

Cancer Treatment:
Fertility Preservation Options
For Women

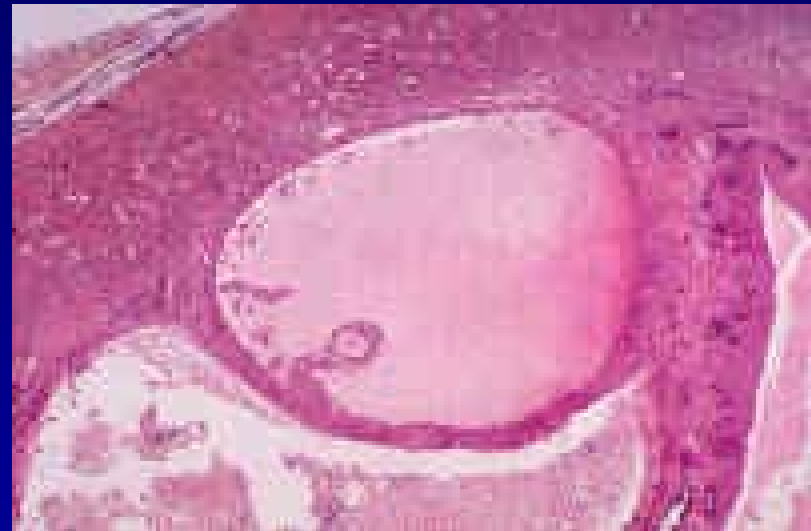
Andrea Vidali MD

Cases

- M.P 34 y/o recently diagnosed with CML, awaiting TBI and BMT. Single
- J.K. 18 y/o with acute progressive autoimmune myelitis, now on a wheelchair, to start cyclophosphamide soon. Single
- S.T. 39 y/o recently diagnosed with rectal cancer. Single.

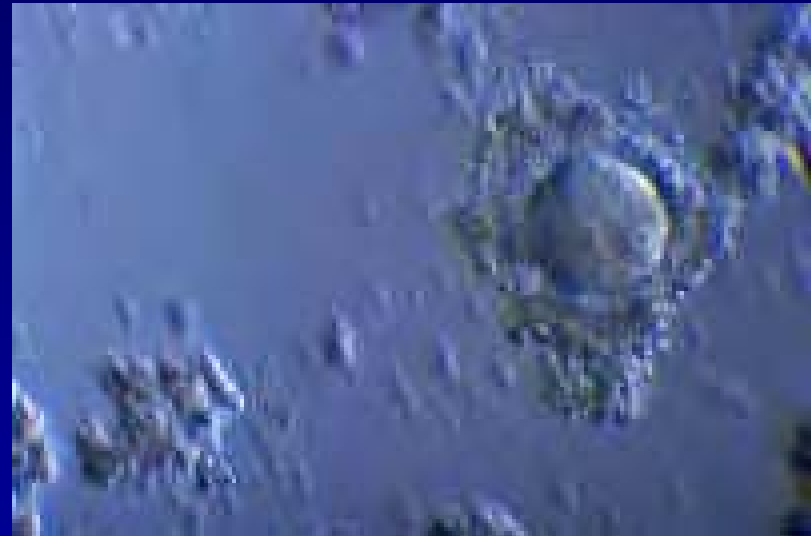
The Oocyte

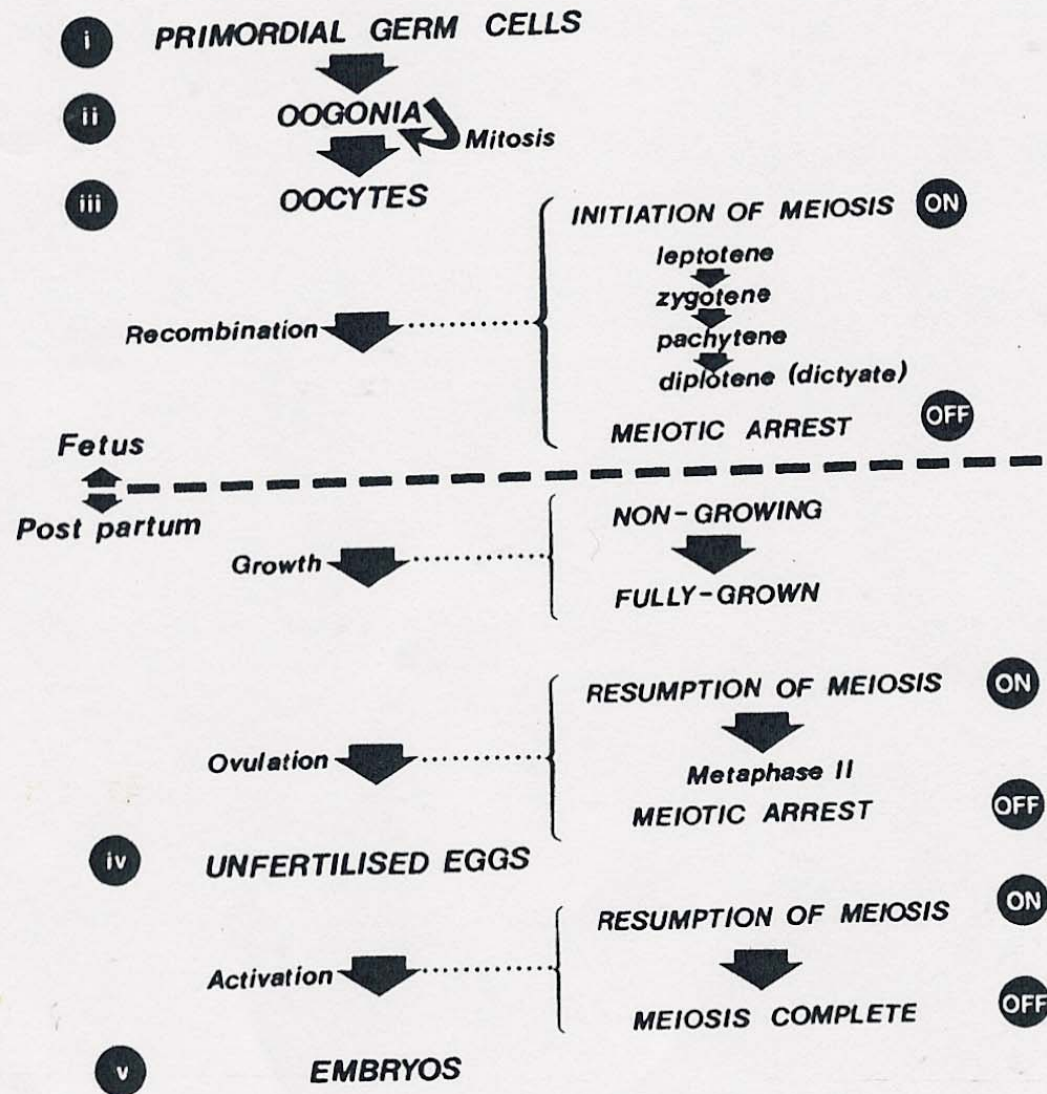
- De Graaf (1670) thought that entire follicle was an egg.
- Von Baer 1870 identifies and describes the oocyte
- Waldeyer (1870) oocytes are limited in number



The Oocyte

- Understanding of ovarian organogenesis only in the 1960'.
- 2million at birth.
- 200,000 at puberty
- Once population falls below a key threshold number: infertility first and menopause





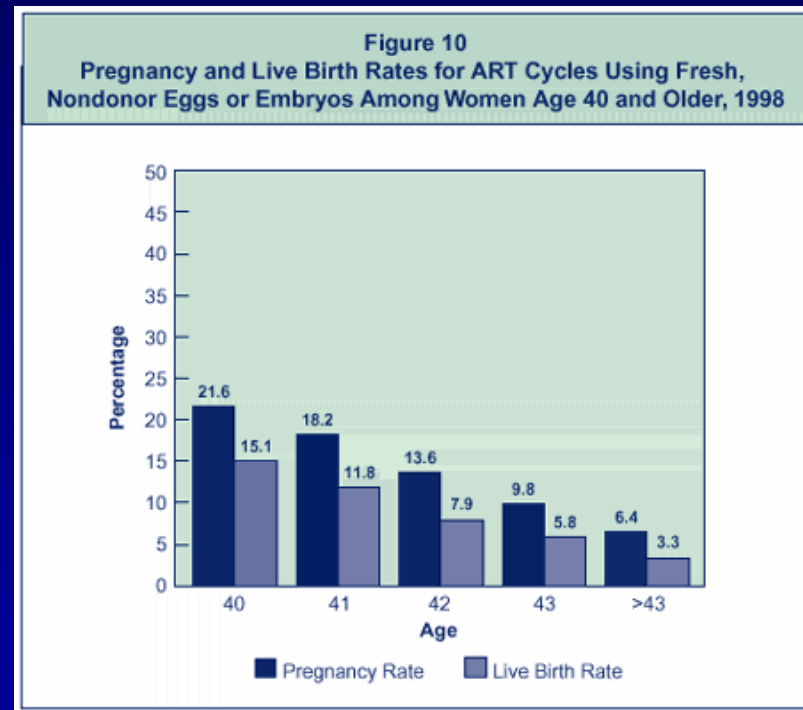
Ovarian Functions

- Endocrine: replaceable
- Exocrine: irreplaceable



Threats to the Ovary

- Age
- Genetic
- Iatrogenic



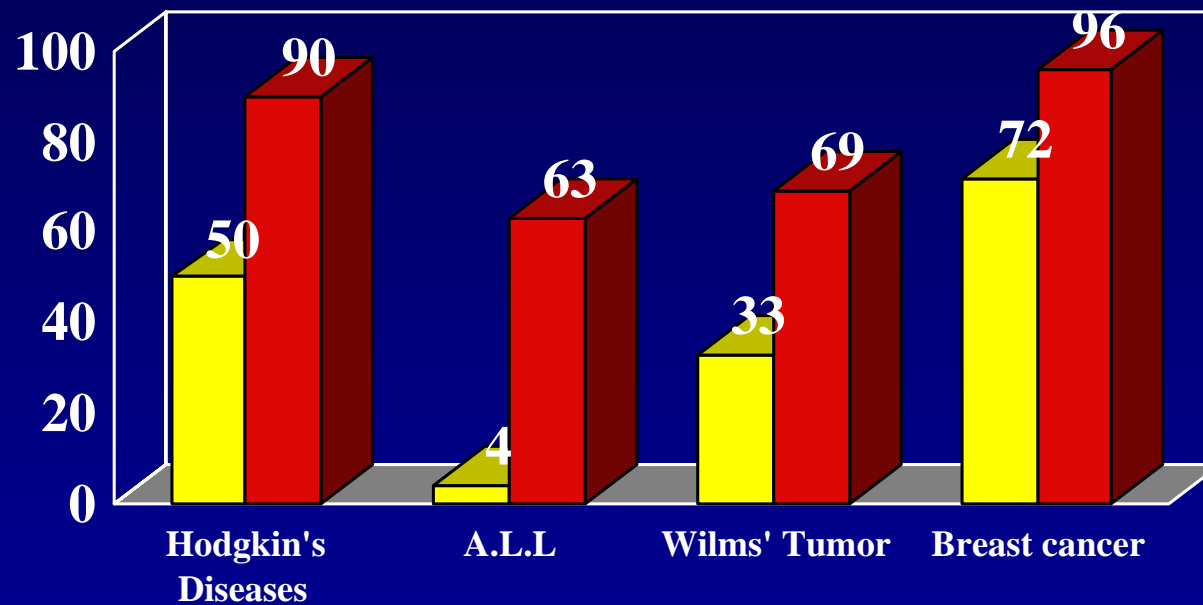
Reproductive Age Cancer

- More than 4000 children/year are exposed to sterilizing cancer treatments
- Approximately 9100 males/year aged 15 to 35 are diagnosed with testicular cancer, Hodgkin's disease, lymphoma and leukemia
- Approximately 8600 women younger than 40 are diagnosed annually with breast cancer
- Approximately 3000 women of reproductive age are treated with radiotherapy for cervical carcinoma

Cancer: Success Stories in Children

- Leukemias: 5 yr survival up to 80% from 33% in 1975
- Hodgkin's: 5 yr survival 90%
- ALL and Non Hodgkin's: 5yr survival 75%
- Sarcomas: Survival rates doubled to 60%

5 Year Survival



■ Year 1960 ■ Year 1997

• *American Cancer Society Surveillance Research, 2002*
• *National Cancer Institute*

Treatments that Threaten Ovarian Function

- Chemotherapy
- Radiation Therapy
- Surgery
- Other

Ovarian Toxicity

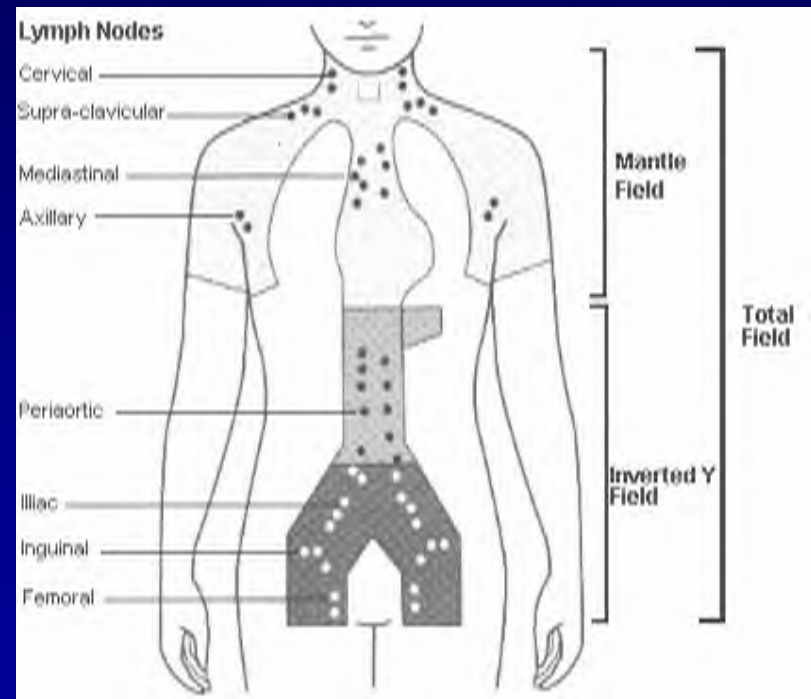
- Prolonged Survival.
- Therefore long-term psychological and physical effects of treatment have been subjected to wider attention.
- Ovarian toxicity is now recognized as an important side effect of chemo/radiotherapy.

Radiation Therapy

- Adverse effects on gonadal function at all ages
- Cervical, Rectal, CNS.
- Hodgkin's
- Total body radiation prior to bone marrow transplantation

Radiation Therapy

- Dose: estimated LD50 is 4Gy.
- 12/19 patients who received a total dose of 30Gy developed ovarian failure
 - (1GY=100RAD)
 - Wallace et al: Brit J Radiol 1989, 62:995



Radiation Therapy: Effects Long Term Survivors

- NCI study, 2283 patients (1945-1975)
- Diagnosis before age 20
- Relative fertility 0.85 (CI 0.78-0.92)
- Radiation Therapy depressed fertility 25%
 - » N Engl J Med 1987 Nov 19;317(21):1315-21
- 40% of 20yo and 90% of 35yo with an incident radiation of 5 Gy.
 - » Devita Jr VT, Hellman S, Rosenberg SA, eds. *Cancer: Principles and Practice of Oncology*. 5th Edn. Philadelphia: Lippincott-Raven, 1997: 2758-73.

Ovarian Transposition

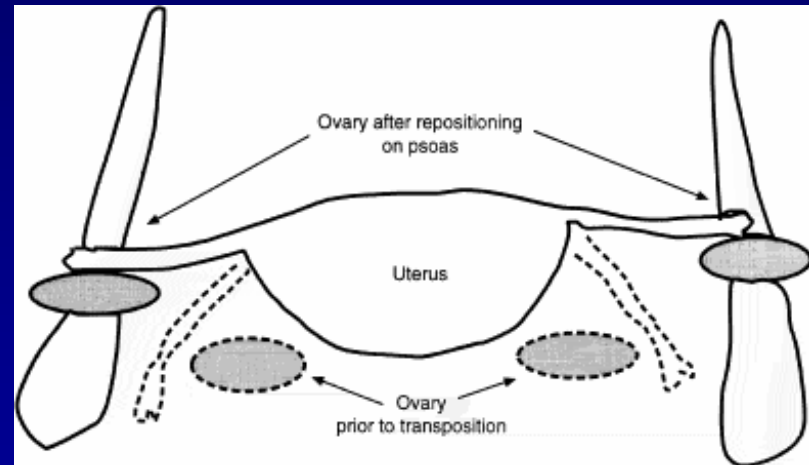
- Repositioning of the ovaries out of the radiation field.
- 90% preservation after vaginal brachytherapy.
- 60% after external beam

» Morice et al Fertil Steril 2000, 74;743-748

Ovarian Transposition: Technique

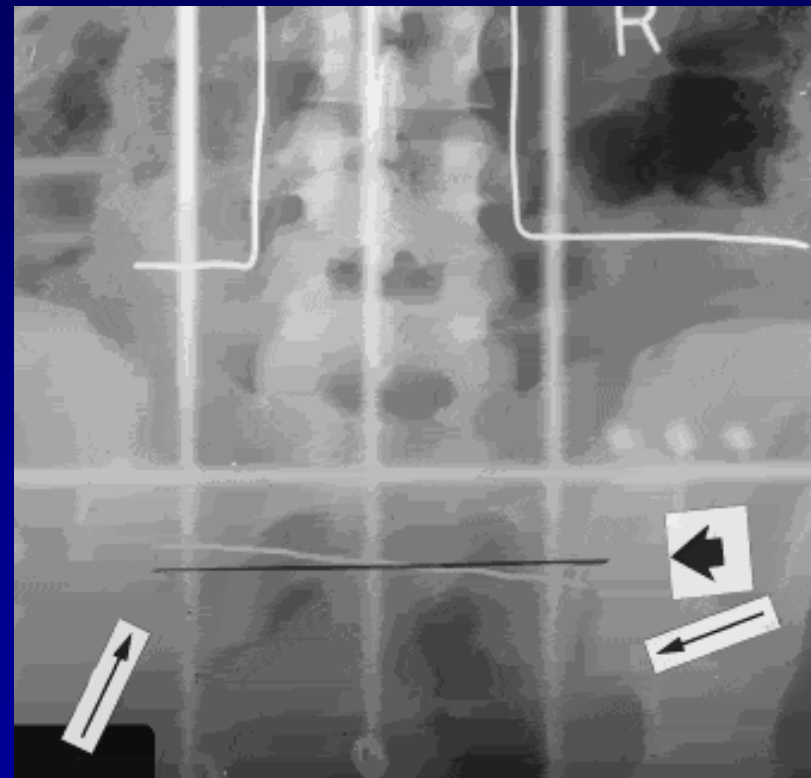
- Section of the tube and utero-ovarian ligament
- Pediculisation of the infundibulopelvic ligament and attachment of the ovary to the parietocolic sulcus
- Identification of the ovary with a metal clip

- Clough et al: Cancer, 1996, 77;2638
- Rhart. GYN Endoscopy 1999



Ovarian Transposition: Indications

- Age < 35 yo.
- Cervical cancer.
 - < 3cm (risk of ovarian mets), no adeno or ectocervical.
- Hodgkin's disease.
- Other solid tumors requiring RT.



Chemotherapy

- Late complications associated with chemotherapy are assuming greater significance.
- Secondary malignancies.
- Adverse effects on the gonads.
- Patients need to be aware.
- Physicians need to understand parameters.

Bone Marrow Transplant

- 20000 cases in 1997
- Extreme example of gonadal damage.
- In a series of 38000 patients only 129 pregnancies reported
 - » Appereley et al . Blood Rev. 1995, 9;93-116
- Most of these patients are young.

Chemotherapy: Breast

- 109 premenopausal patients. Early-stage. MTX or anthracycline based protocol (both Cyclophosphamide)
 - » Lower 1999, Women Health Gemnd Med
- No difference between two groups. 45% amenorrhea
- 28% of patients < 35 menstrual disturbances.
- CMF amenorrhea 68%
 - » Bines JCO 1996

Chemotherapy: Other Malignancies

- Hodgkins: ovarian failure 38-57%
 - » Howell EMCNA 1998 ,26;927-943
- Non Hodgkin's lymphoma 10%
- Often combined chemoradio can reduce ovarian toxicity
 - » Brusamolino.Hematologica 2000,85;1032

Chemotherapy: Amenorrhea

- Leukemia (AML): 15%.
- Non Hodgkin's Lymphoma: 44%
- Hodgkin's: 32%.
- Breast: 50%.
- Average age of patients with normal function: 27 ± 8.3

» Meirou. Fertil Steril 1997.

Gonadal Effects of Combination Chemotherapy

Disease	Regimen	N	%Amenorrhea
Ovarian Ca.	P+others	57	6-8
Breast Ca.	CMF	549	54-96
	L-pam+FU	98	21-72
	Mito	15	26
Hodgkin's	MOPP (adult)	95	55-71
	(pubertal)	15	0
	MVPP	72	36-28
	COPP	14	57
	ABVD	24	0
Lymphoma	High dose	Case reports	

Chemotherapy: Agents

- Alkylating Agents : 4
- Cisplatin :2
- Antibiotics :0.2
- Anti Metabolites:0.3
- Plant Alkaloids:1.2

» Meiorowicz Fertil Steril

Chemotherapy: Autoimmune Disease

- Cyclophosphamide pulse therapy for SLE, thalassemia, nephrotic syndromes, periarteritis nodosa.
- 26-39% ovarian failure.
 - » Boumpas Ann Inter Med 1993,1;119
 - » Mok Arthritis Rheum 1998,41;831

Chemotherapy: Biology of Ovarian Failure

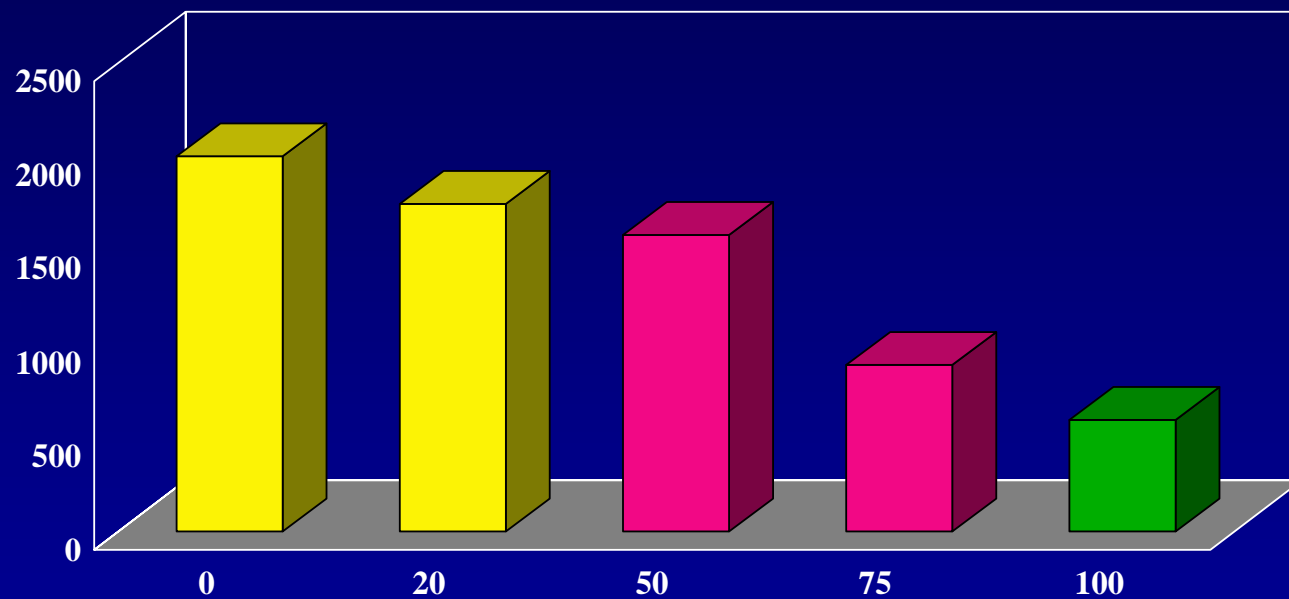
- The direct mechanisms of chemotherapy induced ovarian failure are poorly understood.
- In vitro study has demonstrated that in the human ovary chemotherapy acts on primordial follicles.
- Induction of apoptotic changes in pregranulosa cells which lead to follicle loss.
 - » Meiorow Hum Reprod (Abstr)13 ,1998

Chemotherapy: Biology of Ovarian Failure

- Effect of chemotherapy is not an “all or none” phenomenon.
- A dose-response effect exists.
- A dose of chemotherapy strong enough to destroy 50% of the ovarian primordial reserve does not affect reproductive performance.

» Meirov 1999

Effect of Alkylating Agent Dosage on Murine Primordial Follicle Loss



**Dose of Cyclophosphamide
(mg/kg)**

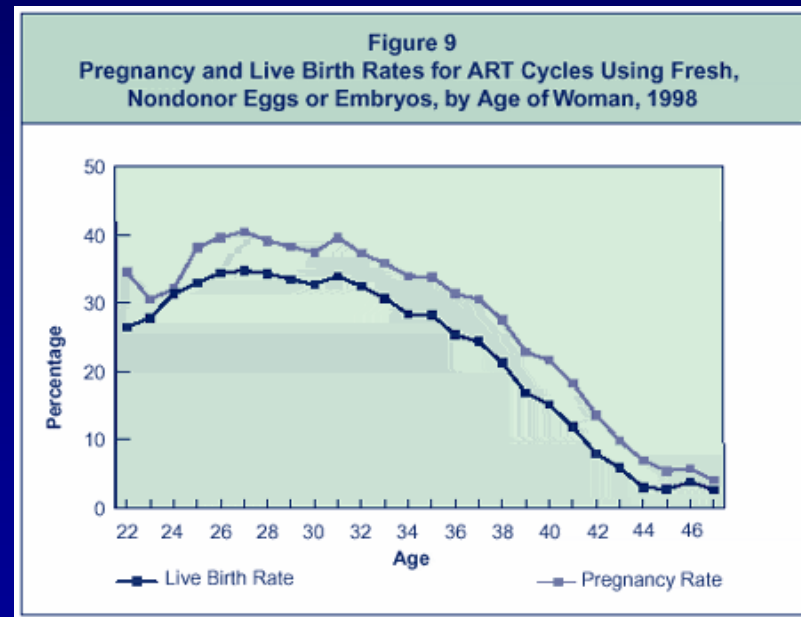
Meirow D., 1999

Effect Of Age on Ovarian Damage

- Older women; much higher incidence of complete ovarian failure.
 - » Moore :Curr Oncol Rep 2000 Nov;2(6):587-93.
- Cutoff may be as young as 26
- Resistance is related to follicle stores prior to treatment.
 - » Sandres: J Clin Oncol 1988 May;6(5):813-8

Age and Ovarian Reserve

- Among women in their twenties, both pregnancy and live birth rates are relatively stable
- Both rates decline sharply from the mid-thirties onward as fertility declines with age
 - CDC2002 (1998data)



Ovarian Preservation

- Ovarian Transposition
- GnRh agonists
- Embryo cryopreservation
- Oocyte cryopreservation
- Ovarian cryopreservation
- Others

GnRh agonists

- Inhibition of the pituitary-ovarian axis may protect ovaries from damage.
- Effect may exist for chemo but not for RT.
- Studies are conflicting. Only randomized trial shows no effect. (Waxman1987)
- Adverse effect on ER+ breast cancer pts
- Biologically no clear explanation since primordial follicles are not under the effect of gonadotropines.

» Blumenfeld J SGI 2000,8

» Meiorow 1999
CancerFertility

IVF for Cancer Survivors

- Sporadic reports.
- If systemic treatment delivery rate 13% vs 40% for local treatments (skin ca)
 - » Ginsburg et al FertilSteril: 2001, 75;705

IVF Post-Chemotherapy

- Possible risk of performing IVF and embryo cryopreservation to preserve fertility in females already exposed to chemotherapy.
- An animal study has raised serious concerns regarding the consequences of chemotherapy on future pregnancies.
 - Meirov2000
- High abortion and malformation rates related to the different stages of oocyte maturation at the time of exposure to chemotherapy were demonstrated.
- These results should be taken into account when considering the use of IVF .

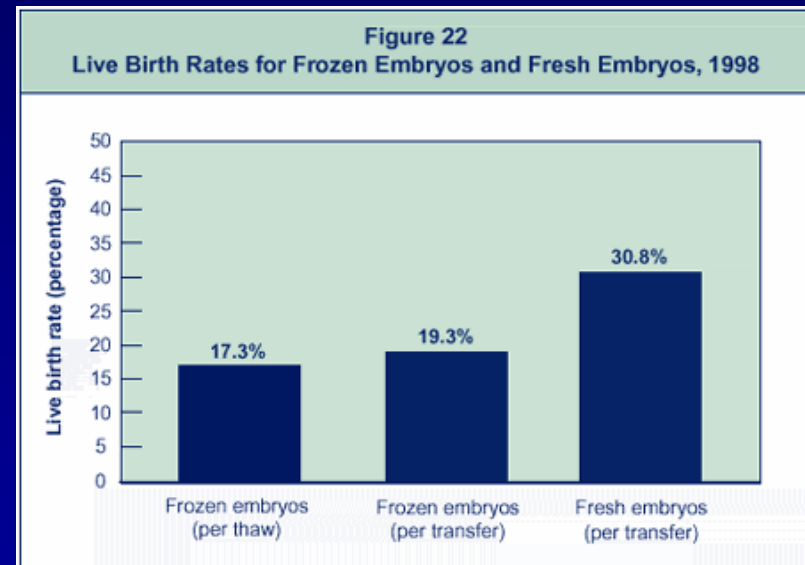
IVF and Embryo Cryo

Prior to treatment

- Most efficient option to date.
- Up to 30% delivery rates (based on age).
- Need to have a partner or use donor sperm.
- Must be post puberal.

Embryo Cryopreservation

- First 1983
 - Trounson et al Nature 305
- Upper limit of storage still controversial.
- Recent report of 9 year old embryos
- Ethical Issues
- Donor sperm if single



Ovarian Hyperstimulation

Prior to treatment

- Delay of treatment 2-4 weeks: probably inconsequential but may create problems in case of recurrence.
 - » Ginsburg: Fertilsteril.
- Do Fertility treatments increase risk of cancer?

Ovarian Hyperstimulation

Prior to treatment

- Does not appear to increase the incidence of breast cancer.
- In fact Clomifene had a protective effect (RR 0.5).
 - » Rossing et al: Gynecol Oncol 1996,60;3-7.
- Would a short term elevation in estradiol increase the risk of recurrence?

Ovarian Hyperstimulation

Prior to treatment

- Clomiphene
- Tamoxifen
- Letrozole
- Gonadotropins

Non Gonadotropin Protocols are not ideal

- Average # of embryos per patient
 - 1.6 Oktay
 - 1.4 Vidali

Cryopreservation

- First tissue freezing experiment 200 yrs ago
 - John Hunter Royal College of Surgeons
- Breakthrough was use of glicerol for sperm freezing by Audrey Smith in 1940
- New Cryoprotectants: dimethylsulfoxide (DMSO), ethylene glycol, propylene glycol.
- Still much easier to freeze cells than tissue.

Ovarian Tissue Cryopreservation (OTCP)

- Partial or total laparoscopic oophorectomy.
- Isolation of ovarian cortex.
- Cryopreservation utilizing standard cryoprotectants.
- Future utilization for reimplantation.

OTCP : Animal Experience

- Sheep studies report successful pregnancies (n=2).
- Autotransplants implanted in round ligament orthotopic
- Continued ovulation for 2 years.
- Ongoing experiments with heterotopic implantation
 - » Godsen et al : Hum Reprod:1994 ,9
 - » Aubard et al: Hum Reprod: 1999

OTCP: Animal Experience

- Human xenografts in SCID mice.
- Growth of implants in different locations (subcapsular, skin, muscle)
- Stimulation with exogenous FSH.
- Identification of antral follicles

» Oktay Hum reprod
1998, 69,1-6

» Oktay et al NEJM
2000 , 342;1919

» Revel et al : CancerFertility
abstract Eur Soc
Inf Bologna 2000



OTCP: Human Trials

- 2 approaches: orthotopic and heterotopic.
- With vascular anastomosis
 - » Leporrier et al: Cancer, 1987,60;2201-2204
- Without vascular anastomosis: loss of 75% of the follicular reserve
 - » Marconi et al: Fertil Steril, 1997, 68;364-366
 - » Nugent et al: Hum Reprod (abstract) 1998

OTCP: Human Trials

- 2 Patients with orthotopic graft
- #1 had combined ortho and heterotopic: follicles noted in forearm. Pelvic implant not visible.
- #2 pelvic implant using laparoscopic pelvic sidewall flap: ovulation observed after exogenous administration of gonadotropines

» Oktay 2000

OTCP: Human Trials

- Embryo development after heterotopic transplantation of cryopreserved ovarian tissue
 - *Kutluk Oktay lancet 2004*

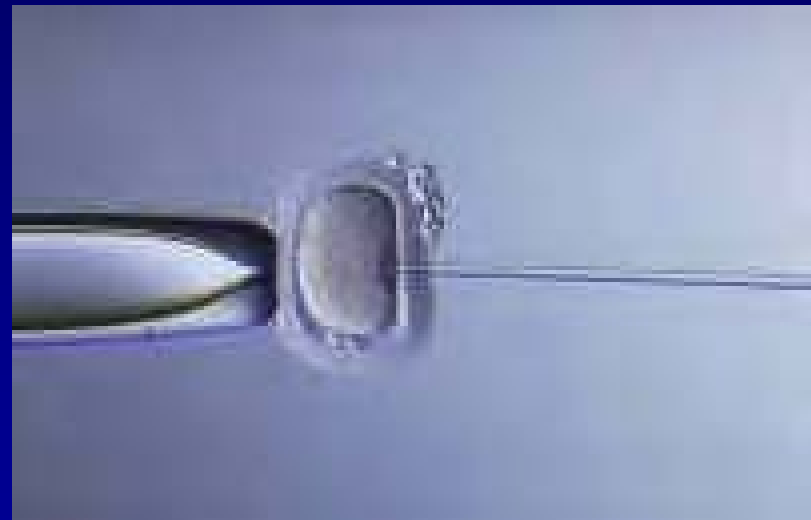
OTCP: Safety Concerns

- Reseeding of cancer cells.
- Aggressive leukemia, neuroblastoma, and Hodgkin's carry a significant risk of ovarian mets (up to 50%)
 - » Scully et al Blaunstein's
- Breast Cancer, Cervical adeno carry a moderate risk (up to 11%)
- Xenografts in SCID mice resulted in cancer recurrence
 - » Shaw 1996
 - » Oktay 2001

Oocyte Cryopreservation

- First case report 1997.
- 12 oocytes:4 survived
- ICSI:2 fertilized
- 1 transferred
- Healthy female delivered

– Porcu et al Fertil
Steril 1997
Oct;68(4):724-6



Cryopreservation Methods

- **Slow Cooling**
 - Conventional cryoprotectant : glycerol , dimethylsulfoxide (DMSO)
- **Vitrification**
 - High concentration of cryoprotectant mixtures
- **Delicate balance between several parameters**

Slow Cooling

- Slow cooling of cells to prevent intracellular ice formation
- Long exposure to increased electrolyte concentrations can damage cells



Vitrification

- High cryoprotectant concentrations are toxic to cells



Oocyte Damage

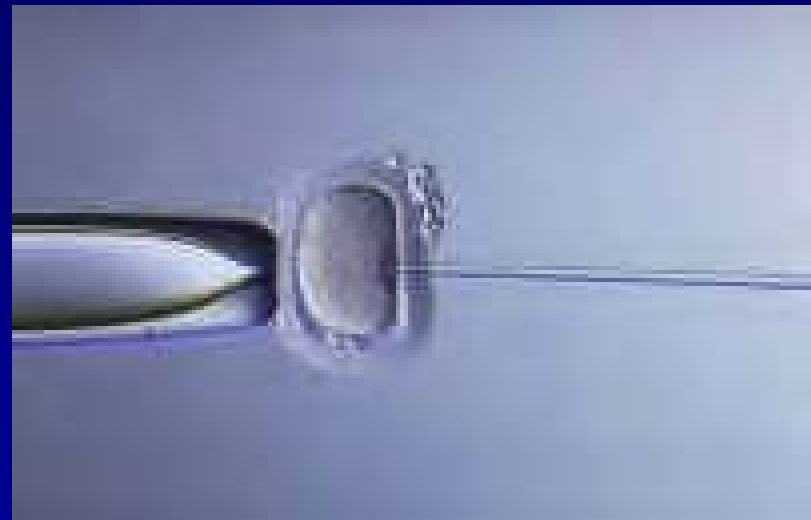
- Cell Lysis
- Disruption of Cytoskeleton and spindle microtubules
- Parthenogenetic activation
- Polyploidy



Membrane Damage

- The Zona Pellucida is damaged by the process.
- Inability of sperm to penetrate ZP
- Solution to problem: ICSI

» Hum Reprod.
1996
Jul;11(7):1513-
5.



Microtubules and Cytoskeleton

- Increased incidence of aneuploidy due to loss of chromosomes at the first maturation division.
- Microfilaments are essential for polar body extrusion and pronuclear migration.
- Alterations in the trafficking and organization of molecules and organelles

Current Protocol

- 1,2-propanediol added with sucrose
- Slow-freezing-rapid-thawing programme
- Cumulus covered cells

MII Oocytes: Literature Results

- Porcu et al 23 pregnancies
- Gianaroli:1
- Al Hasani:1
- Chen:1
- Van Uem :1
- Young:1 (triplet)
- Vidali:2



Patients

- 48 Patients
- Age was $27.2+3.7$ yrs.
- Average number of oocytes retrieved $20.8+3.2$

Indications

- Ethical/religious 87%,
- Inability to produce a sperm sample 6.5%
- Severe ovarian hyperstimulation 6.5%.

Results

- 471 oocytes were thawed.
- Post thaw survival was $62.5 \pm 0.2\%$.
- Fertilization rate was $56 \pm 0.2\%$.
- Average number of embryos transferred 3.0 ± 2.1 per ET.
- Birth rate was 4% per initiated cycle.
 - 13% for cryopreserved embryos (CDC 1998)

Germinal Vesicle Oocytes

- Do not have a spindle: less risk of cytogenetic errors.
- Still risk for zona hardening
- Can be obtained from unstimulated ovary.
- One pregnancy so far.
 - » Tucker et al 2000

Breast Cancer Survivors and Pregnancy

- The issue of subsequent pregnancy after breast carcinoma treatment is of paramount importance to young survivors and their oncologists
- The survival of women with breast carcinoma is not decreased by subsequent pregnancy in any of the published series.
 - » Surbone, Petreck Cancer 1997 Apr 1;79(7):1271-8

The Future

- Cryopreserved oocytes can also serve as a source for stem cell creation.
- If cryopreserved with Granulosa cells that's all the material needed for therapeutic cloning.



Resources for Patients

- www.fertilehope.org
- Simple and valid info.
- No other major resources available online.

