Fertility preservation for young people with cancer: what are the remaining challenges?

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Summary of Talk

* Who is at risk of infertility ?
* What can be offered to those at high risk of infertility ?
* Our Edinburgh experience of ovarian cryopreservation
* Ethical and consent issues
* Future developments and research

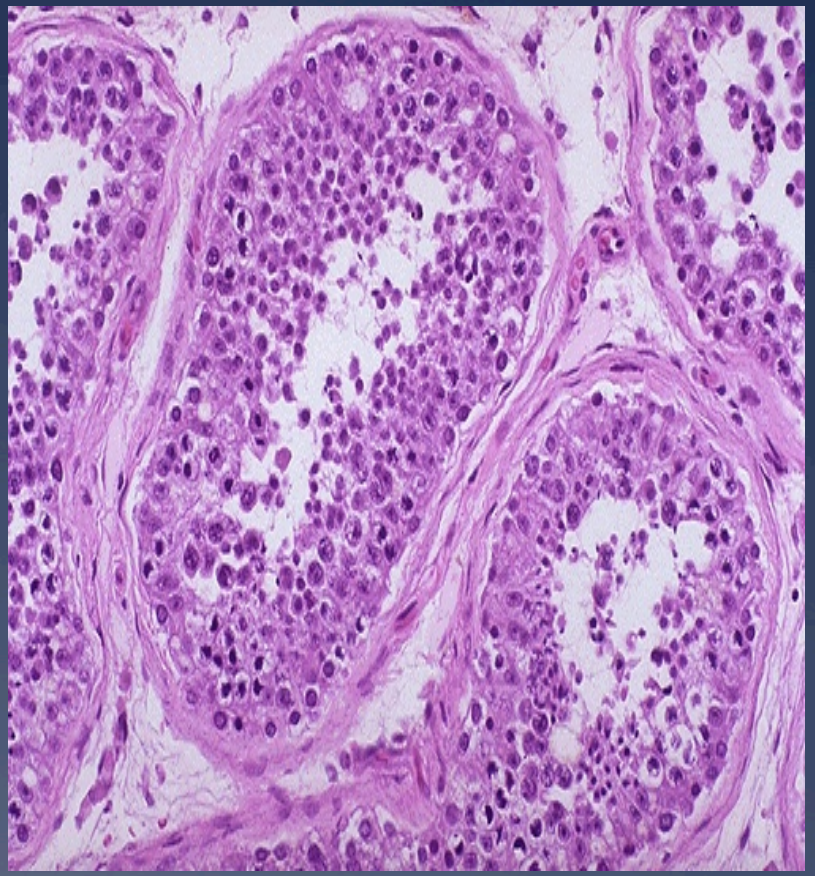
Infertility - Risk Factors

* RT to HPA or a field that includes testes/ovaries
* Busulphan
* BCNU
* CCNU
* Cyclophosphamide
* Ifosfamide
* Melphalan
* Mustine
* Nitrogen mustard
* Procarbazine
* Thiotepa
* Chlorambucil
* Cytarabine

The pre-pubertal gonad is not protected

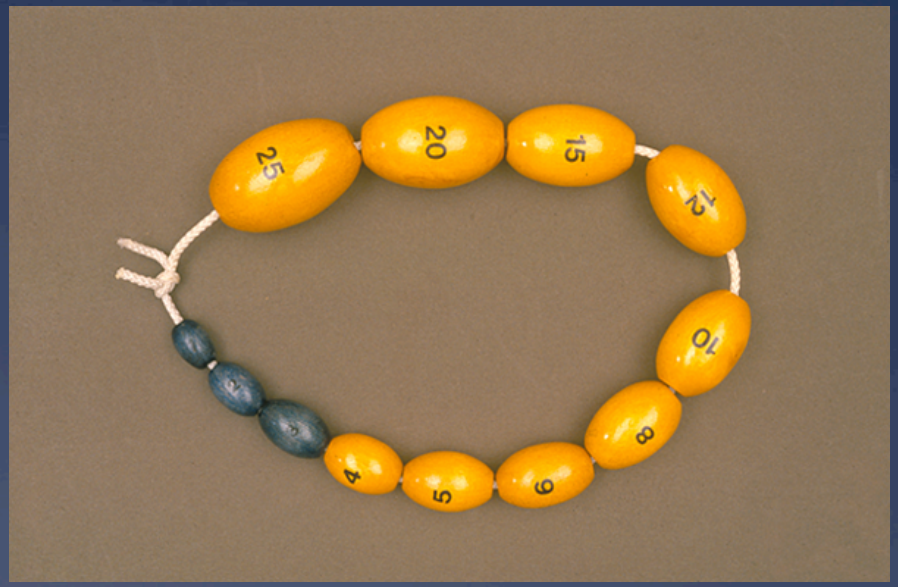
Testicular function

- Spermatogenesis
  - production of mature sperm
- Steroidogenesis
  - production of steroid hormones
    - testosterone



Testicular damage

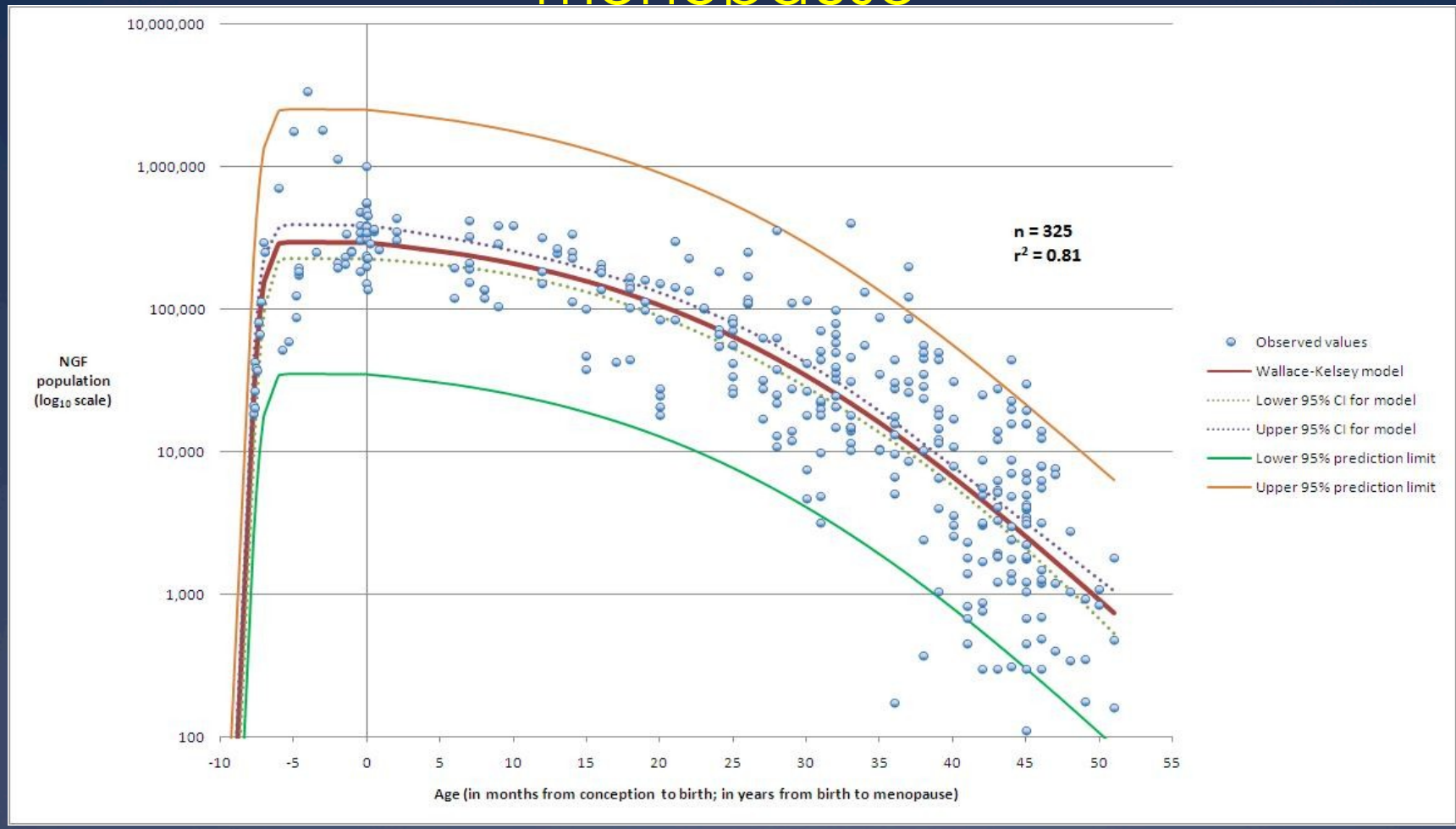
- Leydig cell
  - reduced testosterone
  - elevated LH
- Germinal epithelium
  - elevated FSH
  - low inhibin B
  - impaired spermatogenesis



Radiation-induced testicular damage

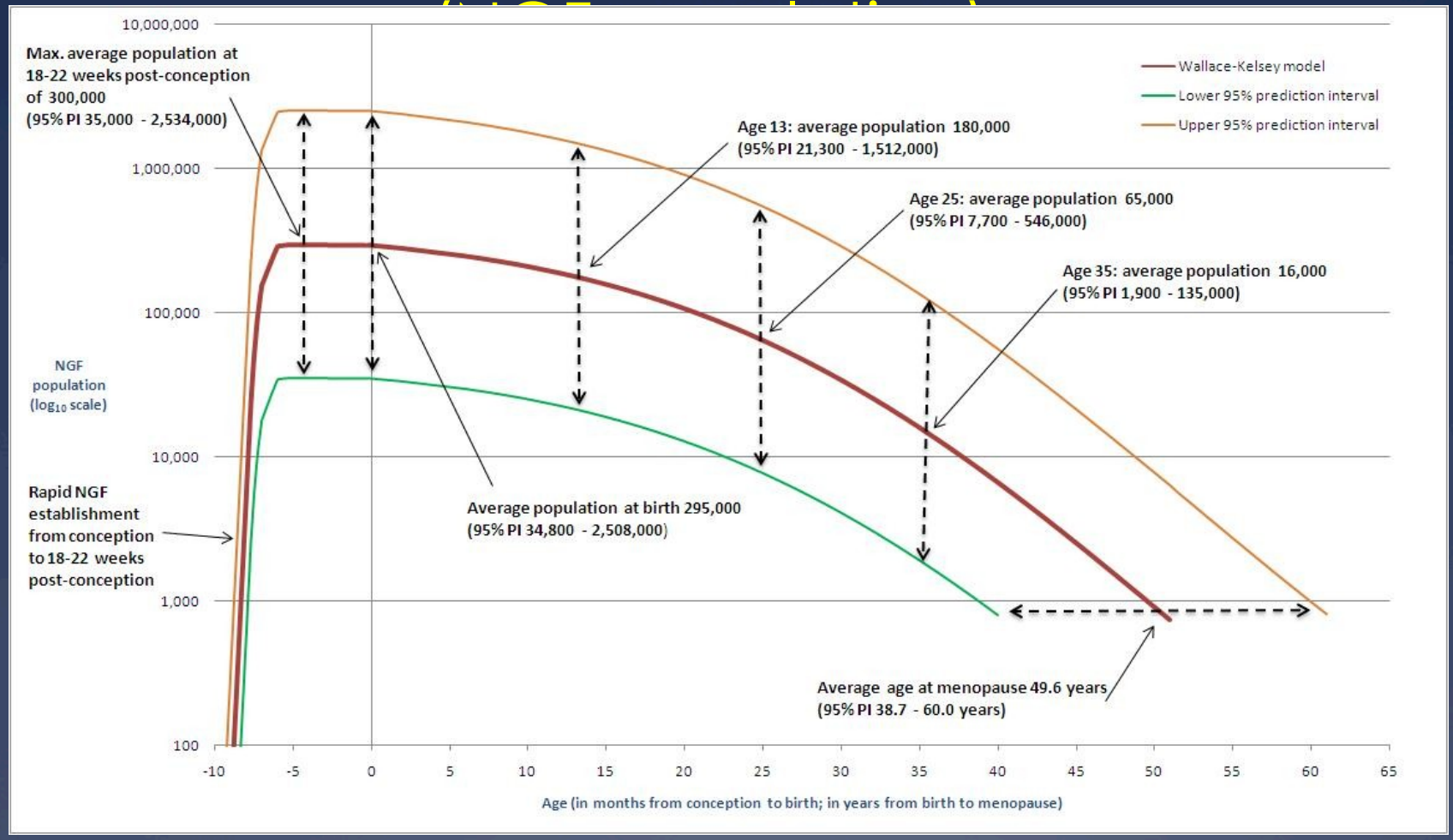
- Germinal epithelium
  - >1.2Gy azoospermia
- Leydig cells
  - >20Gy pre-pubertal
  - >30Gy post-pubertal

Ovarian reserve:conception to menopause



Wallace &Kelsey (2010) PloS ONE

Ovarian reserve: Conception to Menopause



Wallace &Kelsey (2010) PloS ONE

Prediction of ovarian reserve

- AMH is an important product of the adult ovary, produced by the granulosa cells of small growing follicles
- with little variation across and between menstrual cycles, AMH is the best currently available marker of the number of small-growing follicles in the ovary

Prediction of ovarian reserve

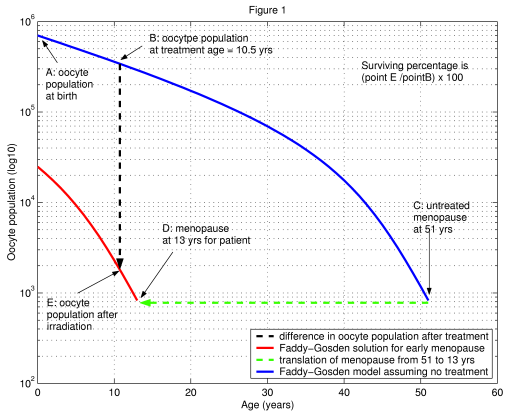
- There is increasing information on the ability of AMH to detect chemotherapy-induced loss of ovarian reserve in survivors of cancer in childhood
- and limited data from prospective studies illustrate its ability to reflect acute gonadotoxicity
- There is a need for more research on markers of ovarian reserve to improve our assessment of the individual patient before the onset of potentially gonadotoxic treatment

Radiation-induced ovarian damage

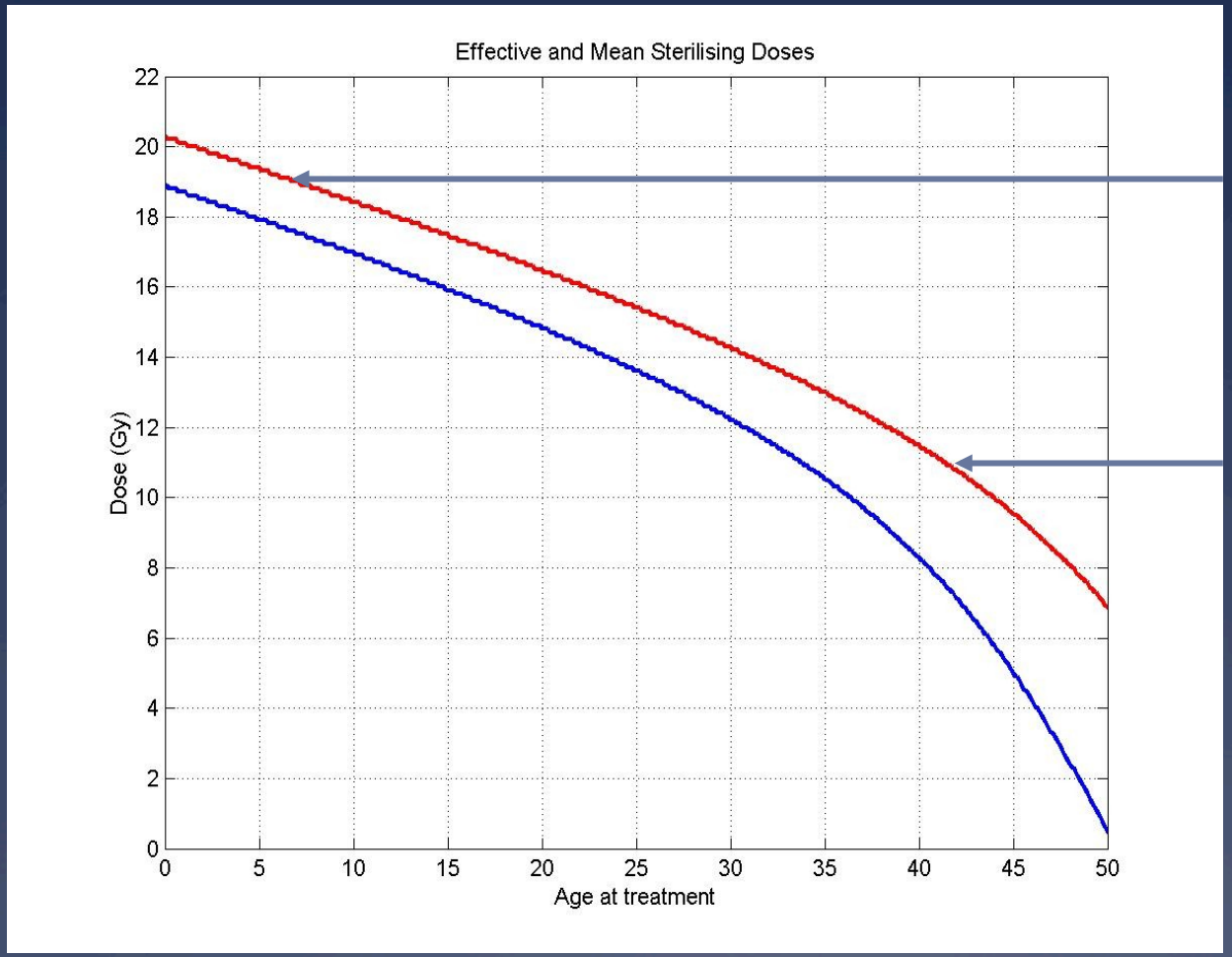
Human oocyte
(Primordial follicle)

* LD_{50} < 2 Gy

Wallace et al. (2003) Hum Reprod.



Effective and mean ovarian sterilizing doses of radiotherapy at increasing age

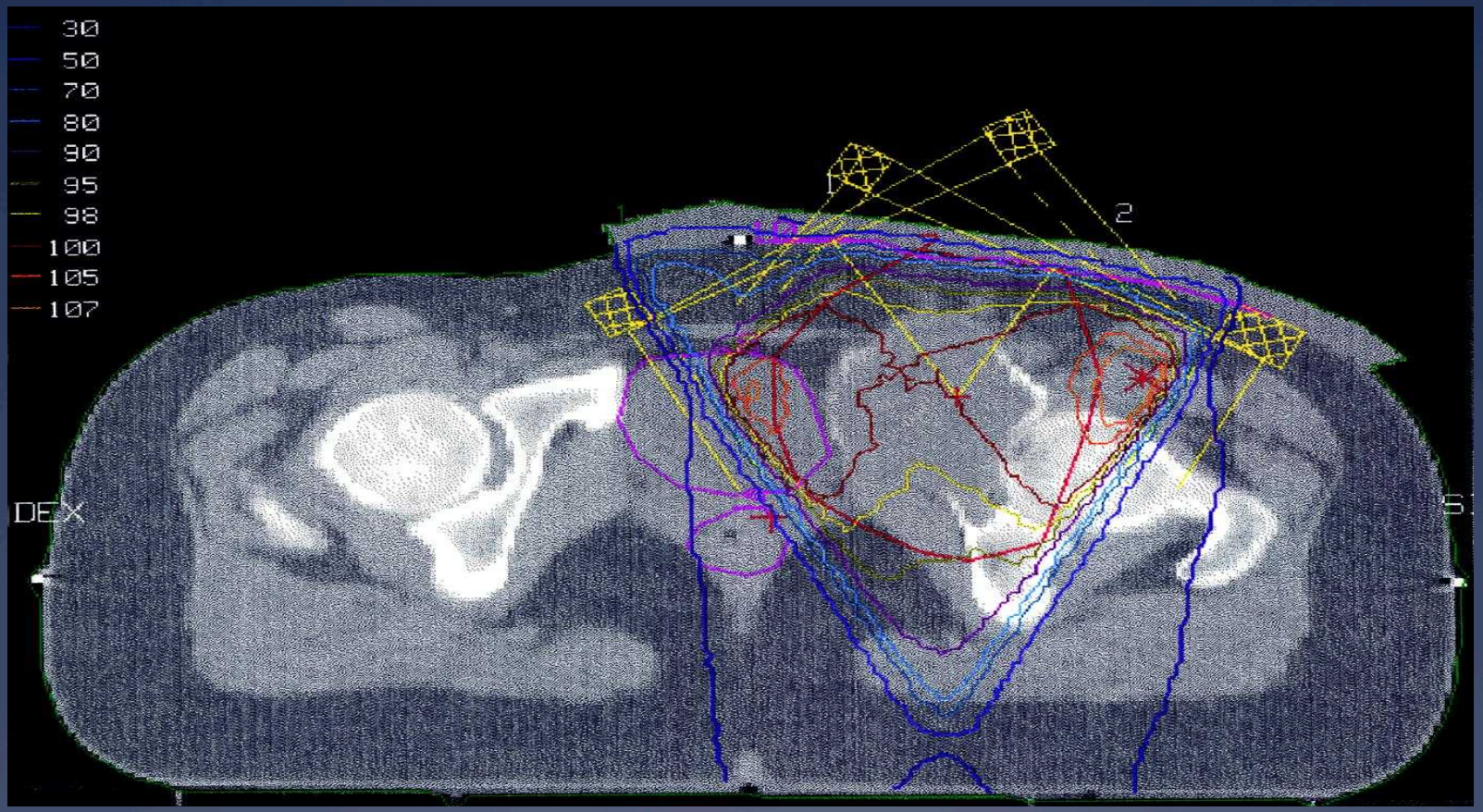


19 Gy will sterilize at 7 years

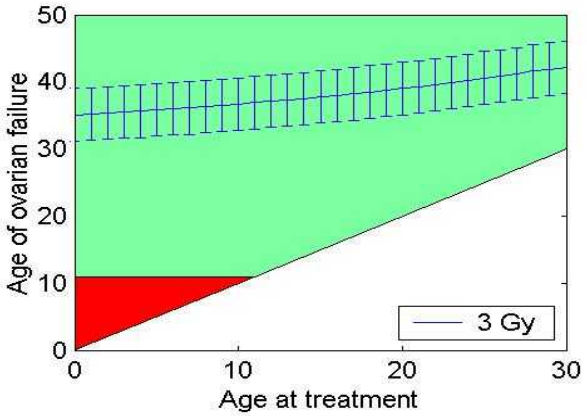
11 Gy will sterilize at 42 years

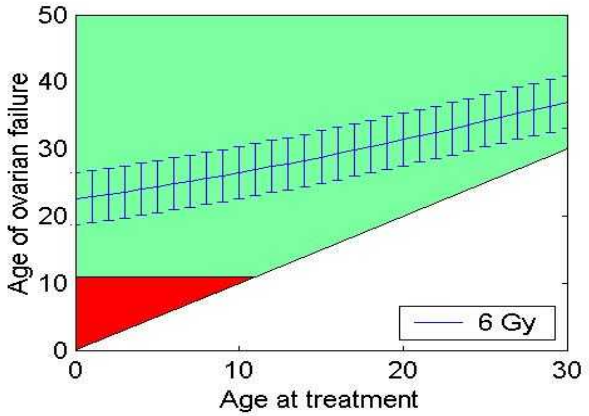
Wallace WH et al.
IJRBP (2005)

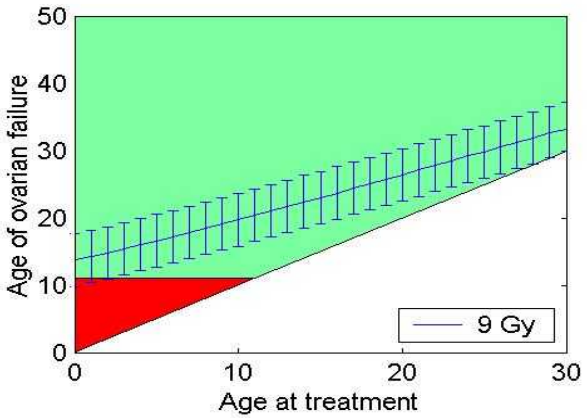
CT planning for pelvic Ewing's sarcoma

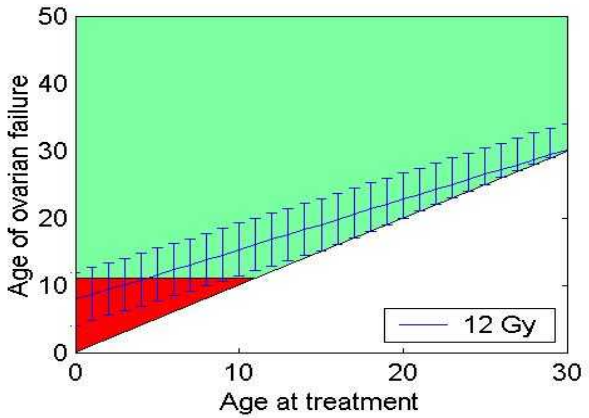


Age prediction for development of ovarian failure after known dose of

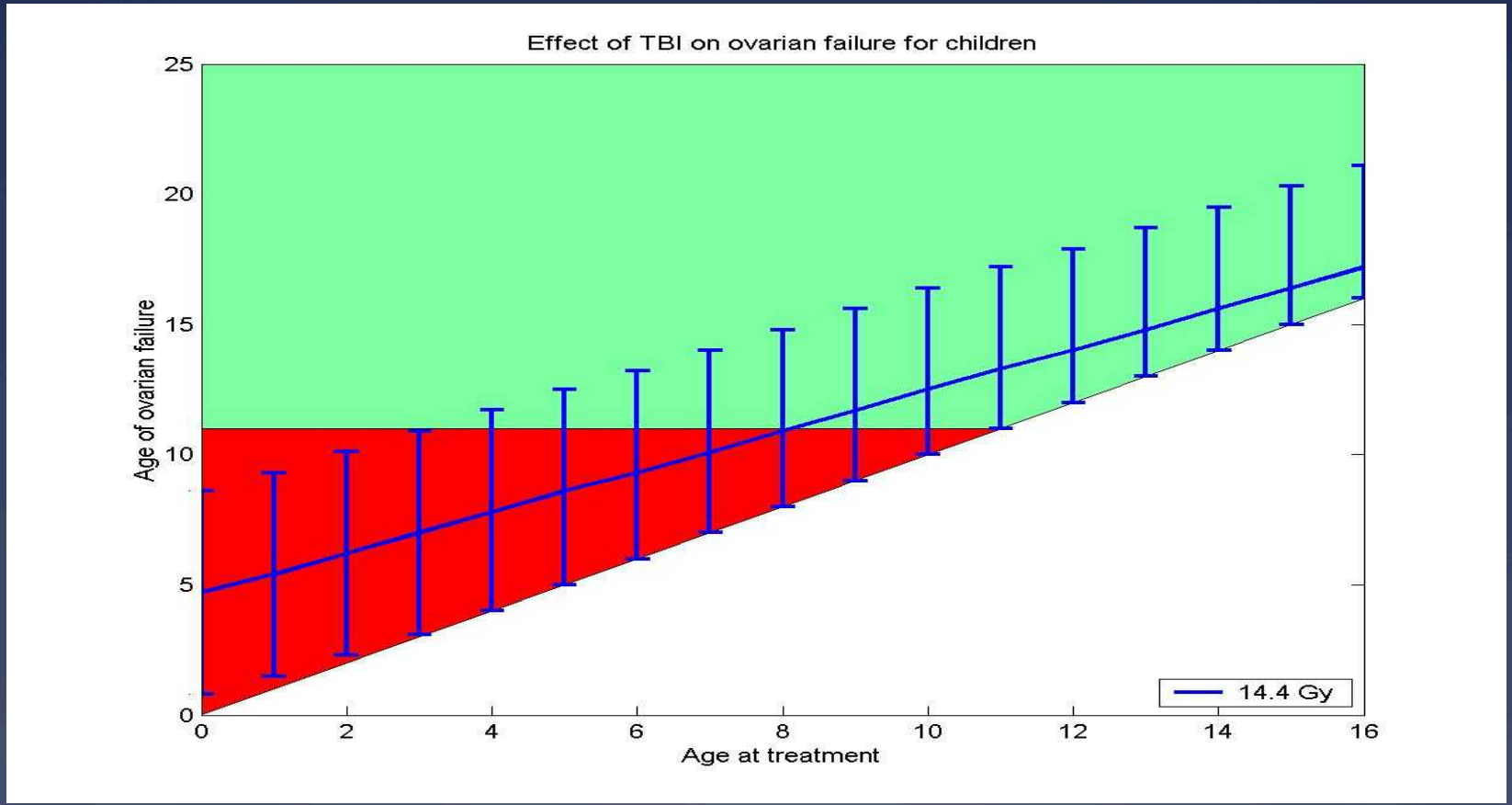








Age at ovarian failure after TBI



Premature menopause in survivors of childhood cancer

Childhood Cancer Survivor Study (CCSS)

- Diagnosed cancer <21 yrs, 1970-86, Five year survivors.
- 2819 eligible subjects, 1065 sibling controls
- Non-surgical menopause: Cumulative Incidence 8% vs 0.8 % (RR 13.21)
- Risk factors:
  - attained Age
  - Increasing doses of radiation to the ovaries
  - Increasing alkylating agent score (dose)
  - Hodgkin's Lymphoma

Sklar et al. JNCI 2006;98:890-6

Strategies for fertility preservation in males undergoing treatment for cancer

- Clinical practice
  - Sperm banking
    - Ejaculation
    - Rectal electrostimulation?
    - Testicular/epididymal aspiration

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 option to preserve fertility in the prepubertal boy

Pilot interviews with adolescent males (Glaser, Crawshaw et al.)

- 7 young men aged 14 to 17 at diagnosis
  - with cancer
  - offered sperm banking
  - (Aged: 16 to 20 at interview)
- Focus on retrospective perceptions of:
  - communication
  - decision-making
  - management of sperm banking

‘I was just walking round with it, walking up and down the corridor until I saw a doctor…..It was a bit weird explaining to them what I’d just been through and what do I do with it…and then they said just leave it in the room……I didn’t know if it was safe or not because the doctor… walked off in a different direction…’

‘it could have been put more kindly I think…”if you were to die”….I had just found out I was diagnosed with it and the question comes up “if you were to die”……’

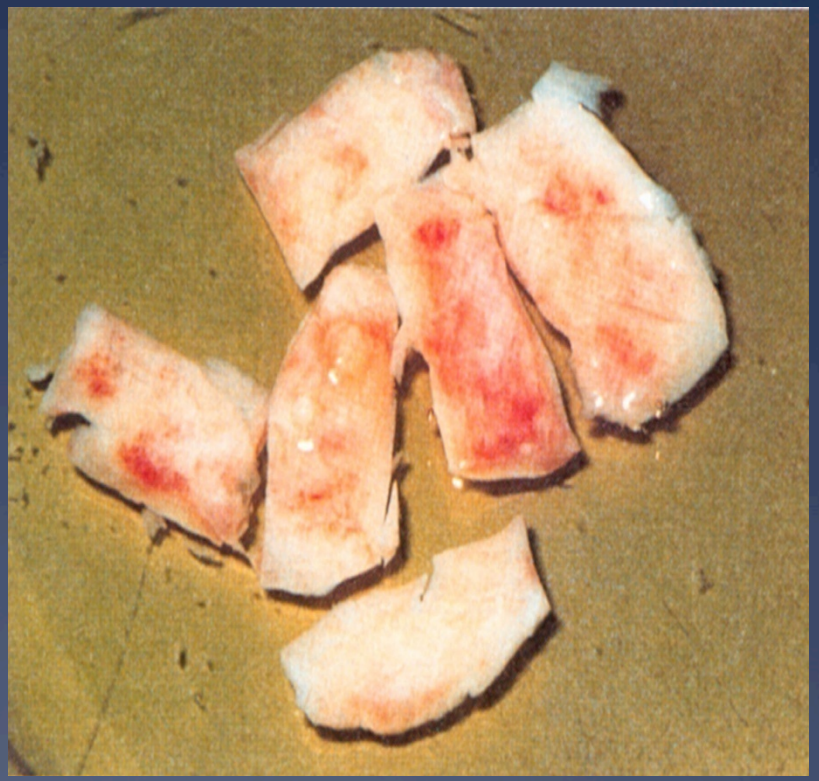
‘to be honest, it went in one ear and out the other. It was just a load of jargon. I didn’t understand it at all. All I understand was if I didn’t sign this, it, the sperm, wouldn’t be stored’

Strategies for fertility preservation in females undergoing treatment for cancer

- Clinical practice
  - Oophoropexy
  - Embryo cryopreservation

Strategies for fertility preservation in young females undergoing treatment for cancer

- Experimental strategies
  - Cryopreservation of oocytes
  - Gonadotrophin suppression
    - (Elnashar et al. ESHRE 2008)
  - Cryopreservation of ovarian tissue

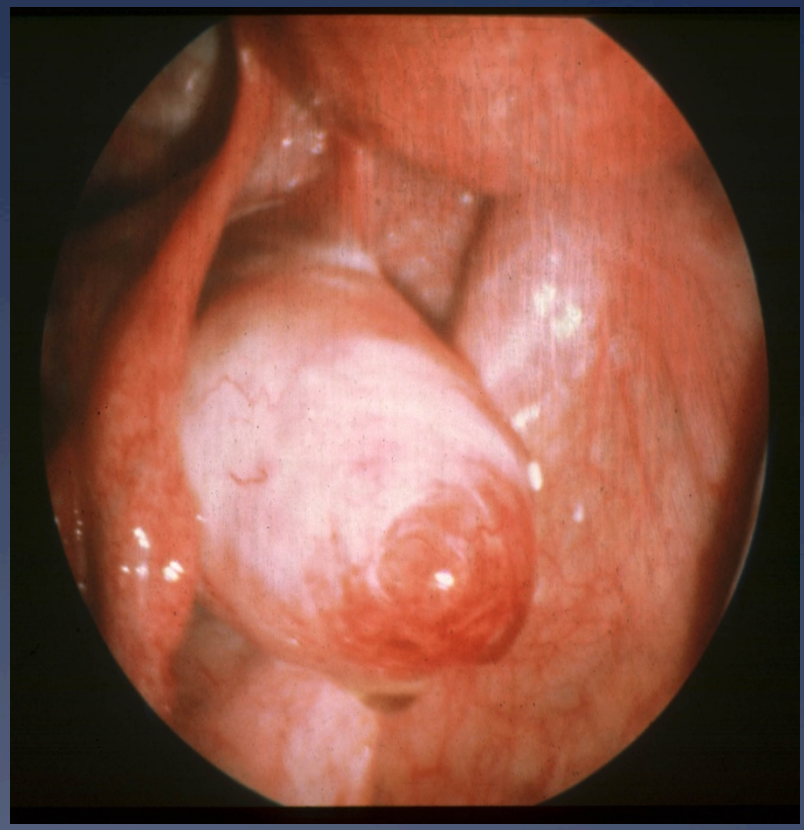


Ovarian cortical strips

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

METHODS OF COLLECTION AND STORAGE

- laparoscopic collection of 6 - 10 strips of ovarian cortex under direct visualisation from 1 ovary
- tissue manipulated in sterile environment
- stored in Leibovitz medium with 10% DMSO and 10% autologous serum at < −135 ^{o}C in vapour phase of liquid nitrogen



Cortical strip or whole ovary?

- Should a whole ovary be removed or are cortical strips sufficient as is the majority practice?
- The most important consideration is "*primum non nocere*"
- If it remains difficult to predict which patients are at high risk of an early menopause, then conservative surgery seems sensible

Cortical strip or whole ovary?

- Laparoscopic ovariectomy is a standard gynaecological operation that can be performed in peripheral centres
- the ovary can be transported on ice to the specialized centre where it can be prepared and frozen 4–5 hours after excision
- There have been live births reported from reimplantation of both cortical strips and sections prepared from whole ovaries

Cryopreservation: World-wide experience

- At least 10 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

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

Prediction of those at risk

- First-line treatment of patients with ALL is associated with an excellent prognosis for future fertility
- survivors are able to have their own children naturally
- their offspring are not at increased risk of congenital abnormalities or cancer in childhood

Prediction of those at risk

- The option of harvesting ovarian tissue or freezing harvested eggs remains if the patient relapses and is due to receive potentially sterilizing chemo-radiotherapy, there is sufficient time, and the patient's health and general condition are satisfactory
- Uterine factor:
- Radiation-induced damage to the uterus and surrounding structures may impair the ability of the uterus to carry a pregnancy to term

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
- in the event of the patient's death the material is disposed of or, 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)

Cryopreservation of ovarian cortical tissue

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

Cryopreservation of ovarian cortical tissue

Edinburgh experience

* Offered to 36 women aged 19.2 (5.0-35) yrs

Diagnosis	No. of patients
Lymphoma	7
Leukaemia	4
Sarcoma	10
Cervical Ca	3
Sacral Ependymoma	1
Breast Carcinoma	3
Wilms' tumour	1
SLE	5
Other Rheumatological disorders	2

Anderson, Wallace and Baird, 2008
Reproduction 2008 Dec;136(6):681-9.

Cryopreservation of ovarian cortical tissue

Edinburgh experience

- Of the 36 women, 15 (42%) < 16 years
- 11 (31%) of the women have died
- Current age of survivors 24.4 years(8.5-43.6)years
- Median duration since cryopreservation 7.1 years
- Overall n=20 >18 yrs old
  - 7 (35%) have had pregnancies
  - 5 live births, one induced, one spontaneous abortion
- The first patient treated, who underwent high dose chemotherapy and TBI aged 19, retains a regular menstrual cycle with early follicular phase FSH concentration <10 IU/L 13 years later.
- Nobody has requested re-implantation of the tissue
- Two have confirmed ovarian failure

Anderson, Wallace and Baird, Reproduction 2008 Dec;136(6):681-9

Reimplantation?

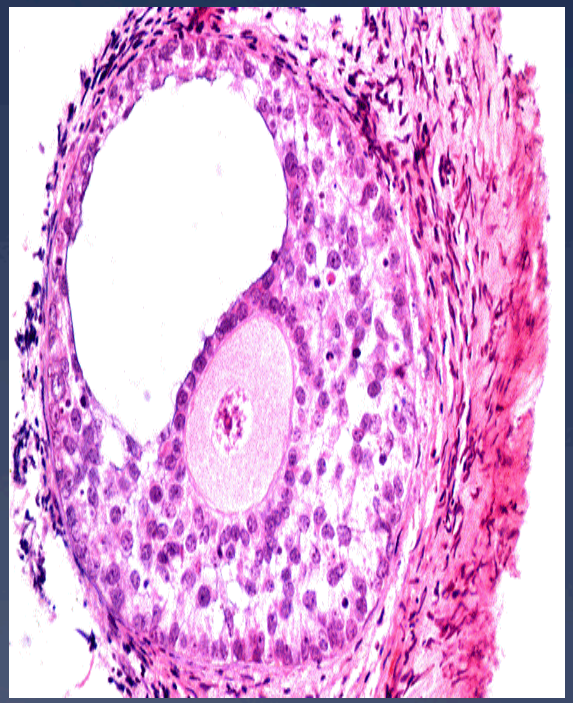
- It is important to be aware that reimplantation of ovarian cortical tissue is a separate procedure
- at a time distant from the treatment of the original cancer
- Consent for harvesting ovarian tissue from children often will have been obtained from their parents
- informed consent for reimplantation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

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

- Cortical strip biopsies from healthy women during LSCS
- Culture in serum free medium for 6 days
- Pre-antral folicles dissected (n=74, mean 100 microm)
- Placed individually in serum free medium (n=38 in presence of activin A, n= 36 without), grown for 4 days
- Human activin A: grew larger than control (143 vs 111 microm, $p<0.005$)
- 30% showed normal morphology with intact oocytes and antral formation



Telfer EE et al. Hum Reprod 2008

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

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

Case history:KB

- Ewing's sarcoma L sup pubic ramus, non metastatic 07.96, Age 15
- EICESS,92; 14 courses of ifos based CT and RT (55Gy)
- Lap cortical strips before treatment
- Completed Rx 04.97
- Premature ovarian failure: FSH 23.6, LH 19.5 E2<37 11.97
- Radiation cystitis and vaginitis
- HRT complicated by breakthrough bleeding
- Uterus 4.7x4.5x2.7cm, normal hysteroscopy

Case history:KB

- Became pregnant on HRT!!
- MRI: distortion of pelvic inlet
- Elective section at 37 weeks, uncomplicated
- Male infant 2.94 Kg

Bath et al. Human Reproduction 2004

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?
- Ovarian grafts will survive for at least 5 years
- Several groups have reimplanted ovarian tissue once the initial graft has failed

HRT and pubertal induction

- To our knowledge reimplantation of frozen–thawed ovarian tissue has not yet been reported in the context of the management of pubertal induction
- Our view is that this precious tissue should only be reimplanted if fertility is requested
- Pubertal induction using hormonal treatment is well established, but there remain questions about which sex steroid replacement regimen is appropriate for the young woman who may face many years of therapy

Summary

Males

* Sperm banking must be considered in all males before treatment that carries a risk of long-term gonadal damage
* There is currently no option to preserve fertility in the pre-pubertal boy (more research required)

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.
- Acccelerated IVG of human oocytes is likely to become a realistic possibility.
- Research-based egg and ovarian tissue storage facilities be developed at a number of collaborating sites in Europe

Thank you