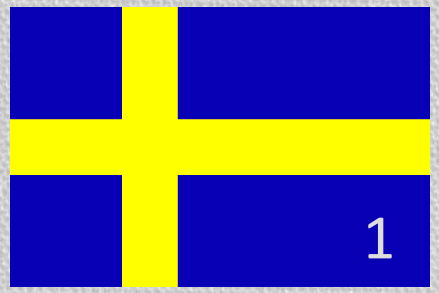

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