Fertility preservation for young people with cancer: Who is at risk and what can be offered ?

Professor W Hamish Wallace
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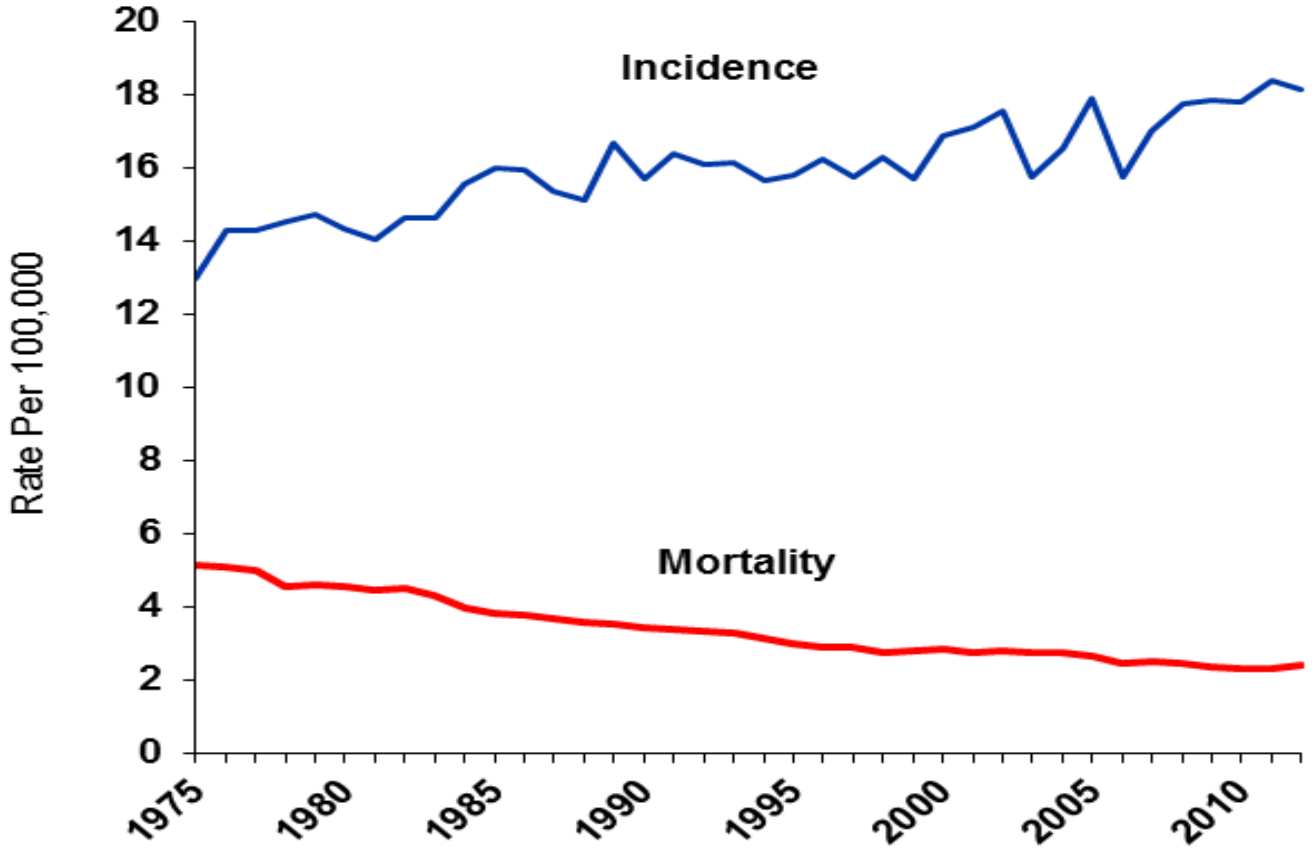
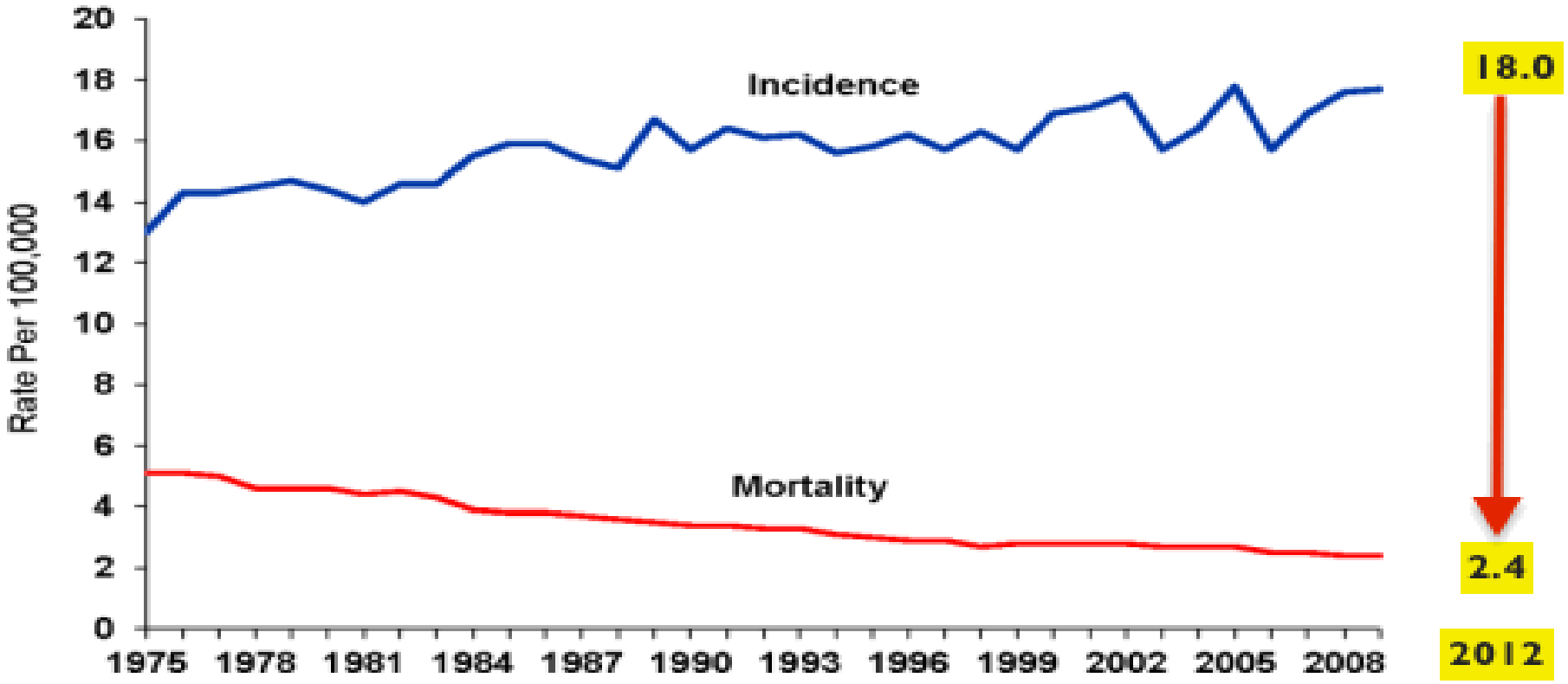
Endocrine Masterclass, Utrecht,
13th April 2017

Trends in Cancer Incidence and Death Rates* in Children and Adolescents (0-19 Years), 1975-2012



*Age-adjusted to the 2000 standard population. Incidence rates are adjusted to account for delays in reporting.
Sources: Incidence – Surveillance, Epidemiology, and End Results (SEER) Program, National Cancer Institute, 2015. Mortality – National Center for Health Statistics, Centers for Disease Control and Prevention, 2015.

Cancer Incidence and Death Rates* in Children 0-19 Years, 1975-2009

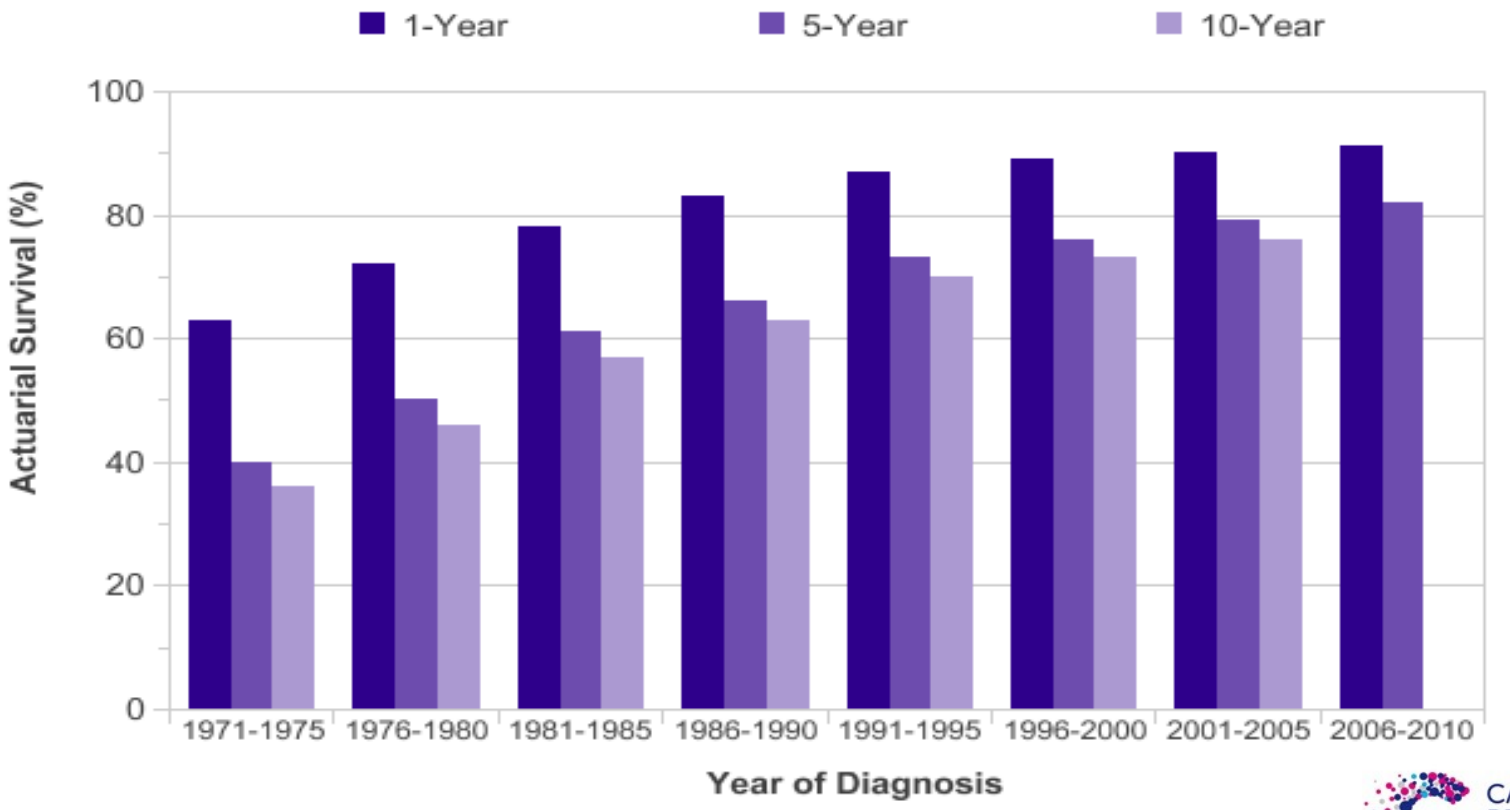


*Age-adjusted to the 2000 Standard population.

Source: Incidence -Surveillance, Epidemiology, and End Results Program, Delay-adjusted Incidence database: SEER Incidence Delay-adjusted Rates, 9 Registries, 1975-2009, National Cancer Institute, 2012.
Mortality – National Center for Health Statistics, 2012.

Childhood Cancer 1971-2010

One-, Five- and Ten-Year Actuarial Survival (%), Children (Aged 0-14), Great Britain



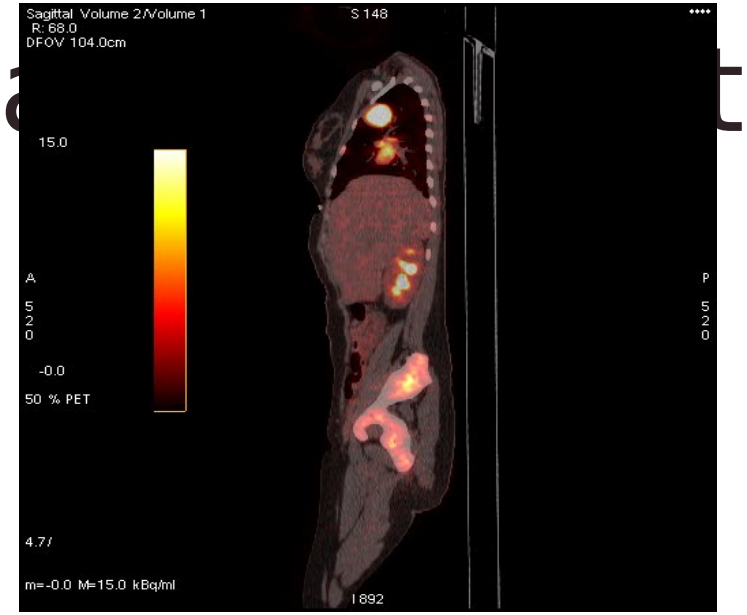
CANCER RESEARCH UK

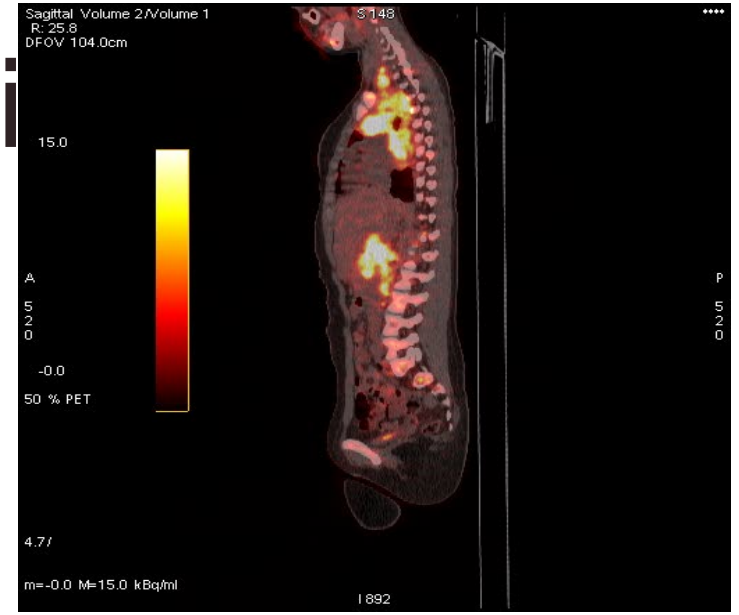
A Patient

March 2011 (age 15 years)

- Six month H/O of intense pruritis of her feet
- Three month H/O fever, night sweats, lethargy, pallor, poor appetite and weight loss
- Widespread LN – lower cervical, mediastinum, abdomen

La tati


Sagittal Volume 2/Volume 1
S 148
R: 68.0
DFOV 104.0cm
15.0
A
P
-0.0
50 % PET
4.7/
m=-0.0 M=15.0 kBq/ml
I 892


Sagittal Volume 2/Volume 1
S 148
R: 25.8
DFOV 104.0cm
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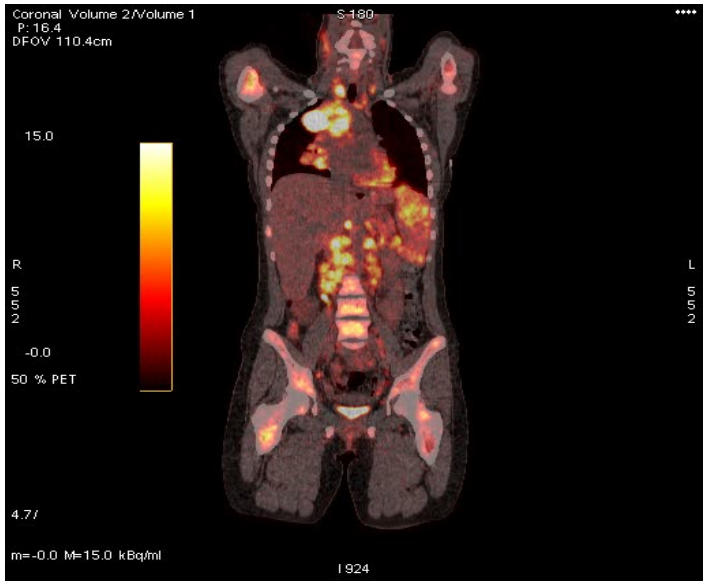
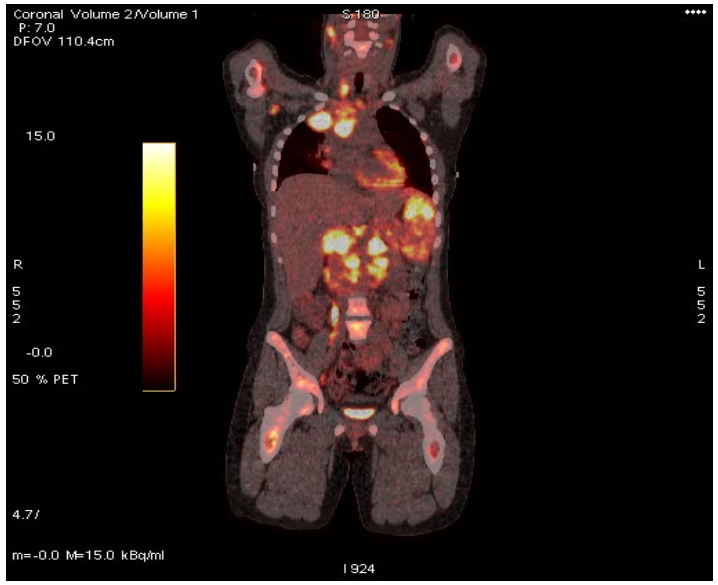
A
P
-0.0
50 % PET
4.7/
m=-0.0 M=15.0 kBq/ml
I 892


Coronal Volume 2/Volume 1
S 180
P: 16.4
DFOV 110.4cm
15.0
R
L
-0.0
50 % PET
4.7/
m=-0.0 M=15.0 kBq/ml
I 924


Coronal Volume 2/Volume 1
S 180
P: 7.0
DFOV 110.4cm
15.0
R
L
-0.0
50 % PET
4.7/
m=-0.0 M=15.0 kBq/ml
I 924

Diagnosis and Staging

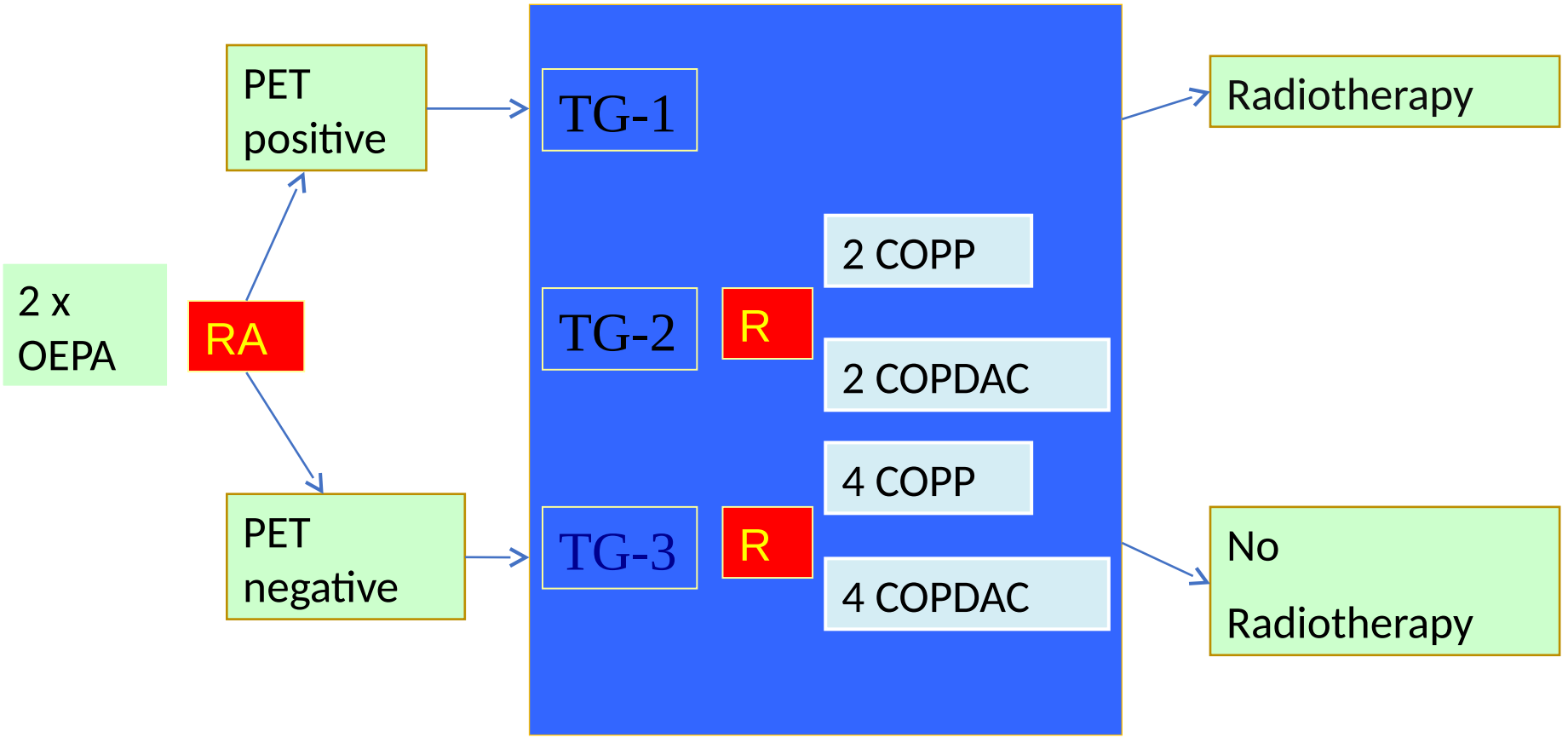
- Mediastinal lymph node biopsy
  - Hodgkin's lymphoma
- Insertion of double lumen portacath

Laparoscopic ovarian biopsy and cryopreservation of ovarian cortical strips

Laura

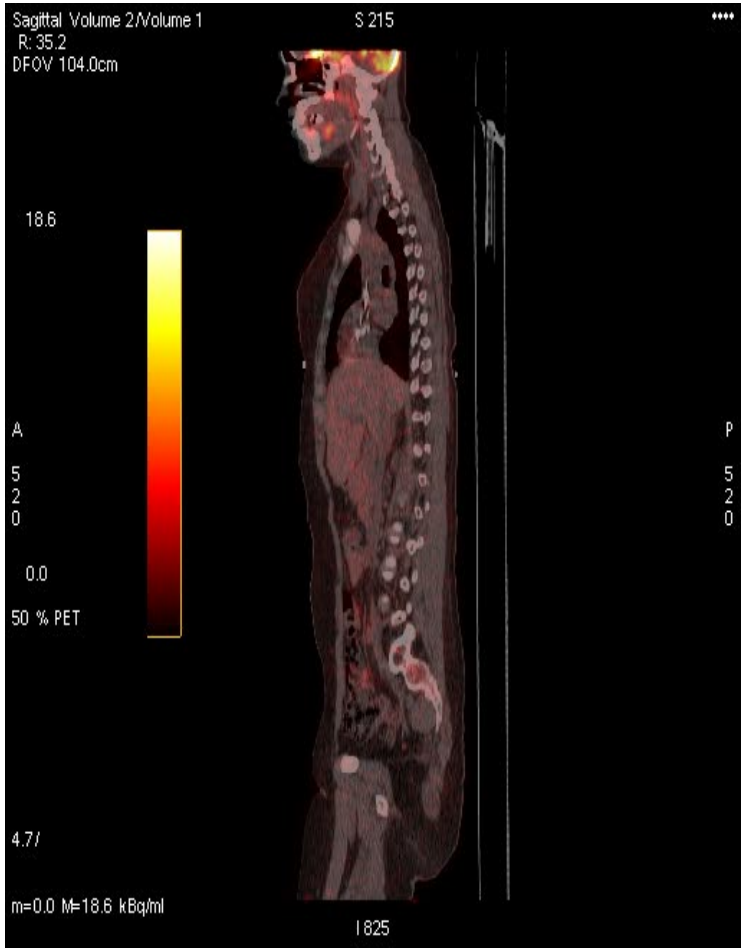
- EuroNet-PHL-C1 Protocol:
- Treatment Group 3 (TG3)
- Two cycles of OEPA
- Four cycles of COPDAC or COPP

EuroNet-PHL-C-1



Wallace WH. UK Chief Investigator CRUK support 400K

Early Response Assessment PET scan



Radiotherapy Field and estimated doses to organs at risk

Organs at risk	Maximium dose received	Mean Dose
- spinal cord	2139.7 cGy	1916.2 cGy
- heart	2116.1 cGy	1701.4 cGy
- left kidney	2169.1 cGy	1439.8 cGy
- right kidney	2022.2 cGy	639.3 cGy
- lung	2148.5 cGy	1168.9 cGy
- right breast	2195.1 cGy	476.7 cGy
- left breast	2156.4 cGy	654.6 cGy
- liver	2153.4 cGy	830.2 cGy
- thyroid	2047.2 cGy	1999.0 cGy

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)

Wallace et el. TLO 2005, Skinner et al TLO 2017, van Dorp et al JCO 2016

Panel 1: Intrinsic and extrinsic factors for fertility preservation strategies in children and young adults[9]

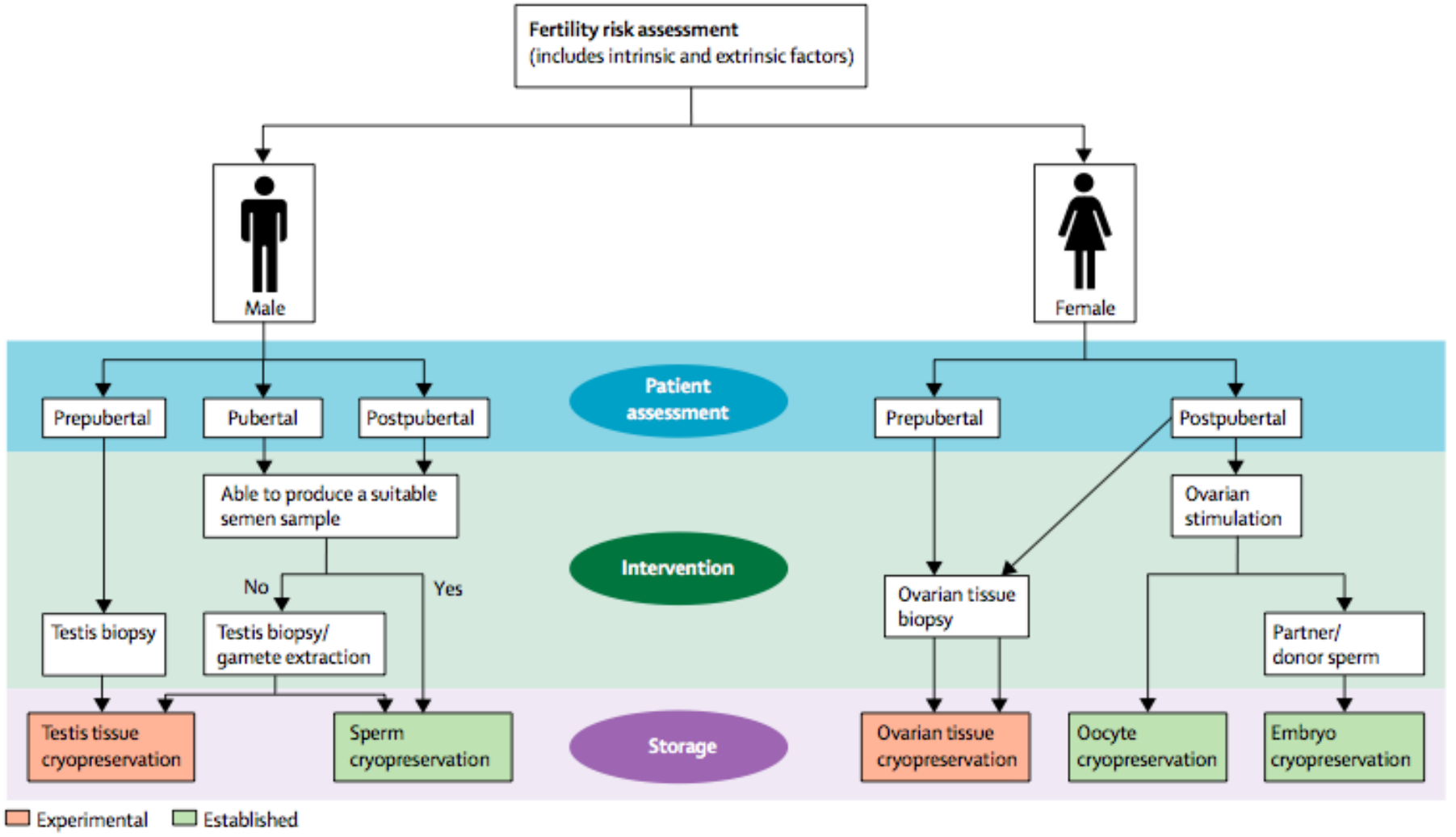
Intrinsic factors

- Health status of patient
- Psychosocial factors
- Consent (patient or parent)
- Assessment of pubertal status
- Assessment of ovarian reserve (female patients)

Extrinsic factors

- Risk of predicted treatment (high, medium, low, or uncertain risk)
- Time available
- Expertise and technical options available

Anderson RA…Wallace WH. Lancet Diabetes Endocrinol. 2015



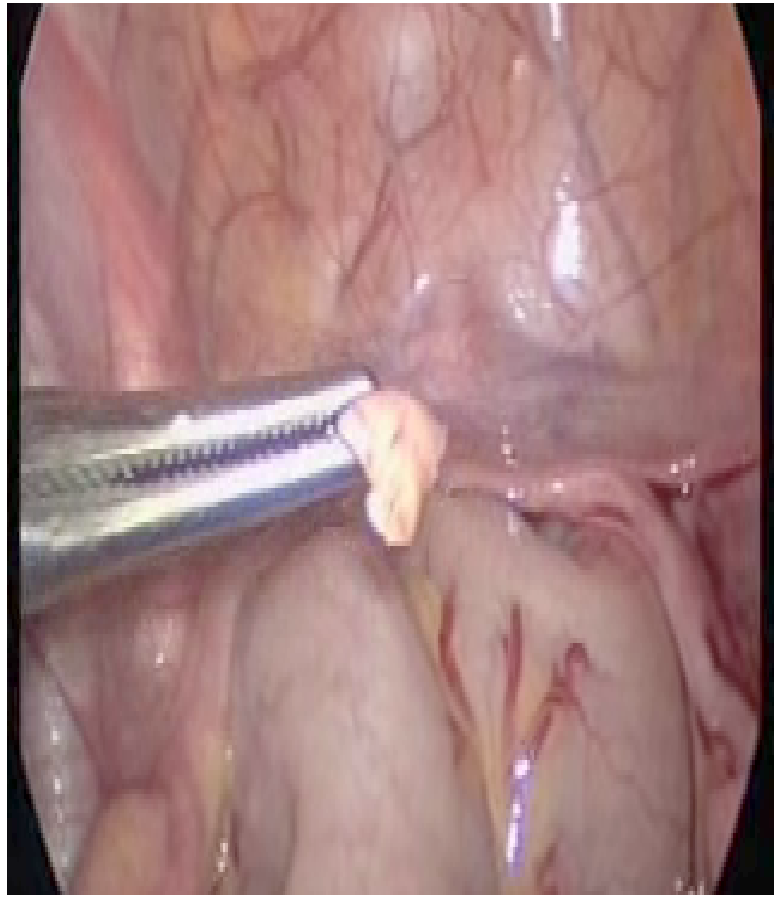
Anderson RA…Wallace WH. Lancet Diabetes Endocrinol. 2015

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

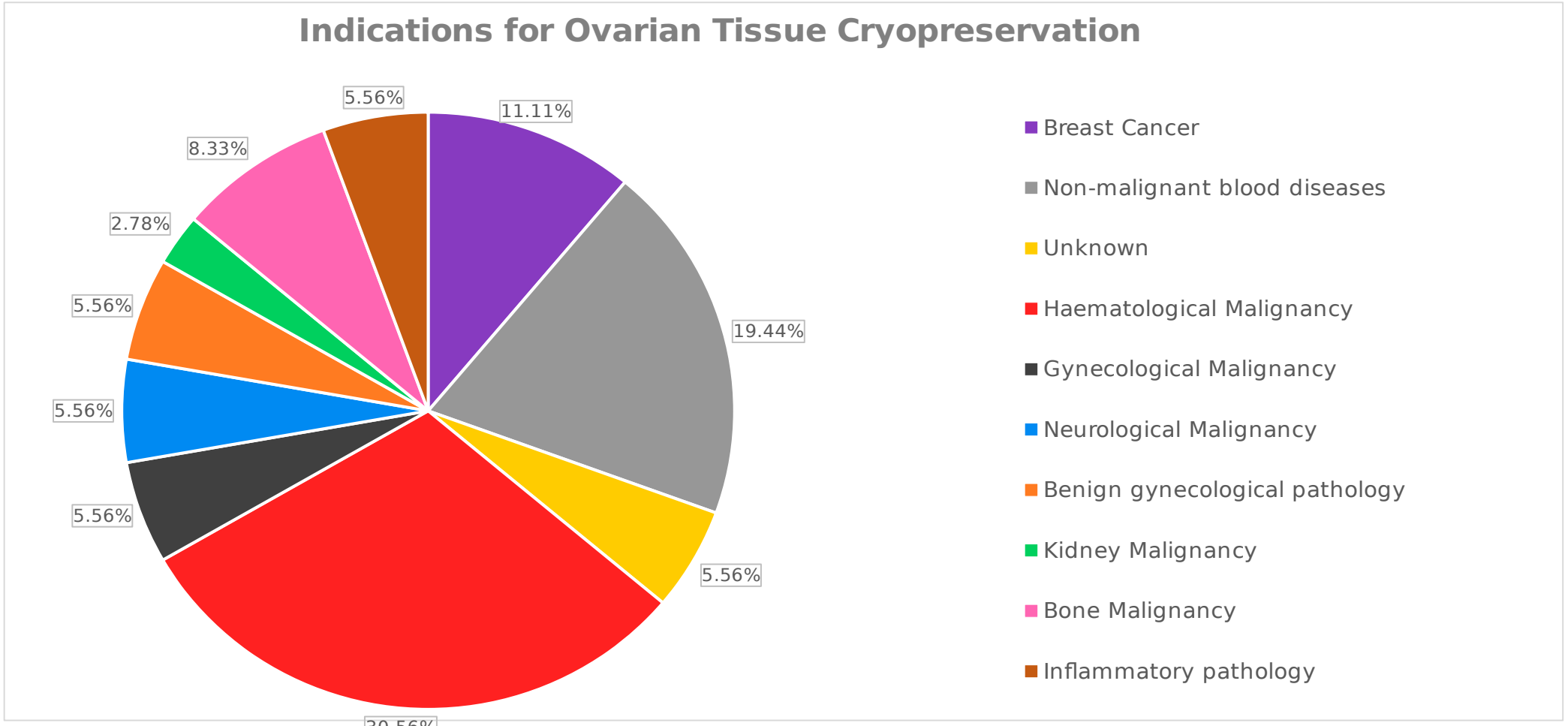
Ovarian tissue cryopreservation: World-wide experience

* At least 60 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



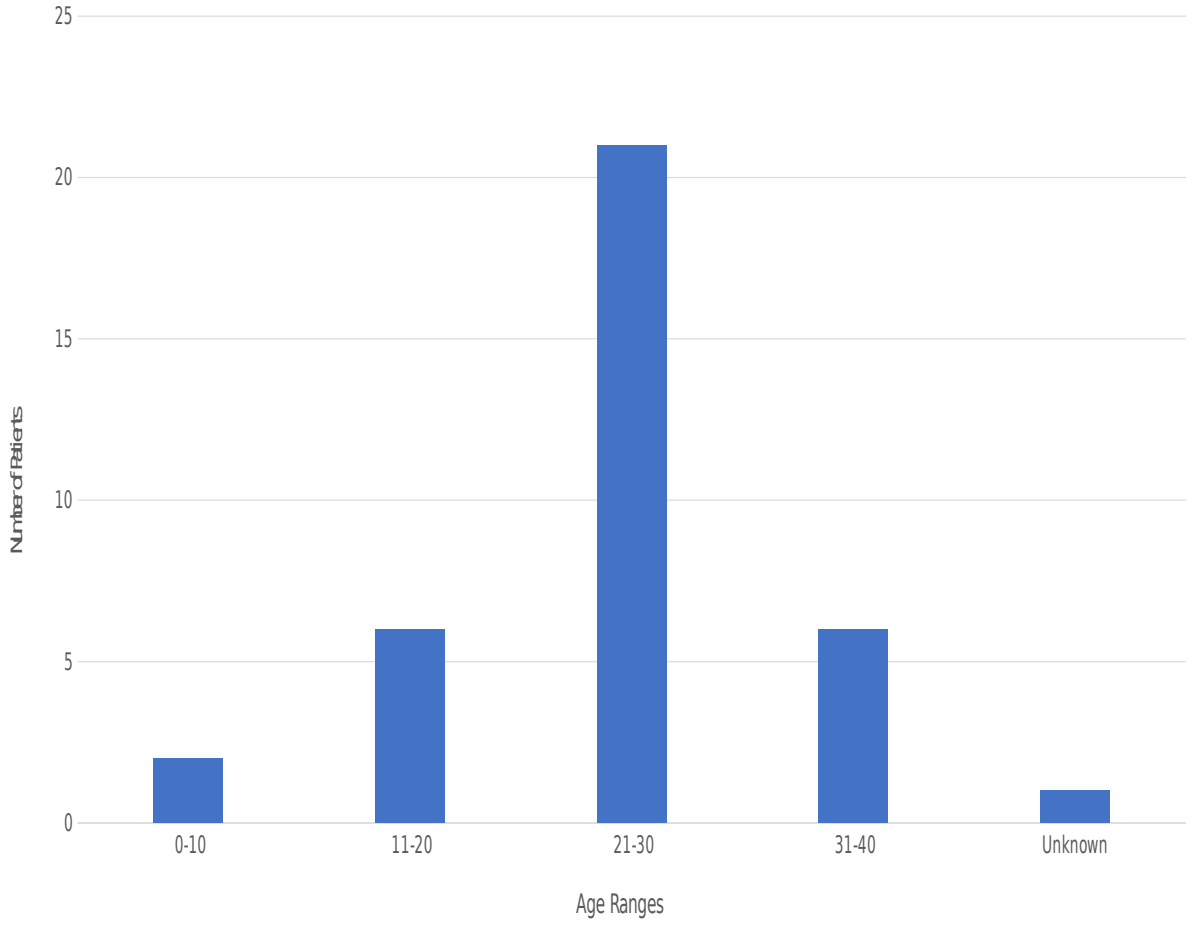
Donnez, J. & Dolmans, M.-M. *Nat. Rev. Endocrinol.* 9, 735–749 (2013)

Indications for ovarian tissue cryopreservation (n=36).



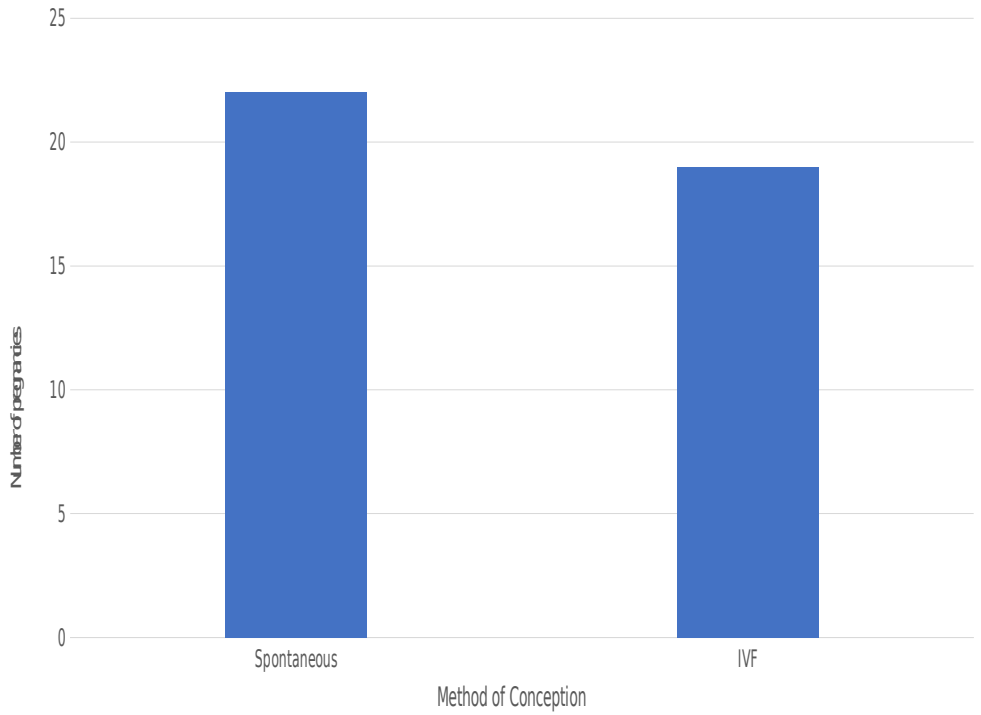
Chalk K & Wallace WH (unpublished)

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



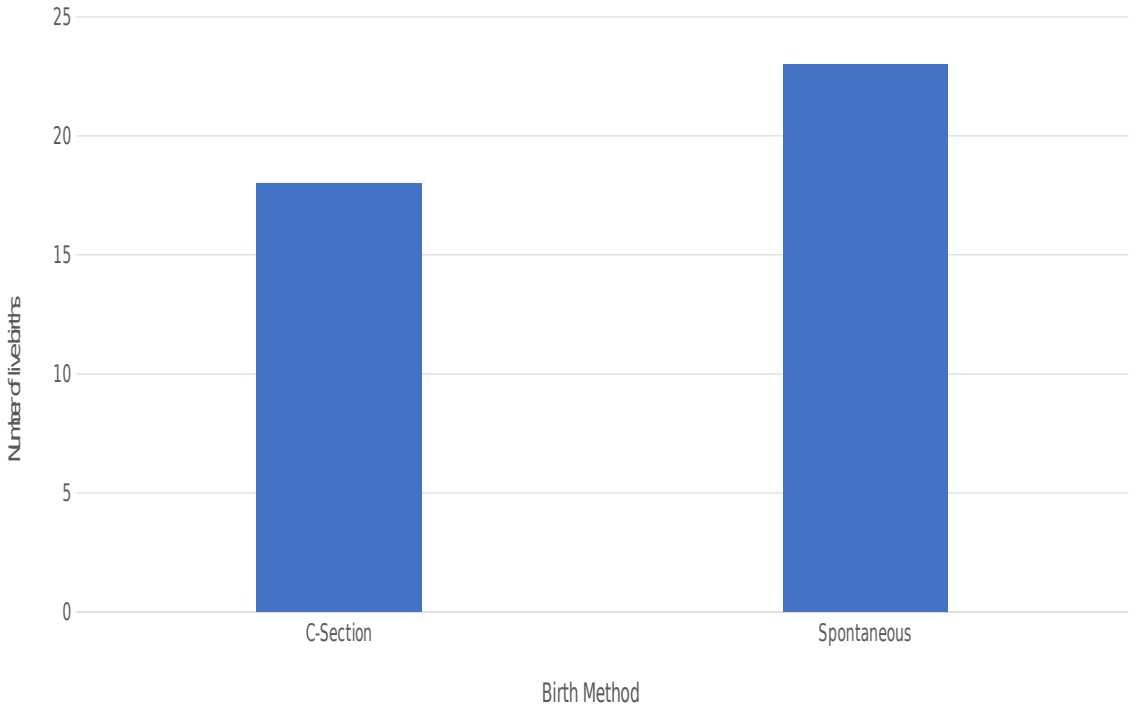
Chalk K & Wallace WH (unpublished)

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



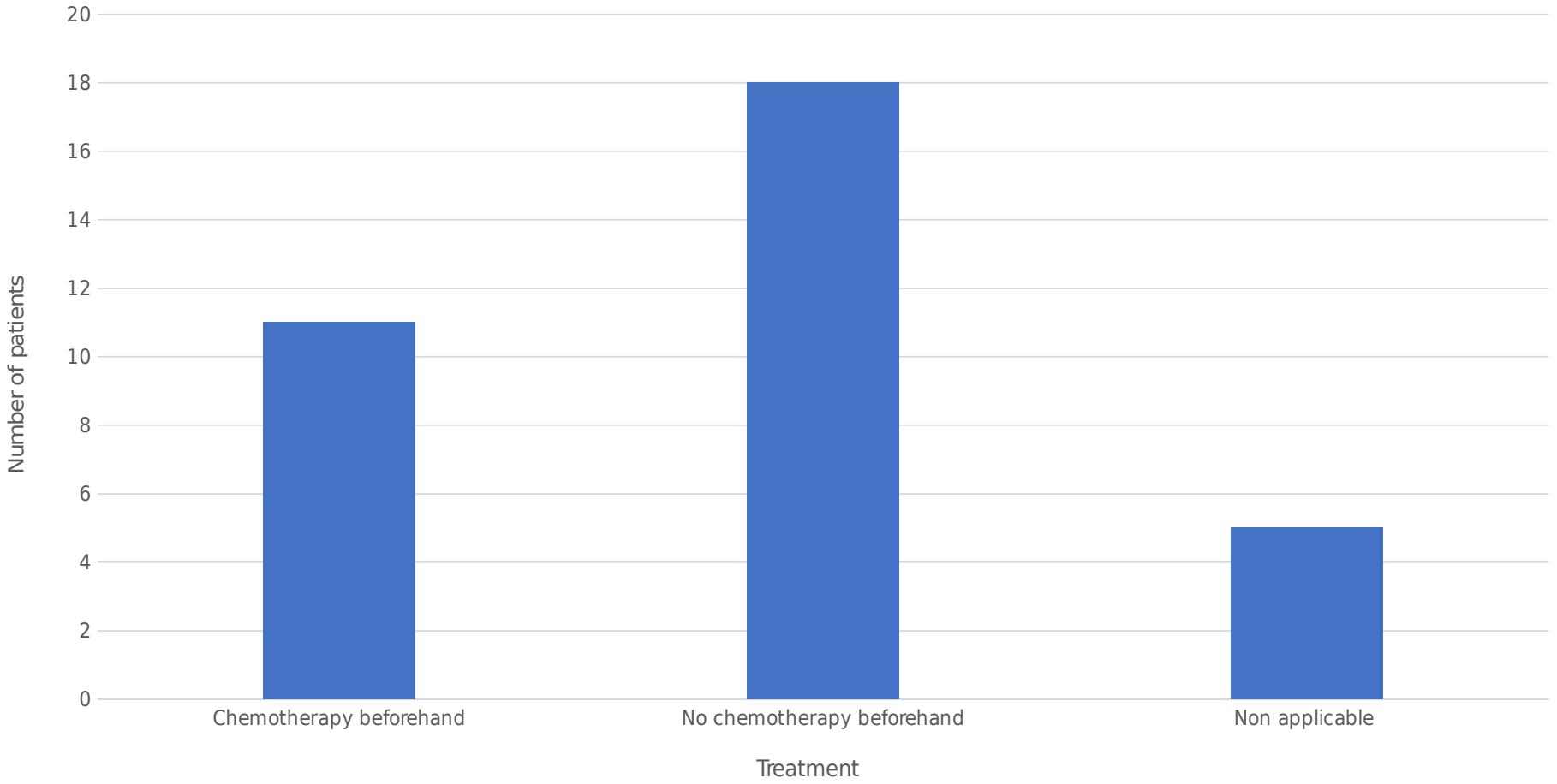
Chalk K & Wallace WH (unpublished)

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)

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).

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)

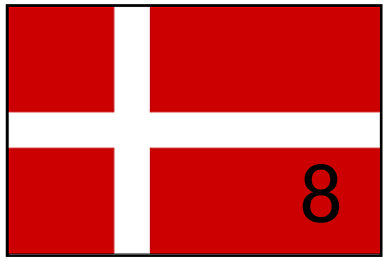
Jensen AK…Andersen CY Hum Rep 2015

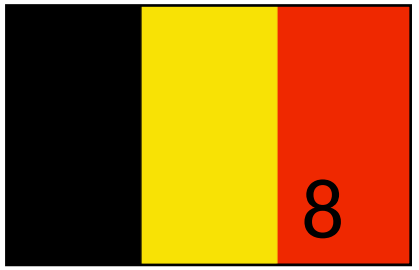
Transplantation of Ovarian Tissue - The Israeli experience

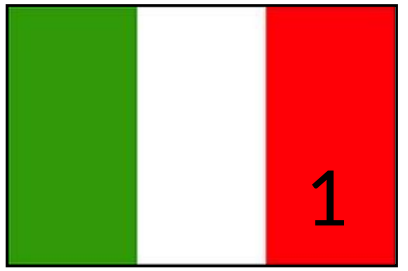
- N= 20 cancer survivors
- Ovarian Tissue harvested 14-39 years
- N=15 haematological malignancies
- N=10 exposed to pre-harvest chemotherapy
- 93% reported endocrine recovery
- N=16 pregnancies(10: IVF, 6 spontaneous)
- 32% had at least one live birth and 53% had a pregnancy
- No cancer relapses
- **Safe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

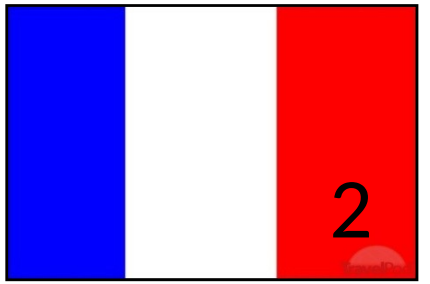
Meirow et al., Fertility and Sterility 2016

Children born from transplantation of frozen/thawed ovarian tissue









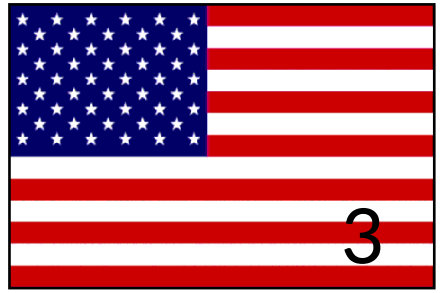
All Normal Babies

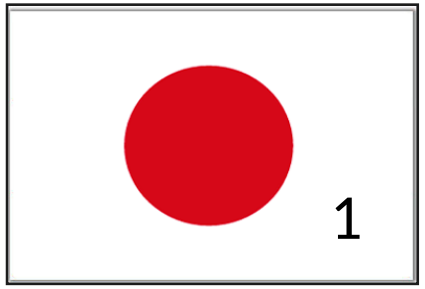
weight and duration

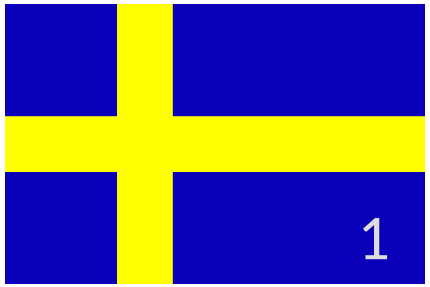
Orthotopic >> heterotopic

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

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

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

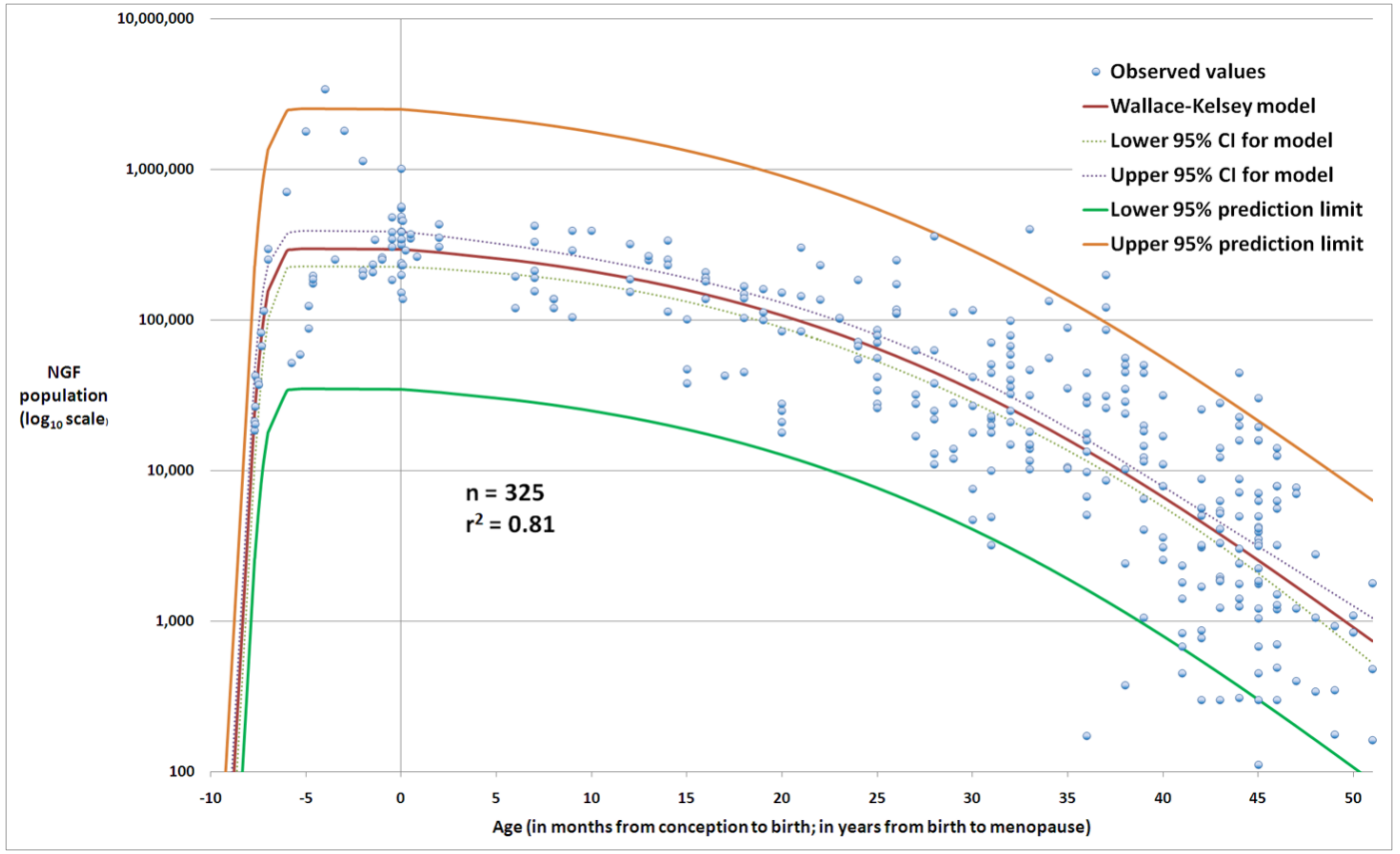
Aged 25 ovarian tissue replaced. After five months menstruation, continued for two years. Assisted conception due to male factor. No pregnancy

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

Demeestere I et al Hum Rep 2015

Ovarian Reserve?

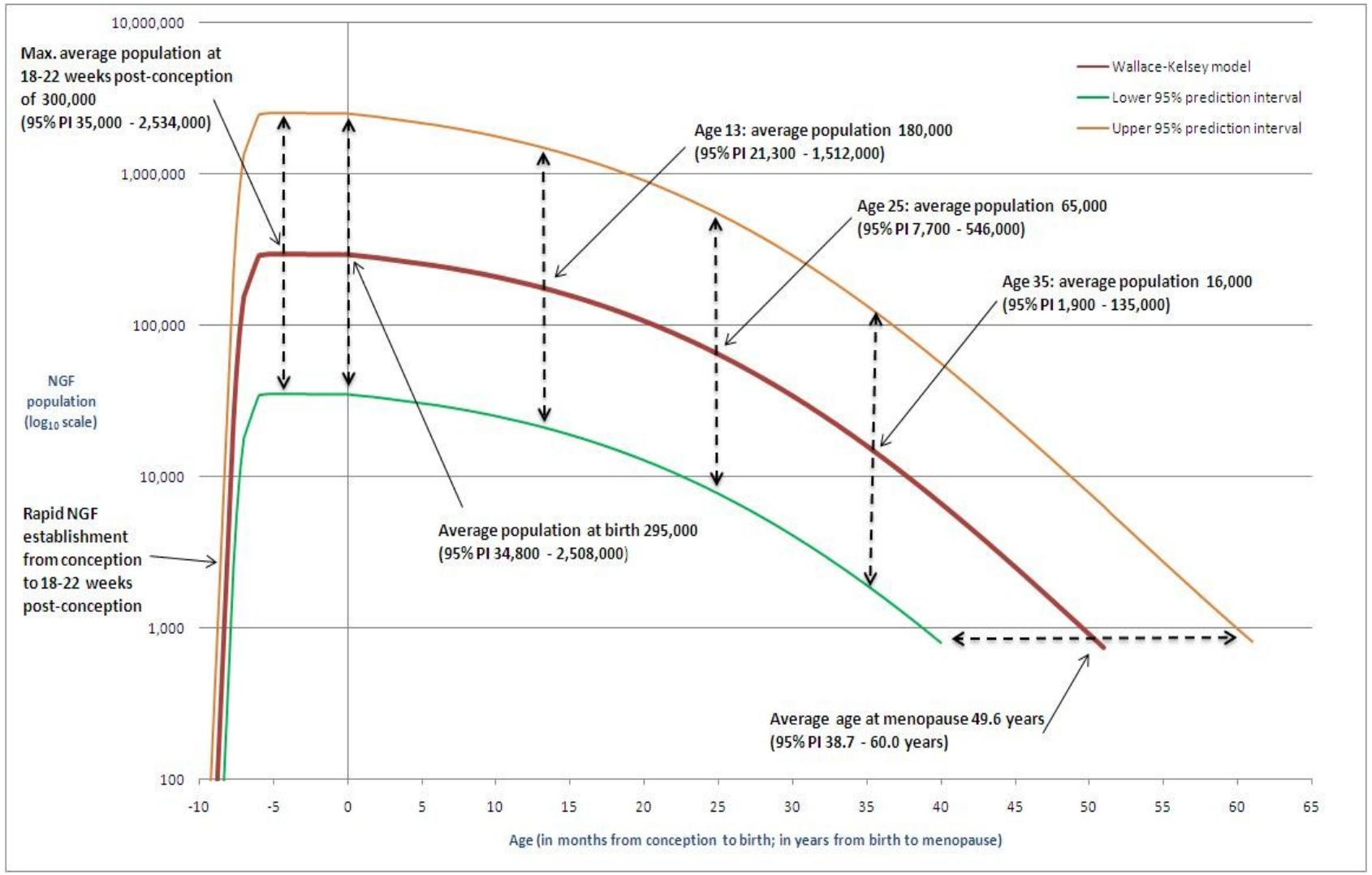
The Wallace-Kelsey Model
(Five parameter asymmetric double-Gaussian cumulative curve)



$$log_{10}(y) = \frac{a}{4}\left[1 + \mathrm{Erf}\left(\frac{x+b+\frac{c}{2}}{d\sqrt{2}}\right)\right]\left[1 - \mathrm{Erf}\left(\frac{x+b-\frac{c}{2}}{e\sqrt{2}}\right)\right]$$

Wallace &Kelsey (2010) PloS ONE

Ovarian reserve: Conception to Menopause

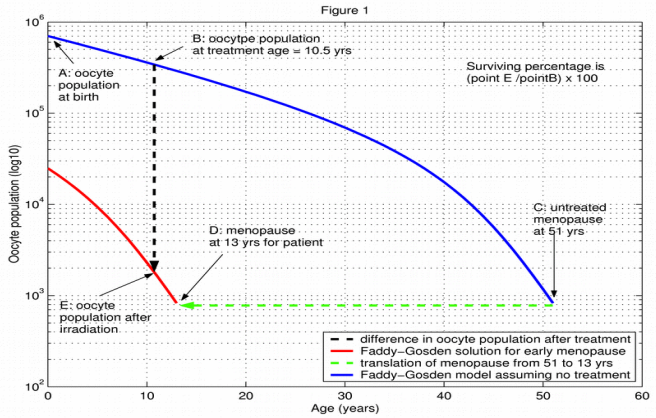


Wallace &Kelsey (2010) PloS ONE

Radiation-induced ovarian damage

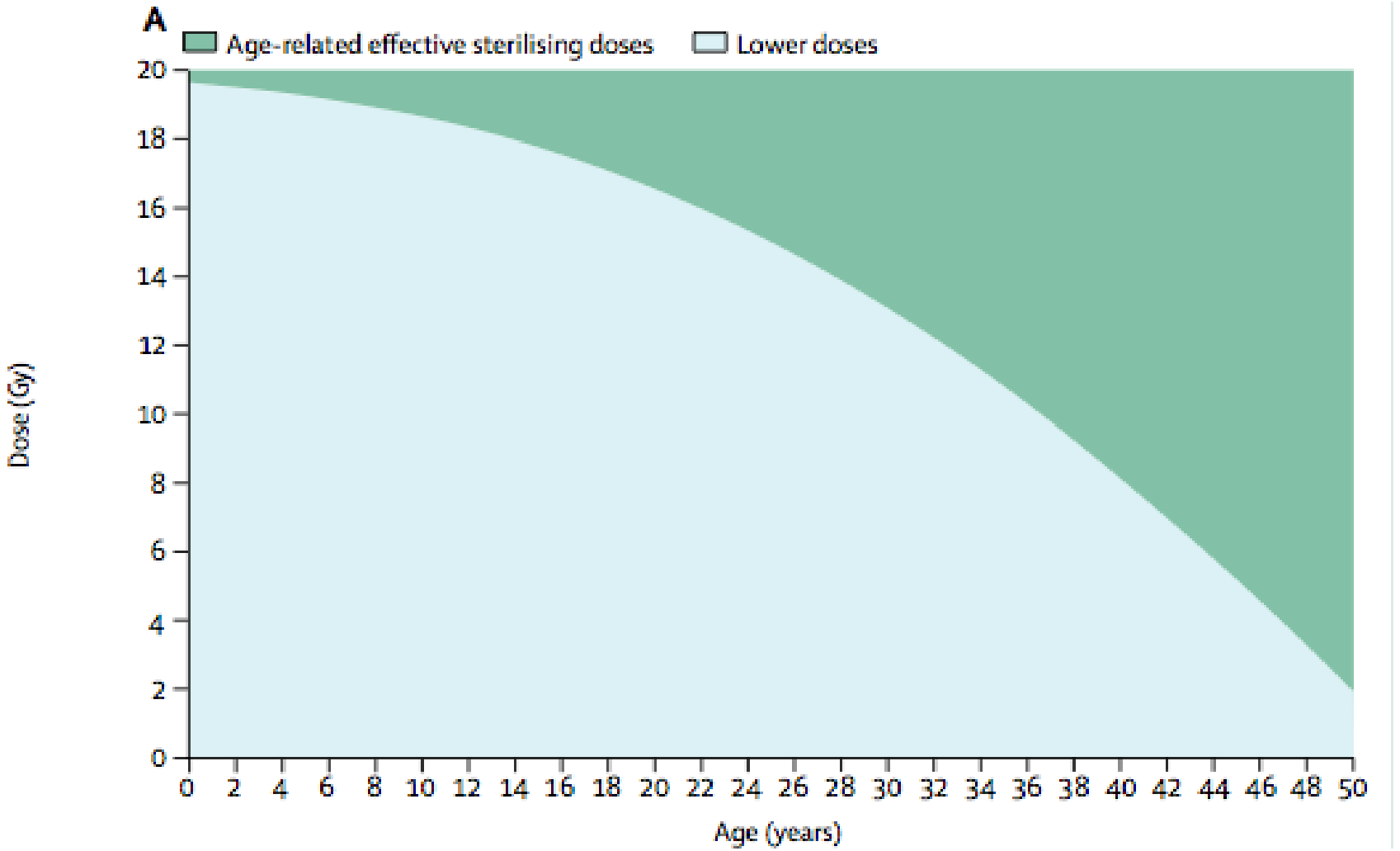
Human oocyte
(Primordial follicle)

- $LD_{50} < 2$ Gy

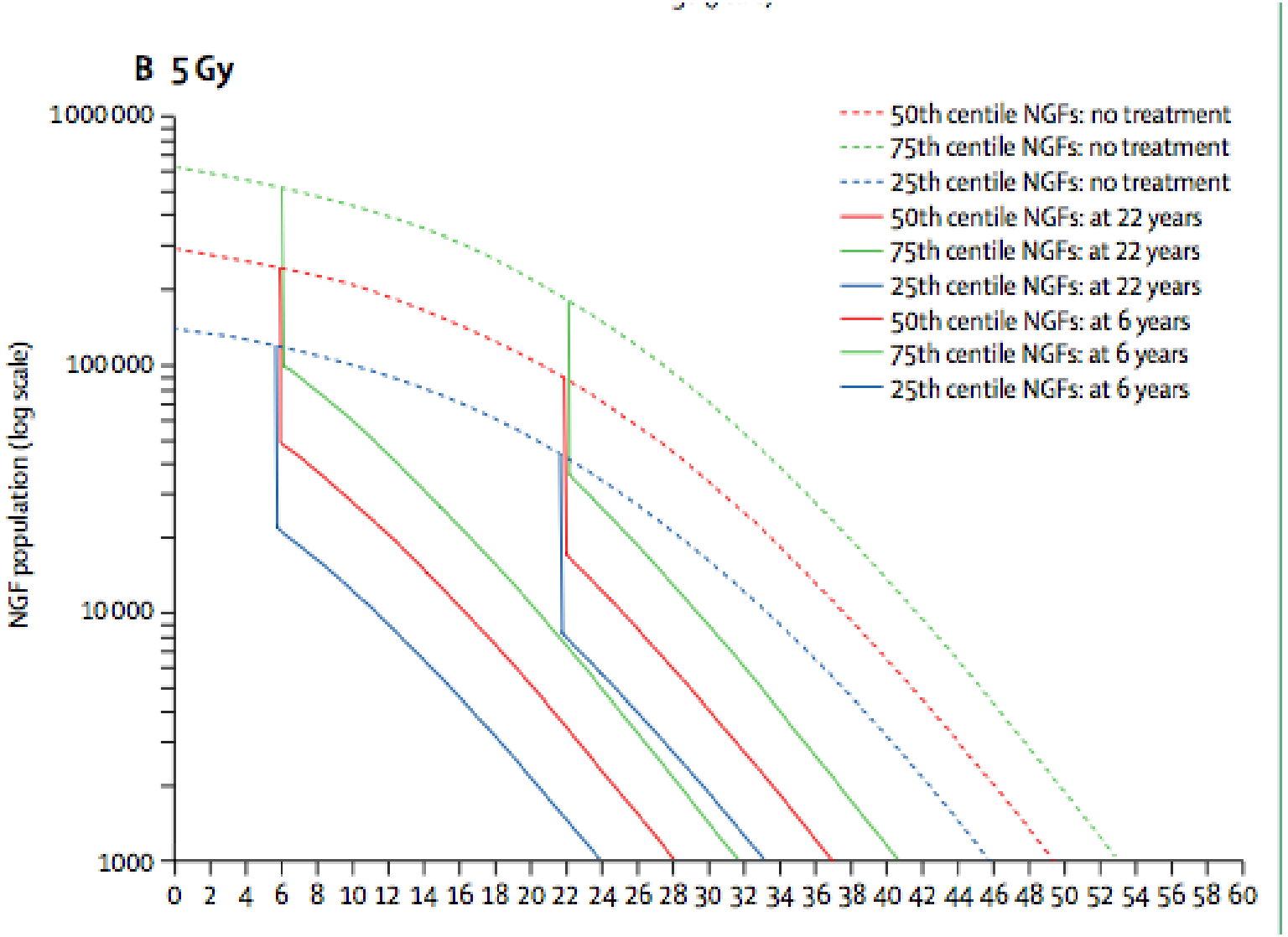


**Wallace, Thomson, Kelsey.
(2003) Hum Reprod.**

Effective ovarian sterilizing doses of radiotherapy with increasing age



Anderson RA…Wallace WH. Lancet Diabetes Endocrinol. 2015



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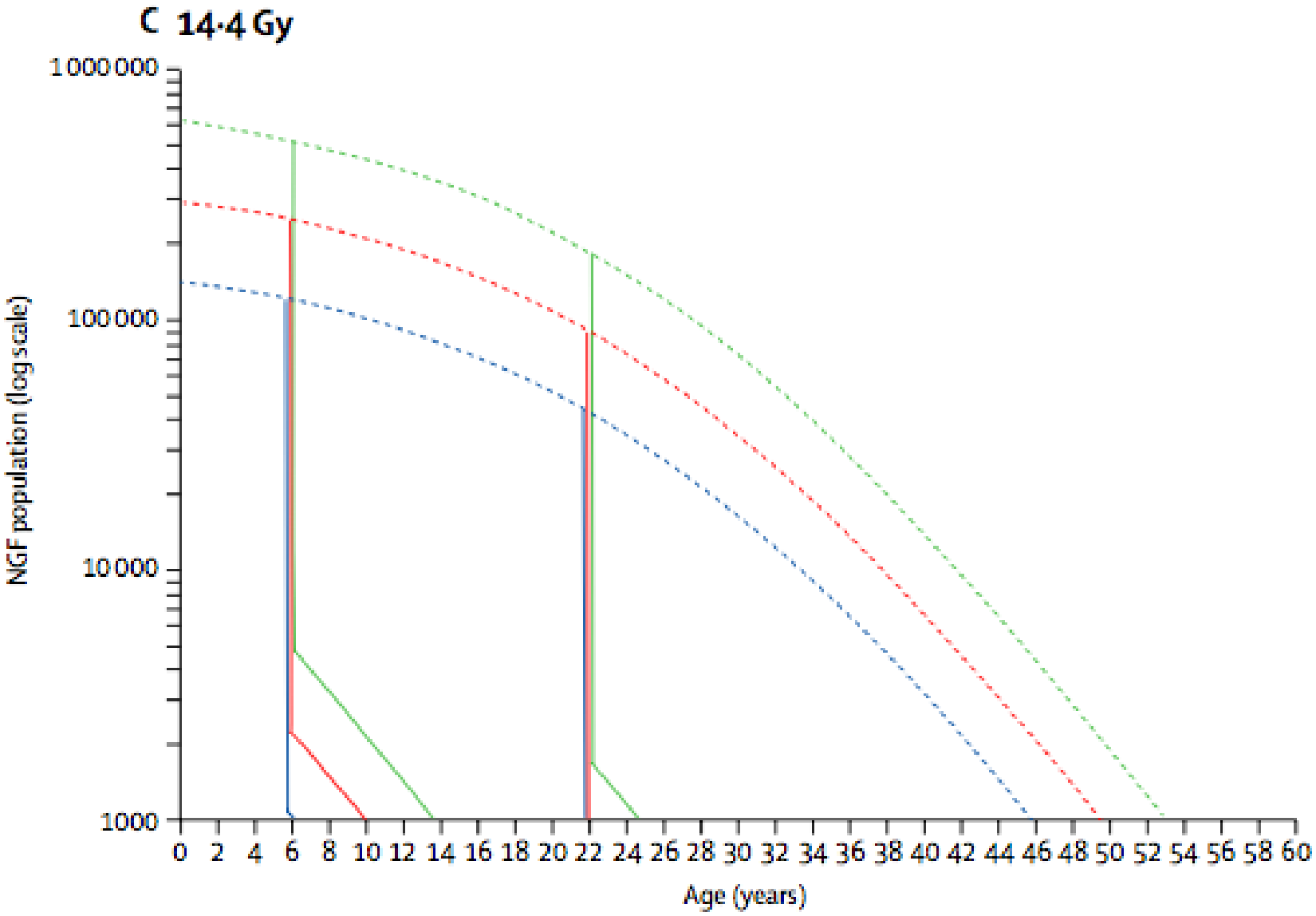


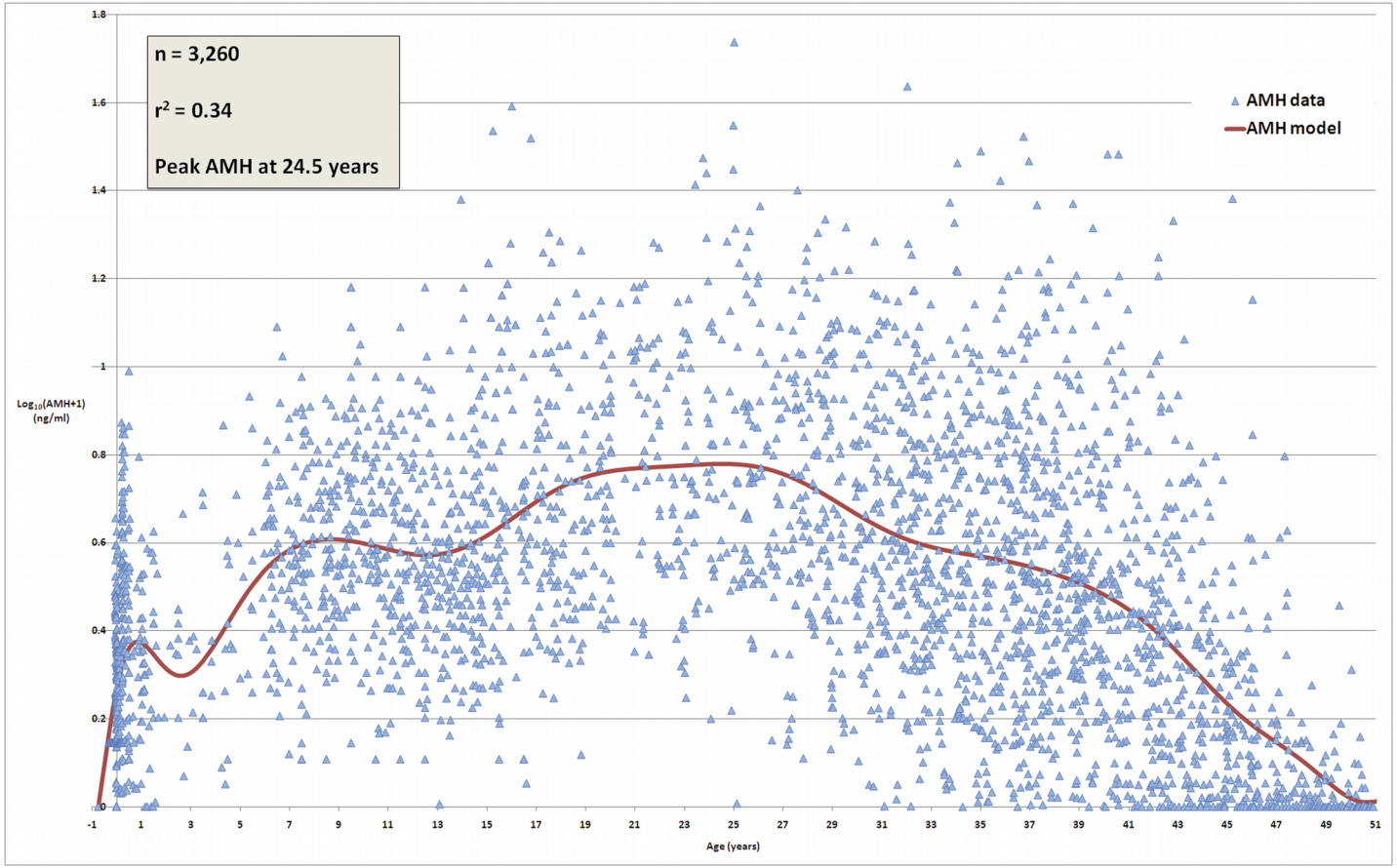
Anderson RA…Wallace WH. Lancet Diabetes Endocrinol. 2015

Prediction of Ovarian Reserve (AMH)

- Anti Mullerian Hormone (AMH) is an important product of the adult ovary, produced by the granulosa cells of small growing follicles
- AMH has little variation across and between menstrual cycles
- AMH is the best currently available marker of the number of small-growing follicles in the ovary
- But there was no validated reference model for AMH available

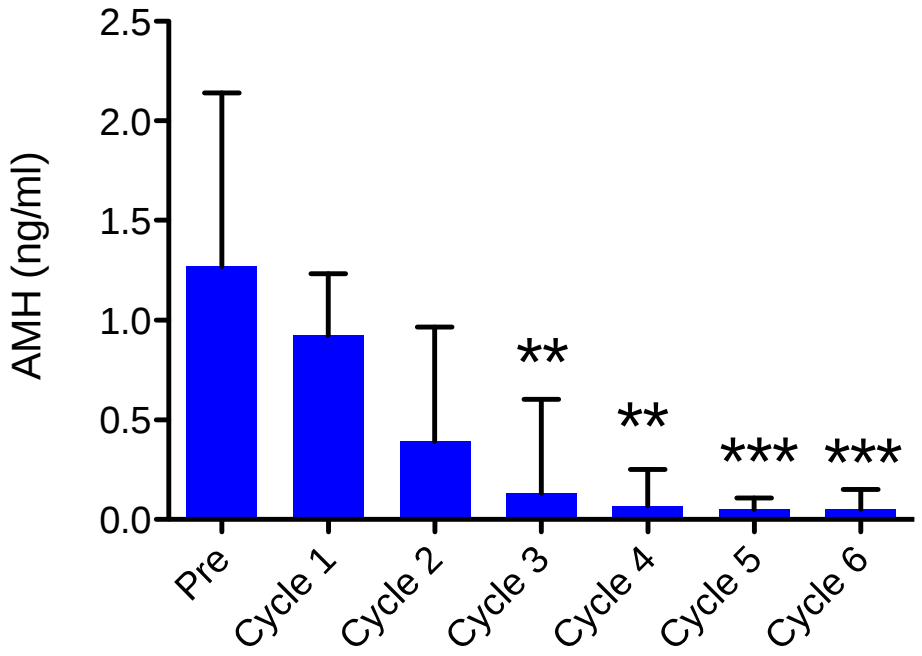
Anderson, Nelson, Wallace (2011) Maturitas

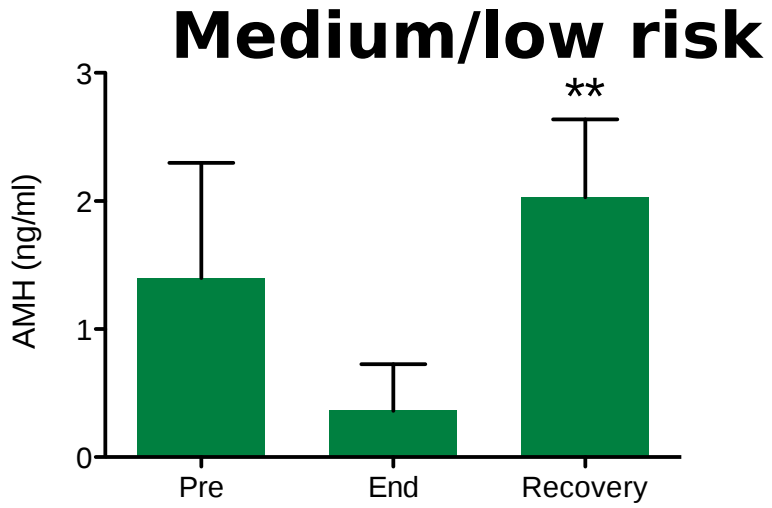
A validated model of serum anti-Mullerian hormone (AMH) from conception to menopause

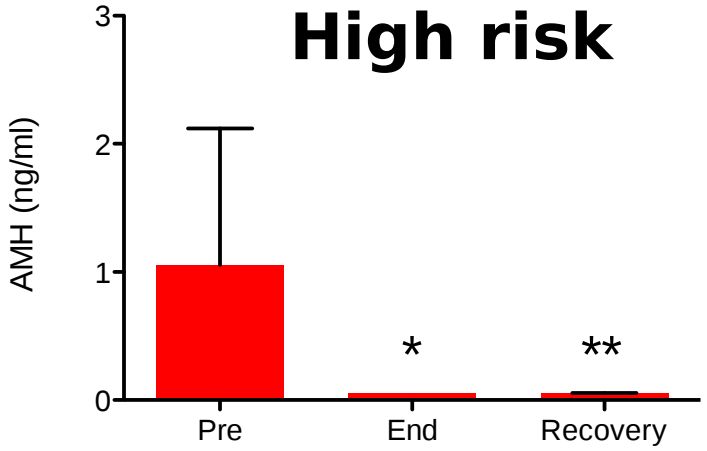


Kelsey et al. PLoS ONE 2011

AMH in childhood cancer



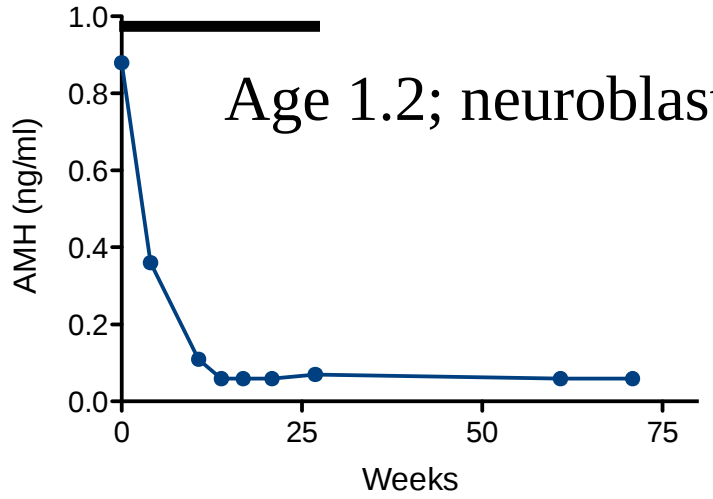




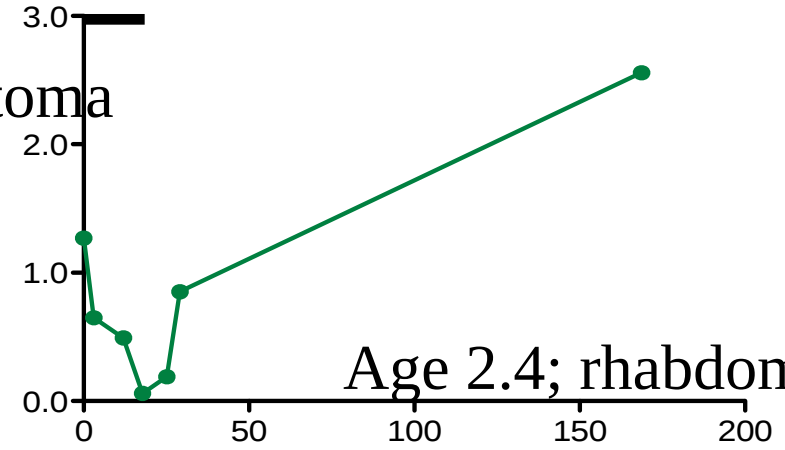
22 girls age 0.3-15yr
17 prepubertal

Brougham et al 2012 JCE&M

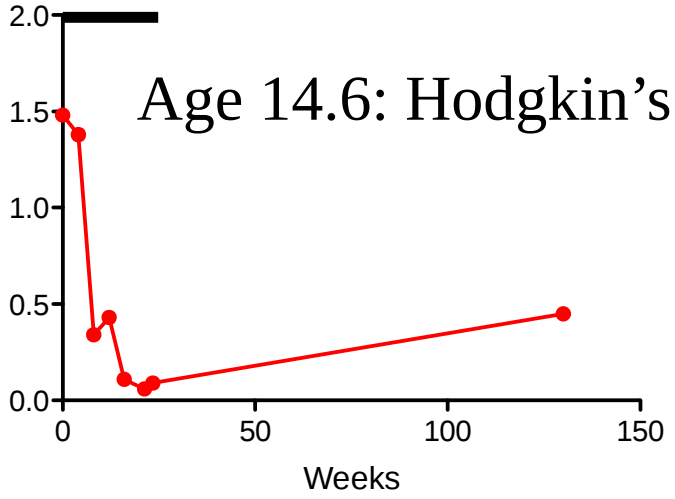
AMH in 3 girls with cancer



Age 1.2; neuroblastoma



Age 2.4; rhabdomyosarcoma



Age 14.6: Hodgkin's lymphoma

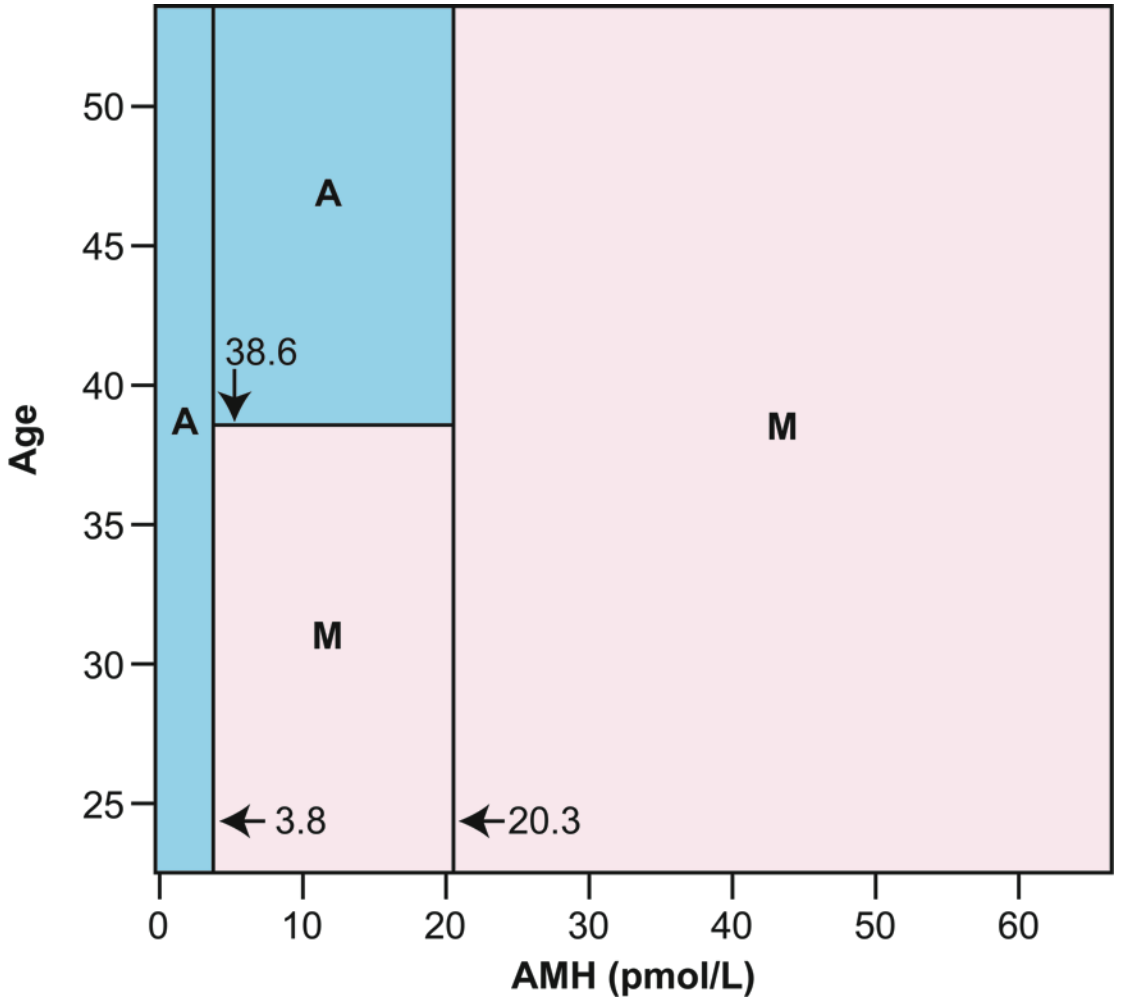
Brougham et al 2012 JCE&M

Summary

- AMH is detectable before puberty
- AMH falls rapidly during cancer treatment in both pre-pubertal and pubertal girls
- AMH levels recover in those patients at low/medium risk of gonadotoxicity
- AMH fails to recover in those at high risk. This could be indicative of future reproductive impairment

Brougham et al 2012 JCE&M

Pretreatment anti-Müllerian hormone predicts for loss of ovarian function after chemotherapy for early breast cancer.

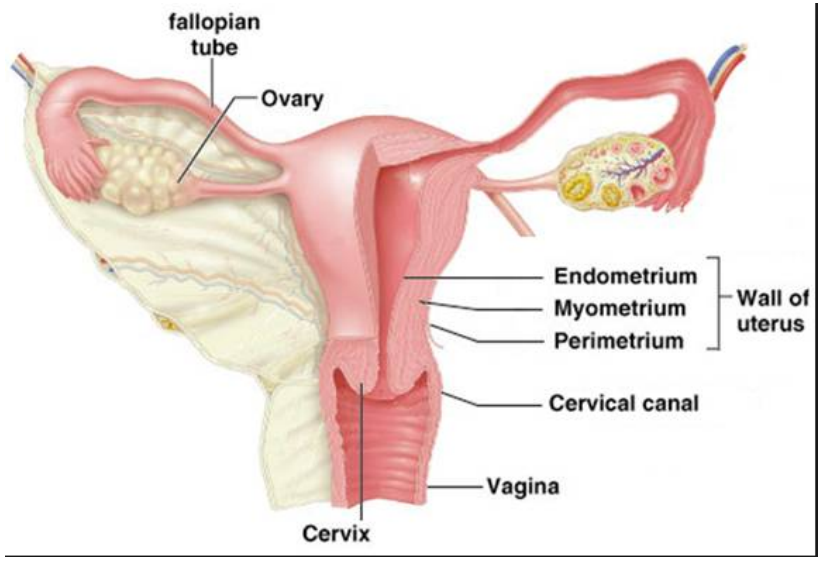


sensitivity 98.2%
specificity 80.0%
for correct classification
of amenorrhoea

n=75

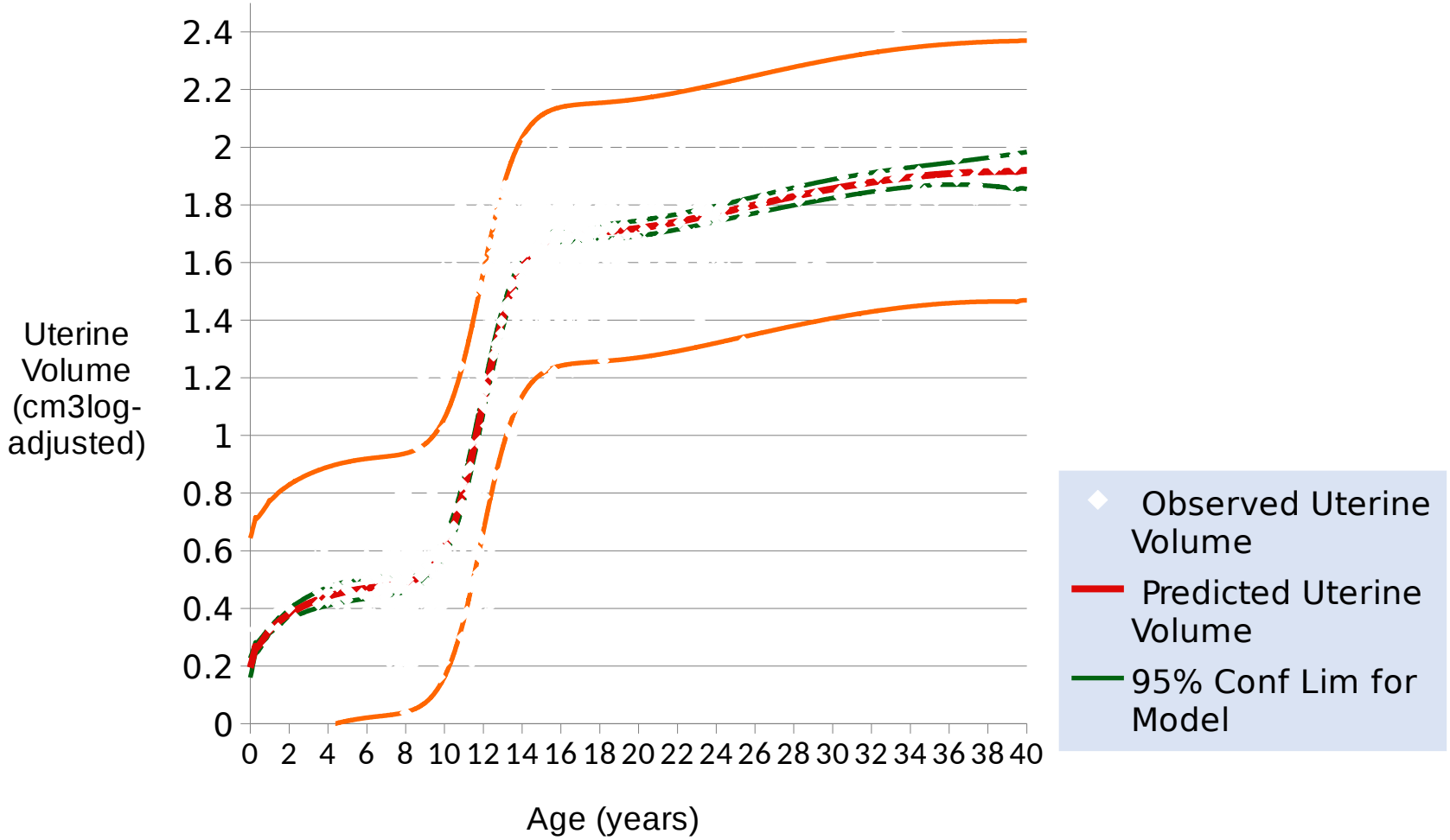
Anderson and Cameron 2011 JCE&M
Anderson et al 2013 Eur J Cancer

The Uterus


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

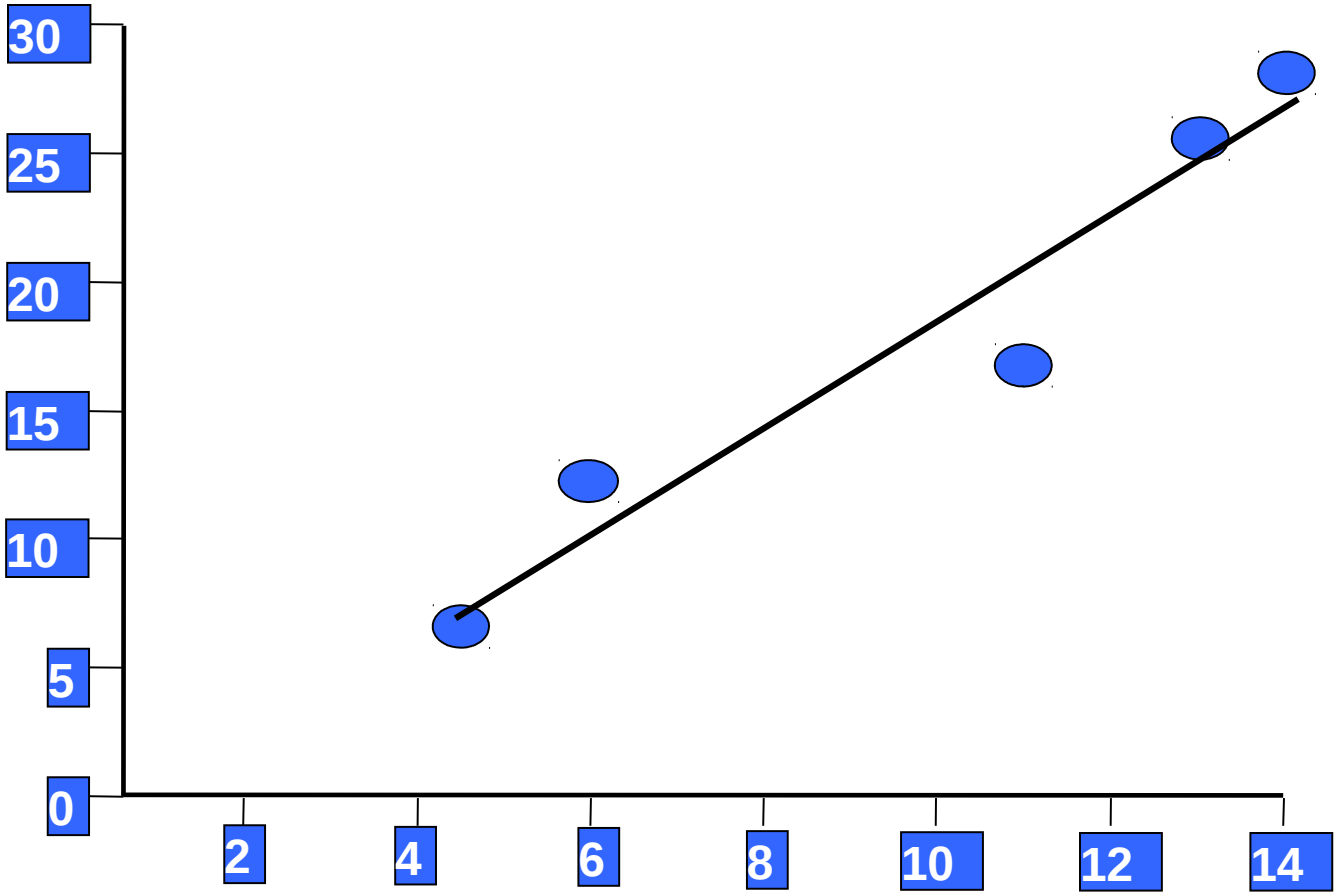

A

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



Kelsey, T. W et al (2016). *PloS One*, *11*(6), e0157375.

Uterine volume and age at irradiation (TBI)



Age at Irradiation (years)

Bath et al. BJOG (1999)

Uterine function after cancer treatment

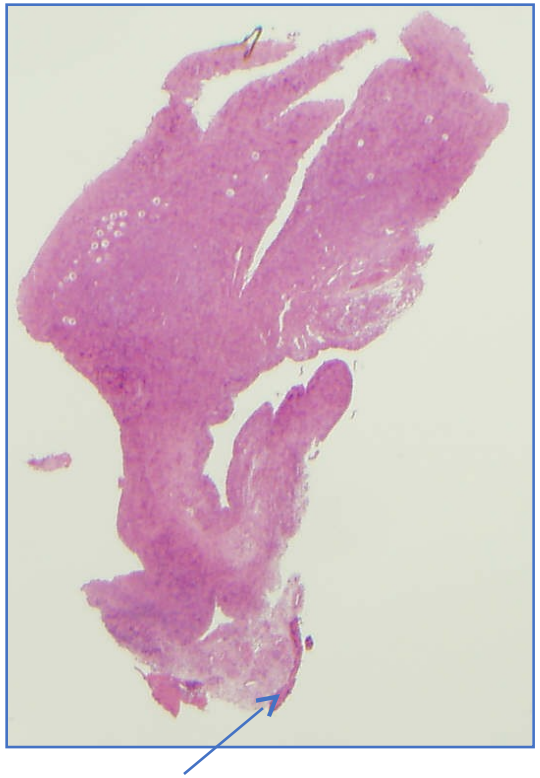
- No reports of uterine damage due to chemotherapy

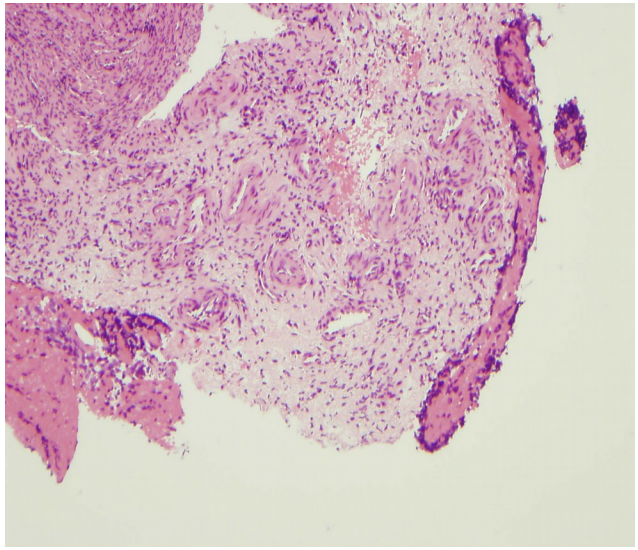
- Radiotherapy:
- Uterine damage, manifest by impaired growth and blood flow.
- Uterine volume correlates with age at irradiation.
- Exposure of the pelvis to radiation is associated with an increased risk of miscarriage, mid-trimester pregnancy loss, PPH, pre-term birth and low birth weight.

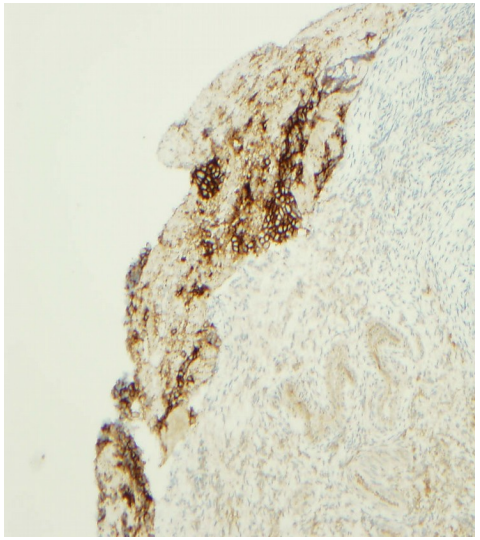
Reimplantation?

- 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





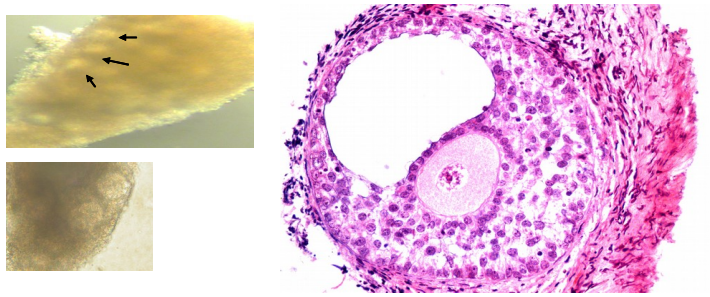


CD99

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

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

Telfer et al. (2008) Human Reproduction

Ovarian Cryopreservation & Ovarian Function

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

Panel 2: The Edinburgh Selection Criteria for gonadal tissue cryopreservation

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

Female patients[112]

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

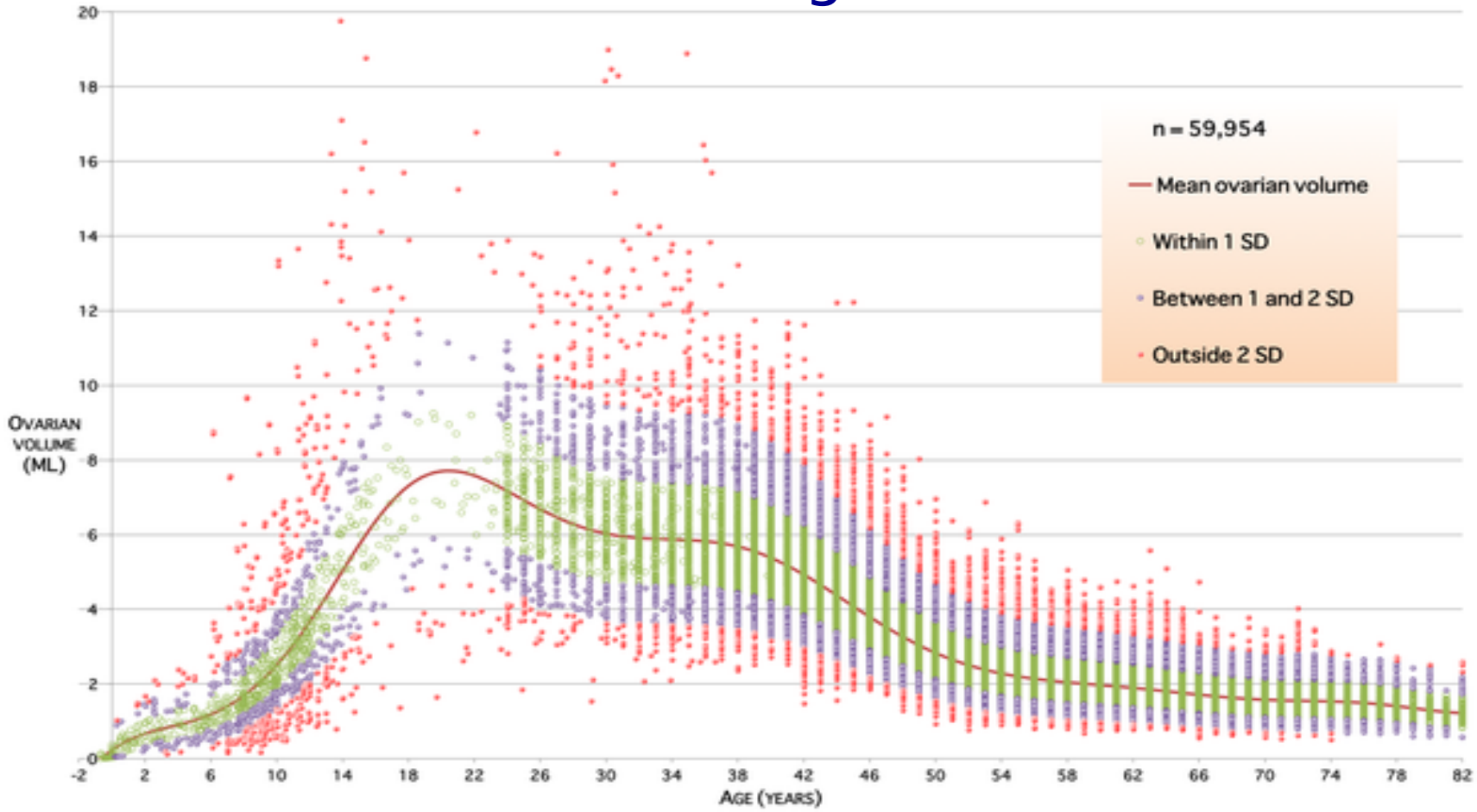
Male patients

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

Anderson RA…Wallace WH. Lancet Diabetes Endocrinol. 2015

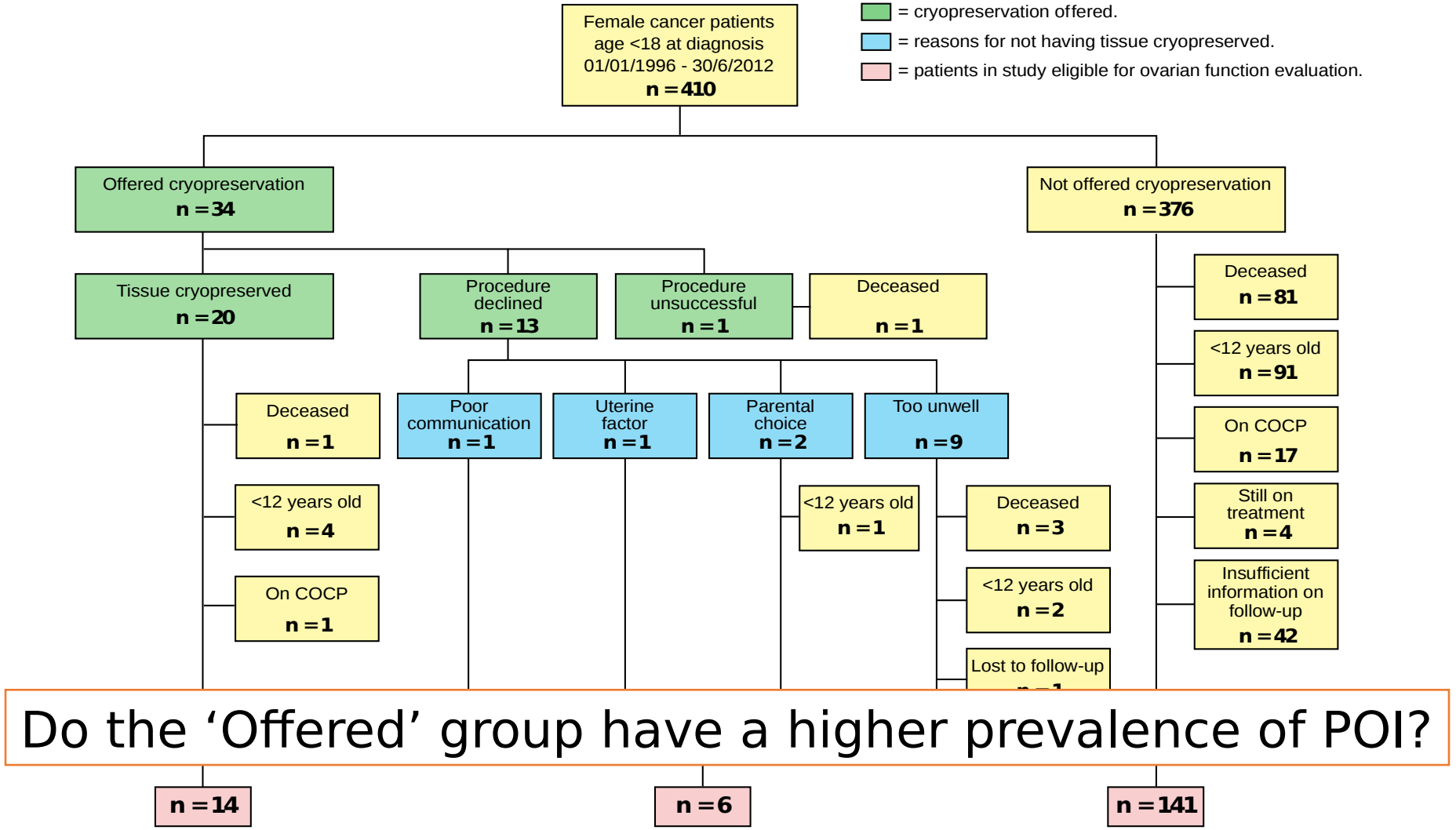
Patient No.	Diagnosis	Age at cryopreservation (years)	Method of ovarian tissue collection	Complications from procedure	Duration since cryopreservation (years)	Age at last assessment (years)	Current Ovarian Function
1	Hodgkin's Lymphoma[◊]	14.9	Laparoscopic Cortical Strip	None	15.8	30.2	Not POI
2	Ewing's Sarcoma (pubic bone)	14.9	Laparoscopic Cortical Strip	None	16.6	25.6	POI (+1 child)
3	Sacral Ependymoma	11.3	Laparoscopic Cortical Strip	None	15.8	24.5	Not POI
4	Hodgkin's Lymphoma	13.7	Laparoscopic Cortical Strip	None	15.6	28.9	Not POI
5	Hodgkin's Lymphoma	11.0	Laparoscopic Cortical Strip	None	14.7		On COCP
6	Chronic Granuloeytic Leukaemia	9.9	Laparoscopic Cortical Strip	None	12.2	21.7	Not POI
7	Rhabdomyosarcoma	5.3	Laparoscopic Cortical Strip	None	8.2	13.1	POI
8	Ewing's Sarcoma (pelvic)	9.8	Laparoscopic Cortical Strip	None	6.7	15.6	POI
9	Uterine Cervix Rhabdomyosarcoma*	16.4	Laparoscopic Cortical Strip	None	5.1	17.5	Not POI
10	Hodgkin's Lymphoma[◊]	14.0	Laparoscopic Cortical Strip	None	3.2	17.2	POI
11	Abdominal Embryonal Rhabdomyosarcoma	7.9	Laparoscopic Cortical Strip	None			Deceased
12	Ewing's Sarcoma	12.1	Laparoscopic Cortical Strip†	None	3.9	15.2	POI
13	Hodgkin's Lymphoma	12.7	Laparoscopic Cortical Strip	None	3.3	14.3	POI
14	Metastatic Medulloblastoma	8.1	Laparoscopic Cortical Strip	None	2.9		Not assessed
15	Hodgkin's Lymphoma	15.2	Laparoscopic Cortical Strip	None	1.9	16.9	Not POI
16	Alveolar Rhabdomyosaccoma	10.5	Laparoscopic Cortical Strip	None	1.4		Not assessed
17	Embryonal Rhabdomyosarcoma	3.0	Oophorectomy	None	1.4		Not assessed
18	Ewing's Sarcoma	12.0	Laparoscopic Cortical Strip	None	1.4	13.5	Not POI
19	Undifferentiated Sarcoma	12.3	Laparoscopic Cortical Strip†	None	1.0	13.4	Not POI
20	Wilm's Tumour	1.2	Oophorectomy	None	0.6		Not assessed

The normative validated model of ovarian volume throughout life



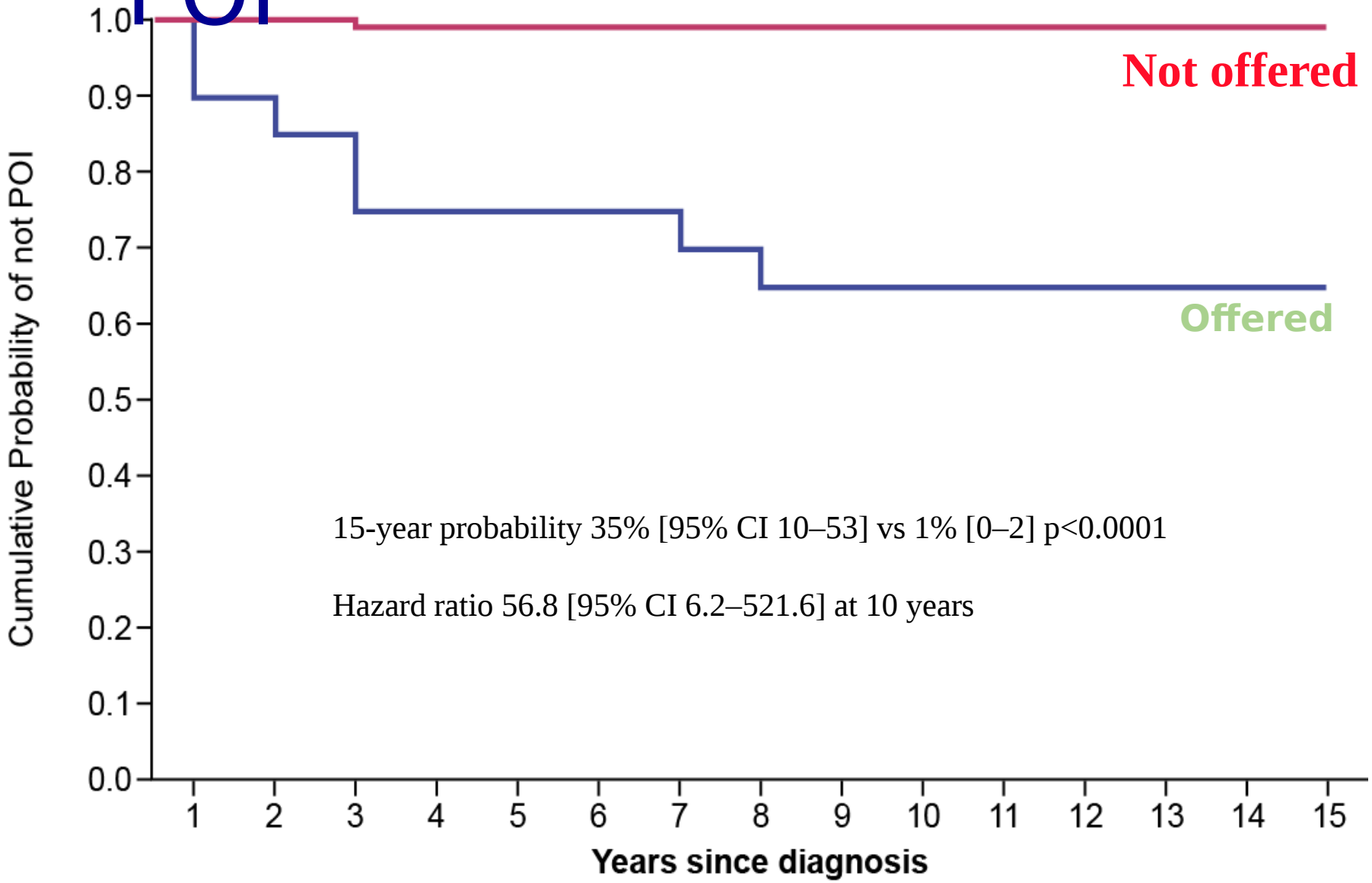
Kelsey TW, Dodwell SK, Wilkinson AG, Greve T, Andersen CY, et al. (2013) Ovarian Volume throughout Life: A Validated Normative Model. PLoS ONE 8(9): e71465. doi:10.1371/journal.pone.0071465

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



Walllace WH et al. 2014 Lancet Oncology

Cumulative incidence of POI



15-year probability 35% [95% CI 10–53] vs 1% [0–2] p<0.0001

Hazard ratio 56.8 [95% CI 6.2–521.6] at 10 years

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

Challenges

- Provide fertility counseling to all young patients with cancer
- Cryopreserve ovarian and pre-pubertal testicular 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

nowledgements

- Richard Anderson
- David T Baird
- Tom Kelsey
- Evelyn Telfer
- Marie McLaughlan
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- Rod Mitchell
- Louise Bath
- Angela Edgar
- Mark Brougham
- Fraser Munro

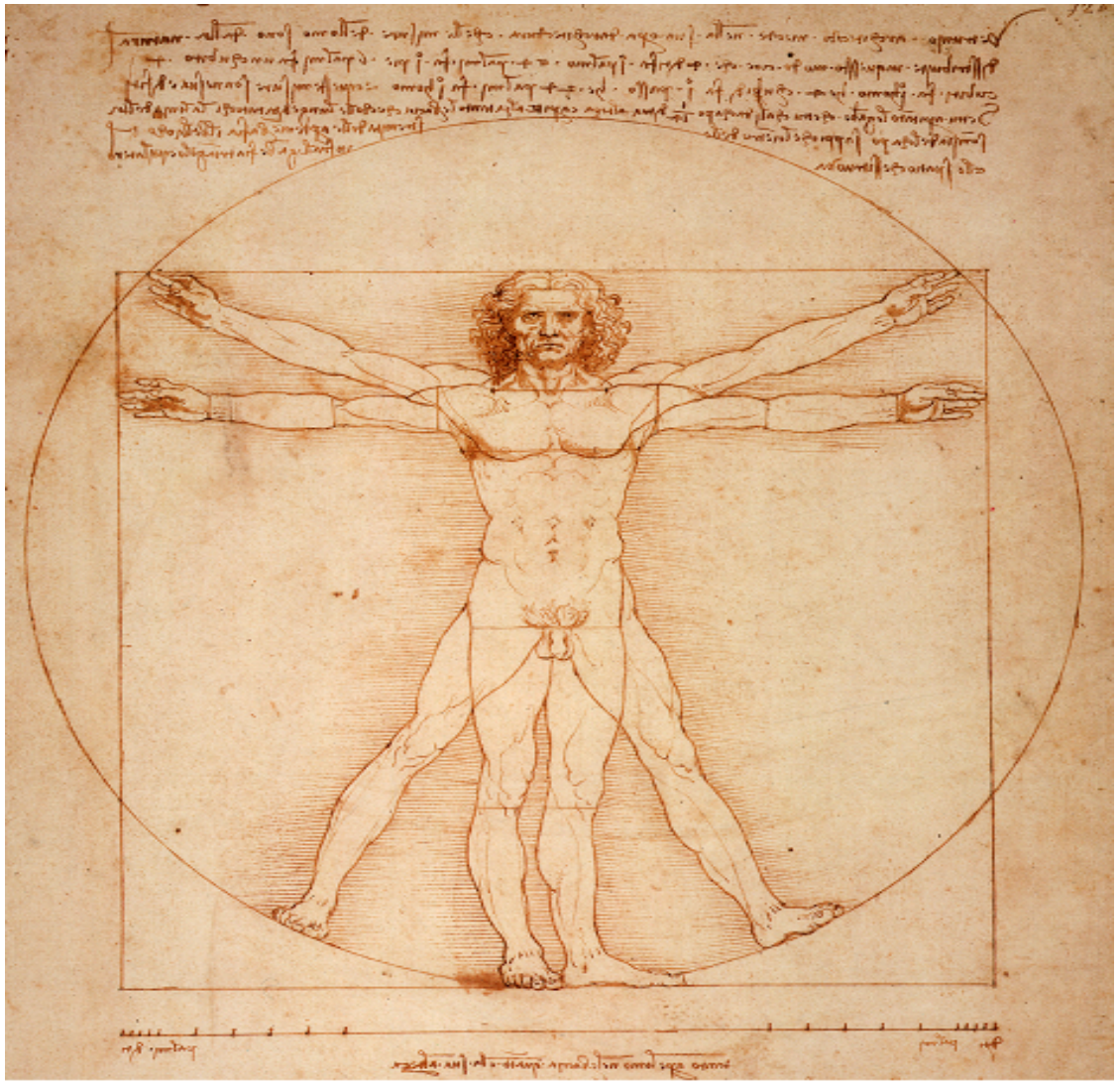
Thank You

Edinburgh Fertility Preservation

www.ed.ac.uk/Edinburgh-fertility-preservation

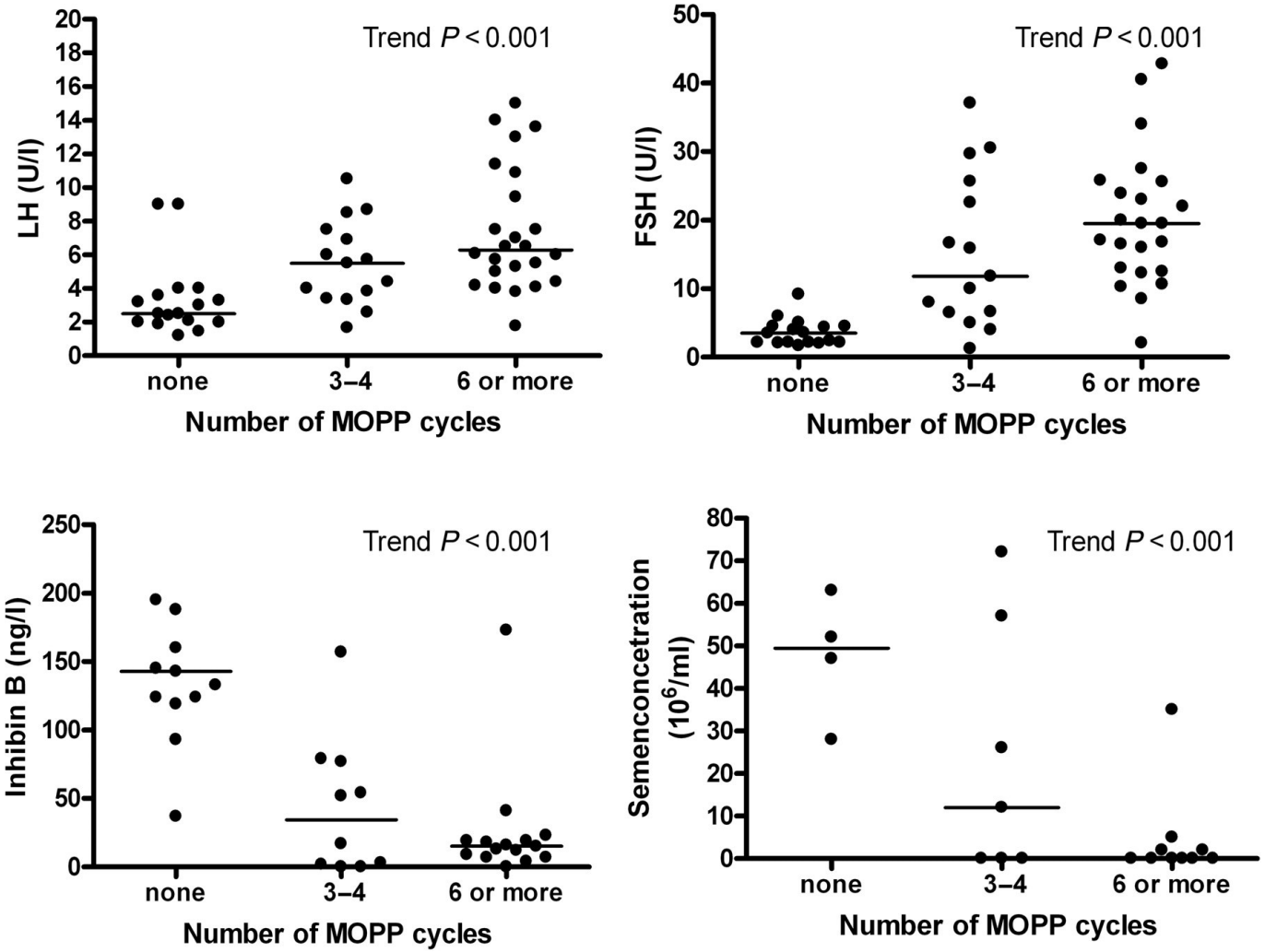
@edinfertility

Vitruvian man



Leonardo da Vinci 1490

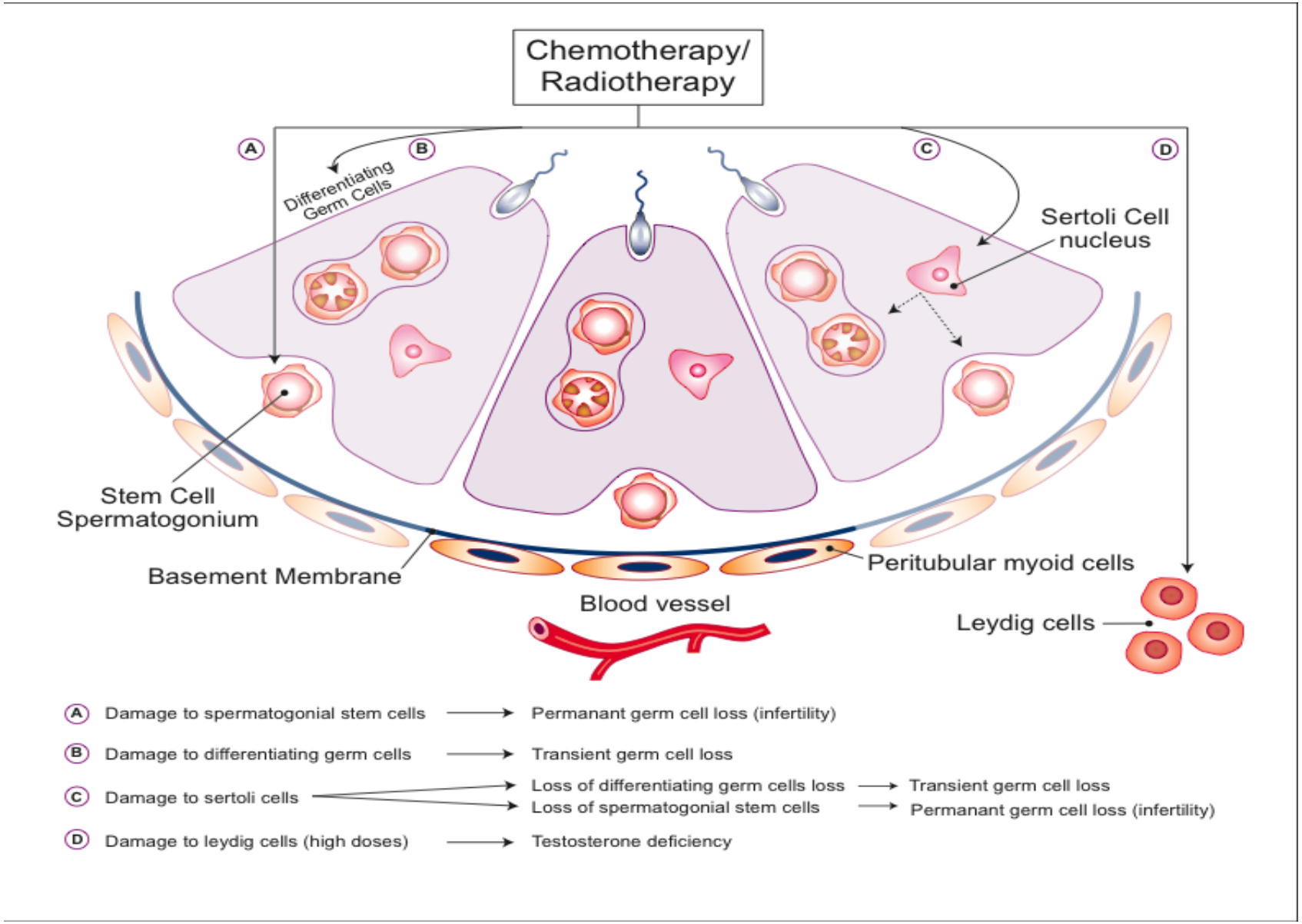
Hormone levels and semen concentration in relation to the number of MOPP cycles in male long-term survivors of childhood Hodgkin's.



van Beek R D et al. Hum. Reprod. 2007;22:3215-3222

human reproduction

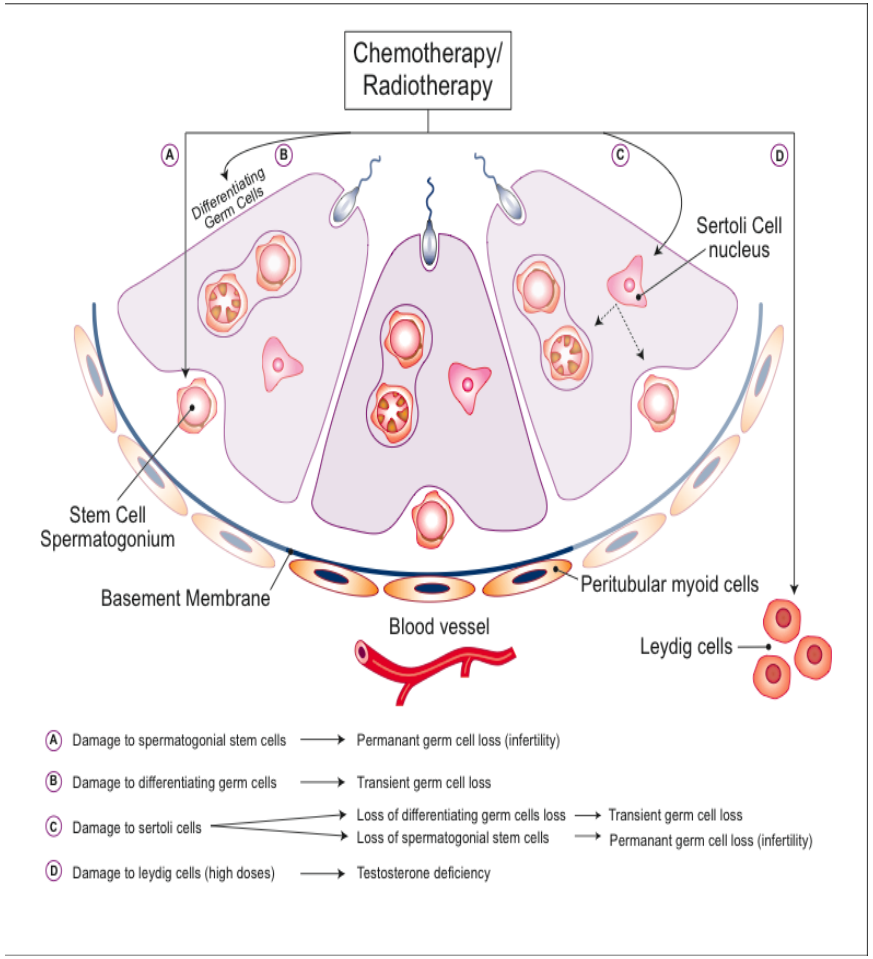
Sertoli Cell



Radiation-induced testicular damage

Germinal epithelium

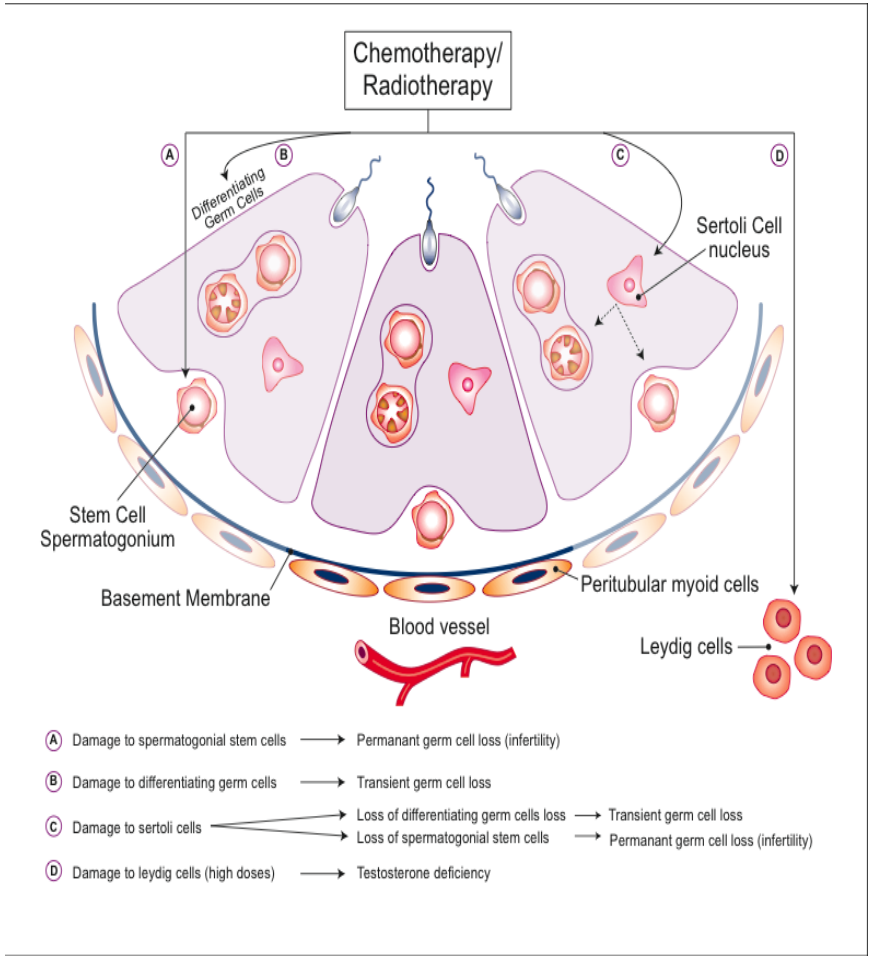
>1.2Gy azoospermia



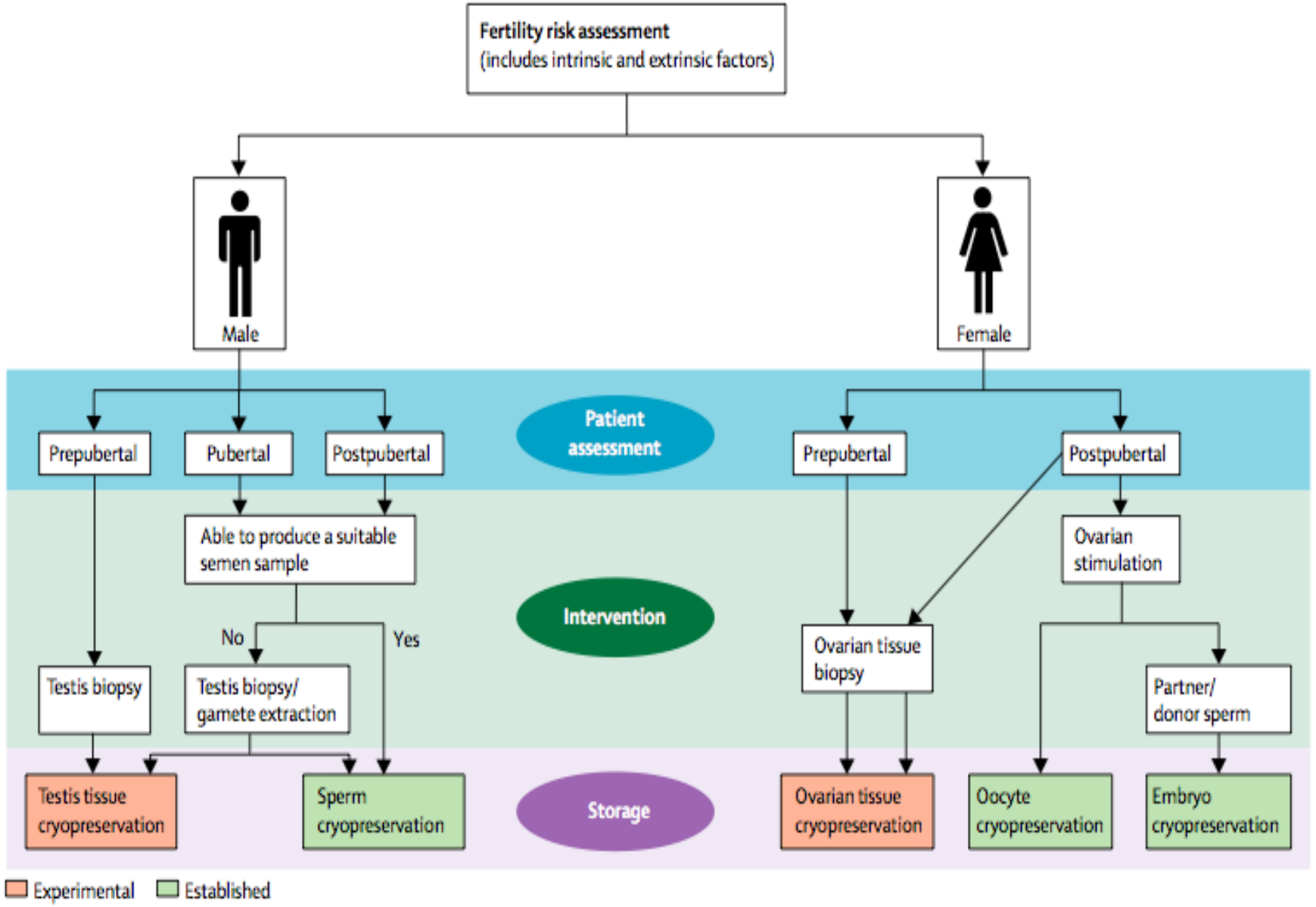
Radiation-induced testicular damage

Leydig cell function

- Dose received by testis P <0.05
- Time Interval after radiotherapy P <0.05
- Age at treatment NS



Li, Kelsey, Wallace (unpublished data)



Anderson RA…Wallace WH. Lancet Diabetes Endocrinol. 2015

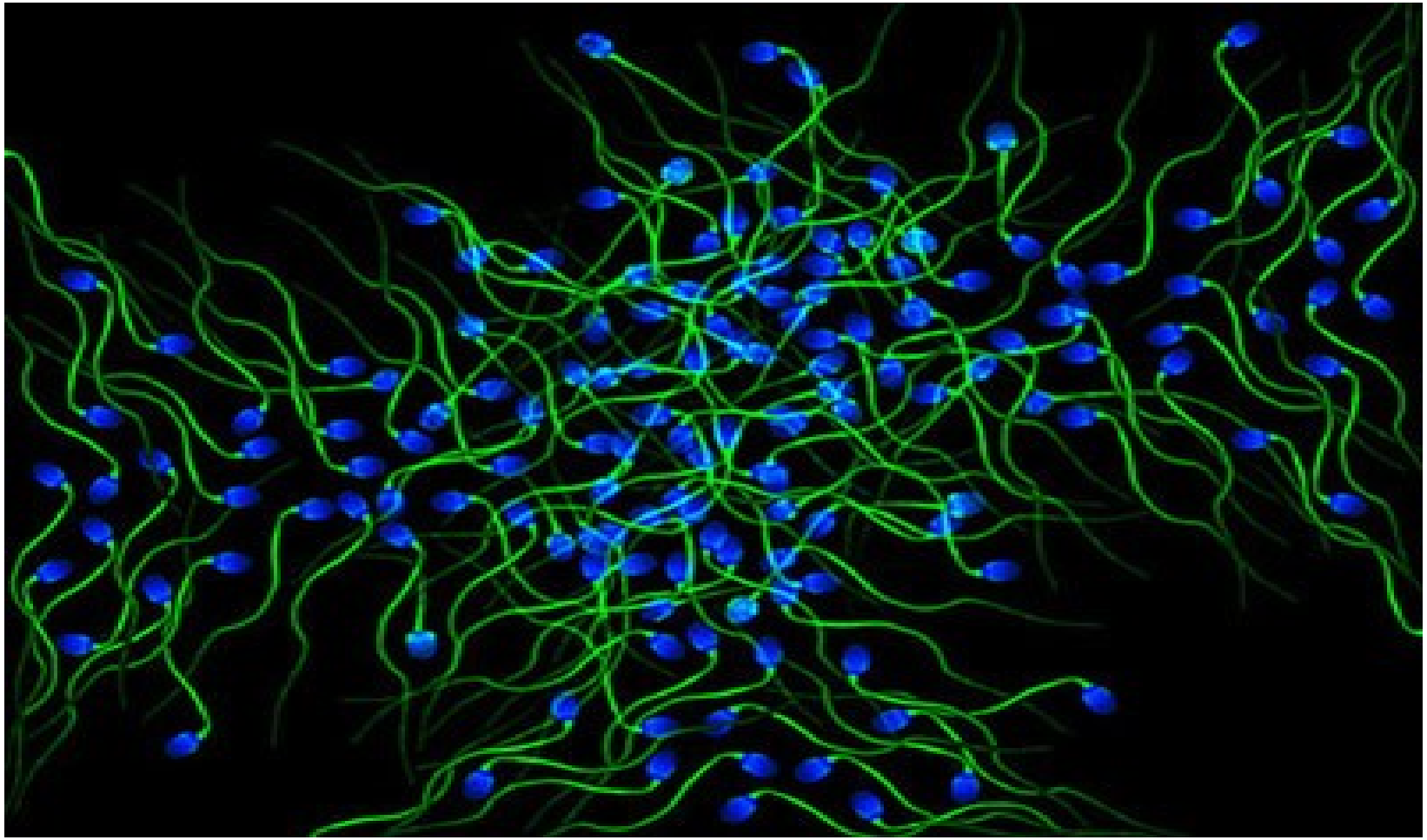
Males: Fertility preservation

- Young men who can produce semen should have the opportunity of sperm banking before treatment begins
- Sperm retrieval should be considered if the chances of infertility are high and the testes are >10mls
  - Storage of gametes is governed by the HFE act 1990
  - Written informed consent from a competent male is required

- There is currently no established option to preserve fertility in the pre-pubertal boy….

Isolated human sperm cells (1500x)

Albert Tousson – Nikon Small world

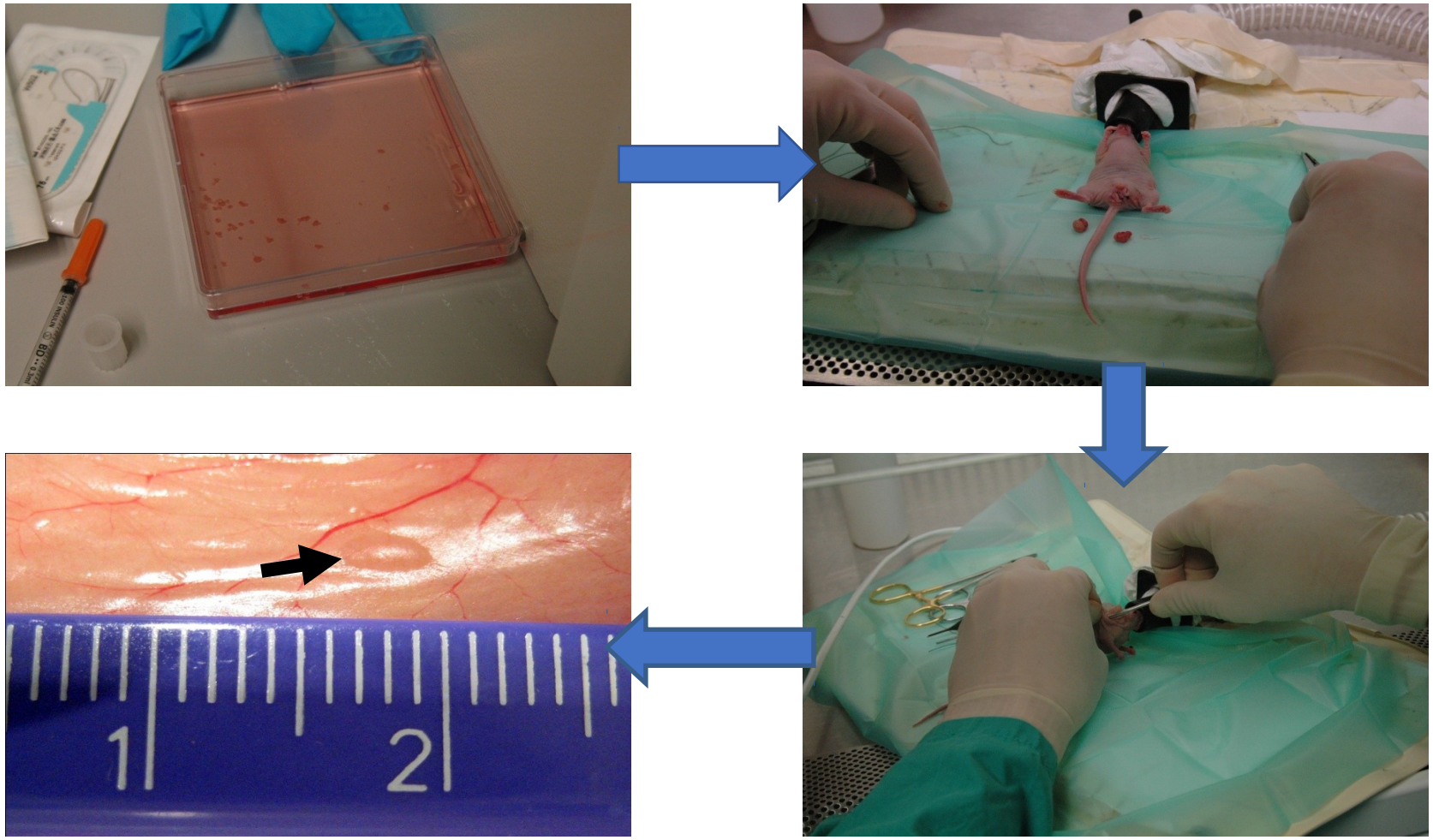


Cryopreservation of pre-pubertal testis tissue prior to cancer treatment

- Boys undergoing cancer treatment with >80% risk of infertility
- Biopsy to be taken with routine procedure
- Storage by Tissue Services according to ‘mature’ or ‘immature’ protocol
- Small piece of tissue to be used for research

Ethical Approval Granted – September 2013

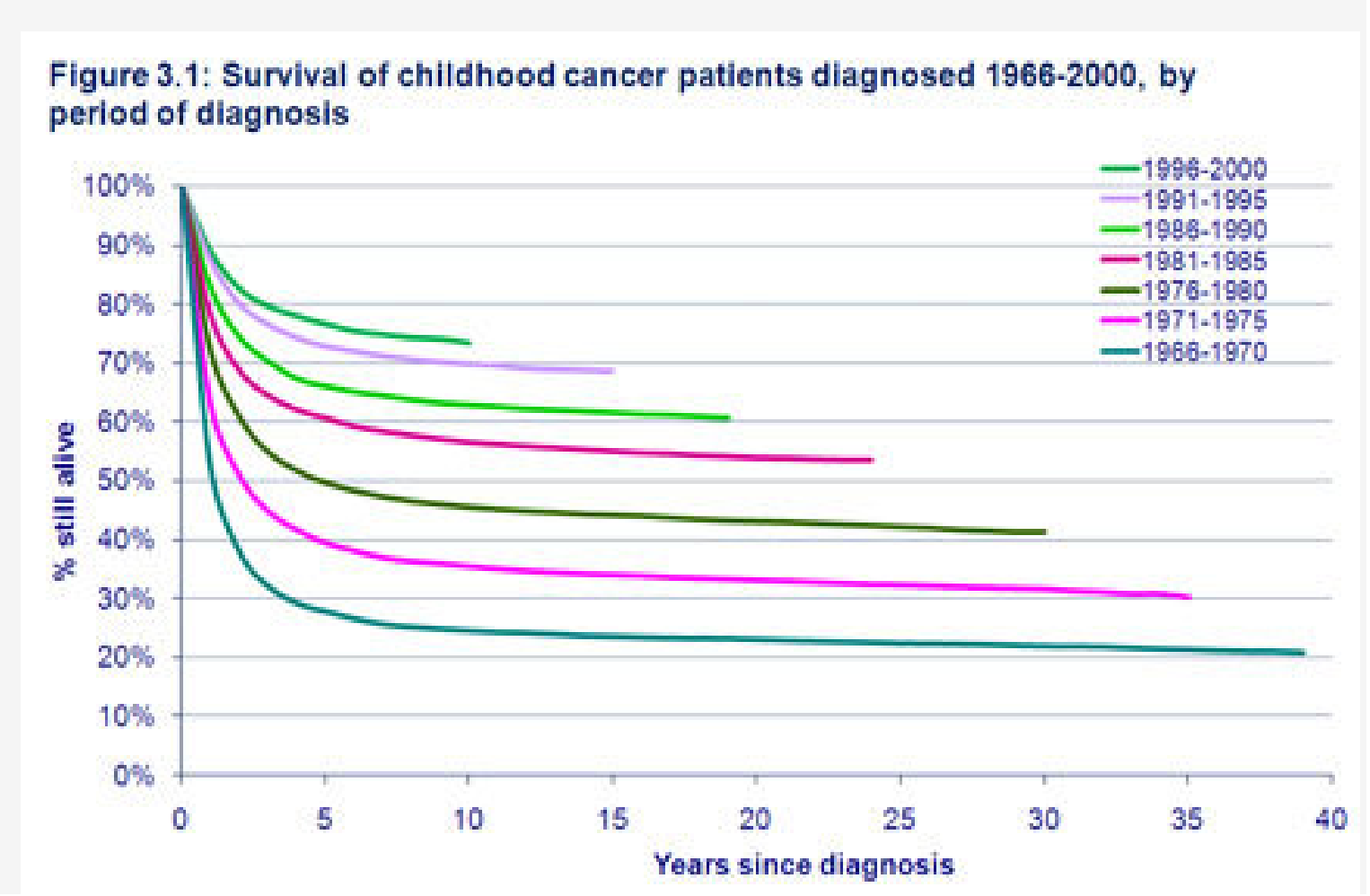
Human Testis Xenografting



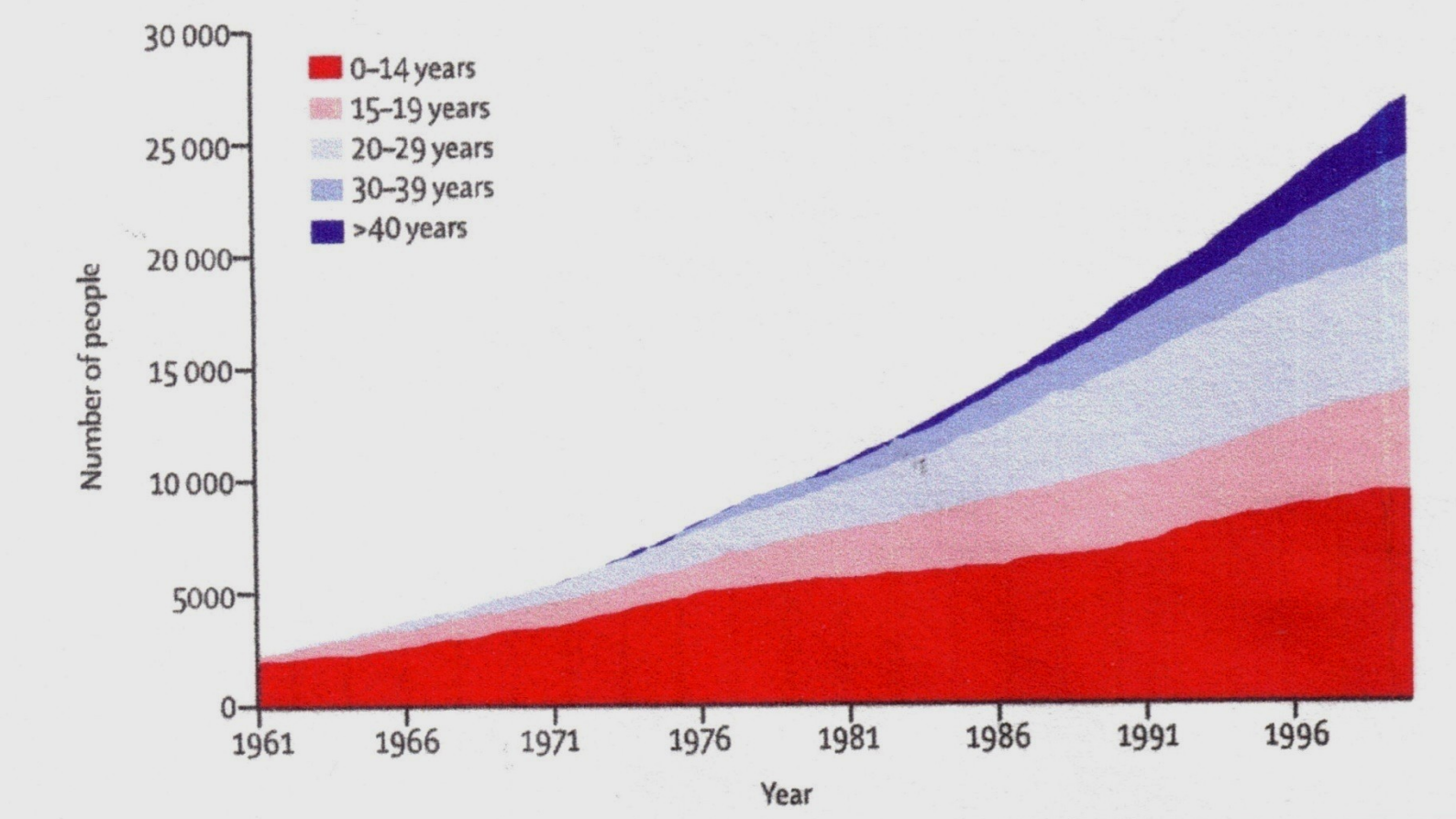
Challenges

- 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

Improved Five Year Survival (1966-2000)

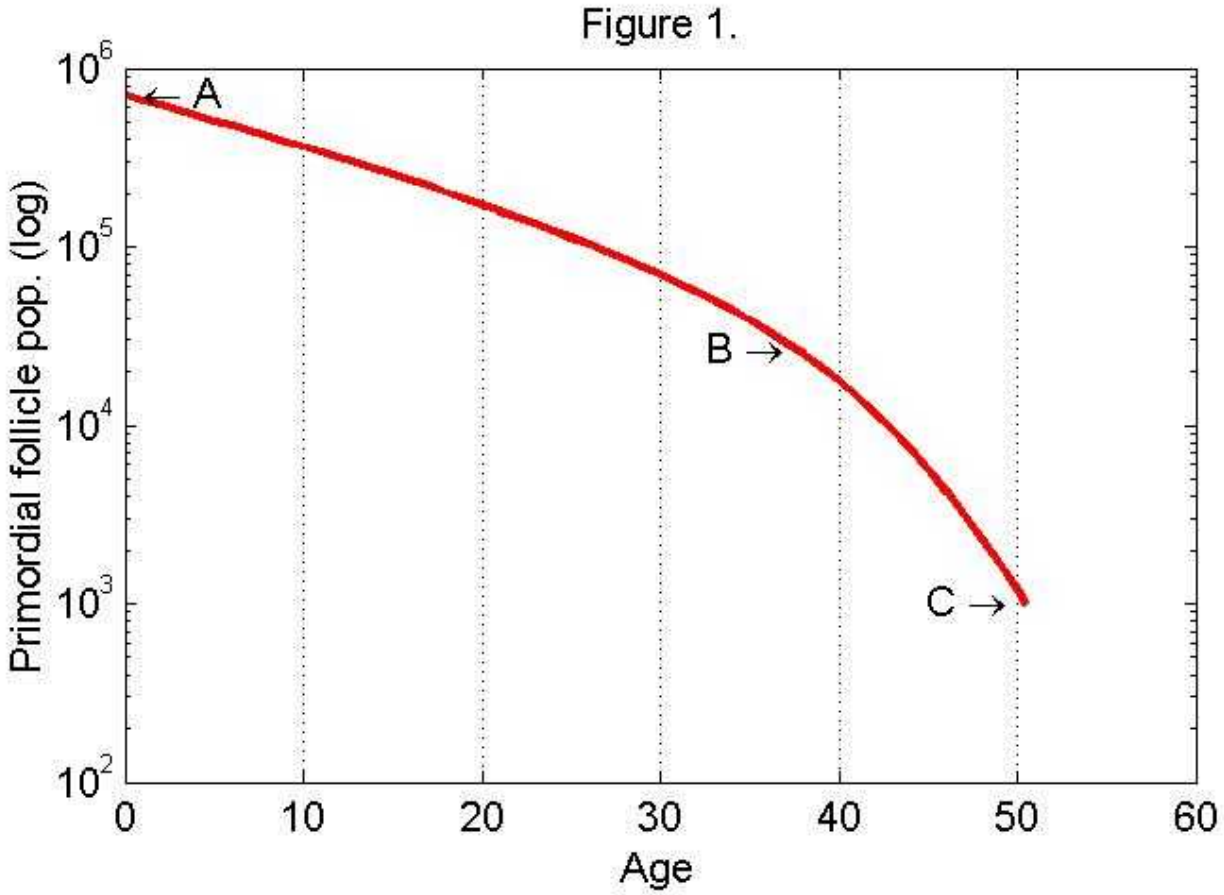


Increasing numbers of five year UK survivors by current age



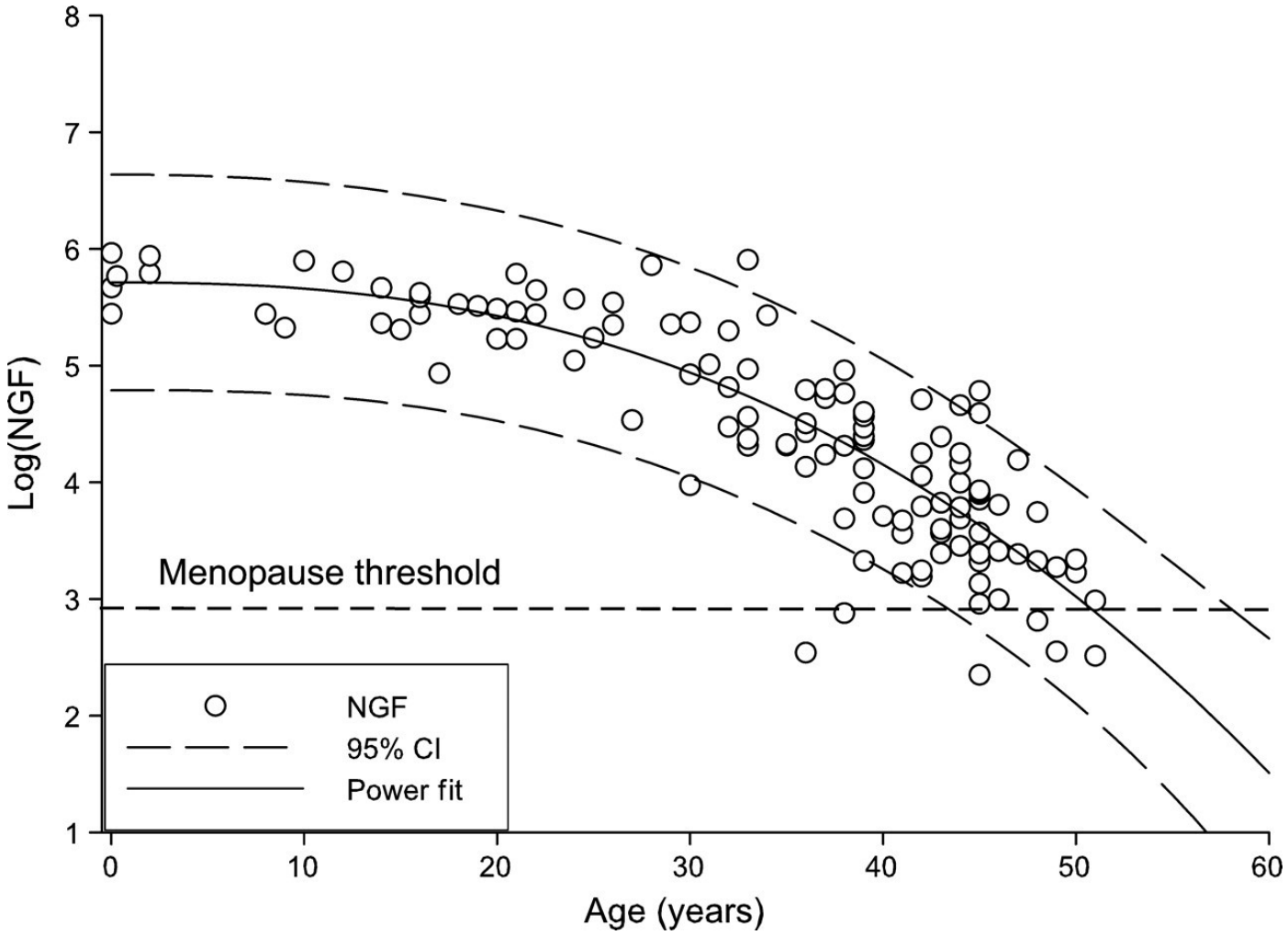
Skinner et al, Lancet Oncology, 2006

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.

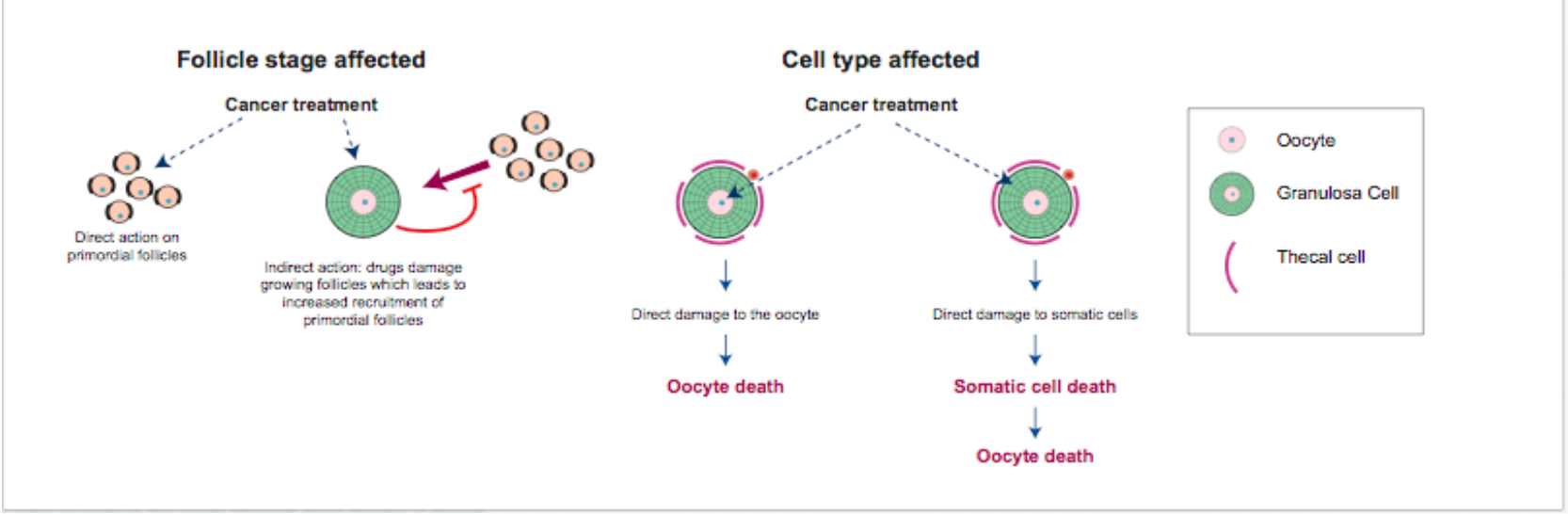
Power-model of human ovarian NGF decay



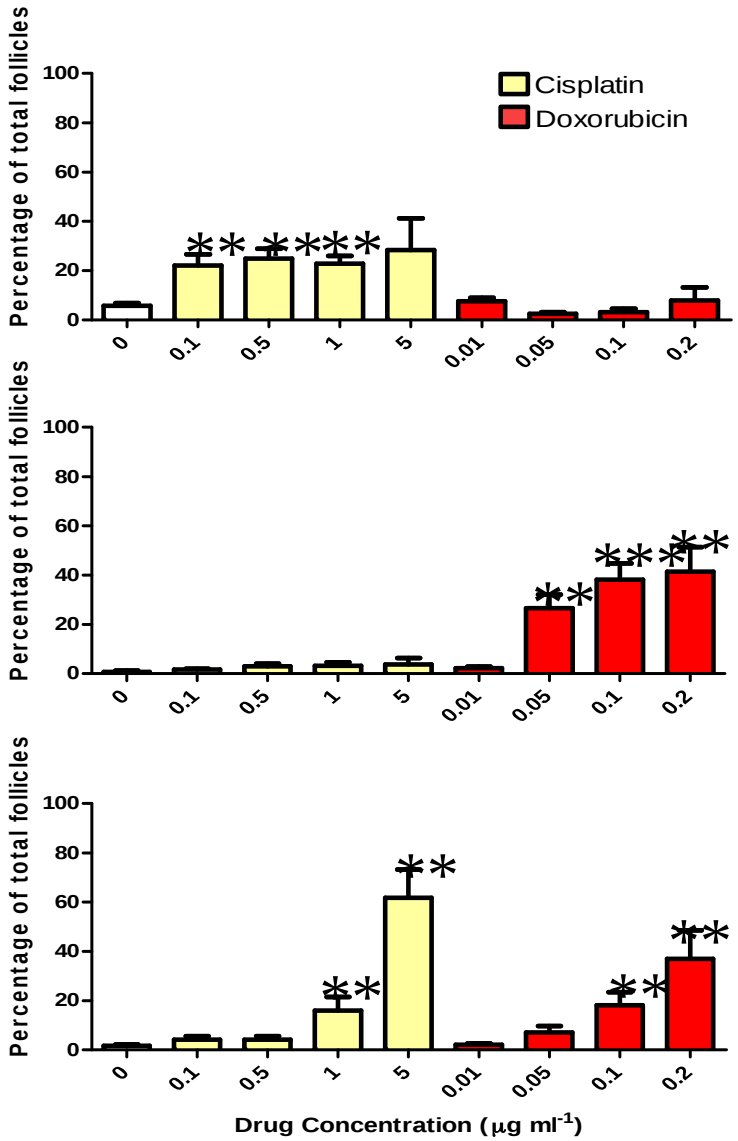
Hansen, K. R. et al. Hum. Reprod. 2008 23:699-708

Oocyte or granulosa cells?

- Newborn mouse ovary culture system
- Morgan et al. 2013, PLoS ONE



Cisplatin and doxorubicin: a mouse ovary culture system



Unhealthy oocytes

Unhealthy granulosa cells

Unhealthy oocytes and granulosa cells

Morgan et al, 2013, PlosOne

Cisplatin and Doxorubicin (Mouse ovary)

- Cisplatin showed oocyte-specific damage
- Doxorubicin preferentially caused damage to the granulosa cells
- Suggestion:
- Imatinib protected the mouse ovary against damage by cisplatin but not doxorubicin

Morgan et al, 2013, PlosOne