Update Fertility Preservation (Cancer Patients) and Fertility Postponement (Social Reasons)

Pasquale Patrizio, M.D., MBE
Department Obstetrics & Gynecology
Yale University Fertility Center
New Haven, CT-USA
Topics

• Epidemiology of Cancer
• Risk Factors
• Options to preserve Fertility for Women
• Updates:
  ➢ In Vitro Folliculogenesis
  ➢ Whole Ovary Perfusion and Directional Freezing
• Fertility Postponement
Young Women Exposed to Sterilizing Cancer Treatment/Year in USA

4% of Cancers (~55,000/Year): diagnosed in women under the age of 35

- 3,000 Cervix ca
- 3,500 Leukemia and 3,000 Lymphomas
- 15% of Breast cancer (~40,000/year)
- Bone Marrow – Stem Cell Transplantation
- SLE, Glomerulonephritis, Behcet, Sickle cells, etc.
## Incidence & Survival

<table>
<thead>
<tr>
<th>Lymphoma/leukemia (female)</th>
<th>Total number women newly diagnosed with cancer in 2011</th>
<th>Number and percentage women under age 34 with newly diagnosed cancer in 2011</th>
<th>5 Year relative Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL</td>
<td>4,000</td>
<td>1,760 (44%)</td>
<td>90-95%</td>
</tr>
<tr>
<td>NHL</td>
<td>30,300</td>
<td>1,650 (5.5%)</td>
<td>80-85%</td>
</tr>
<tr>
<td>ALL</td>
<td>2,410</td>
<td>1,750 (70.6%)</td>
<td>64%</td>
</tr>
<tr>
<td>CLL</td>
<td>600</td>
<td>20 (0.3%)</td>
<td>78%</td>
</tr>
<tr>
<td>AML</td>
<td>6,120</td>
<td>810 (12.7%)</td>
<td>23%</td>
</tr>
<tr>
<td>CML</td>
<td>2,150</td>
<td>220 (10.3%)</td>
<td>57%</td>
</tr>
</tbody>
</table>

Chemo/Radiotherapy are **Gonadotoxic** and **Risk** of Early Menopause

- **Type** of chemotherapy drug
- **Cumulative dose of chemotherapy**
- **Concomitant use and dose of radiation**
- **Age** of patient (>35 high risk)

# Chemo Drugs Risks for Gonadotoxicity

<table>
<thead>
<tr>
<th>High Risk</th>
<th>Low Risk</th>
<th>Intermediate Risk</th>
<th>Unknown Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide</td>
<td>Methotrexate</td>
<td>Cisplatin</td>
<td>Oxaliplatin</td>
</tr>
<tr>
<td>Chlorambucil</td>
<td>5-Fluorouracil</td>
<td>Adriamycin</td>
<td>Irinotecan</td>
</tr>
<tr>
<td>Melphalan</td>
<td>Vincristine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Busulfan</td>
<td>Bleomycin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrogen Mustard</td>
<td>Actinomycin D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procarbazine</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Lee S et al. ASCO guidelines, JCO (2006 and 2013)
Factors responsible for gonadotoxicity are **Age**, **Dose** and **Number of cycles** of the Alkylating agent

- **Six cycles of CMF** (cyclophosphamide, methotrexate, fluorouracil): **33% of Amenorrhea**

- **Six cycles of FEC** (fluorouracil, epirubicin, CTX): **50-65% of Amenorrhea**

*After 6 cycles of CTX containing polychemotherapy, ovarian age can be advanced up to 10 years.*

Kim et al., 2011, Fertil Steril
Overview Fertility preservation Strategies

- Hormonal suppression (evidence inconclusive)
- Surgery: Ovarian transposition/Trachelectomy (established)
- Oocyte freezing (established)
- Embryo freezing (established)
- Ovarian freezing and Transplantation (experimental)
  - Cortical strips
  - Whole Ovary
- In vitro folliculogenesis (experimental)
- In vitro ovary perfusion (experimental)
Oocyte Cryopreservation

- Single women
- Young (<40 years old)
- Ethical objections to embryo freezing
- Need time (about 2 weeks) before start of chemo or radiotherapy
- No contraindications to hormonal stimulation
- Should be offered prior to starting potentially sterilizing cancer treatment
# Results-Oocyte Cryo

**Vitrification is the Winner!!**

<table>
<thead>
<tr>
<th></th>
<th>Survival Rate/thawed oocyte</th>
<th>Fertilization Rate/thawed oocyte</th>
<th>Implantation Rate/thawed oocyte</th>
<th>Pregnancy Rate/thawed oocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Slow Freezing</strong></td>
<td>71.9% [67.44, 75.89] 95% CI</td>
<td>51.2% [42.2,60.1] 95% CI</td>
<td>7% [4.3-11.2] 95% CI</td>
<td>4.2% [3.08, 5.56] 95% CI</td>
</tr>
<tr>
<td><strong>Vitrification</strong></td>
<td>78.6% [70, 85.18] 95% CI</td>
<td>55.96% [47.4, 67.1] 95% CI</td>
<td>7.7% [5.35, 11] 95% CI</td>
<td>7.6% [4.98, 11.4] 95% CI</td>
</tr>
</tbody>
</table>
Embryo Cryopreservation

- Need time (about 2 weeks) before start of chemo or radiotherapy
- Need partner
- No contraindication to hormonal stimulation
- Should be offered prior to starting sterilizing cancer treatment
Embryo Freezing Stages

• **Pronuclear (1, 2 Propanediol)**
  – Single cell-No Spindle
  – Easy to assess survival – most viable divide

• **Cleavage (1, 2 Propanediol)**
  – Can freeze at all cleavage stages
  – No time urgency
  – Survival considered if > 50% blastomeres viable

• **Blastocyst (Vit Protocols) ↑↑**
  – More than 100 cells
  – Loss of some cells does not compromise the entire embryo

Courtesy Noyes N MD, NYU
Breast Cancer: Protocols for Egg/Embryo Freezing

- Natural cycle IVF
- Tamoxifen
- Tamoxifen + FSH
- **Letrozole + FSH + GnRH antagonist**
  - (5 mg)  (150/225IU)

** The winner!!**
Ovarian Tissue Cryopreservation

• Cancer patients who do not have enough **time** for ovarian stimulation (2 weeks) or not **safe**
• Have **no** partner (and/or wants to freeze more than few oocytes)
• **Pre-pubertal** girls
Pre-Freezing Evaluation (Safety)

- Realistic Chance of long term survival
- Cancer work-up negative for metastasis
- Oncologist approves procedure
- Pelvic exam and ultrasound normal
- Negative histological biopsies
  - Light microscopy and Molecular Markers
Risk of Ovarian Involvement in Cancer patients - Safety

<table>
<thead>
<tr>
<th>Low Risk (&lt;1%)</th>
<th>Mod. Risk (1%-11%)</th>
<th>High Risk (&gt;11%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilm’s Tumor</td>
<td>Stage III-IV</td>
<td>Leukemia</td>
</tr>
<tr>
<td>Lymphomas</td>
<td>Breast Cancer</td>
<td></td>
</tr>
<tr>
<td>Stage I-II</td>
<td>Adeno Cancer Cx</td>
<td>Neuroblastoma</td>
</tr>
<tr>
<td>Breast Cancer</td>
<td>Colorectal Cancer</td>
<td></td>
</tr>
<tr>
<td>Nongenital-Rhabdomyosarcoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteogenic Sarcoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous Cell Cx Cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ewing Sarcoma</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

What to do with Patients with Leukemia?

- No Time
- High risk of ovarian metastastic disease
- What is the best option?
- In Vitro Folliculogenesis from cortical strips
- In vitro Whole Ovary perfusion and Freeze
- Artificial Follicles
In vitro Folliculogenesis

Follicle culture performed using fresh cortical strips (IRB-approved protocol) [collaboration with E. Telfer, Edinburgh]

Hypothesis: Manipulation of the Target of Rapamycin (TOR) kinase allows control of follicle survival and growth (should improve likelihood of generating fertilizable mature eggs)
Multi-step Culture system to support human oocyte development

1) Preparation of the tissue (a) is crucial step: underlying stroma should be removed and the tissue flattened.

2) Multilaminar follicles (b) can be removed within 6 days of culture.

3) Once antral formation has been achieved (c), oocyte complexes can be removed and grown for a further period.

Approx 30% of oocytes that complete culture process can reach Metaphase II: Epigenetic Status and Fertilisation potential?

McLaughlin, Albertini, Wallace, Anderson & Telf...
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
Ovarian cryopreservation strategies and the fine control of ovarian follicle development *in vitro*

Joshua Johnson and Pasquale Patrizio
Akt/mTOR signaling and Growth Activation

Key:
- Suppression of follicular activation
- Maintenance of primordial follicle survival

(a) Suppressors
- AMH
- PTEN
- Tsc1/2
- Foxo2
- Foxo3a
- PDK1
- rpS6

(b) Maintainers
- AMH
- PTEN
- Tsc1/2
- Foxo2
- Foxo3a
- PDK1
- rpS6

Follicular activation

Follicle death
mTOR kinase controls granulosa proliferation and thus follicle growth (Yaba et al., 2008).

PTEN/Akt/mTOR pathway has been shown to be a key regulator of the rate of primordial follicle growth activation in mice (Liu group) and humans (Hsueh group).

Can this pathway by manipulated to maximize the growth activation, survival, and oocyte maturation in human cortical strip cultures?
Rapamycin treatment of **mouse** follicles in vitro: dose-dependent reduction in follicle growth

**Ultra-low dose @ 0.00001nM increases follicle size and improves morphology**
Distribution of Bovine Ovarian Follicles: Effect of Rapamycin

Distribution of HUMAN Ovarian Follicles: Effect of Rapamycin

120-360 Follicles Assessed per TREATMENT
Oocyte “Quality” in Human Ovarian Strip Cultures

% OOCYTES WITH HEALTHY APPEARANCE

- Non-growing
- Primary
- Secondary
Ultra-low dose Rapamycin and Ovarian Follicles in Vitro

a) Enhances primordial follicle growth activation and oocyte 'viability' (mouse, cow, and human)

b) Picomolar dose significantly alters granulosa cell gene expression at the level of transcription

c) Clinically attractive (? in vitro oocyte production)
In vitro perfusion apparatus
Sheep Ovaries perfused 36 hrs.
Sheep ovaries after 36 hrs: after hMG and hCG (retrieval)
Whole Sheep Ovaries Perfused in Vitro

6 Follicles between 7-8 mm

4 Oocytes retrieved

1MII and 1 MI and 2 GV
Estradiol secretion during in Vitro Perfusion

Estradiol, nmol/l

- Höger, hMG
- Vänster, kontroll

0  1  3  17  24  26 timmar
Whole Ovary Cryo new data
Beneficial effect of Directional Freezing

MTG directional freezing device

Sketch of directional freezing apparatus

Gradient T along the track

Arav et al RBMO (2010); Patrizio and Bromer Semin Reprod Med 2009
Study design
[Maffei S et al, Hum Reprod 2013]

experimental groups*
(n=10 each)

DIRECTIONAL FREEZING
• whole ovary (DFwo)
• Cortical tissue (Dfof)

CONVENTIONAL FREEZING
• whole ovary (CFwo)
• Cortical tissue (Cfof)

controls (CTR)
(n=8)

Follicle Culture 7 days

Morphological Analysis
Tunel Assay
Immunohistochemistry
Western blot

*Same freezing/thawing solutions applied for both protocols
Development of PMF into primary follicles *p<0.05

- Whole ovarian cryo provided higher yields of primary follicles development
- Directional freezing leads to higher rates of follicle growth
Result (ii) - cell Proliferation and Apoptosis

DFwo shows comparable proliferation rate (Ki67) to CTR.

More Ki67-cells in Dfof compared to Cfwo and Cfof (p<0.05)

At day 0 apoptotic rate comparable between groups.

After 7 days of follicle culture Cfwo shows a significant increase of apoptotic cells (p<0.05)
Result (iii) - expression of HS proteins

**HSP70** = most abundant heat shock protein in cells

Conventional freezing whole ovary induces the **activation of proteins involved in stress-response pathways**

No differences in other groups
Summary Directional Freezing

- DF significantly improve the integrity of follicular structure from primordial to secondary transition; is able to remove the latent heat produced by ice crystal formation (most likely the cause of tissue cryoinjury), and decreases rate of intracellular ice formation.

- Functional analysis showed that ovarian viability is well preserved in DF of Whole Ovaries: 
  A) higher follicular proliferation rate; 
  B) lower expression of HSP and 
  C) capacity to activate DNA repairing system.
Fertility Preservation in Women with Cancer

Ovarian Involvement Unlikely
- Chemo Cannot Be Delayed
  - Ovarian Cryo

Ovarian Involvement Likely
- Chemo Can Be Delayed for 2 weeks
  - Embryo/Oocyte Freezing
  - Ovarian Cryo
- Cryo Ovarian Tissue for Future In Vitro Folliculogenesis
  - Whole ovary in vitro Perfusion

Summary
Egg Freezing for “Social” Reasons
Postponement of Fertility
Average Age of First Time Mothers in USA
Now age **26.3 first pregnancy** (2013 data)
<table>
<thead>
<tr>
<th>Birth rates</th>
<th>20-24 y</th>
<th>25-29 y</th>
<th>30-34 y</th>
<th>35-39 y</th>
<th>40-44 y</th>
<th>45-49</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>96/1000</td>
<td>110/1000</td>
<td>97.7/1000</td>
<td>46.5/1000</td>
<td>10.1/1000</td>
<td>0.7/1000</td>
</tr>
<tr>
<td>2008</td>
<td>103/1000</td>
<td>115/1000</td>
<td>99.3/1000</td>
<td>46.9/1000</td>
<td>9.8/1000</td>
<td>0.7/1000</td>
</tr>
<tr>
<td>Variation</td>
<td>-7% declining last 20 yrs</td>
<td>-4%</td>
<td>-2%</td>
<td>-----</td>
<td>+1% increasing last 20 yrs</td>
<td>-----</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.3 in 1992</td>
<td></td>
</tr>
</tbody>
</table>
### Fresh (non-donor) IVF Cycles 1999-2008 (CDC)

<table>
<thead>
<tr>
<th>Year</th>
<th>&lt;35</th>
<th>35-37</th>
<th>38-40</th>
<th>41-42</th>
<th>&gt;42</th>
<th>Total Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>29,682</td>
<td>15,291</td>
<td>12,848</td>
<td>5,302</td>
<td>2,628</td>
<td>65,751</td>
</tr>
<tr>
<td>2000</td>
<td>33,453</td>
<td>17,284</td>
<td>14,701</td>
<td>6,118</td>
<td>3,401</td>
<td>74,957</td>
</tr>
<tr>
<td>2001</td>
<td>35,984</td>
<td>17,791</td>
<td>16,283</td>
<td>7,044</td>
<td>3,762</td>
<td>80,864</td>
</tr>
<tr>
<td>2002</td>
<td>37,591</td>
<td>19,110</td>
<td>17,454</td>
<td>7,733</td>
<td>3,938</td>
<td>91,032</td>
</tr>
<tr>
<td>2003</td>
<td>39,852</td>
<td>20,056</td>
<td>18,660</td>
<td>8,185</td>
<td>4,279</td>
<td>91,032</td>
</tr>
<tr>
<td>2008</td>
<td>43,296</td>
<td>23,326</td>
<td>21,793</td>
<td>9,783</td>
<td>4,907</td>
<td>103,105</td>
</tr>
<tr>
<td>2004</td>
<td>40,853</td>
<td>21,019</td>
<td>19,174</td>
<td>8,487</td>
<td>4,709</td>
<td>94,242</td>
</tr>
</tbody>
</table>

| % Change | +45.8 | +52.5 | +69.6 | +83.0 | +86.2 | +56.6 |

Notes: CDC, Centers for Disease Control and Prevention.
Reasons for Postponement

• Most common reason women give for their decision to postpone pregnancy is **uncertainty** about the stability of their relationships

• Another common reason for delaying pregnancy are **future goals and aspirations**
  – Women wait until reaching certain academic and career achievements
  – Women do not want to fall behind in the workplace
  – Desire to be financially secure when having a child
Need to Educate Women

Most women unsure what age infertility begins to take effect and how quickly it advances
  - Estimates suggest that as few as 75% of women understand that fertility decreases between ages 30-40

Believe that ART can overcome infertility
Risks of Postponing Fertility

- Older women have more trouble naturally becoming pregnant
  - Fertility starts to decline after age 30 with rapid decrease after age 35

- Even with reproductive technologies older women have a low chance of pregnancy
  - Only 8.8% of women over the age of 42 who use IVF will become pregnant
  - Only 4.1% of them will actually give birth to a child
Moving Forward

• General practitioners and gynecologists who see women at an early age should have a discussion with their patients about:
  – The risks of fertility postponement
  – Options Oocyte and Embryo cryopreservation

• Societal practices that encourage women to postpone fertility need to be addressed

• We must not think of age-related infertility as a disease but rather a social harm
SOLUTION to the PROBLEM

OOCYTE FREEZING
(by Vitrification)
One more consideration......

Cost of Fresh EGG donation: $32,000

Cost of Frozen EGG donation: $18,000

Cost of storing own EGGS: $6,500 (plus storage $600/year)
Fertility Postponement

• **51 cycles** (42 patients) cryopreserved for social reasons: **Total oocytes = 487**

• **Mean age**: 38 (range 31-42)

• **Job classification**: 13 Businesswomen-5 MD-5 Teachers-2 Psychologists-2 Lawyers-1 Minister-1 Chemist-3 Students.
Fertility Postponement

- Total of 487 oocytes cryopreserved
- 134 by slow freezing
- 353 by vitrification
- So far only two patients utilized oocytes: [41 years old, minister- now 43, had 13 oocytes by slow freezing-9 (69%) survived-2 fertilized (22%)-NP] and [39 years old, teacher-now 42, 12 oocytes SF-9 survived, 4 fertilized, NP]
<table>
<thead>
<tr>
<th>Age (y)</th>
<th>≤34 (n = 41)</th>
<th>35 - 37 (n = 129)</th>
<th>≥38 (n = 329)</th>
<th>P (anova)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2 day of OT (pg/ml±SD)</td>
<td>2612±1285</td>
<td>2416±1424</td>
<td>2248±1291</td>
<td>.07</td>
</tr>
<tr>
<td>LH morning after LA OT (IU/L; range) pt n=22</td>
<td>126 (70-170)</td>
<td>109 (45-201)</td>
<td>90 (19-211)</td>
<td>NS</td>
</tr>
<tr>
<td>Number oocytes retrieved n (range)</td>
<td>21 (4-59)</td>
<td>17 (3-47)</td>
<td>14 (2-74)</td>
<td>.0001</td>
</tr>
<tr>
<td>Number MII oocytes retrieved and frozen n (range)</td>
<td>15 (2-35)</td>
<td>12 (1-36)</td>
<td>10 (1-55)</td>
<td>.0001</td>
</tr>
<tr>
<td>Number MII per total number of oocytes</td>
<td>73%</td>
<td>74%</td>
<td>71%</td>
<td>NS</td>
</tr>
<tr>
<td>Peak E2 per retrieved oocyte (pg/ml±SD)</td>
<td>153±81</td>
<td>162±83</td>
<td>196±118</td>
<td>.001</td>
</tr>
</tbody>
</table>

All values are means.

Werner, Knopman, Arslan, Noyes, ISFP, 2011
Conclusions Fertility Postponement

- The majority of patients are older than 35 yrs
- So far low utilization rates (eggs still frozen)
  - The number of women that are using oocyte cryopreservation for fertility postponement is still low
- Although ASRM has removed the label experimental, it is not encouraging Oocyte freezing for fertility postponement
The TEAM

- J Johnson (Cell Biology, Yale)
- A Arav (Tel Aviv, Israel)
- M Brannstrom, M Milenkovich (Goteburg, Sweden)
- E Telfer (Edinburgh, Scotland)

References

Johnson & Patrizio Ann NY Acad Sci (2011)