FERTILITY PRESERVATION STRATEGIES IN PEDIATRIC CANCER PATIENTS

OZGUR OKTEM, MD
Koc University School of Medicine
Department of Obstetrics and Gynecology
Division Reproductive Endocrinology and Infertility
American Hospital Women's Health Center Assisted Reproduction Unit
Istanbul TURKEY

- No conflict of interest
- Nothing to declare

FACTS ABOUT CANCER

1

FEMALE CANCER

Breast	182,460	26%
Lung & bronchus	100,330	14%
Colon & rectum	71,560	10%
Uterine corpus	40,100	6%
Non-Hodgkin lymphoma	30,670	4%
Thyroid	28,410	4%
Melanoma of the skin	27,530	4%
Ovary	21,650	3%
Kidney & renal pelvis	21,260	3%
Leukemia	19,090	3%
All Sites	**692,000**	**100%**

2

Survival rates of childhood and adult cancers have INCREASED with modern multi-agent chemo/ radiotherapy regimens

Childhood cancers

Adult cancers

5-year survival rates (%)

1974	2010
58% →	81%
50% →	66%

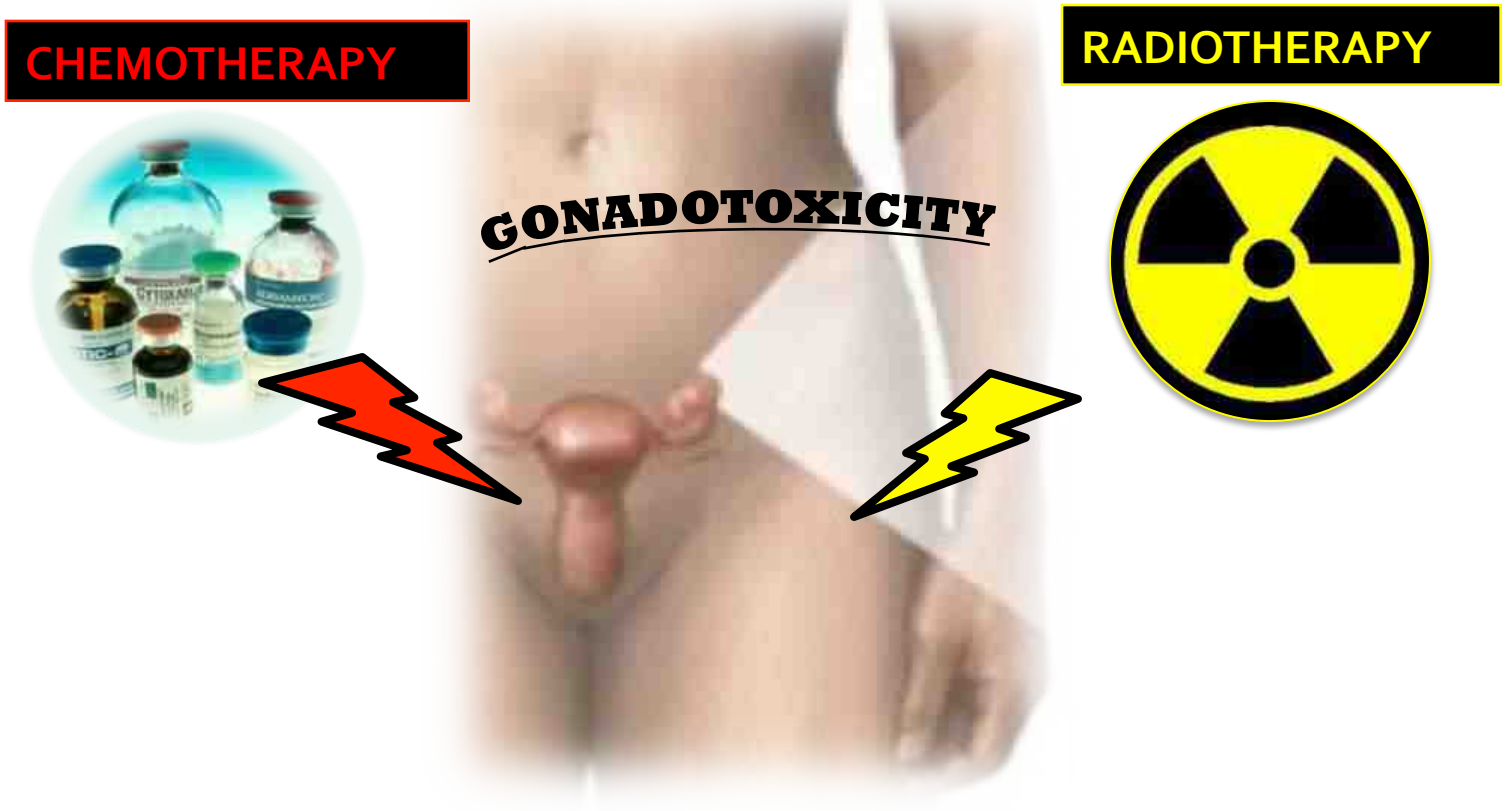
Jemal et al. CA Cancer J Clin 2013

THE MOST COMMON CANCERS IN THE PEDIATRIC AGE GROUP (NATIONAL STATISTICS 2008-2012 per 100,000)

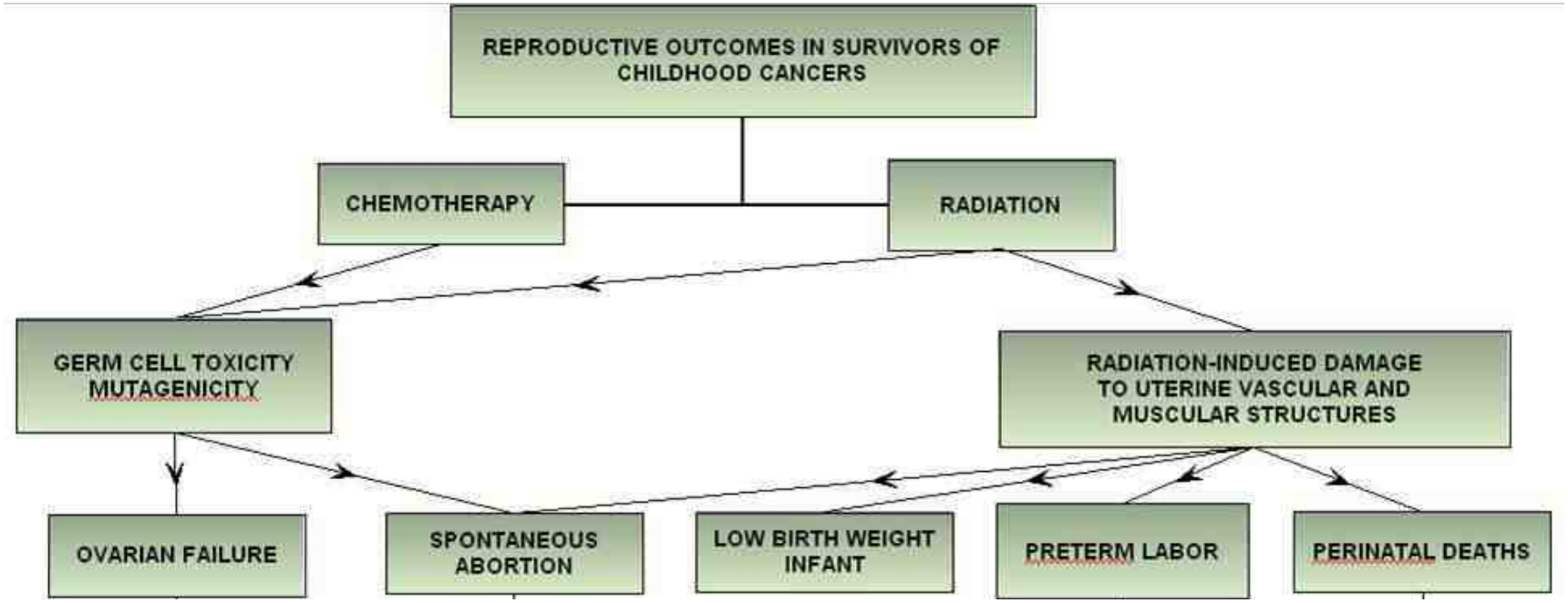
1. Leukemia-Lymphoma (7)
2. CNS tumors (2.1)
3. Urinary system tumors (1.9)
4. Sof tissue sarcomas (1.7)
5. Endocrine system tumors (1.6)
6. Eye (1.2)

QUALITY OF LIFE ISSUES IN CANCER SURVIVORS

FERTILITY PRESERVATION

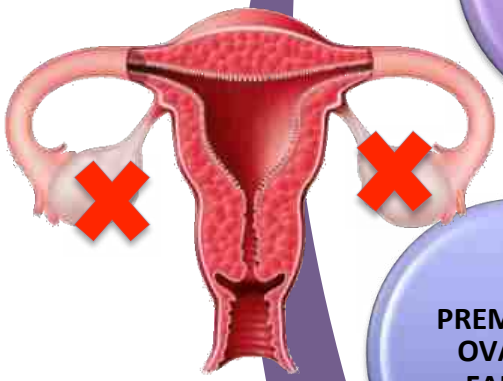


Adult survivors of childhood cancers



Oktem et al Ann N Y Acad Sci 2008
Oktem et al Pediatr Blood Cancer 2009
Oktem et at. Obstet Gynecol Surv 2010

PRE AND POSTPUBERTAL EXPOSURE MAINLY DUE TO <u>OVARIAN</u> DAMAGE INDUCED BY BOTH CHEMO AND RADIATION



CHEMOTHERAPY
RADIATION

INFERTILITY

PREMATURE OVARIAN FAILURE

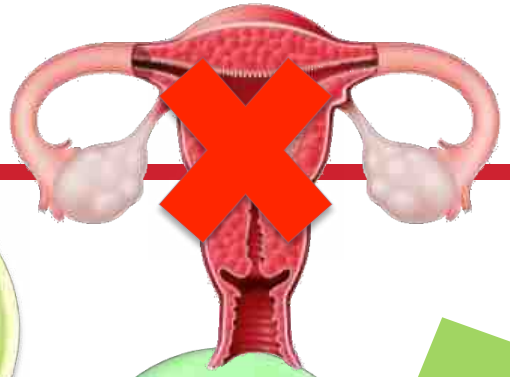
FETAL GROWTH RESTRICTION

INCREASED BREECH

PLACENTATION ABNORMALITIES

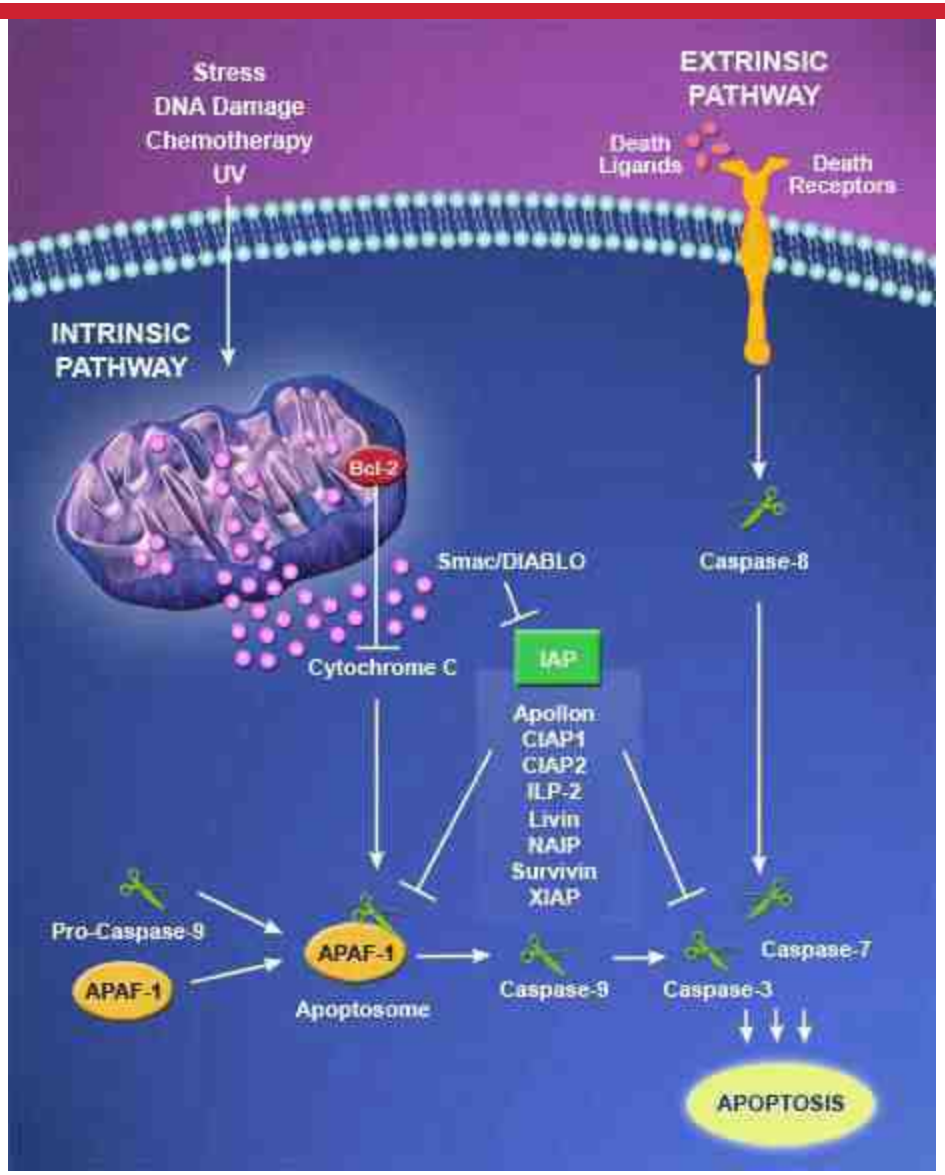
ABORTION

PRETERM BIRTH

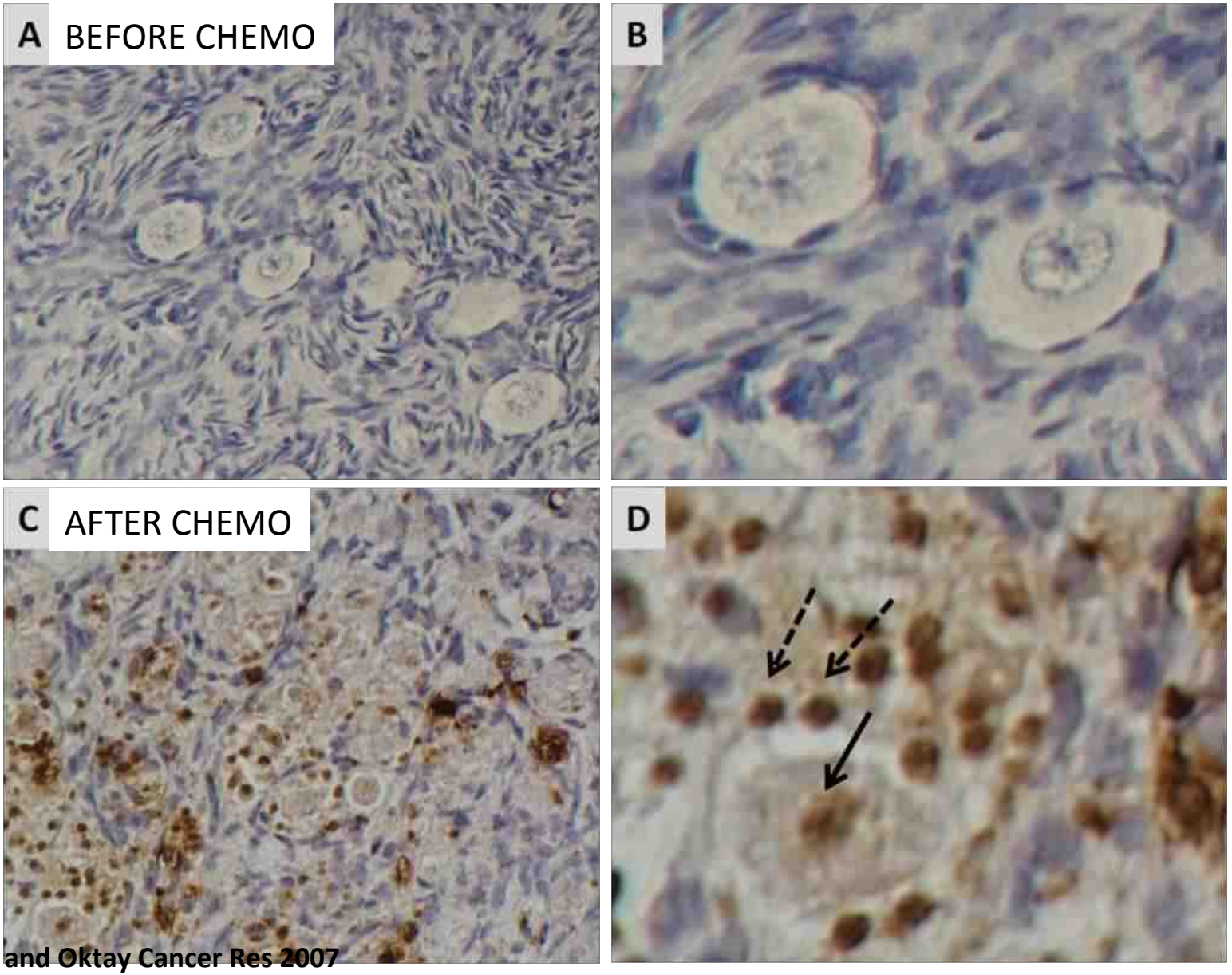


PREPUBERTAL EXPOSURE MAINLY DUE TO <u>UTERINE</u> DAMAGE INDUCED BY RADIATION

Chemotherapy induced cytotoxicity

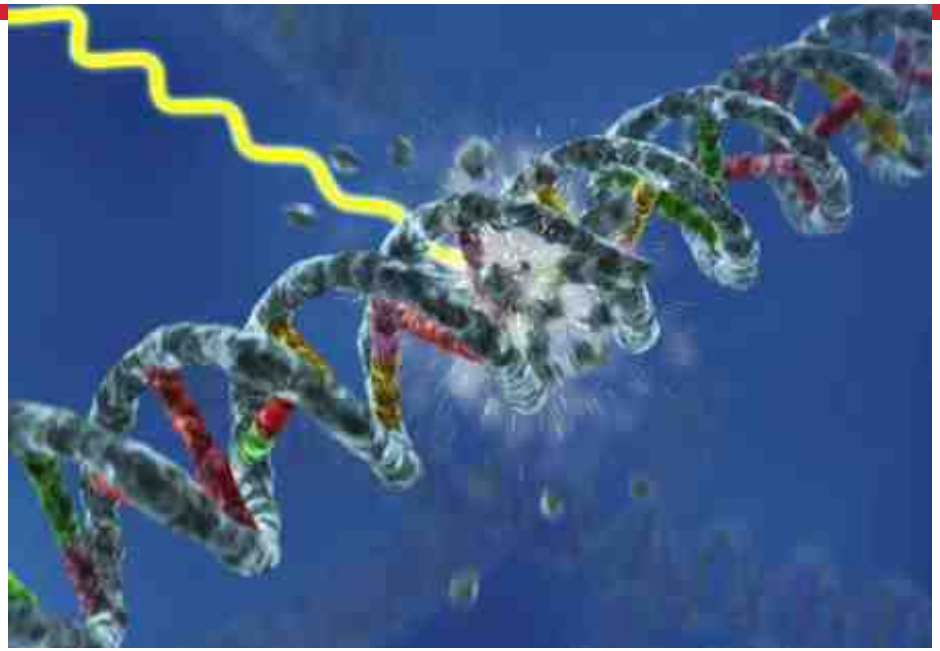


APOPTOTIC OOCYTE DEATH AFTER CHEMOTHERAPY (TUNNEL STAINING)



Oktem and Oktay Cancer Res 2007

RADIATION and GONADOTOXICITY



- **DAMAGE TO DNA.**
  as neutrons and particles

- Indirect actions due to formation of free radicals and DNA damage. This mechanism is particularly true for sparsely ionizing radiation such as x-rays.

The higher the dose of radiation

The higher the risk of premature ovarian failure !

Single dose is more toxic than fractionated dose.

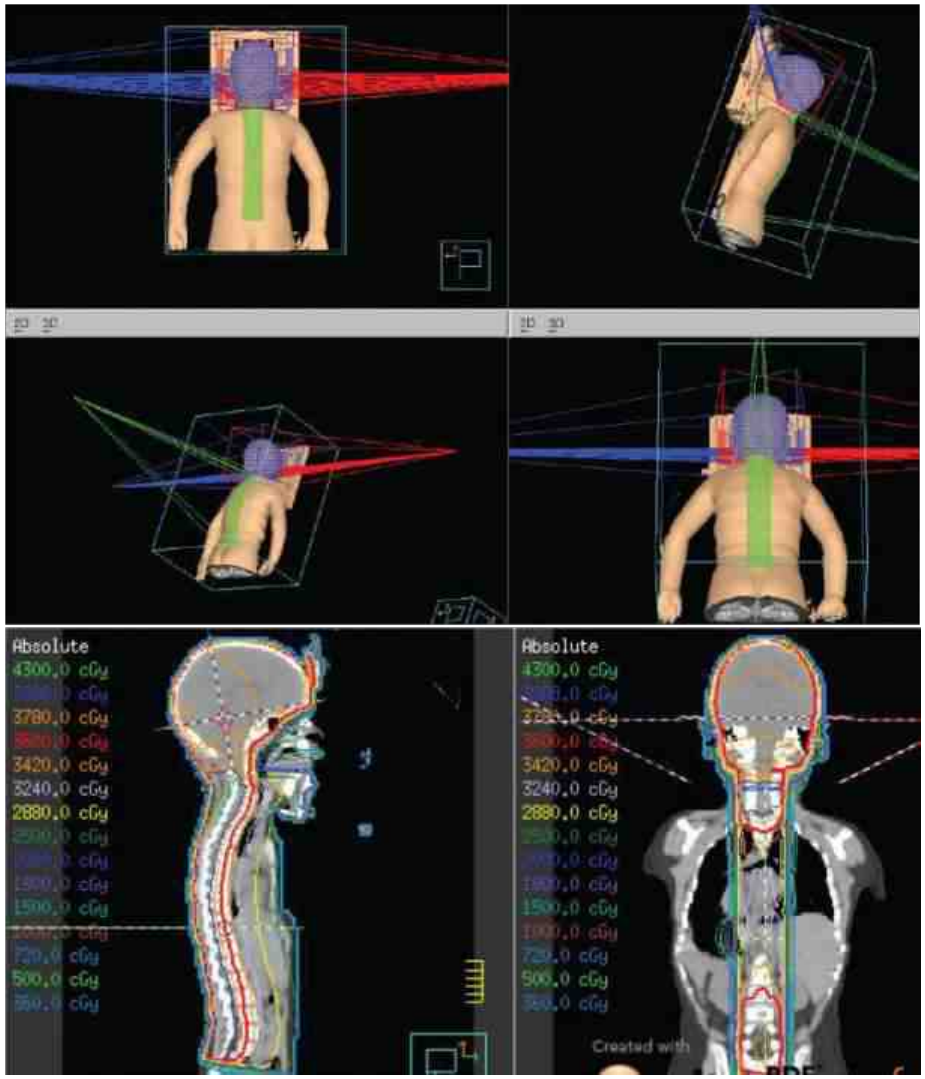
The LD_{50} of the human oocytes may be 1.99 Gy*;less than the previously thought (4 Gy)**

100cGy=1Gy=100 Rad

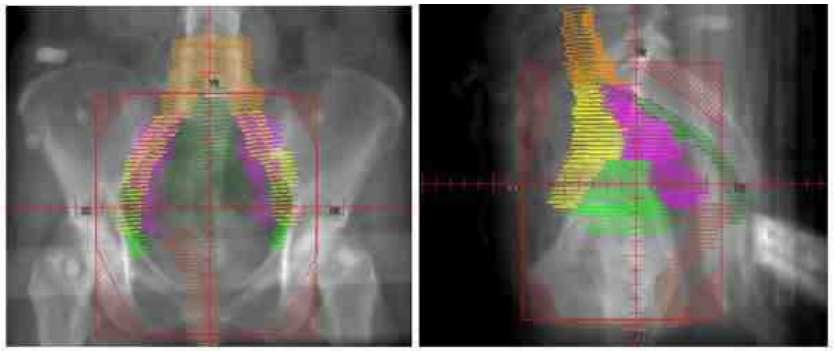
HSCT

- **TBI**
  - **20-30 Gy⇒37/38 Ovarian failure**
- **TBI + Cyc**
  - **OR:~1 (1 yıl)**
  - **135/144 patients have POF**

IRRADIATION FIELDS



PELVIC RADIATION

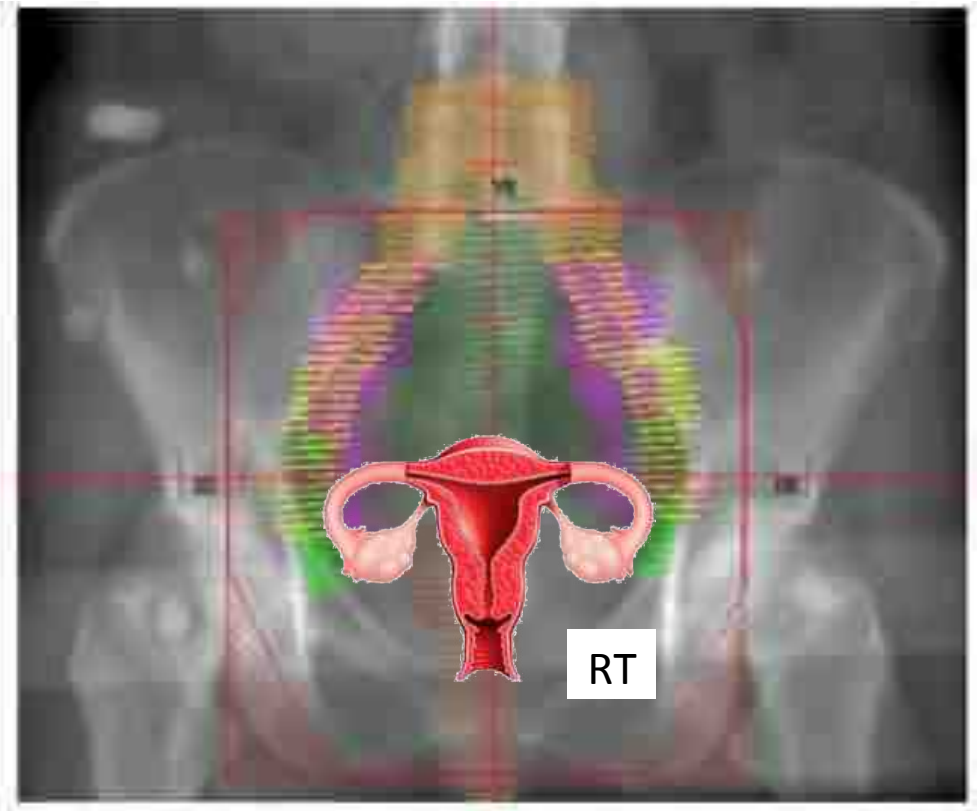


Pelvic radiotherapy (women: adjusted OR 20.24, 95 % CI 4.69–87.29; men: 12.22; 1.18–126.70) than those who were 'fertile/ probably fertile'.

Radiosensitizer → Etoposide, particularly ≥5,000 mg/m^2 in women, and carboplatin and/or cisplatin in both sexes seemed to have independent risk potential for infertility.

The FeCt—survey of childhood **cancer survivors in Germany**
J Cancer Res Clin Oncol (2013) 139:2071–2078

The uterine impact of RT



- Pelvic
- Total body
- Whole abdomen
- Craniospinal

Uterus

- The threshold radiation dose for uterine damage to occur such that pregnancy is not sustainable is unknown.
- To our knowledge no successful pregnancy has been reported after a direct radical dose (>45 Gy) to the whole pelvis.
- It appears that younger age at uterine radiation leads to greater adverse effects on uterine reproductive capacity, particularly in prepubertal girls.
- The radiation-induced uterine injury is also dose and site dependent and with more severe uterine damage occurs with higher dose radiation and radiation directly involving the uterus.

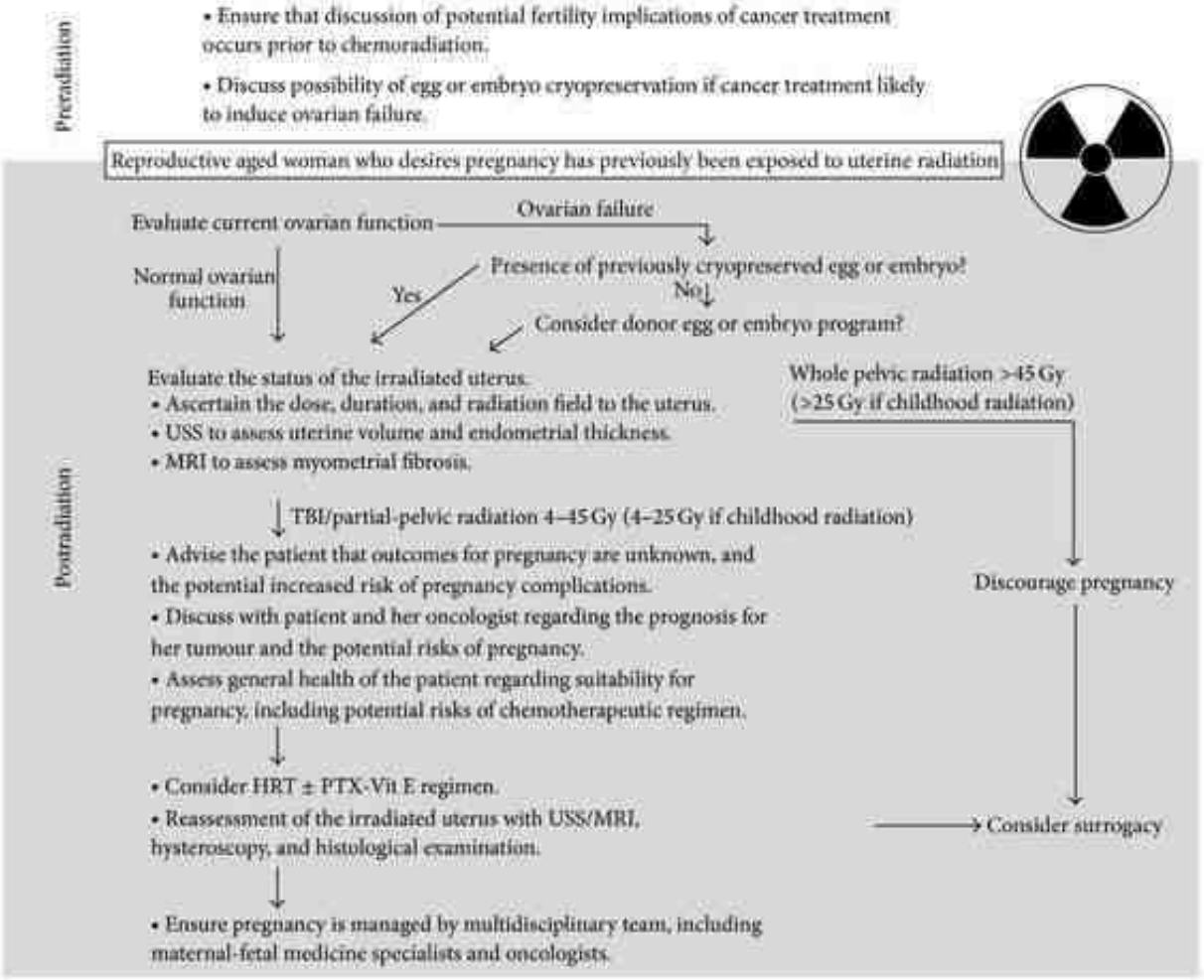
Uterus

- Radiation doses of >25 Gy directly to the uterus in childhood appears to induce irreversible damage.
- Exposure of adult uterus to TBI (12 Gy) is associated with increased risk of miscarriage, preterm labour, and low birth weight babies.
- The mechanisms of impaired uterine function following radiotherapy are not clearly defined; however, impaired uterine blood supply, defective endometrial function, and poor uterine distensibility have all been implicated.

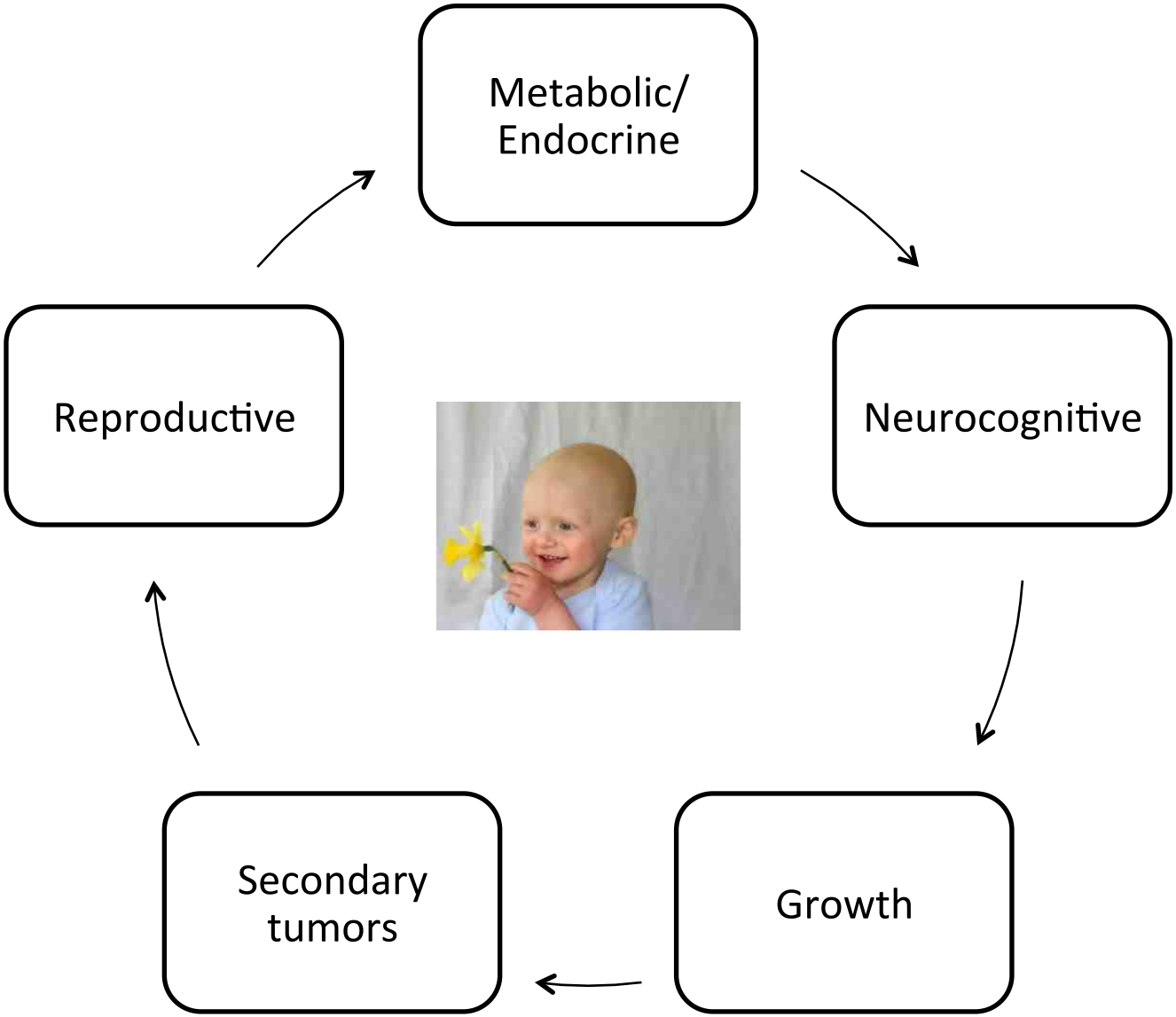
Uterus

- It has been suggested that the uterine damage from radiation is related to
  - damage to the endometrium, therefore impairing normal decidualization and interference with placentation.
  - damage to the uterine vasculature and impairment of future trophoblast invasion, which ultimately can decrease fetal-placental blood flow causing fetal growth restriction.
  - development of myometrial fibrosis which reduces uterine elasticity and volume. This can lead to preterm labour and delivery.

Uterus


Preradiation
• Ensure that discussion of potential fertility implications of cancer treatment occurs prior to chemoradiation.
• Discuss possibility of egg or embryo cryopreservation if cancer treatment likely to induce ovarian failure.
Reproductive aged woman who desires pregnancy has previously been exposed to uterine radiation
Evaluate current ovarian function
Ovarian failure
Normal ovarian function
Yes
Presence of previously cryopreserved egg or embryo?
No
Consider donor egg or embryo program?
Postradiation
Evaluate the status of the irradiated uterus.
• Ascertain the dose, duration, and radiation field to the uterus.
• USS to assess uterine volume and endometrial thickness.
• MRI to assess myometrial fibrosis.
Whole pelvic radiation >45 Gy (>25 Gy if childhood radiation)
TBI/partial-pelvic radiation 4–45 Gy (4–25 Gy if childhood radiation)
• Advise the patient that outcomes for pregnancy are unknown, and the potential increased risk of pregnancy complications.
• Discuss with patient and her oncologist regarding the prognosis for her tumour and the potential risks of pregnancy.
• Assess general health of the patient regarding suitability for pregnancy, including potential risks of chemotherapeutic regimen.
Discourage pregnancy
• Consider HRT ± PTX-Vit E regimen.
• Reassessment of the irradiated uterus with USS/MRI, hysteroscopy, and histological examination.
Consider surrogacy
• Ensure pregnancy is managed by multidisciplinary team, including maternal-fetal medicine specialists and oncologists.

Long term adverse health outcomes in the survivors of pediatric cancer patients



The Childhood Cancer Survivor Study (CCSS)

- Childhood cancer survivors diagnosed between 1970 and 1986 were identified for this long-term, retrospective cohort study from participating centers in the United States and Canada.
- More than 14,000 survivors were surveyed and followed for long-term health outcomes.
- In addition, about 4,000 of their siblings were recruited as comparison subjects.
- Due to the significant changes in therapy for children with cancer over the past 30 years, a second group of about 10,000 survivors diagnosed between 1987 and 1999 and about 1,000 of their siblings were also recruited for the study.

The Childhood Cancer Survivor Study (CCSS)

- 3531 survivors and 1366 female sibling controls
- Compared with their siblings, survivors had
  - an increased risk (relative risk [RR] 1.48 [95% CI 1.23–1.78];p<0. 0001) of clinical infertility (ie, >1 year of attempts at conception without success),
  - Increasing doses of uterine radiation and alkylating agent chemotherapy were strongly associated with infertility.
  - Although survivors had an increased time to pregnancy compared with their siblings (p=0·032), 292 (64%) of 455 participants with self-reported clinical infertility achieved a pregnancy.

Barton SE. Lancet Oncol 2013; 14: 873–81

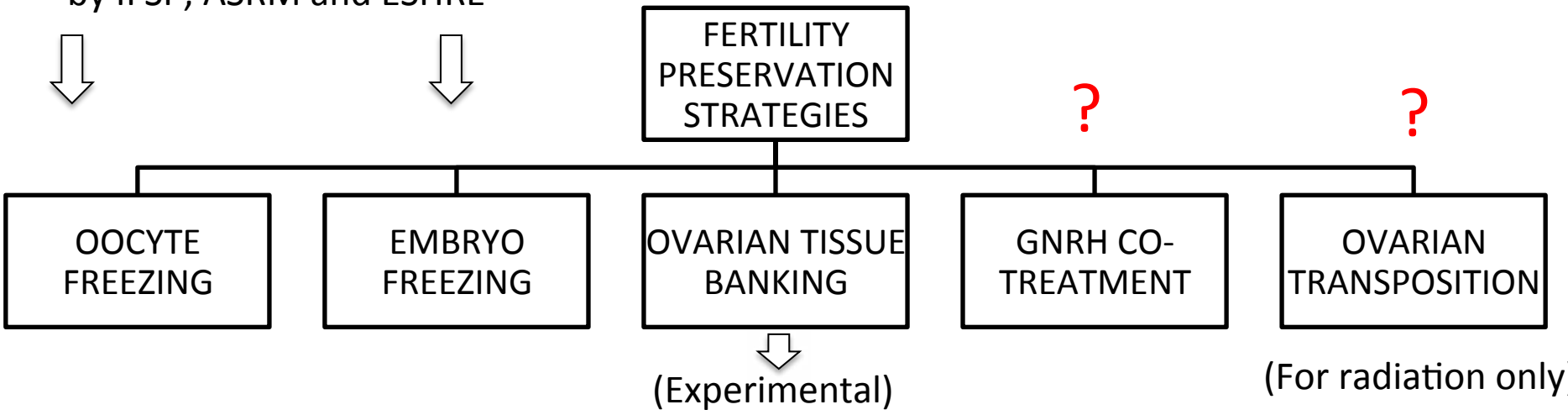
ADULT SURVIVORS OF CHILDHOOD CANCERS

- Survivors cured with minimal gonadotoxic treatment had significantly higher AMH and AFC compared with survivors cured with either potentially gonadotoxic treatment or treatment including alkylating chemotherapy and ovarian irradiation
  - (20.0, 5.8 and <3 pmol/l, $P < 0.001$; and 15, 9 and 2, $P = 0.03$, respectively).
  - Lower AMH (median 13.0 versus 17.8 pmol/l)

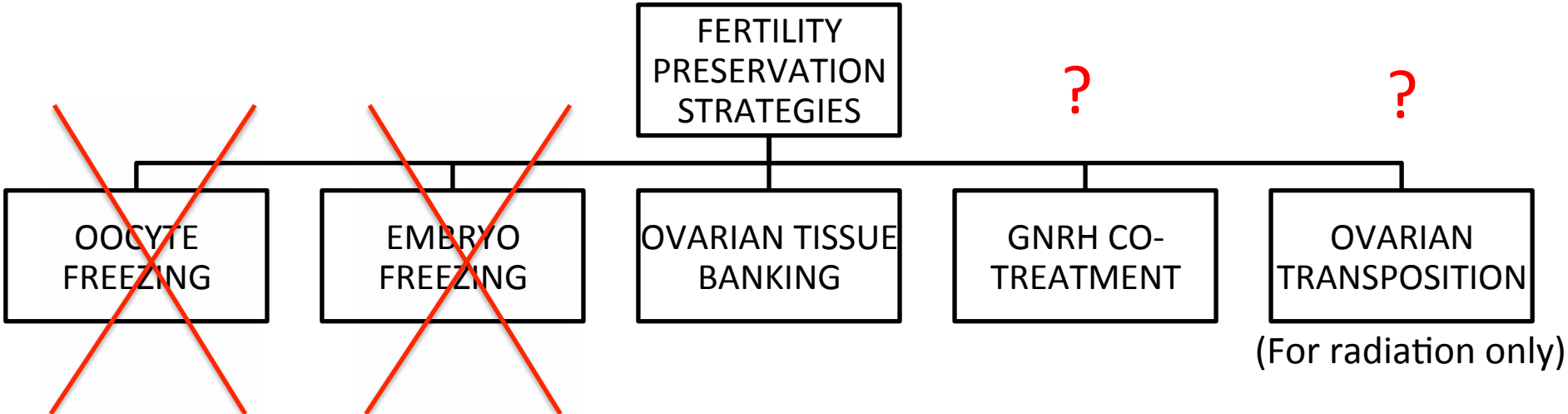
Nielsen et al. Reproductive BioMedicine Online (2013) 27, 192– 200

Considered as the only established methods by IFSP, ASRM and ESHRE

ADULTS



CHILDREN

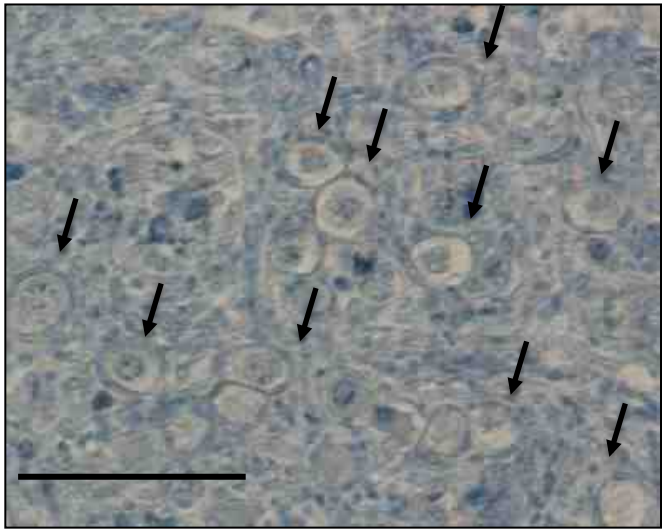


OVARIAN TISSUE BANKING TO PRESERVE FERTILITY

- **Ovarian tissue cryopreservation** is the only available option of fertility preservation in pediatric age group.
- Ovarian stimulation for oocyte/embryo freezing is not possible due to sexual immaturity.
- Ovarian tissue cryopreservation, if the risk of ovarian failure after cancer treatment is high enough to justify the procedure such as HSCT.

ISFP Practice CommitteeRecommendations for fertility preservation in patients with lymphoma, leukemia, and breast cancer. J Assist Reprod Genet. 2012 Jun;29(6):465-8.

AGE 10

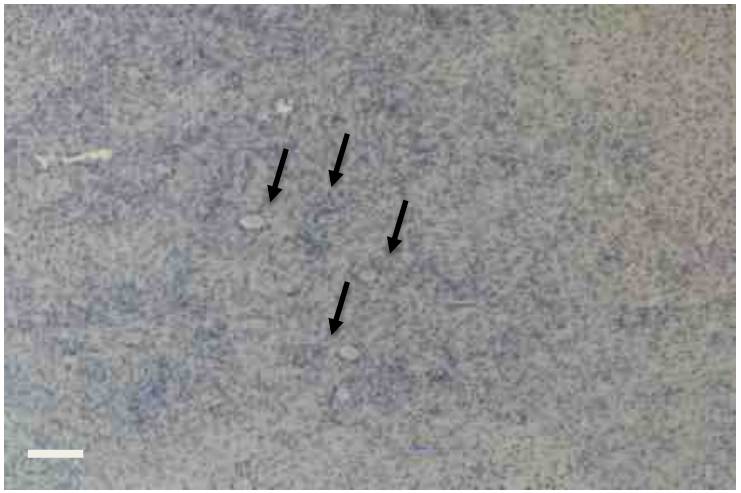


HIGHER CHANCE OF RECOVERING OVARIAN FUNCTION

HIGHER RESIDUAL OVARIAN RESERVE POST EXPOSURE TO CHEMO/RT

LOW-RISK FOR POF

AGE 37



LOWER CHANCE OF RECOVERING OVARIAN FUNCTION

LOWER RESIDUAL OVARIAN RESERVE POST EXPOSURE TO CHEMO/RT

HIGH-RISK FOR POF

CASE-1

- Age:6
- Dx: Medulloblastoma
- Tx option:
  - Cyclophosphamide+Temazolamide
  - Carboplatin, cisplatin, cyclophosphamide, etoposide, vincristine
  - Craniospinal RT:CSI doses of 23.4 Gy (for “standard risk” disease), the ovaries received approximately 1Gy and in those receiving CSI doses between 36 and 39.6 Gy (for “high risk” disease), the ovaries received approximately 2Gy.

OR

HIGH DOSE CHEMOTHERAPY PRIOR TO AUTOLOG STEM CELL RESCUE

CASE-1

- Age:6
- Dx: Medulloblastoma
- Tx option:
  - Cyclophosphamide+Temazolamide
  - Carboplatin, cisplatin, cyclophosphamide, etoposide, vincristine
  - Craniospinal RT:CSI doses of 23.4 Gy (for “standard risk” disease), the ovaries received approximately
  - 1Gy and in those receiving CSI doses between 36 and 39.6 Gy (for “high risk” disease), the ovaries received approximately 2Gy.

The rate of ovarian function is 60-100%

Balachandar et al. Pediatr Blood Cancer 2015;62:317–321

CASE-1

- Age:6
- Dx: Medulloblastoma
- Tx option:
  - High dose chemotherapy for Autolog Stemm Cell Rescue
    - etoposide,carboplatin, thiotepa or,
    - carboplatin and thiotepa

The rate of ovarian function:0%
Primary ovarian dysfunction:100%
POF:>%60

Balachandar et al. Pediatr Blood Cancer 2015;62:317–321

Risk Factors for POF in Pediatric Age Group

- ASSR, HSCT
- Pelvic RT
- Etoposide, Platinum, Cyclophosphamide

OVARIAN TISSUE BANKING TO PRESERVE FERTILITY

- Pathological examination of removed ovaries is a prerequisite to rule out any microscopic tumoral invasion in the ovaries especially in cancers with a high risk of ovarian metastasis such as
  - leukemia,
  - neuroblastoma
  - genital rhabdomyosarcoma.

ISFP Practice CommitteeRecommendations for fertility preservation in patients with lymphoma, leukemia, and breast cancer. J Assist Reprod Genet. 2012 Jun;29(6):465-8.

Ovarian Freezing in Childhood Cancers

Patient	Age	Diagnosis	Indication for Fertility Preservation
1	4	*Diamond – Blackfan Syndrome*	HSCT
2	6	*Thalassemia Major*	HSCT
3	8	*Diploid – Triploid Mosaicism*	Gonadectomy
4	8	*Wilms tumor*	Chemotherapy
5	8	*Rhabdomyosarcoma*	Radiotherapy
6	10	*Acute Lymphoblastic Leukemia*	HSCT
7	10	*Acute Lymphoblastic Leukemia*	HSCT
8	16	*SLE with glomerulonephritis*	Chemotherapy
9	16	*Turner Syndrome*	POF
10	16	*Turner Syndrome*	POF
11	17	*Hodgkin's Lymphoma*	HSCT
12	17	*Hodgkin's Lymphoma*	HSCT
13	17	*Ovarian Carcinoma*	Gonadectomy
14	17	*Myelodyplasia*	HSCT
15	18	*Ewing Sarcoma*	Chemotherapy
16	18	*Hodgkin's Lymphoma*	HSCT
17	18	*Acute Myelocytic Leukemia*	HSCT
18	18	*Non – Hodgkin's Lymphoma*	HSCT
19	19	*Wegener Disease*	Chemotherapy
20	19	*Immature Teratoma*	Gonadectomy
21	20	*Non – Hodgkin's Lymphoma*	HSCT
22	21	*Acute Myelocytic Leukemia*	HSCT
23	21	*Acute Lymphoblastic Leukemia*	HSCT

Oktem et al Ann N Y Acad Sci. 2008
Oktem et al Pediatr Blood Cancer 2009

CASE SERIES OF OVARIAN TISSUE FREEZING IN THE PEDIATRIC AGE GROUP

Authors	Patients (*n*)	Age (years)		Patients under 16 years		Patients under 10 years	
		Range	Mean	*n*	%	*n*	%
Feigin *et al.* (2007)	23	5–17.5	13.5	NA	NA	NA	NA
Poirot *et al.* (2007)	47	0.8–15	6.1	47	100	38	81
Anderson *et al.* (2008b)	36	5–35	19.2	11	31	3	8
Revel *et al.* (2009)	19	5–20	15.3	8	42	2	11
Oktay and Oktem (2009)	26	4–21	14.3	9	35	6	23
Borgström *et al.* (2009)	57	8–19.8	14.4	40	70	4	7
Jadoul *et al.* (present publication)	58	0.8–15.8	10.4	58	100	21	36

From: Jadoul, P., Hum Reprod Update 16, 617-30 (2010).

Oktay, K. & Oktem, O Pediatr Blood Cancer 53, 267-73 (2009).

Feigin, E. et al.. J Pediatr Surg 42, 862-4 (2007).

Anderson, R.A., Reproduction 136, 681-9 (2008).

Revel, A. Fertil Steril 92, 458-63 (2009).

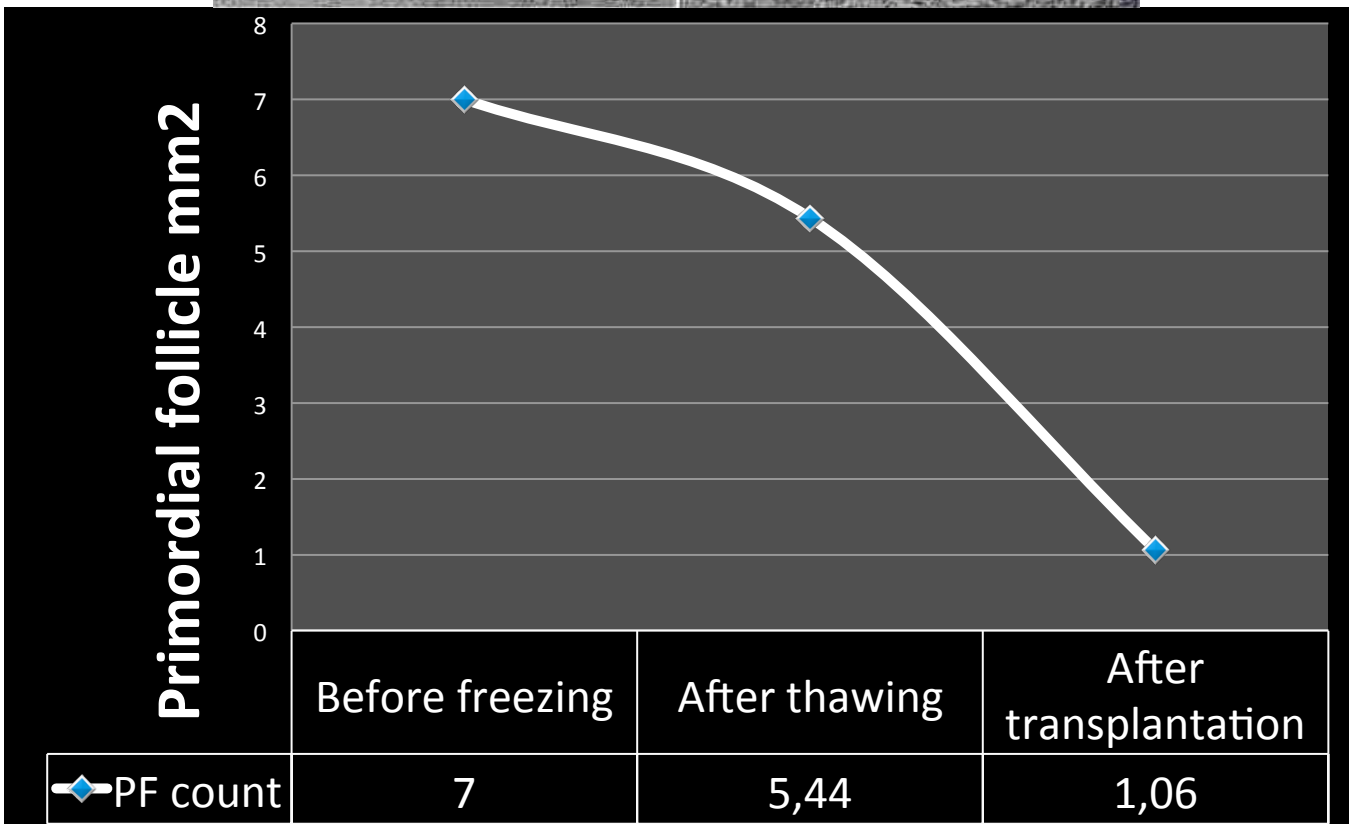
Borgstrom, B. et al. J Clin Endocrinol Metab 94, 74-80 (2009).

Jadoul, P., Hum Reprod Update 16, 617-30 (2010).

Poirot, C.J. et al. Pediatric blood & cancer 49, 74-8 (2007).

ADULT OVARY


BEFORE FREEZING
AFTER THAWING


Primordial follicle mm2
8
7
6
5
4
3
2
1
0
Before freezing
After thawing
After transplantation
PF count
7
5,44
1,06

Any data on the long-term viability and function of prepubertal cryopreserved ovaries?

- Luycyx et al (Fertil Steril 2013) evaluated cryopreserved ovarian tissue from deceased patients
- Five deceased prepubertal patients were selected for this study (alveolar rhabdomyosarcoma, lymphoblastic lymphoma, humerus osteosarcoma, Ewing's sarcoma,acute lymphoblastic leukemia).
- At the time of cryopreservation, the patients were aged between 7.2 and 12.2 years and all were prepubertal.
- Thawed tissues were xenografted into SCID mice.

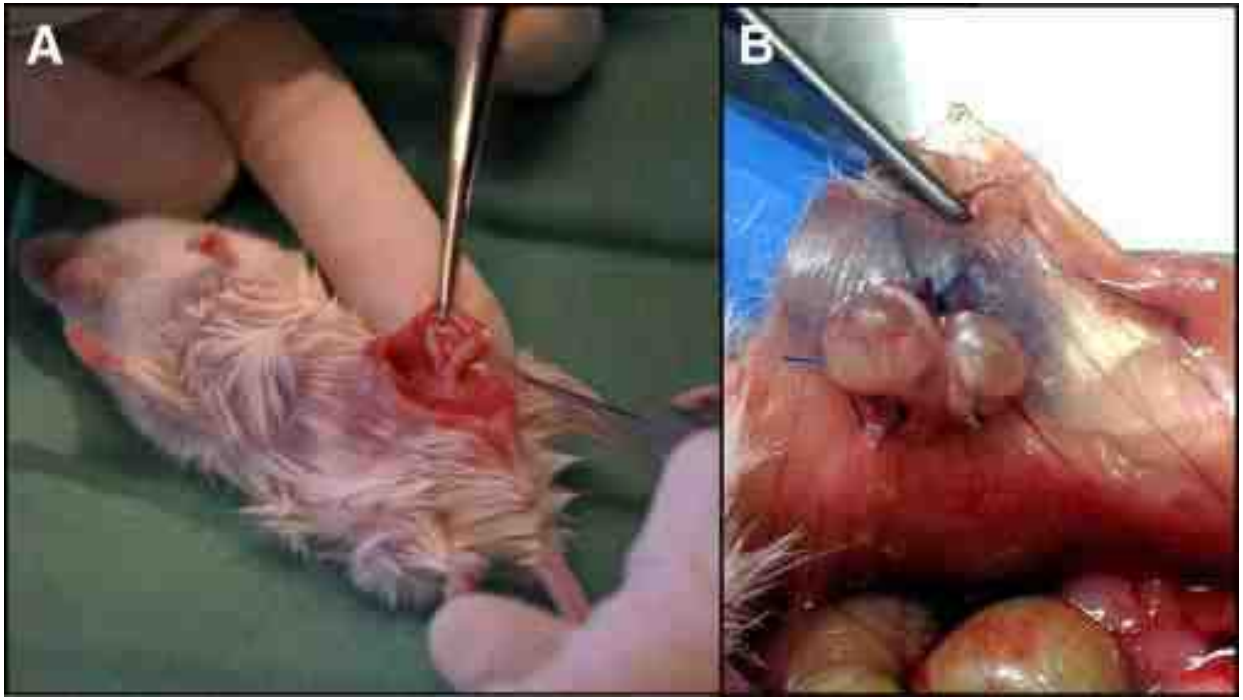


Figure 1. (A) Macroscopic view of the surgical procedure. After thawing, two ovarian fragments were grafted intraperitoneally to a female immunodeficient mouse. (B) After long-term grafting and exogenous stimulation, antral follicles were macroscopically obser...

Valérie Luyckx, Sarah Scalercio, Pascale Jadoul, Christiani Andrade Amorim, Michelle Soares, Jacques Donnez, Marie-Madeleine Dolmans

Evaluation of cryopreserved ovarian tissue from prepubertal patients after long-term xenografting and exogenous stimulation

Fertility and Sterility, Volume 100, Issue 5, 2013, 1350–1357.e3

http://dx.doi.org/10.1016/j.fertnstert.2013.07.202

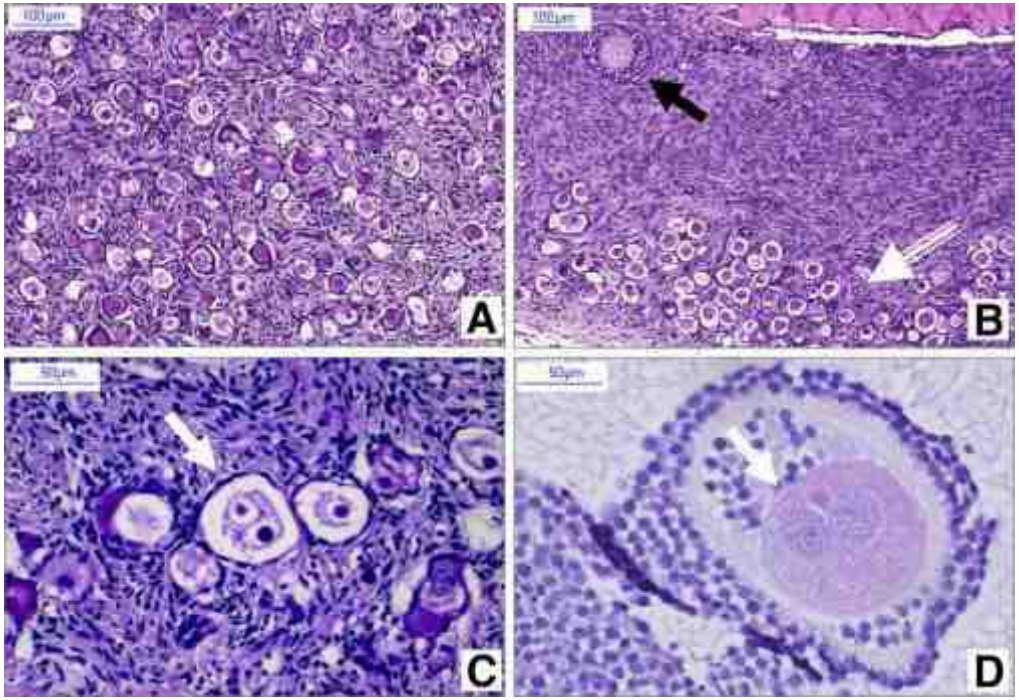


Figure 2. Histologic illustrations of ovarian tissue from prepubertal patients before (A and C) and after (B and D) grafting. (A) Histologic illustrations of ovarian tissue from prepubertal patients before grafting. All follicles were inactive and at the primo...

Valérie Luyckx, Sarah Scalercio, Pascale Jadoul, Christiani Andrade Amorim, Michelle Soares, Jacques Donnez, Marie-Madeleine Dolmans

Evaluation of cryopreserved ovarian tissue from prepubertal patients after long-term xenografting and exogenous stimulation

Fertility and Sterility, Volume 100, Issue 5, 2013, 1350–1357.e3

http://dx.doi.org/10.1016/j.fertnstert.2013.07.202

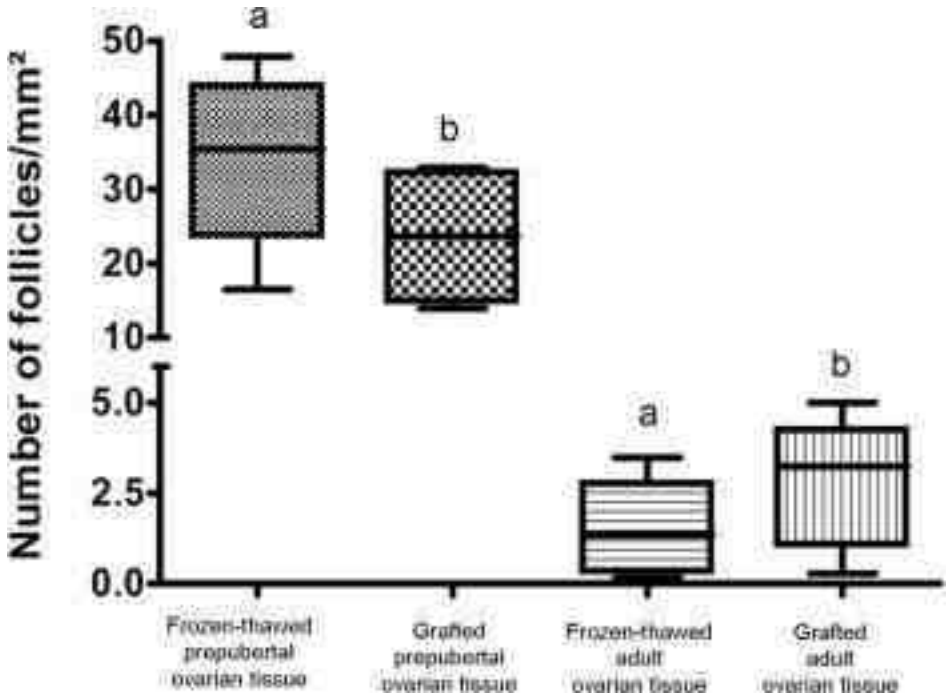


Figure 3. Graph representing follicular density (expressed as the number of ovarian follicles per mm²) in frozen-thawed and grafted ovarian tissue from prepubertal and adult patients. A significant difference (P<.05) was evidenced between frozen-thawed prep...

Valérie Luyckx, Sarah Scalercio, Pascale Jadoul, Christiani Andrade Amorim, Michelle Soares, Jacques Donnez, Marie-Madeleine Dolmans

Evaluation of cryopreserved ovarian tissue from prepubertal patients after long-term xenografting and exogenous stimulation

Fertility and Sterility, Volume 100, Issue 5, 2013, 1350–1357.e3

http://dx.doi.org/10.1016/j.fertnstert.2013.07.202

- Do pediatric cancer patients or their parents receive an expert opinion/counselling about the risk of gonadal failure and FP strategies prior to cancer treatment?

- NO!

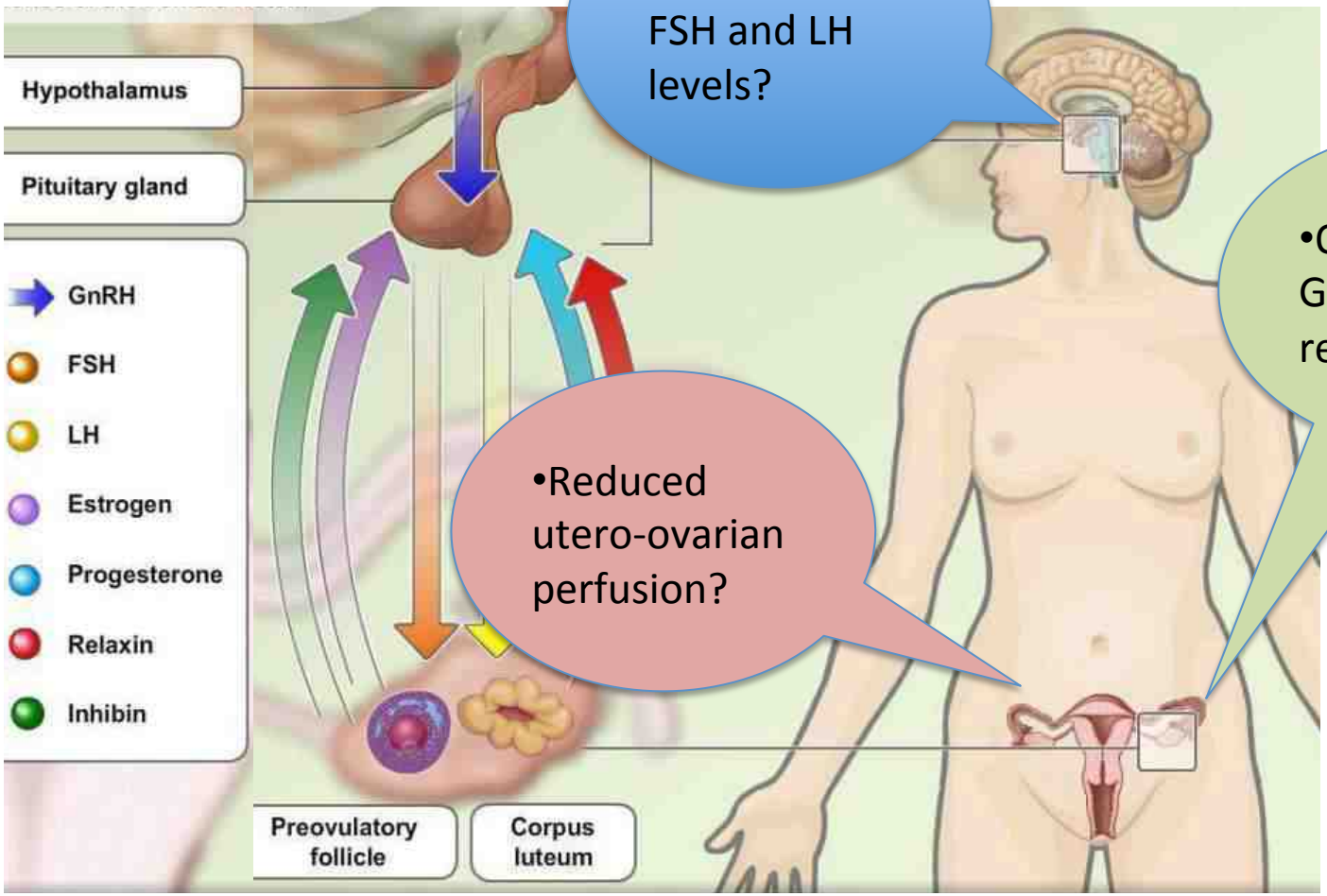
- Only 55% of the patients receive counseling
- White ethnicity
- higher annual income
- higher education level are significantly
- associated with a positive opinion about FP techniques.

Kim et al. Palliative and Supportive Care (2015), 13, 1251–1260.

GnRH agonist for the prevention of ovarian damage induced by chemotherapy

- The administration of gonadotropin-releasing hormone agonists during chemotherapy has been proposed as a potential fertility preservation strategy to preserve ovarian reserve after emergence of the promising findings from anecdotal reports, primate models and non-randomized trials in human.
- However, randomized controlled trials (RCTs) have shown inconsistent results in female patients with cancer.

GnRH agonist for protection against chemotherapy?

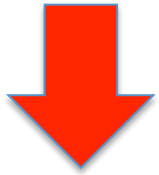


- Prepubertal FSH and LH levels?
- Reduced utero-ovarian perfusion?
- Ovarian GnRH receptors?

Proposed mechanisms of protective action with GnRHa

- Various mechanisms have been suggested,
  - GnRHa-induced decrease in the number of primordial follicles entering the differentiation stage,
  - reduction of ovarian perfusion due to a GnRHa-induced hypoestrogenic state,
  - decreased ovarian cell apoptosis, through either activation of GnRH receptors or upregulation of intragonadal antiapoptotic molecules (GnRHas) during adjuvant chemotherapy.
  - But none of these theories has been validated so far

- Various mechanisms have been suggested,
  - GnRHa-induced decrease in the number of primordial follicles entering the differentiation stage,
  - reduction of ovarian perfusion due to a GnRHa-induced hypoestrogenic state,
  - decreased ovarian cell apoptosis, through either activation of GnRH receptors or upregulation of intragonadal antiapoptotic molecules (GnRHas) during adjuvant chemotherapy.



Hum. Reprod. Advance Access published October 13, 2015

Human Reproduction, Vol.0, No.0 pp. 1–14, 2015

doi:10.1093/humrep/dev257

human reproduction

ORIGINAL ARTICLE *Reproductive endocrinology*

GnRH agonist leuprolide acetate does not confer any protection against ovarian damage induced by chemotherapy and radiation *in vitro*

Gamze Bildik[1], Nazlı Akin[1], Filiz Senbabaoglu[1], Gizem Nur Sahin[1], Sercin Karahuseyinoglu[2], Umit Ince[3], Cagatay Taskiran[4,5], Ugur Selek[5,6,7], Kayhan Yakin[4], Yilmaz Guzel[8], Cem Ayhan[8], Ebru Alper[8], Mustafa Cetiner[5,9], Basak Balaban[8], Nil Molinas Mandel[5,10], Tarık Esen[5,11], Akira Iwase[12], Bulent Urman[4,5,8], and Ozgur Oktem[4,5,8,*]

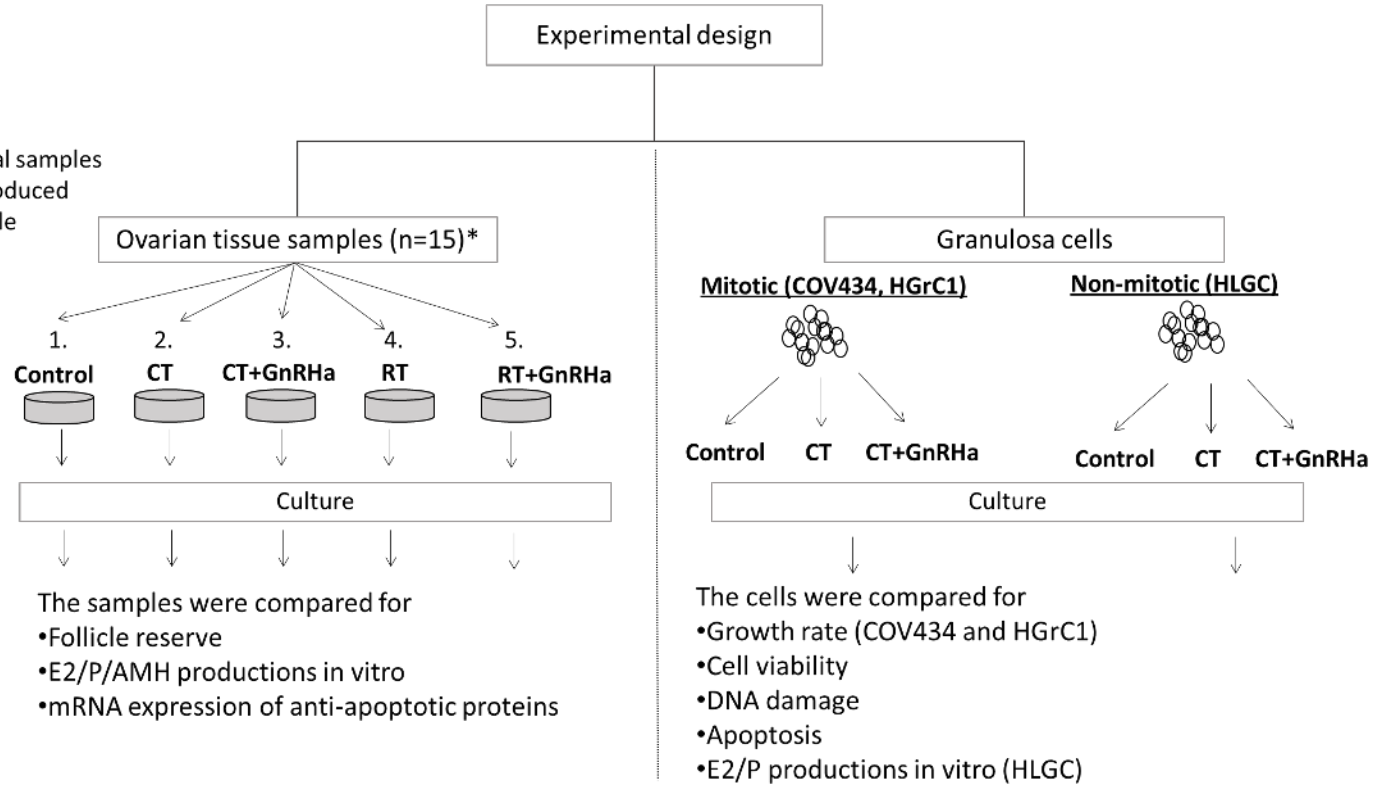
[1]The Graduate School of Health Sciences, Koc University, Istanbul, Turkey [2]Department of Histology and Embryology, Koc University School of Medicine, Istanbul, Turkey [3]Department of Pathology, Acibadem University School of Medicine, Istanbul, Turkey [4]Department of Obstetrics and Gynecology, Koc University School of Medicine, Istanbul, Turkey [5]Comprehensive Cancer Care and Fertility Preservation Programs American Hospital, Istanbul, Turkey [6]Department of Radiation Oncology, Koc University School of Medicine, Istanbul, Turkey [7]Department of Radiation Oncology, University of Texas MD Anderson Cancer Center, Houston, TX, USA [8]Assisted Reproduction Unit, Women' Health Center American Hospital, Istanbul, Turkey [9]Department of Hematology and Oncology, Koc University School of Medicine, Istanbul, Turkey [10]Department of Medical Oncology, Koc University School of Medicine, Istanbul, Turkey [11]Department of Urology and Urologic Oncology, Koc University School of Medicine, Istanbul, Turkey [12]Department of Obstetrics and Gynecology, Nagoya University, Nagoya, Japan

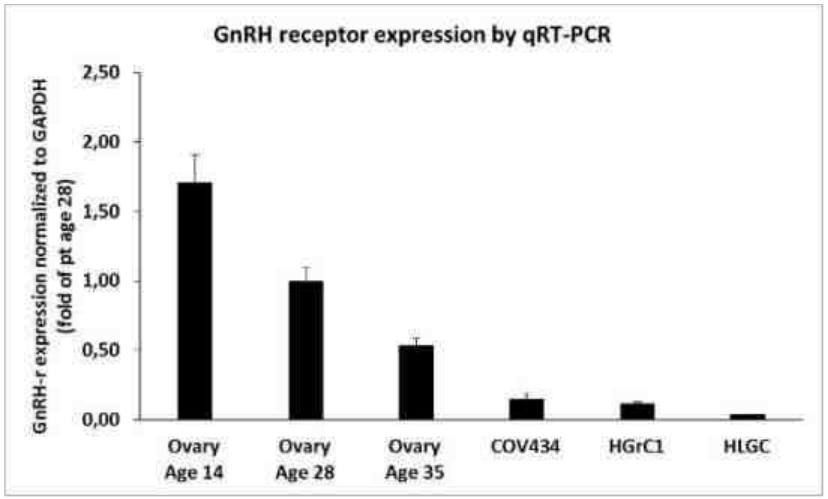
*Correspondence address. Department of Obstetrics and Gynecology, Division Reproductive Endocrinology and Infertility, Koc University Hospital, Davutpasa Topkapı, Istanbul, 34400 Turkey. E-mail: ooktem@ku.edu.tr

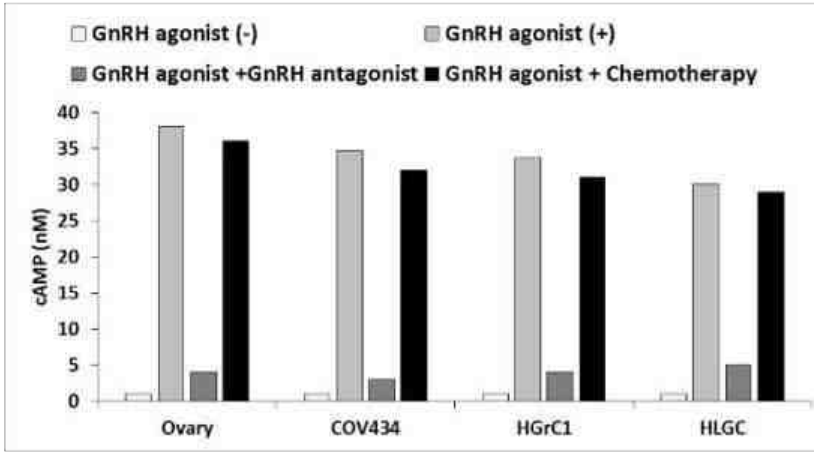
Bildik and Oktem TSRM 2014 Prize and ASRM 2015 prize

Bildik et al. Human Reprod 2015 in press

E2:Estradiol
P:Progesterone
AMH:Anti-mullerian hormone
CT:Chemotherapy
RT: Radiation
*: The total number of ovarian cortical samples
Each treatment arm has 15 pieces produced from equal division of the main sample




GnRH receptor expression by qRT-PCR
GnRH-r expression normalized to GAPDH (fold of pt age 28)
2,50
2,00
1,50
1,00
0,50
0,00
Ovary Age 14
Ovary Age 28
Ovary Age 35
COV434
HGrC1
HLGC


GnRH agonist (-)
GnRH agonist (+)
GnRH agonist +GnRH antagonist
GnRH agonist + Chemotherapy
cAMP (nM)
40
35
30
25
20
15
10
5
0
Ovary
COV434
HGrC1
HLGC

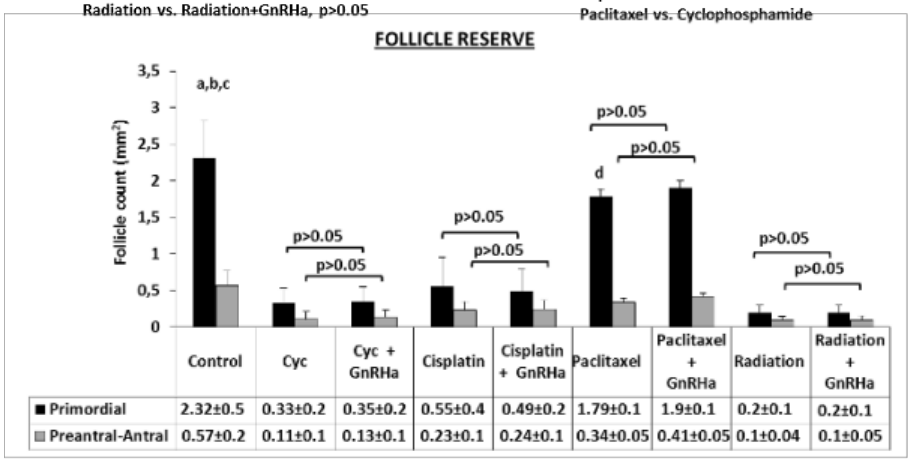
a: p<0.001 (Friedman test)
Primordial and preantral/antral follicle counts
(Wilcoxon paired analysis)
Control vs. Cyc, p<0.01
Control vs. Cyc±GnRHa, p<0.01
Cyc vs. Cyc+GnRHa p>0.05
Control vs. Cisplatin, p<0.05
Control vs. Cisplatin+GnRHa, p<0.05
Cisplatin vs. Cisplatin+GnRHa, p>0.05
Control vs. Radiation, p<0.01
Control vs. Radiation+GnRHa, p<0.01
Radiation vs. Radiation+GnRHa, p>0.05

b: p<0.05 Preantral/antral follicle counts
Control vs. Paclitaxel±GnRHa subgroups

c: p>0.05 Primordial follicle count
Control vs. Paclitaxel±GnRHa subgroups

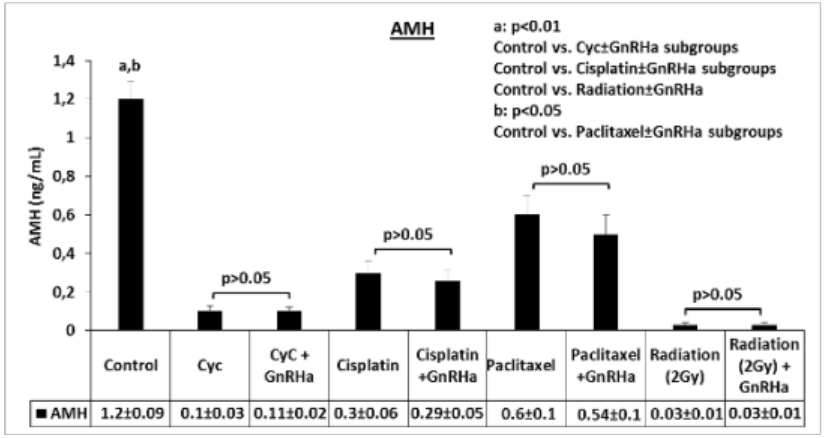
d: p<0.05 Primordial follicle count
Paclitaxel vs. Cyclophosphamide

2A



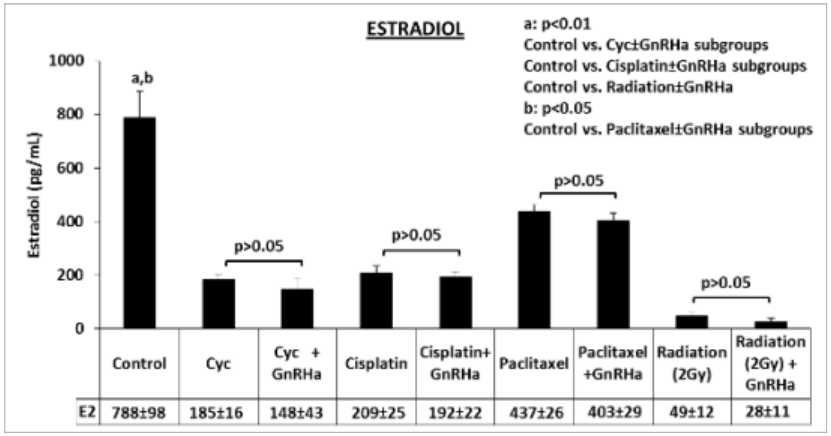
	Control	Cyc	Cyc + GnRHa	Cisplatin	Cisplatin + GnRHa	Paclitaxel	Paclitaxel + GnRHa	Radiation	Radiation + GnRHa
■ Primordial	2.32±0.5	0.33±0.2	0.35±0.2	0.55±0.4	0.49±0.2	1.79±0.1	1.9±0.1	0.2±0.1	0.2±0.1
▒ Preantral-Antral	0.57±0.2	0.11±0.1	0.13±0.1	0.23±0.1	0.24±0.1	0.34±0.05	0.41±0.05	0.1±0.04	0.1±0.05

2B



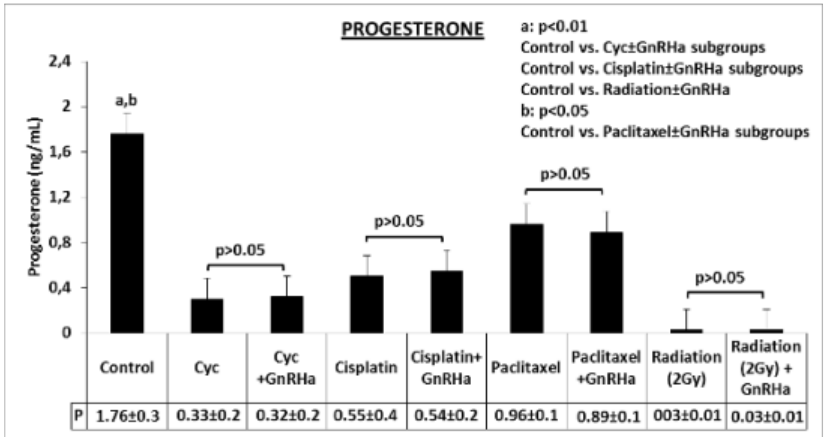
	Control	Cyc	CyC + GnRHa	Cisplatin	Cisplatin +GnRHa	Paclitaxel	Paclitaxel +GnRHa	Radiation (2Gy)	Radiation (2Gy) + GnRHa
■ AMH	1.2±0.09	0.1±0.03	0.11±0.02	0.3±0.06	0.29±0.05	0.6±0.1	0.54±0.1	0.03±0.01	0.03±0.01

2C



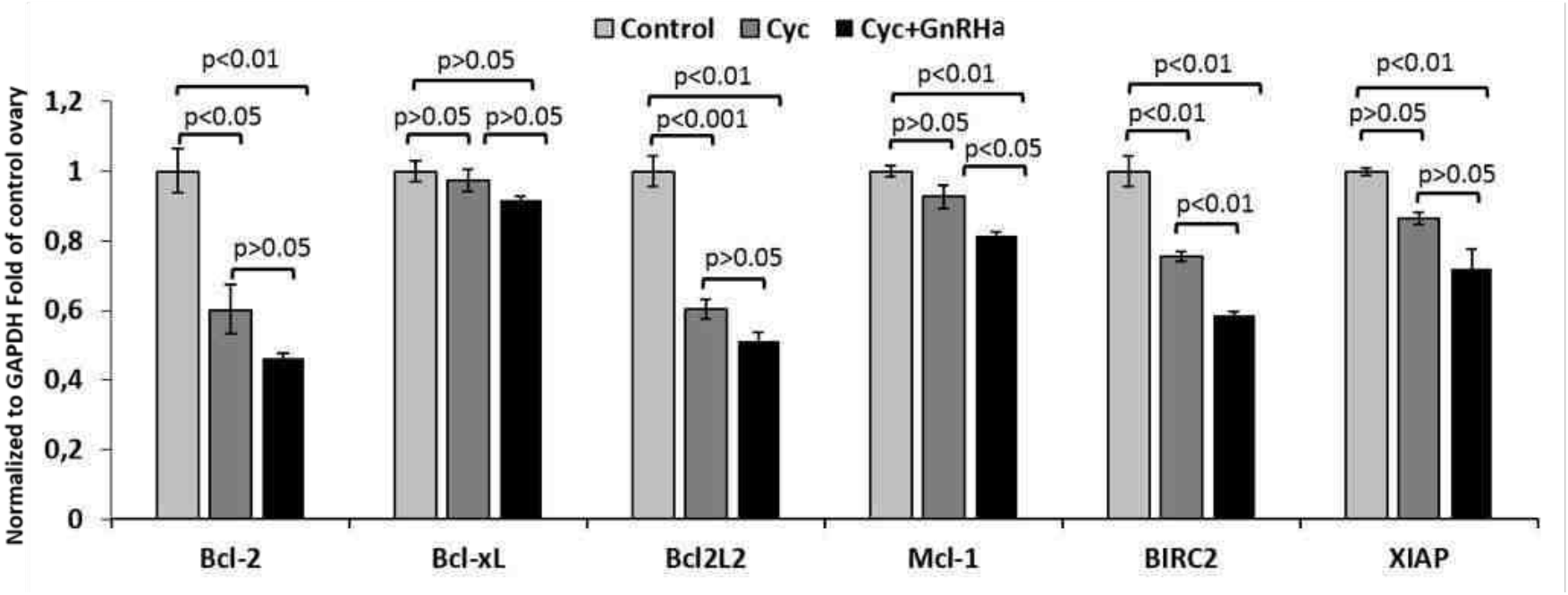
	Control	Cyc	Cyc + GnRHa	Cisplatin	Cisplatin+ GnRHa	Paclitaxel	Paclitaxel +GnRHa	Radiation (2Gy)	Radiation (2Gy) + GnRHa
E2	788±98	185±16	148±43	209±25	192±22	437±26	403±29	49±12	28±11

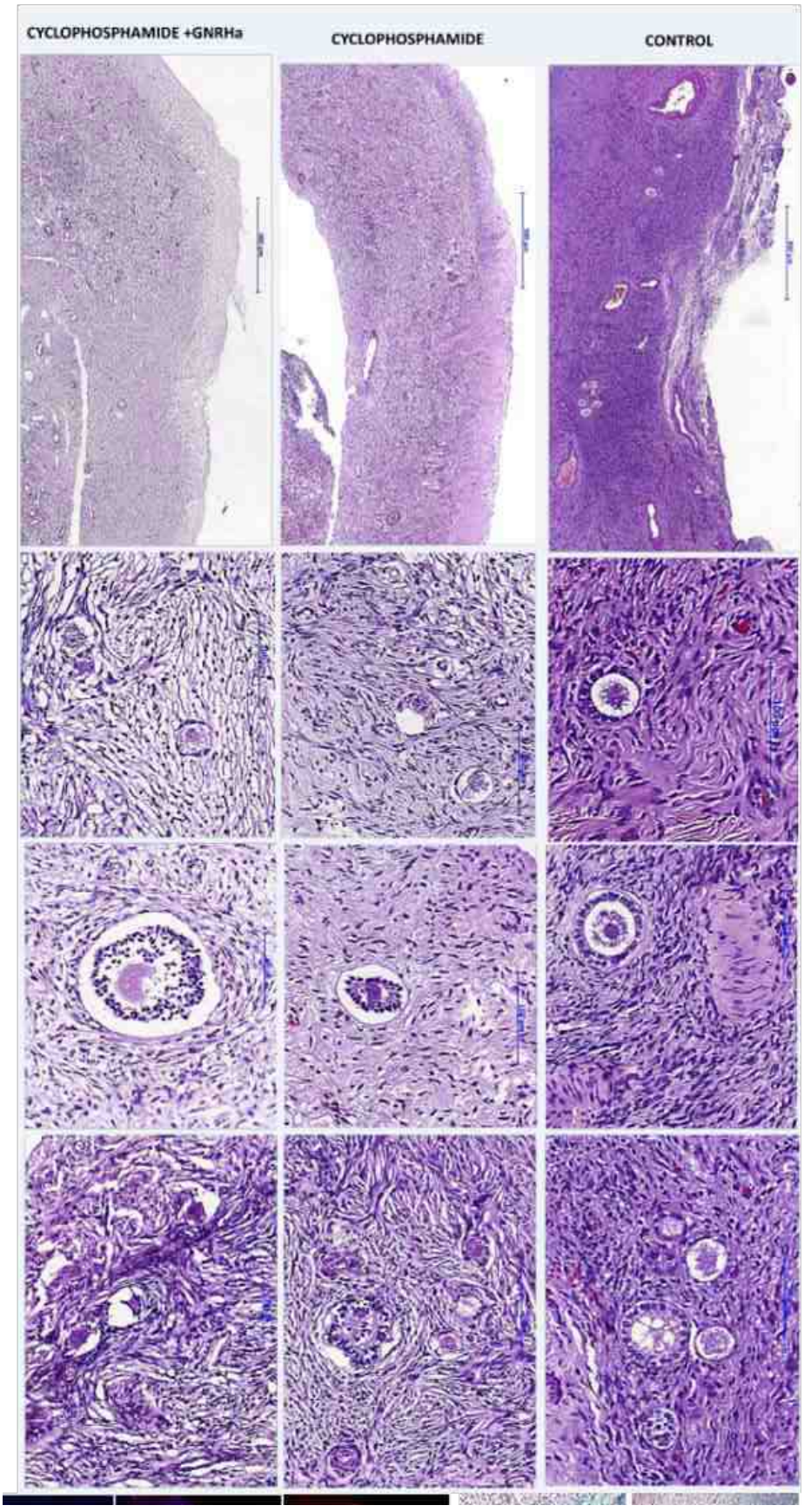
2D



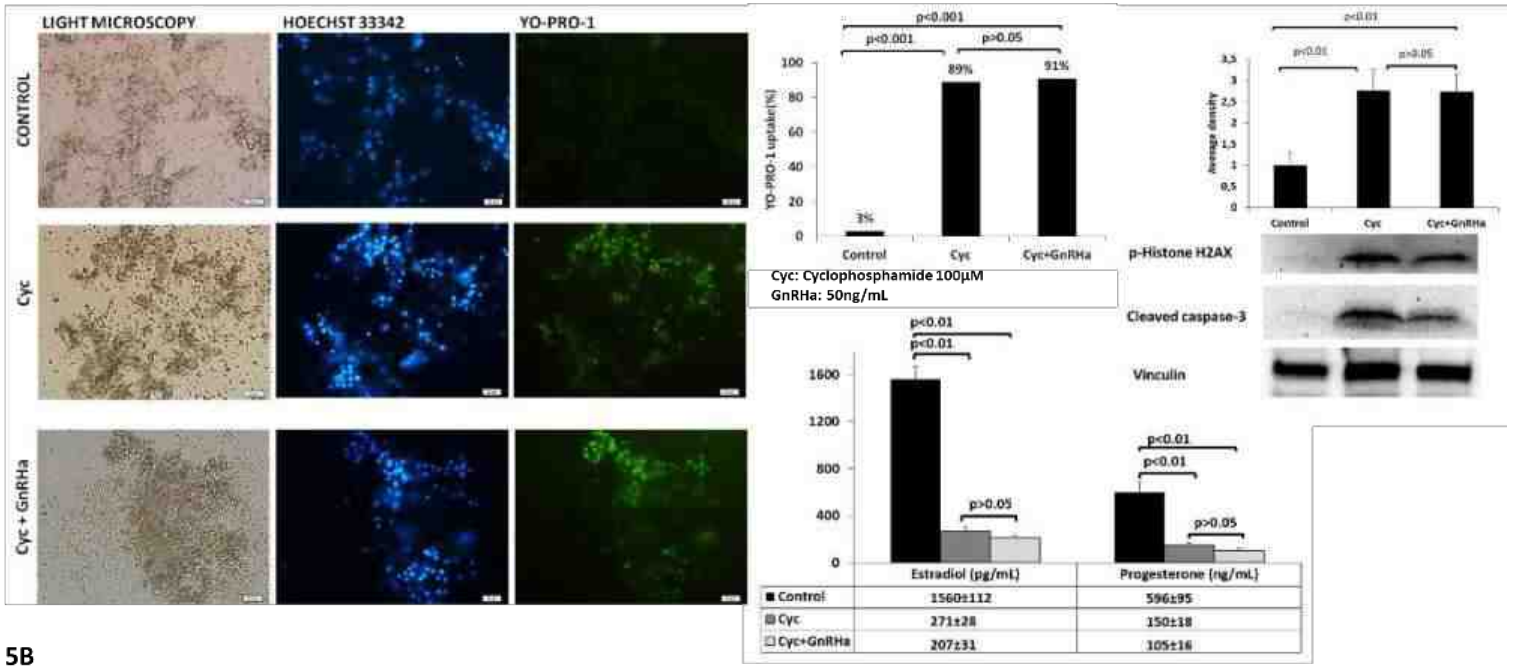
	Control	Cyc	Cyc +GnRHa	Cisplatin	Cisplatin+ GnRHa	Paclitaxel	Paclitaxel +GnRHa	Radiation (2Gy)	Radiation (2Gy) + GnRHa
P	1.76±0.3	0.33±0.2	0.32±0.2	0.55±0.4	0.54±0.2	0.96±0.1	0.89±0.1	003±0.01	0.03±0.01

mRNA expression of the anti-apoptotic proteins

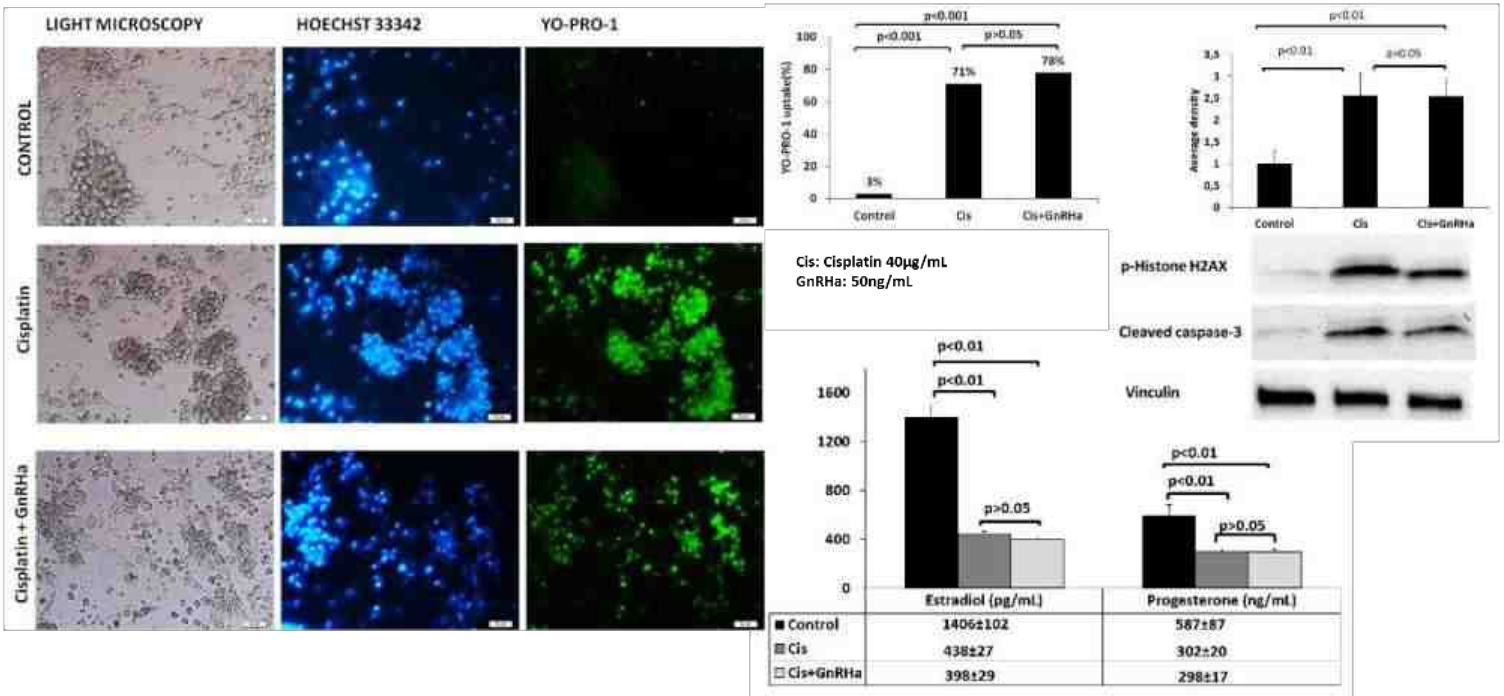


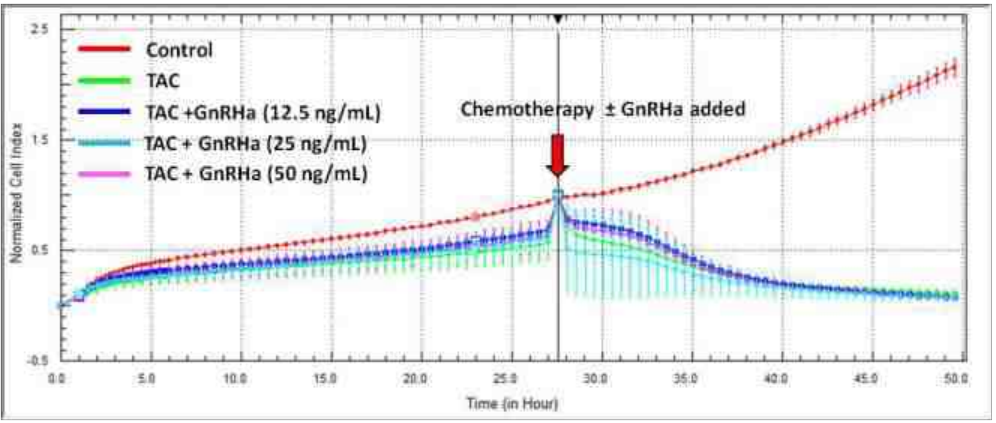

CYCLOPHOSPHAMIDE +GNRHa
CYCLOPHOSPHAMIDE
CONTROL

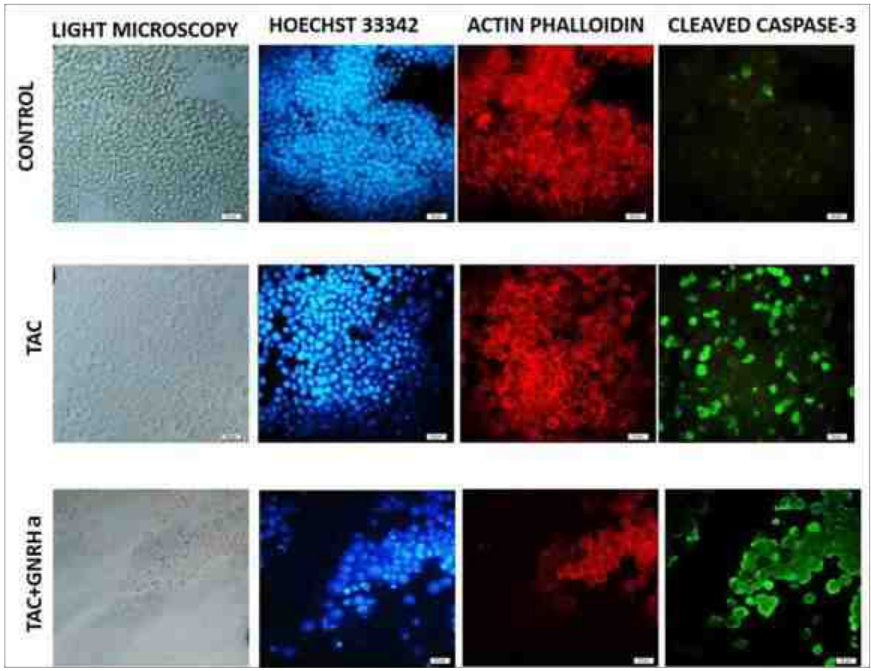
5A

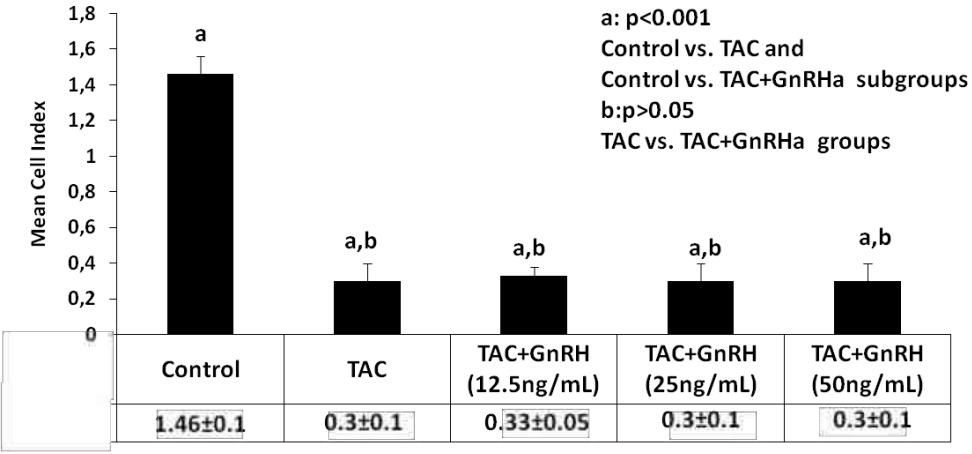

LIGHT MICROSCOPY
HOECHST 33342
YO-PRO-1
CONTROL
Cyc
Cyc + GnRHa
p<0.001
p>0.05
YO-PRO-1 uptake(%)
3%
89%
91%
Control
Cyc
Cyc+GnRHa
Cyc: Cyclophosphamide 100µM
GnRHa: 50ng/mL
p<0.01
Average density
p-Histone HZAX
Cleaved caspase-3
Vinculin
Estradiol (pg/mL)
Progesterone (ng/mL)
Control 1560±112 596±95
Cyc 271±28 150±18
Cyc+GnRHa 207±31 105±16

5B


LIGHT MICROSCOPY
HOECHST 33342
YO-PRO-1
CONTROL
Cisplatin
Cisplatin + GnRHa
p<0.001
p>0.05
YO-PRO-1 uptake(%)
3%
71%
78%
Control
Cis
Cis+GnRHa
Cis: Cisplatin 40µg/mL
GnRHa: 50ng/mL
p<0.01
Average density
p-Histone HZAX
Cleaved caspase-3
Vinculin
Estradiol (pg/mL)
Progesterone (ng/mL)
Control 1406±102 587±87
Cis 438±27 302±20
Cis+GnRHa 398±29 298±17

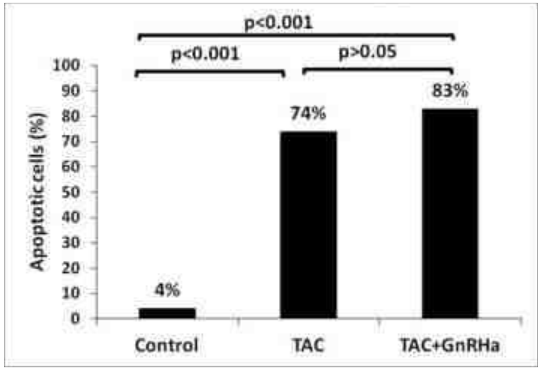






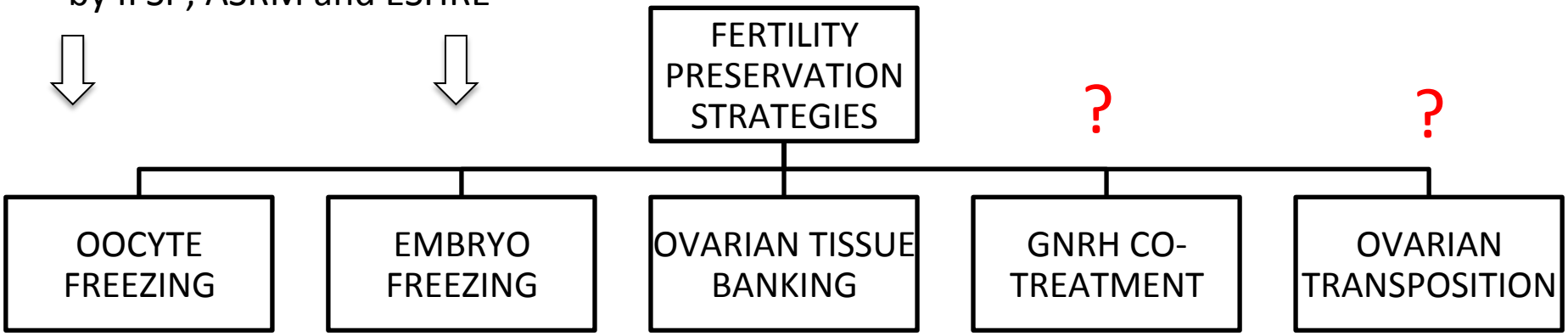
a: p<0.001
Control vs. TAC and
Control vs. TAC+GnRHa subgroups
b:p>0.05
TAC vs. TAC+GnRHa groups

Control	TAC	TAC+GnRH (12.5ng/mL)	TAC+GnRH (25ng/mL)	TAC+GnRH (50ng/mL)
1.46±0.1	0.3±0.1	0.33±0.05	0.3±0.1	0.3±0.1

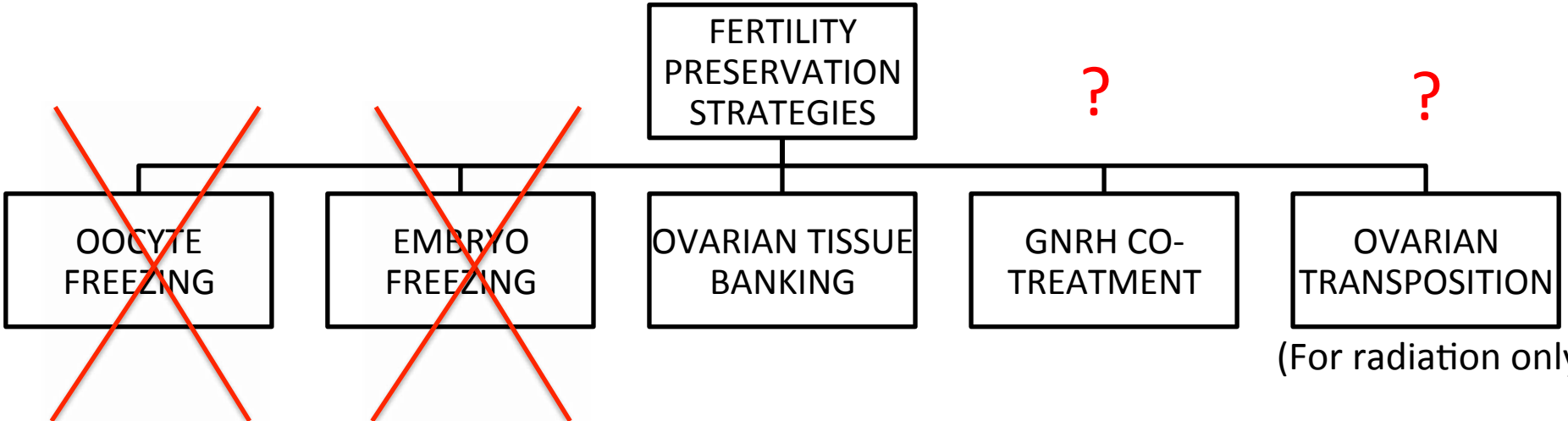


Considered as the only established methods by IFSP, ASRM and ESHRE

ADULTS



CHILDREN



Conlcusion

- Ovarian tissue cryopreservation is the only available method for pediatric cancer patients.
- No need to preserve fertility except
  - Pelvic RT,
  - High dose chemo (etoposide, platinum, cyclophosphamide)
  - HSCT

THANK YOU

KOÇ UNIVERSITY HOSPITAL

AMERICAN HOSPITAL