

# Fertility issues before and after cancer treatment

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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
- What are the attitudes of paediatric oncologists to discussing fertility issues with their patients?
- How should we treat premature ovarian failure?

# 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



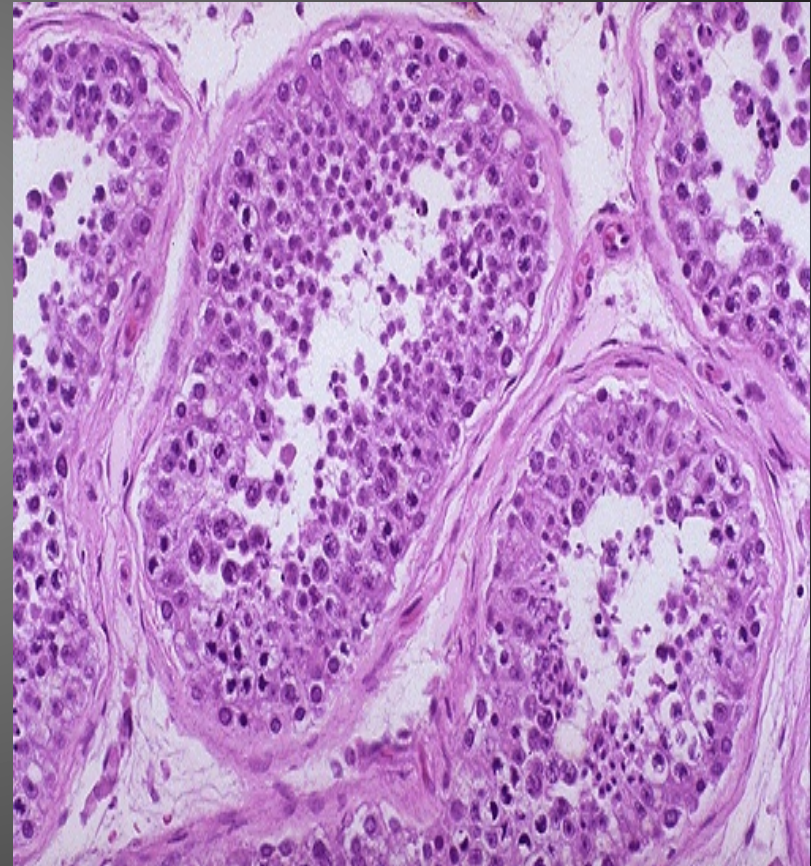
# Testicular function

- **Spermatogenesis**

- ◆ production of mature sperm

- **Steroidogenesis**

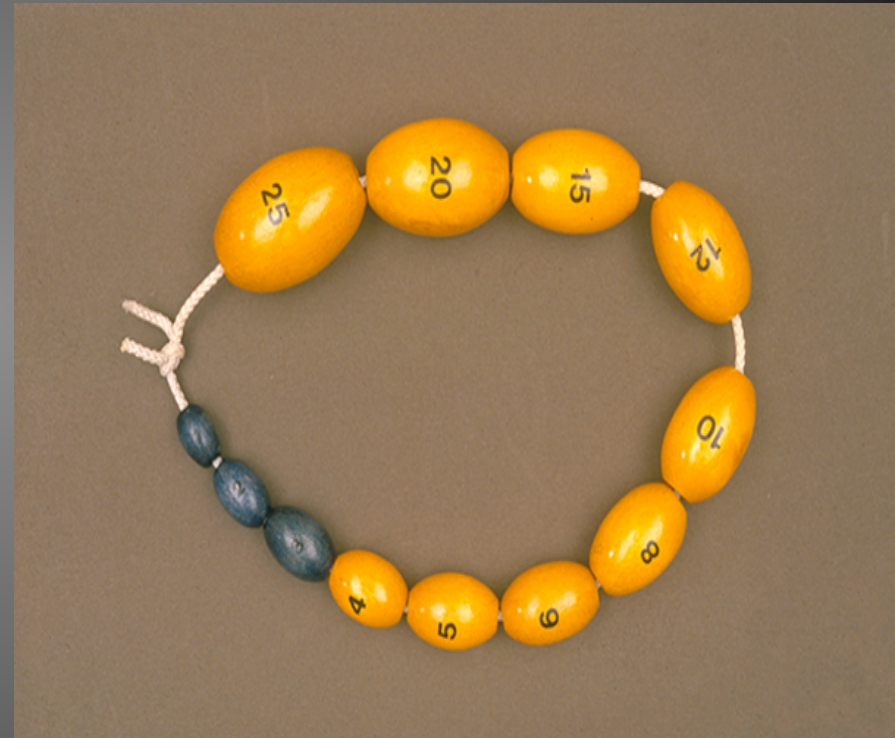
- ◆ production of steroid hormones
  - ◆ testosterone





# Testicular damage

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



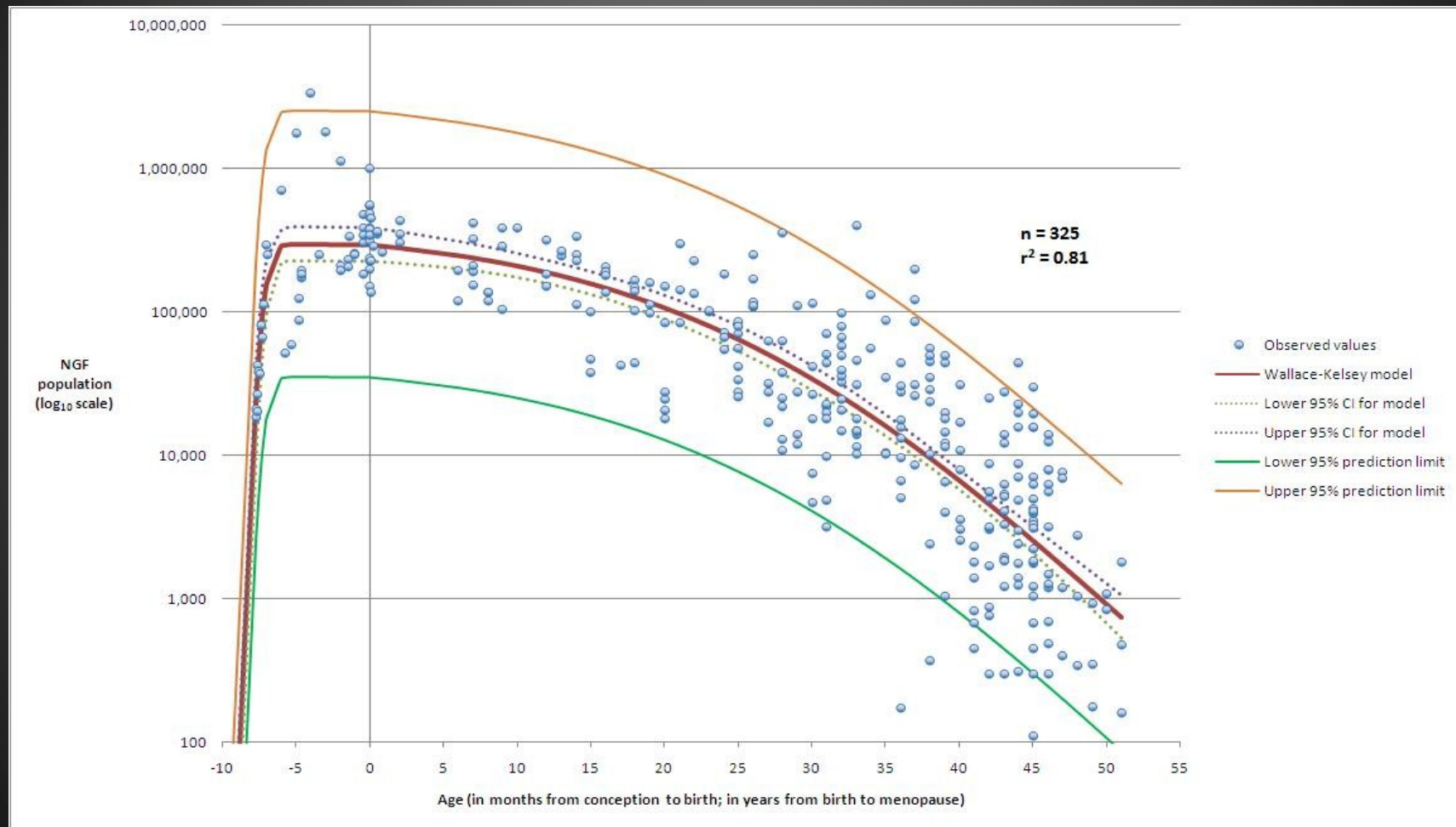
# Radiation-induced testicular damage

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



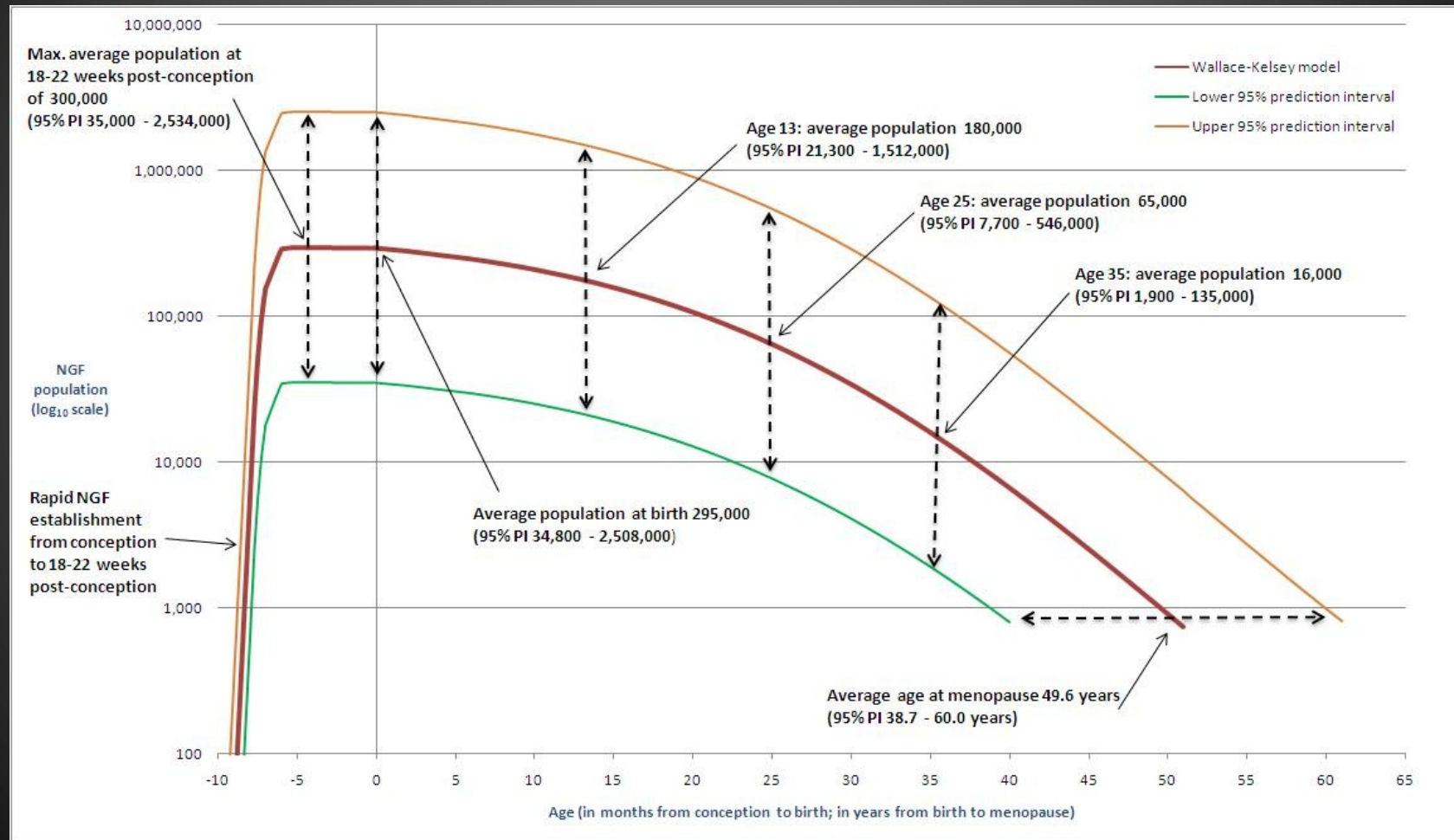


# Ovarian reserve:conception to menopause



Wallace & Kelsey (2010) PloS ONE

# Ovarian reserve: Conception to Menopause (NGF population)

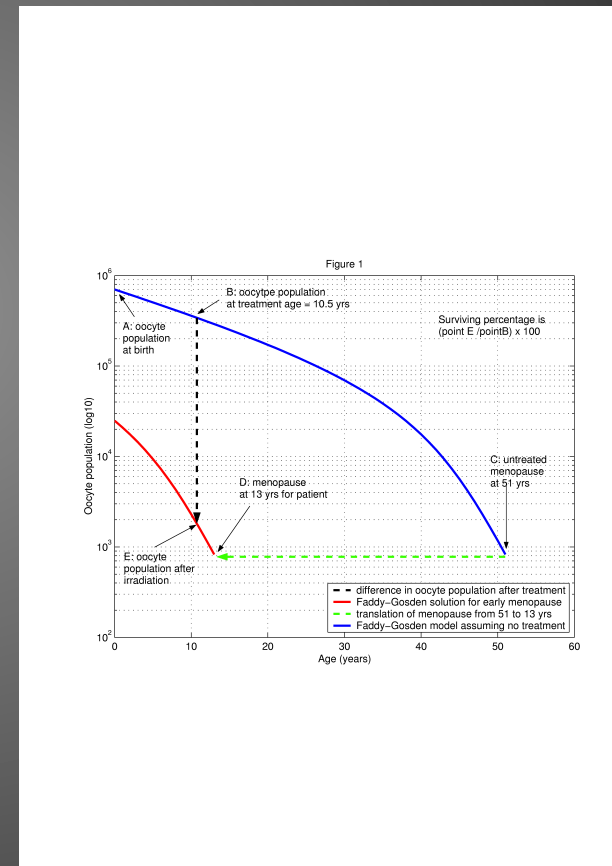


# Radiation-induced ovarian damage

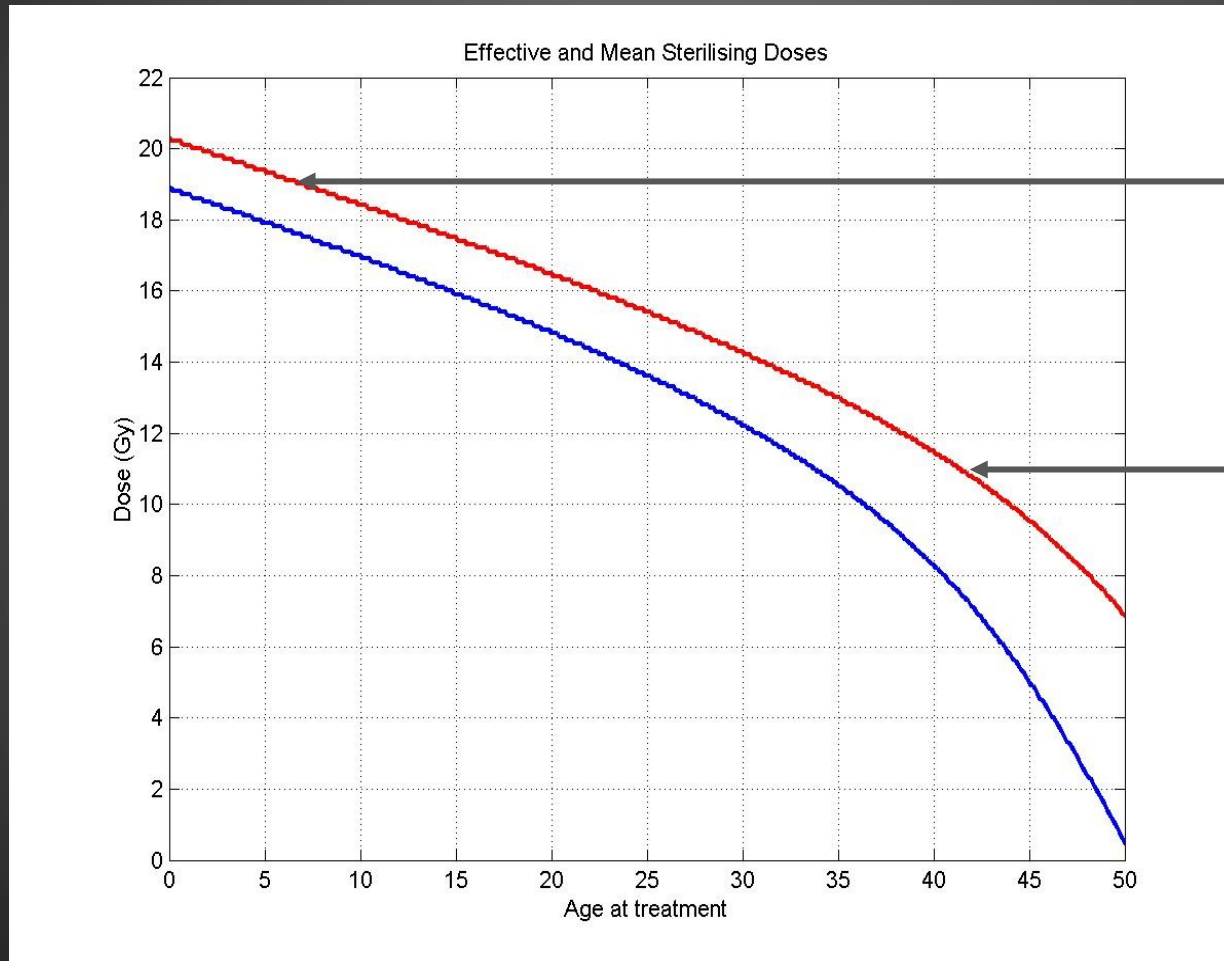
Human oocyte  
(Primordial follicle)

- $LD_{50} < 2 \text{ Gy}$

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)

# 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

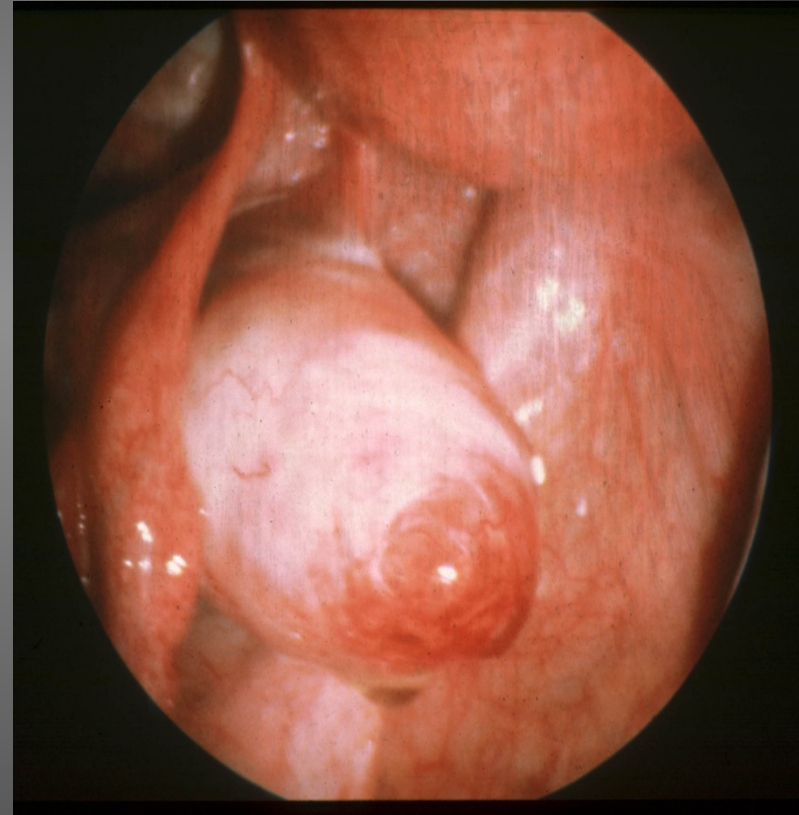
- rich in primordial follicles
- survive cryopreservation
- technique validated in sheep





# 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  $< -135$  °C in vapour phase of liquid nitrogen



# 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

# 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 1 <sup>st</sup> course chemo)	Orthotopic (Both ovaries)	IVF, live birth	Meirow 2005; 2007
Hodgkin's Lymphoma	31	Unilateral ovarian biopsy (after 1 <sup>st</sup> 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

# 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

# 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

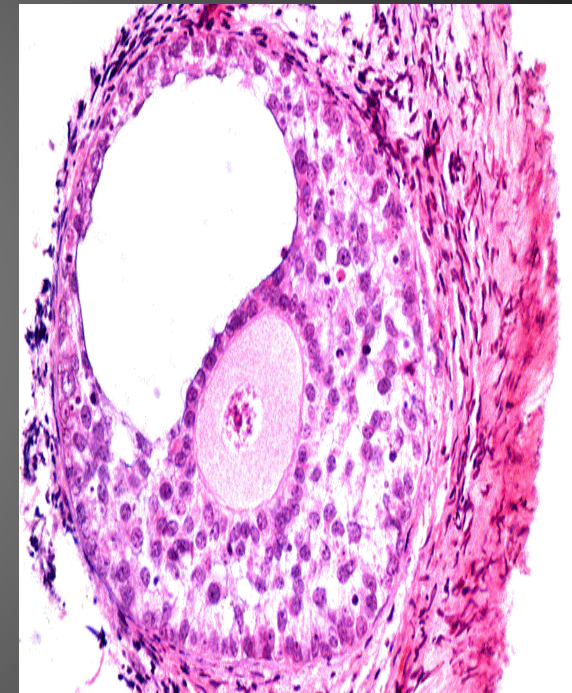


# Re-implantation or IVG and maturation?

- 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 follicles 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

## Do paediatric doctors discuss fertility issues with their patients before cancer treatment?

- Prospective analysis of 1 year of CCLG registrations
- N=1030 (68%) from 17 centres, age and diagnoses representative
- The effect of cancer treatment on fertility was discussed with most patients and/or their parents (63%).
- Techniques were more commonly discussed with boys than girls
- Of the pubertal/post-pubertal children, 27% of boys but fewer than 1% (n=4) of girls were referred to an assisted conception unit.

Anderson R et al. Hum Reprod 2008 Oct;23(10):2246-51.

# 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.
- Accelerated 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



**Optimal management for the  
oestrogen deficient cancer  
survivor?**

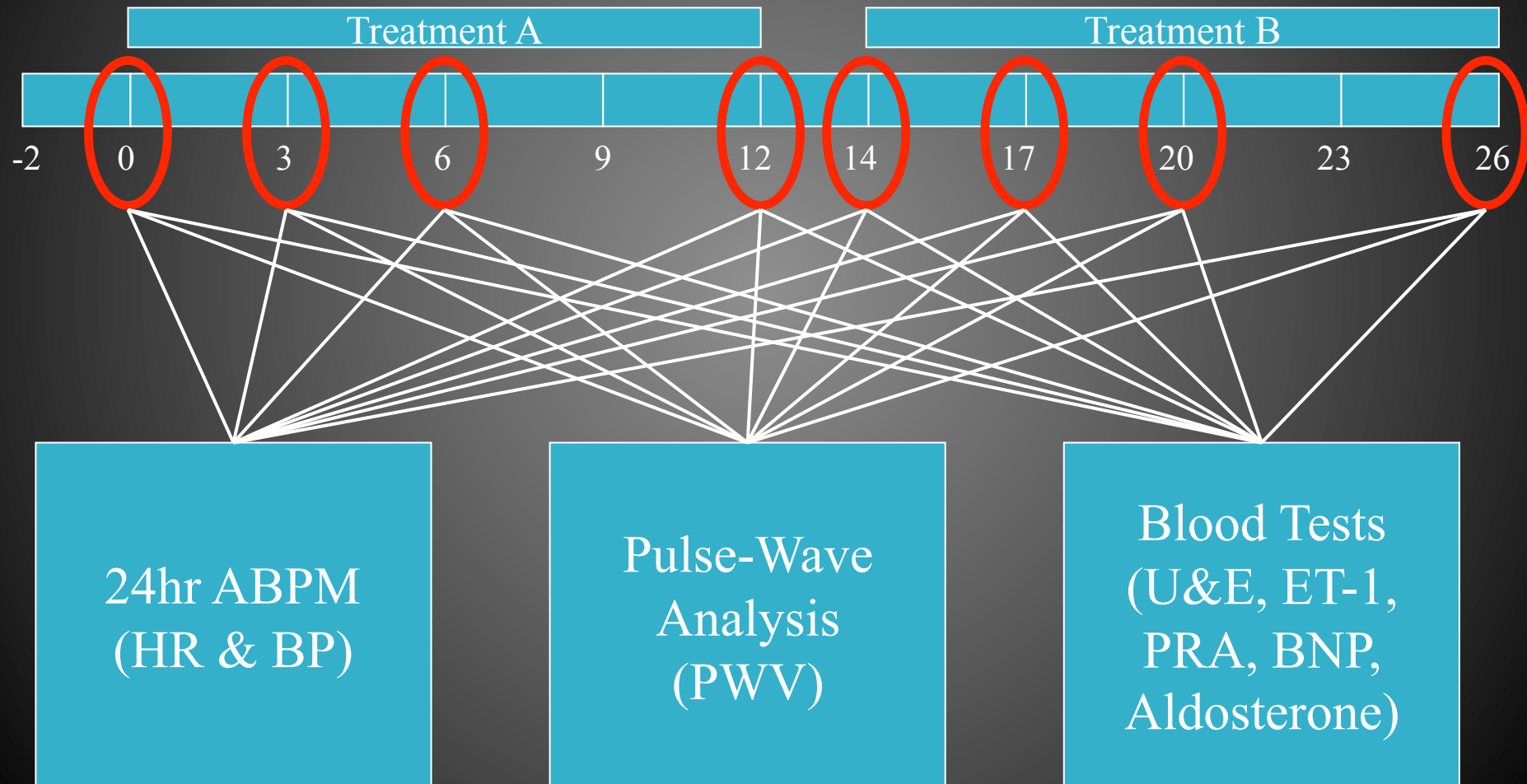
## Physiological versus standard sex steroid replacement in young women with premature ovarian failure

- Eligibility
- Documented premature ovarian failure <40 yrs.
- Aim:
- To establish whether we can improve skeletal, cardiovascular and uterine health with a physiological regimen of SSR in young women with premature ovarian failure due to different causes.

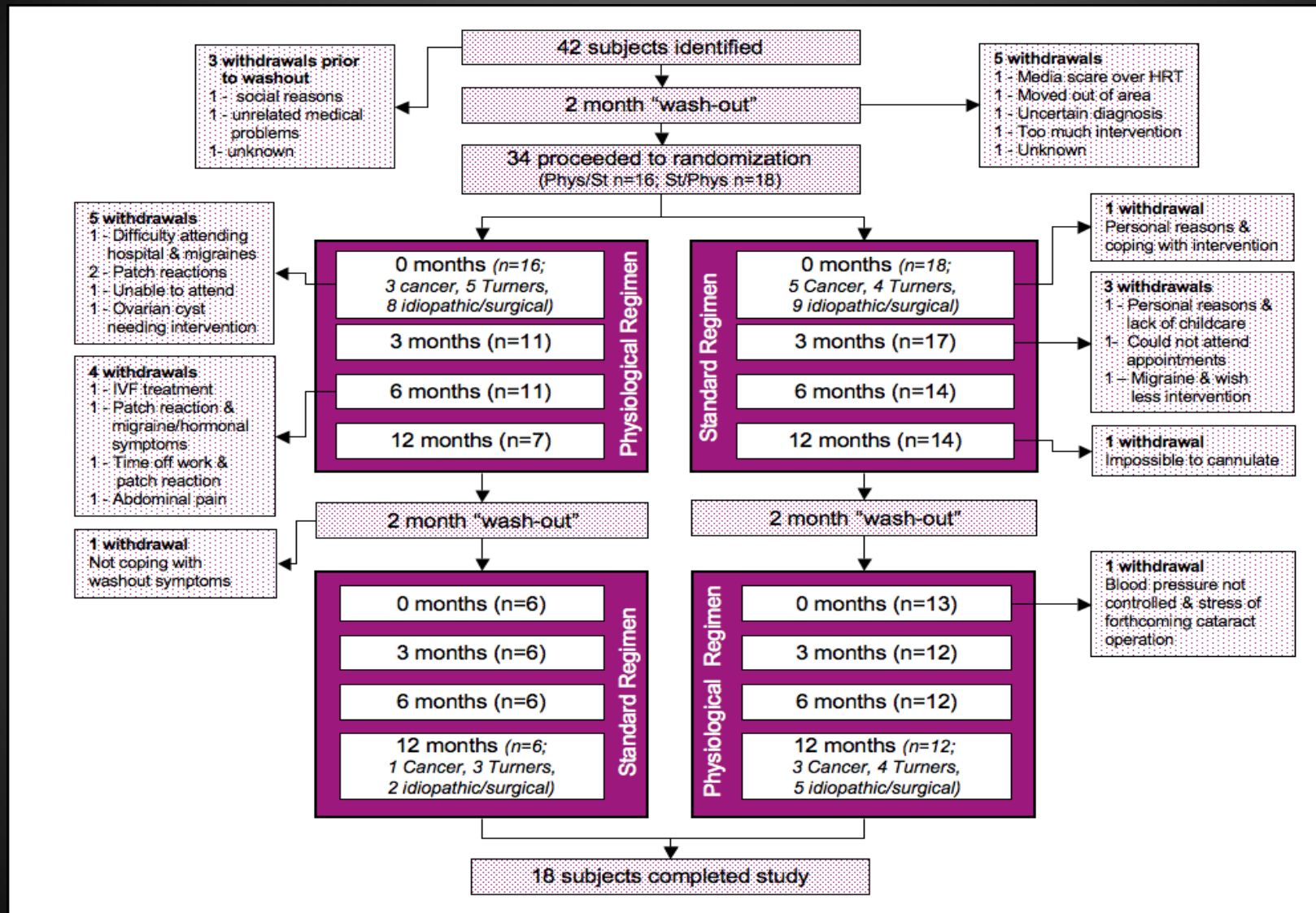
# Sex steroid replacement

- Physiological
- transdermal Oestradiol, 100 $\mu$ g/24h week 1, 150  $\mu$ g/24h weeks 2-4; vaginal progesterone, 200 mg/12 hourly weeks 3-4)
- Standard
- Loestrin 30: (30  $\mu$ g/24h ethinyloestradiol + 1.5mg/24h norethisterone acetate weeks 1-3, week 4 tablet-free)

# Study Design



# Consort flow chart of study participants



	Physiologic / Standard	Standard / Physiologic
Aetiology (Turner : Cancer treatment : Idiopathic/Surgical)	3 / 1 / 2	4 / 3 / 5
Age, years (median, range)	26 (20-34)	24 (19-39)
Height, cm (median, range)	158 (149-163)	162 (148-175)
Weight, kg (median, range)	55 (46-105)	70 (51-102)
BMI, kg/m <sup>2</sup> (median, range)	24 (19-40)	27 (21-37)
24-hr mean systolic blood pressure, mmHg	114 (109-119)	114 (107-121)
24-hr mean diastolic blood pressure, mmHg	70 (68-71)	72 (67-77)
Blood urea nitrogen, mg/dL	13.2 (10.1-16.5)	12.9 (10.4-15.1)
Serum creatinine concentration, mg/dL	0.78 (0.70-0.88)	0.94 (0.83-1.04)
Plasma renin activity, ng/mL/hr	1.9 (1.3-2.5)	2.4 (0.8-4.0)
Plasma angiotensin II concentration, pg/mL	9.6 (6.4-12.8)	11.8 (4.2-19.5)

**Table 1.**

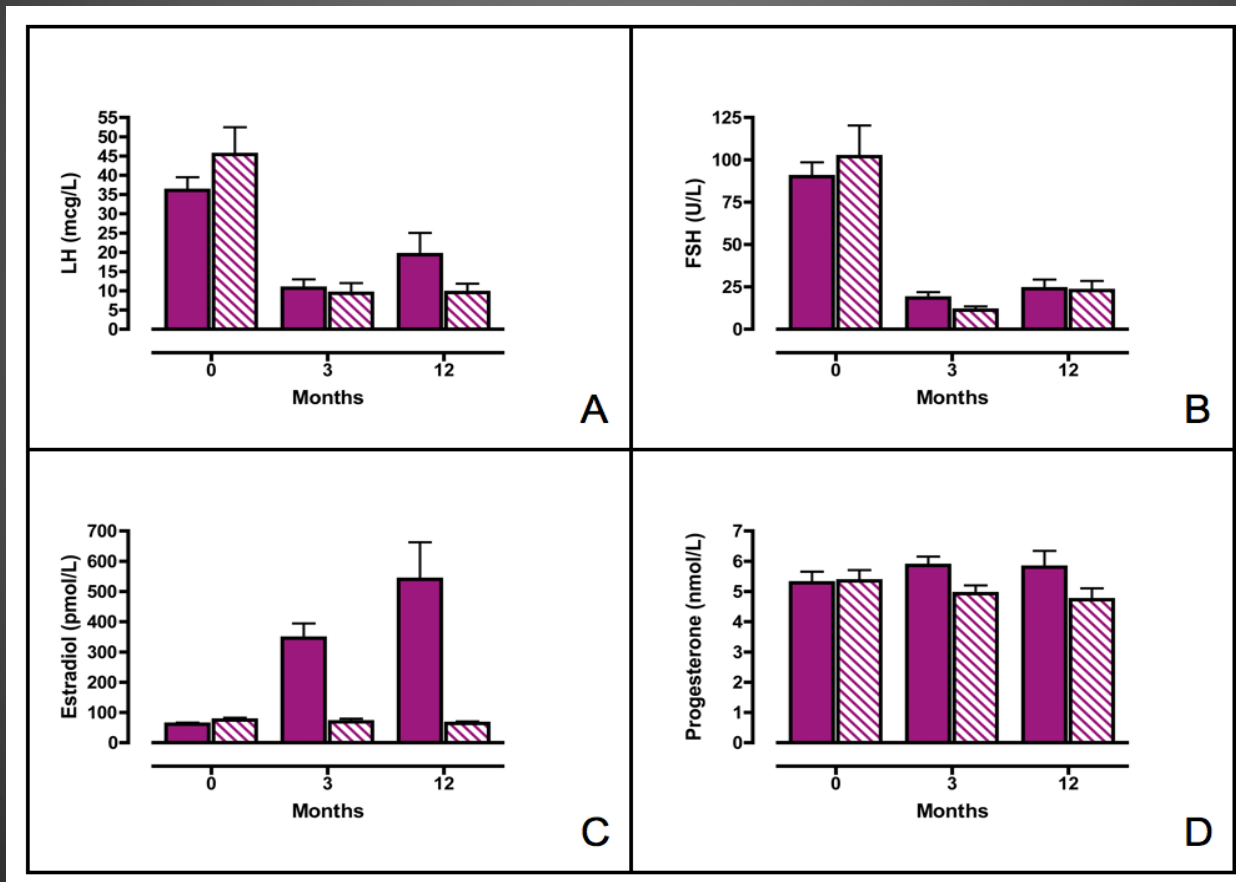
Baseline characteristics of study participants at study entry by treatment order. Mean (95% confidence intervals). Data for 24-hour blood pressure, N=17; renal and humoral markers, N=13.



## Baseline characteristics of subjects who completed the study compared with subjects who subsequently withdrew from the study

Characteristic	Completed	Withdrew
	n = 18	n = 17
Aetiology of ovarian failure: Turner's / Childhood cancer / Adult-acquired	7 / 4 / 7	2 / 4 / 11
Age, years	27 (23 – 30)	31 <sup>a</sup> (28 – 34)
Height, cm	160 (156 – 164)	162 (157 – 168)
Weight, kg	71.4 (62.3 – 80.4)	72.3 (62.7 – 81.9)
BMI, kg/m <sup>2</sup>	27.7 (24.8 – 30.7)	27.1 (24.4 – 29.9)
Treatment order: pSSR-sHRT / sHRT-pSSR	6 / 12	11 / 6

# Hormone levels after first wash-out and during pSSR and sHRT



## Changes in BMD in response to physiological SSR and standard HRT

BMD measurement	pSSR	sHRT
Lumbar spine aBMD z-score	+0.17 <sup>a</sup> (+0.07 to +0.27)	+0.07 (-0.03 to +0.18)
Lumbar spine trabecular vBMD z-score	+0.02 (-0.19 to +0.22)	+0.04 (-0.10 to +0.18)
Femoral neck BMD z-score	+0.12 (-0.05 to +0.29)	+0.11 (-0.04 to +0.25)
Total Hip BMD z-score	-0.04 (-0.16 to +0.08)	0.03 (-0.08 to +0.13)

Data are expressed as mean (95% CI mean)

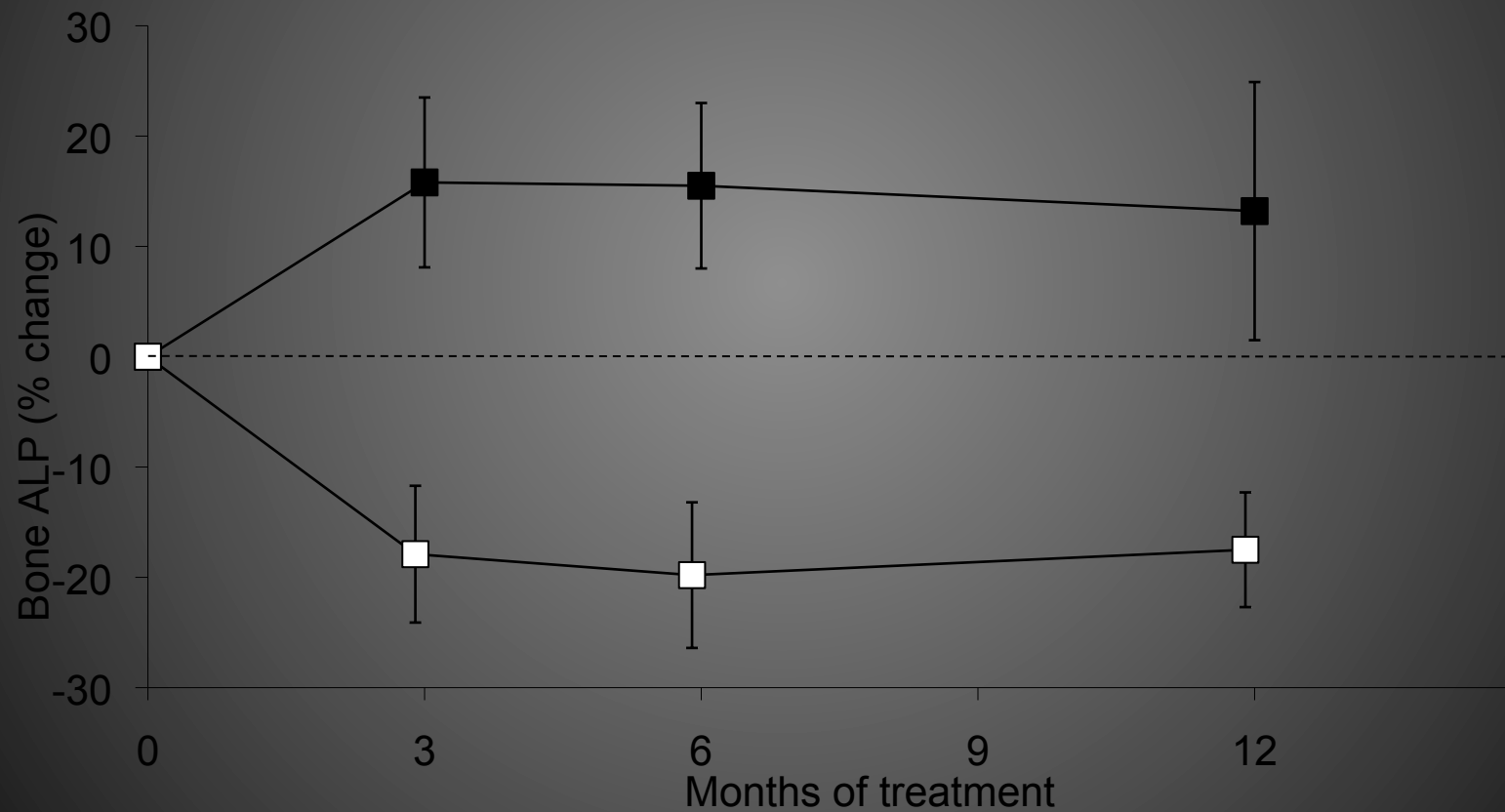
<sup>a</sup> P <0.01 versus baseline aBMD z score

Crofton et al. Clin Endo (in press)

Percentage changes in bone markers compared with post wash-out baseline in response to pSSR (solid squares) and - HRT (open squares).

### Bone ALP

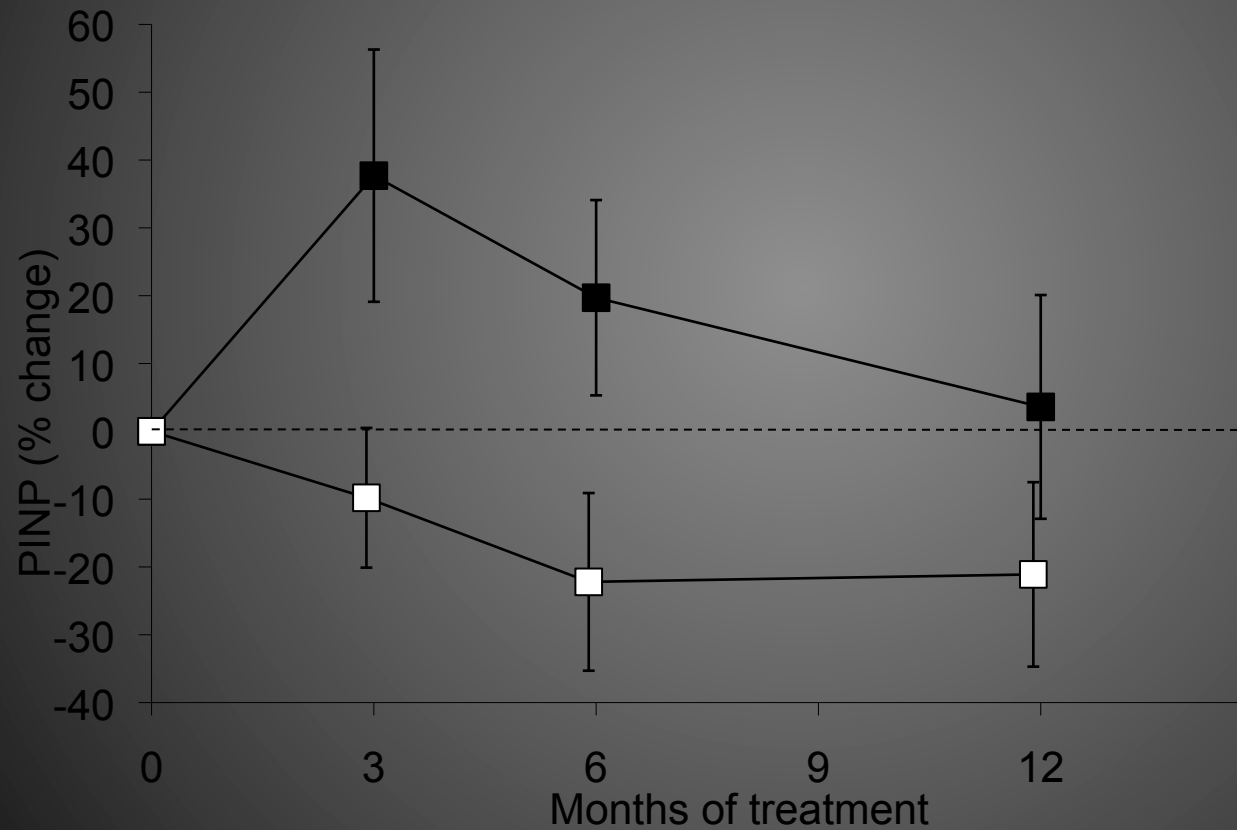
Data are expressed as mean (95% CI).



Percentage changes in bone markers compared with post wash-out baseline in response to pSSR (solid squares) and s HRT (open squares).

PINP .

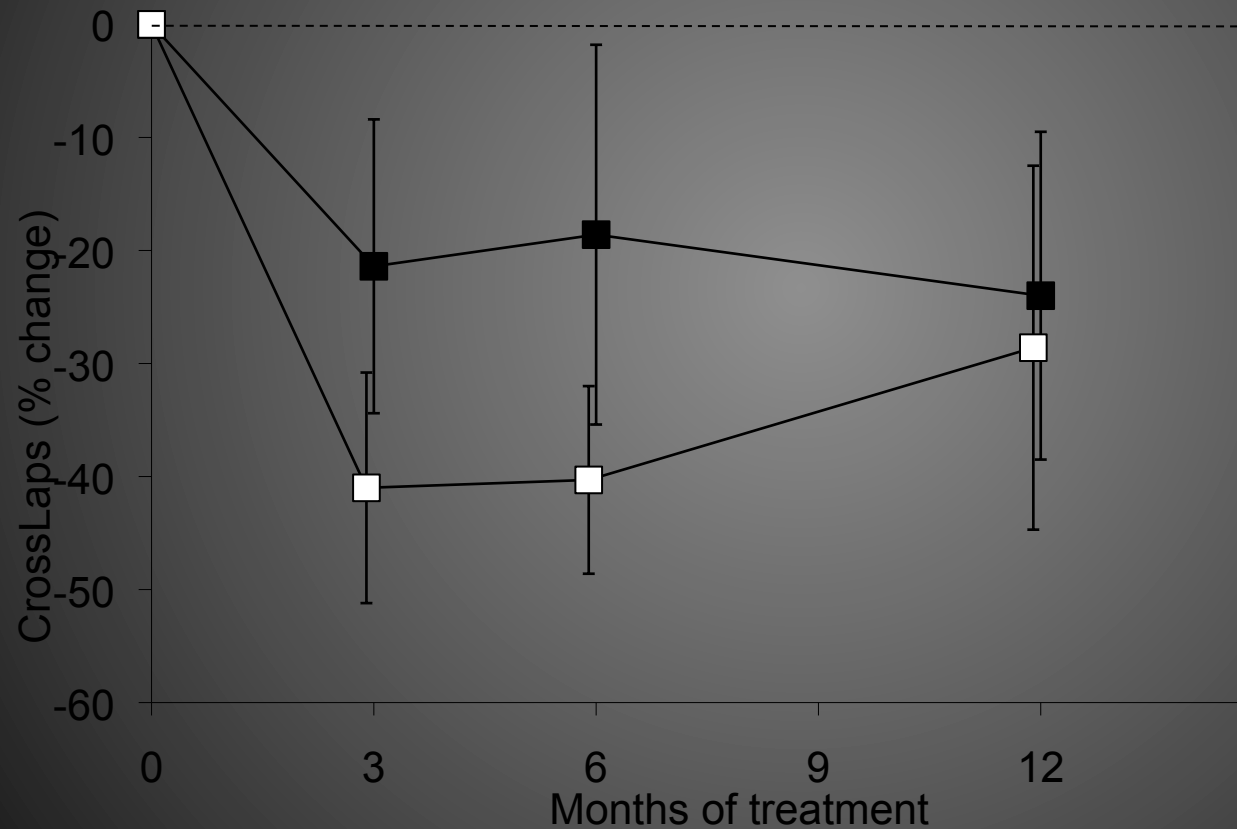
Data are expressed as mean (95% CI).



Percentage changes in bone markers compared with post wash-out baseline in response to pSSR (solid squares) and s HRT (open squares).

CrossLaps.

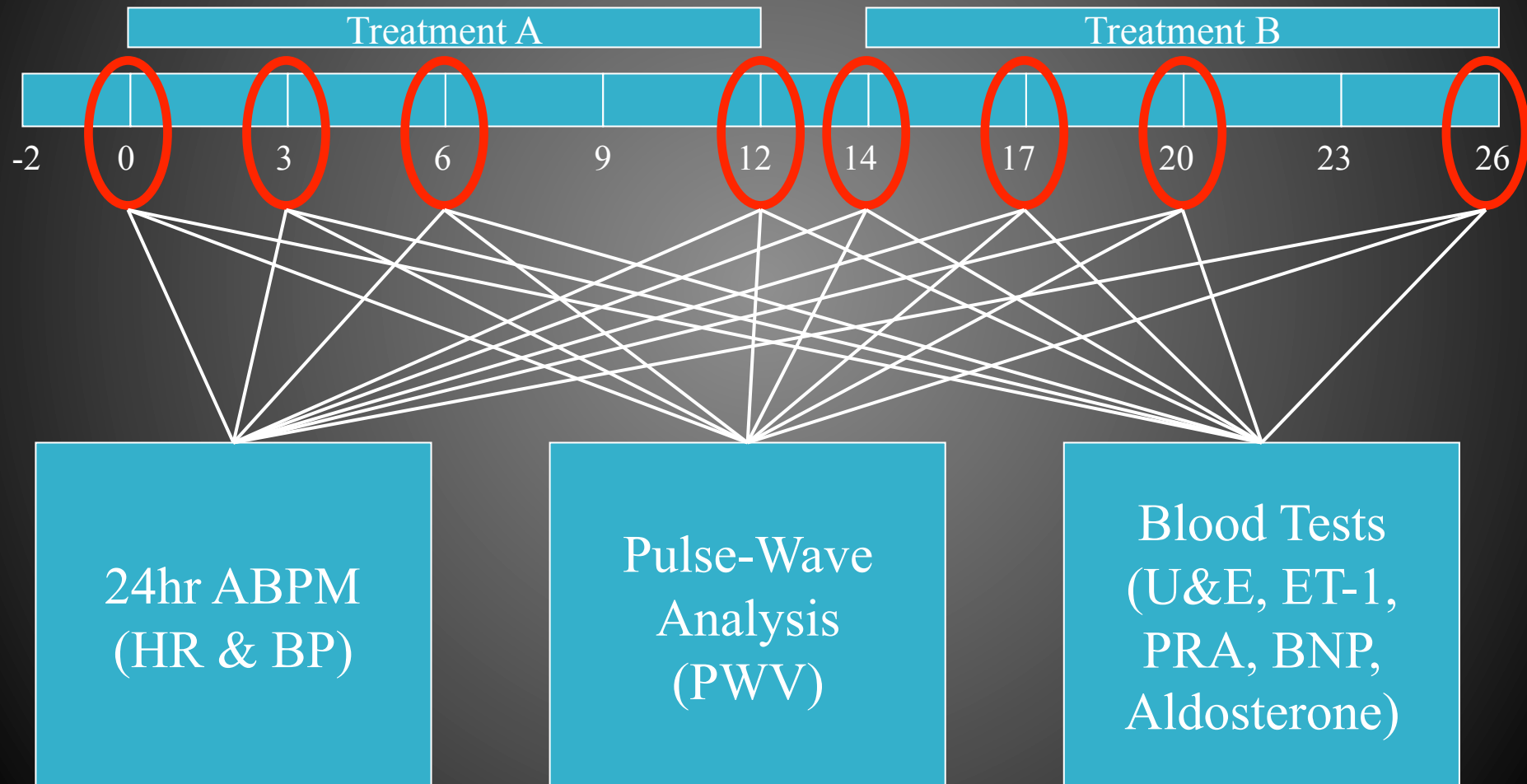
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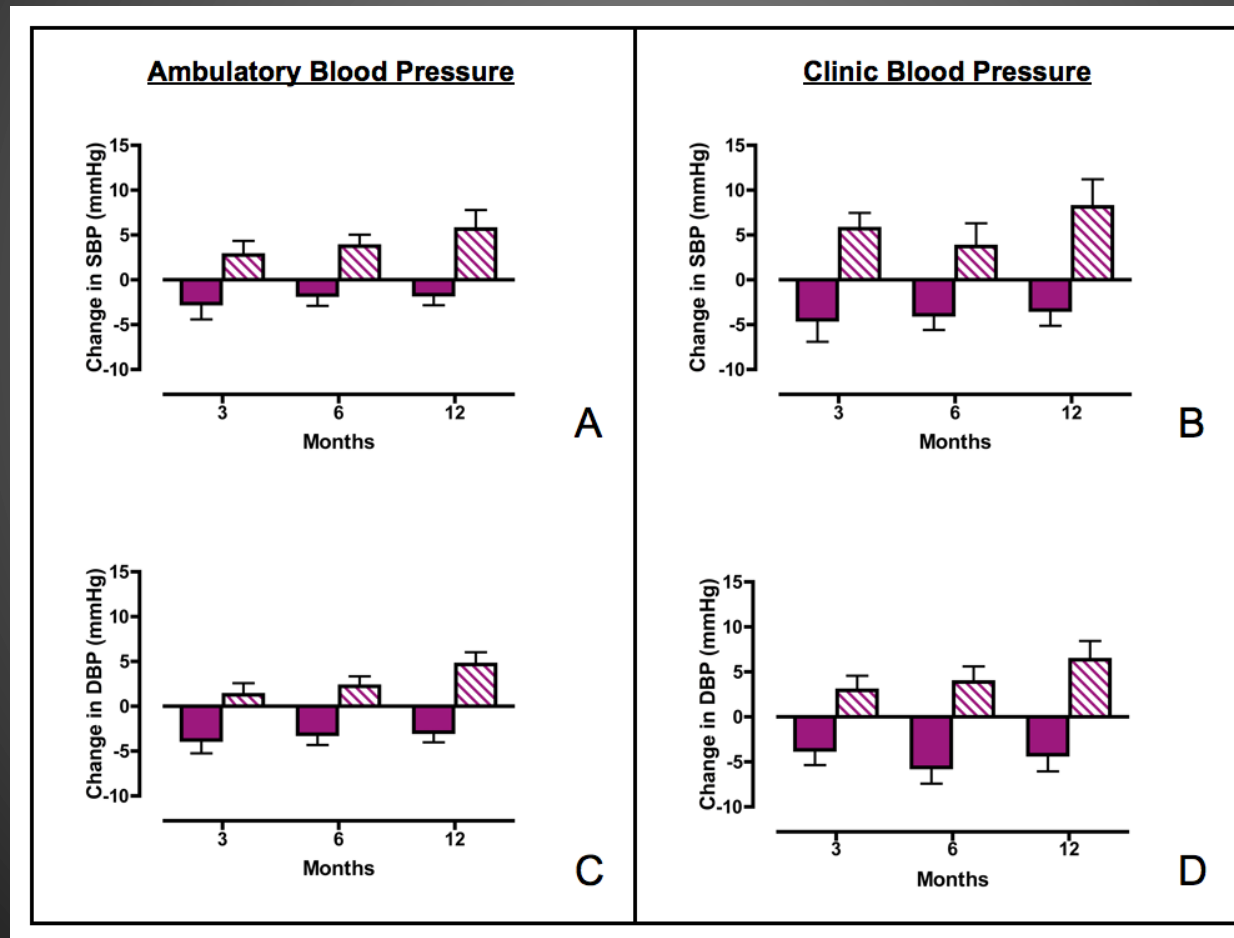


# Haemodynamic Changes with Hormone Replacement

# Study Design



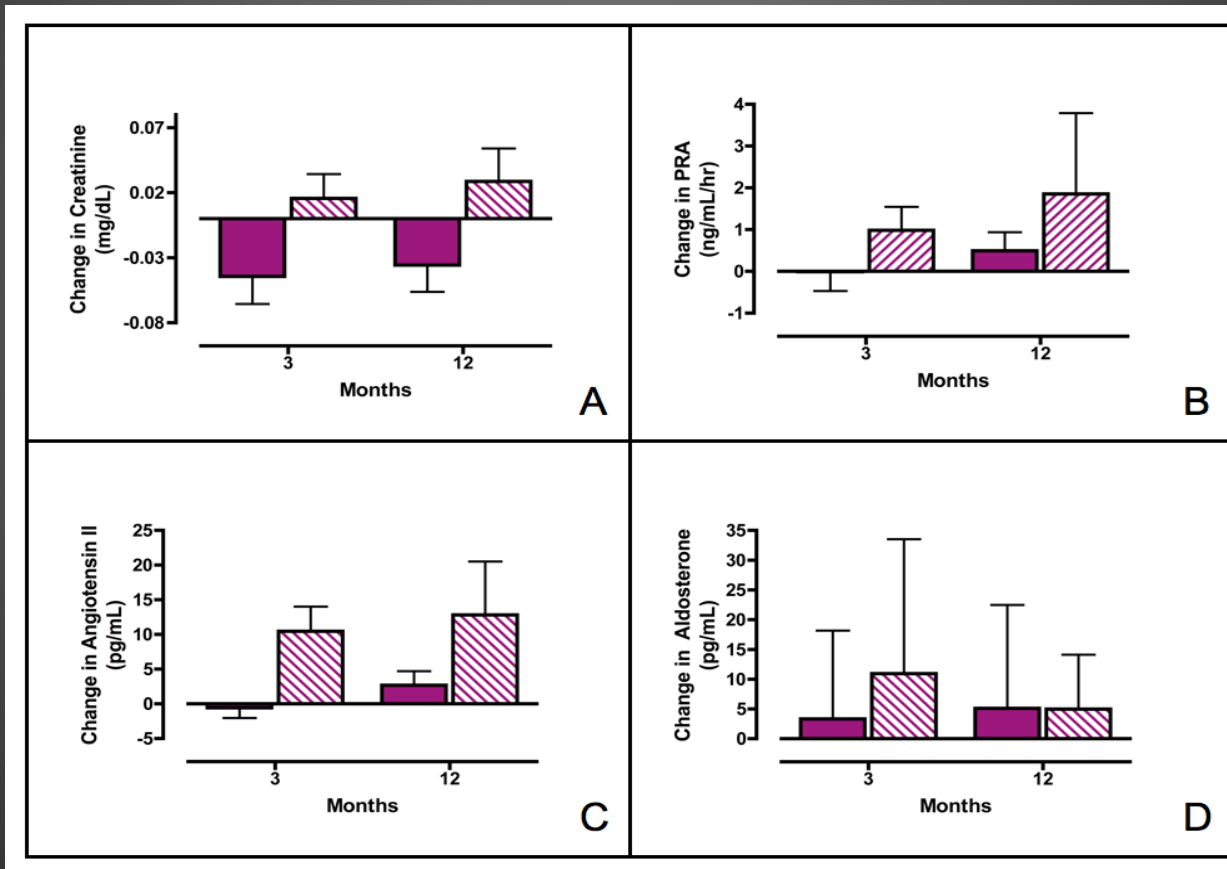
# Blood pressure changes



# Blood Pressure Changes

- In Summary
- At 12 months: 7.3mmHg mean 24-hour Systolic BP and 7.4 mmHg diastolic BP benefit with PSSR
- Langrish et al. Hypertension. 2009;53:805-11

# Renal and Humoral Factors



# Acknowledgements

- *Crofton PM,*
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- *Newby D,*
- *Webb D*
- Richard Anderson
- David Baird
- Tom Kelsey
- CLIC Sargent



Thank you



# Human ovarian tissue cryopreservation: published experience

- Of 51 women assessed, Ovarian tissue harvested in 31.
  - Age 17.9 (2.7-34)yrs
    - Laparoscopy (n=29), laparotomy (n=2); Whole ovary in 24, in 7 approx half.
- Primordial follicles:
  - <7yrs: 20/mm<sup>2</sup> (n=6)
  - 10-15 yrs: 4/mm<sup>2</sup> (n=8)
  - >15yrs: 1.6/mm<sup>2</sup> (n=17)
- Only 9 had **not** received previous CT
- 11 died (7 under 18 yrs); 8 lost to follow up.
- No data on ovarian function of 12 remaining patients

Poirot et al. Human Reproduction 2002, 17:1447-1452

'I haven't had a girlfriend since I was diagnosed...I think if I did get a girlfriend, having to tell them that I'm not going to be able to have kids or anything, that's going to be a bit of a shock to them isn't it, so...Relationships, yeh, that's affected me getting in a relationship, getting the confidence....'

## Baseline anthropometry and BMD according to aetiology of ovarian failure

	<u>Turner</u>	<u>Childhood cancer</u>	<u>Adult-acquired</u>
<u>N</u>	<u>9</u>	<u>8</u>	<u>18</u>
<u>Age (y)</u>	<u>22</u> (20 – 24)	<u>31</u> (27 – 36)	<u>31</u> (28 – 34)
<u>Height (cm)</u>	<u>152</u> (148 – 157)	<u>162</u> (155 – 169)	<u>165</u> (161 – 169)
<u>Weight (kg)</u>	<u>63.4</u> (49.9 – 76.8)	<u>64.4</u> (53.4 – 75.4)	<u>79.3</u> (70.4 – 88.2)
<u>Lumbar spine aBMD z score</u>	-1.13 (-1.90 to -0.37)	-0.46 (-1.19 to +0.26)	-0.16 (-0.77 to +0.45)
<u>Femoral neck aBMD z score</u>	-0.84 (-1.81 to +0.12)	-0.46 (-1.07 to +0.14)	+0.16 (-0.48 to +0.80)
<u>Total hip aBMD z score</u>	-0.62 (-1.52 to +0.27)	-0.38 (-1.16 to +0.41)	+0.21 (-0.32 to +0.75)
<u>Lumbar spine trabecular vBMD z score</u>	<u>-0.29</u> (-0.97 to +0.39)	<u>+0.23</u> (-0.76 to +1.22)	<u>+0.10</u> (-0.31 to +0.51)

Data are expressed as mean (95% CI mean).