The Biologic Clock: Timepieces and Time Machines

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Disclosures

• No conflicts of interest to disclose
Learning Objectives

1. Recognize the process of the normal physiologic depletion of oocytes and its effect on reproductive potential

2. Describe various tests to assess ovarian reserve and their inherent limitations

3. Understand the advantages and appreciate the limitations of options for preserving fertility potential, specifically oocyte cryopreservation
WHY NOW?
Timeline for Egg Freezing

• 1986: 1st pregnancy using cryopreserved oocytes, Chen
• 1999 1st pregnancy with vitrification, Kuleshova
• 2002 Publication of Creating a Life: Professional Women and the Quest for Children, Sylvia Ann Hewlett
• 2013 ASRM Guideline: “fertilization and pregnancy rates are similar” with fresh and vitrified, no longer experimental
• 2016 All the Single Ladies: Unmarried Women & the Rise of an Independent Nation
Creating a Life: Professional Women and the Quest for Children, Sylvia Ann Hewlett

- 2002 book, Time cover, 60 Minutes
- Career women making a mistake by not having children in their 20s
- 40% of women earning $50,000 or more a year are childless at age 45
Saturday Night Live Sketch

Video Not Provided
All the Single Ladies
Unmarried Women & the Rise of an Independent Nation

• 1960: 60% of Americans wed by 29 y.o.
• 2016: 20% wed by 29 y.o.
• 2013: >10,000 had frozen eggs; <1,500 had used them
Why now?

What are the demographic changes that have made this an urgent issue now?

Average age of mother at 1st birth: US 1970-2006

Percentage of 1st births by age of mother: US 1970-2006

SOURCE: CDC/NCHS, National Vital Statistics System
Age at 1\textsuperscript{st} Marriage 1890 - 2000

Age data from the U. S. Bureau of the Census, Current Population Reports (2000), "Estimated Age at First Marriage"
Changes in US Birth Rates by Selected Age of Mother 2007-2009

NOTES: The area of each column represents the group's contribution to the overall change. Column width is proportional to the number of births in 2007 in each group. Data for 2009 are preliminary.

Absolute Increase in Mother’s Average Age at 1st Birth by State
1970 -2006

United States: 3.6 years
- 4.3–5.5 years
- 3.8–4.2 years
- 3.3–3.7 years
- 3.0–3.2 years
- 2.0–2.9 years


Probability of Conceiving (Fecundability)
Peak: Age 22 = 1.0

“Biological Clock”
(Natural Oocyte Decline)

- Largest complement of eggs is at approx. 20 wks gestation: 7 Million
- At birth: 1-2 Million
- By puberty: 3-400,000
- During reproductive lifetime: 400-500 eggs are ovulated
Indicators of Oocyte Depletion

Remaining follicles
pre and post menopause

Ovarian volume

Pregnancy and Live Birth rates
Decline in Oocyte Quality

Aneuploidy Risk Increases with Age

Rate of occurrence (per thousand births)

Oocyte meiotic spindle abnormalities

Trisomy 21
Trisomy 18
Trisomy 13
Aneuploidy in Unfertilized Human Oocytes

- 3,042 unfertilized human oocytes analyzed
- 792 patients

Pellestor et al., 2003

Haploidy and the global incidence of aneuploidy.

Aneuploidies

Maternal age
Ovarian Reserve

- **OVARIAN RESERVE** is “the number and functional competence of the remaining primordial follicles and germ as the cells”

- Functional ovarian reserve decreases with increasing chronological age in an individual.

- Goal of ovarian reserve testing is to predict response to ovarian stimulation.

- Goal of ovarian stimulation is to stimulate an adequate number of oocytes without diminishing oocyte quality.
Proposed Tests for Ovarian Reserve

Ovarian Reserve Testing (ORT)

- Basal levels
  - Day 2-3 FSH & estradiol
  - Inhibin B
  - Anti Mullerian Hormone (AMH/MIS)

- Dynamic testing (stimulated)
  - CCCT
  - GAST
  - EFFORT

- Sonographic measures
  - Ovarian volume
  - Ovarian Blood flow
  - Antral Follicle Counts
So how do we measure ovarian reserve now?

Ovarian Reserve Testing (ORT)

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  - CCCT
  - GAST
  - EFFORT

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  - Ovarian volume
  - Ovarian Blood flow
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Best predictor of oocyte Quality: AGE
Testing & interpreting measures of ovarian reserve: A Committee Opinion

• Currently, there is no uniformly accepted definition of DOR as the term may relate to 3 related but distinctly different outcomes:
  • Oocyte Quality
  • Oocyte quantity
  • Reproductive Potential
• The use of a screening test for DOR in a low risk population will yield a larger # of false + results
• FSH is most commonly used
• Single FSH value has limited reliability

• AMH and AFC have less variability and are promising predictors
• Emerging evidence that Low (undetectable) AMH or AFC <6 is specific for POR but insufficient evidence that it can be used as a screen for failure to conceive
• Evidence of DOR does not necessarily equate with the inability to conceive

FertilSteril 103; 2015
FSH, Day 3

• Most widely used test of ovarian reserve
• Single result of limited value due to significant inter- and intra-cycle variability
• Does not predict poor response to stimulation unless very high thresholds are used
• Unable to predict lack of pregnancy unless even higher thresholds are used
• No evidence to support a benefit of waiting for a cycle with lower FSH values with regard to response or pregnancy.

VanRooij.HumReprod2002:17;3065
Antral Follicle Count (AFC)

- Sum of antral follicles (2-10mm) present in both ovaries
- Good inter-cycle and inter-observer reliability
- AFC directly correlates to # oocytes retrieved in low responders
- While able to predict high responders, these patients might not be maximally stimulated reducing specificity

Bancsi FertilSteril2004:81;35-41
AMH

- AMH can be measured at any time during the cycle
- Linear correlation between AMH levels and response to gonadotropin stimulation
- AMH also correlated with AFC and # oocytes retrieved
- No significant correlation between AMH level and pregnancy
- Decline in AMH over time precedes FSH, Inhibin B and AFC (by about 1 year!)

Van Rooij IAJ Human Reprod 2002;17:3065-71
Anti Mullerian Hormone (AMH)

- Dimeric glycoprotein of TGF-β family
- Exclusive expression in granulosa cells
- Greatest production in preantral and small antral follicles ≤ 2 – 6 mm

Weenen et al. Mol Hum Reprod 2000
AMH Assay Variability
Sources of AMH assays over past 3 years

1. Manual: Immunotech (Europe/limited US)
5. Manual: Beckman, Fix 2 (premix)
6. Manual: Ansh 1
8. Automated: Roche (Europe)
   Soon...
9. Automated: Beckman Access
10. Automated: Ansh
Inter-Lab AMH Variation

CAP 2014
45 Labs,
Same two samples

<table>
<thead>
<tr>
<th>Method</th>
<th>No. Labs</th>
<th>Mean</th>
<th>S.D.</th>
<th>C.V.</th>
<th>Median</th>
<th>Low Value</th>
<th>High Value</th>
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<tbody>
<tr>
<td>All Methods</td>
<td>44</td>
<td>1.635</td>
<td>0.337</td>
<td>21.9</td>
<td>1.50</td>
<td>0.63</td>
<td>2.37</td>
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<tr>
<td>All Methods</td>
<td>45</td>
<td>3.944</td>
<td>0.906</td>
<td>22.0</td>
<td>4.00</td>
<td>1.68</td>
<td>5.76</td>
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Even with the same AMH Kit, large differences


10 labs testing the same samples

Up to 80% Different Bias By Lab
Ovarian Reserve Testing

• Limited usefulness for patients wishing to delay fertility
• Benefit for predicting response to stimulation, # of oocytes retrieved and avoiding OHSS
• Educate patients about the effects of age on reproduction
• Discussion may also include oocyte/embryo cryopreservation where appropriate
Ovarian reserve summary

• Age is most important predictor of reserve

• Ovarian reserve testing predicts the number of oocytes that can be retrieved

• Abnormal testing demonstrates a smaller resting follicular pool and is correlated with worse outcomes
# Interpretation of Ovarian Reserve Testing

<table>
<thead>
<tr>
<th></th>
<th>Diminished Ovarian Reserve</th>
<th>Good Ovarian Reserve</th>
<th>Exceptional Ovarian Reserve</th>
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</thead>
<tbody>
<tr>
<td><strong>FSH</strong></td>
<td>&gt; 10-12 IU/L</td>
<td>&lt;8 IU/L</td>
<td>NA</td>
</tr>
<tr>
<td><strong>AMH (ng/ml)</strong></td>
<td>&lt; .8</td>
<td>&gt; 1.0</td>
<td>&gt; 1.6</td>
</tr>
<tr>
<td><strong>Antral Follicle Count</strong></td>
<td>&lt; 8-10</td>
<td>10-20</td>
<td>&gt; 20</td>
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</table>
Oocyte Cryopreservation

- Chen, 1986: First reported live birth
- Advantage: No partner needed
- Disadvantages: time, controlled hyperstimulation required, limited by poor thaw techniques
- Why poor survival of frozen oocytes vs embryo?
  - Initial slow freeze method
  - Fragility of meiotic spindle
  - Ice crystal formation (mature oocyte large H20 vol)
  - Hardening zona pellucida effect fertilization
Oocyte Freezing
Glass formation during vitrification

Two Techniques for Oocyte Cryopreservation

Traditional slow freezing
(Whittingham, Leibo & Mazur, Science; 1972; Wilmut, Life Sci, 1974)

Vitrification
(Rall & Fahy, Nature; 1985)
Indications for Oocyte Cryopreservation

- Oncofertility
- Oocyte donation
- Fertility preservation, "Social" can be oocyte or embryo freezing


### Efficiency of Oocyte Vitrification

<table>
<thead>
<tr>
<th></th>
<th>Vitrified</th>
<th>Fresh</th>
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<tbody>
<tr>
<td># oocytes</td>
<td>186</td>
<td>204</td>
</tr>
<tr>
<td>Survival</td>
<td>148 (79.6%)</td>
<td></td>
</tr>
<tr>
<td>Fertilized</td>
<td>124 (66.6%)</td>
<td>153 (75%)</td>
</tr>
<tr>
<td># good quality day 3 embryos</td>
<td>90 (48.4%)</td>
<td>101 (49.5%)</td>
</tr>
<tr>
<td># blastocysts</td>
<td>59 (55.1%)</td>
<td>65 (53.2%)</td>
</tr>
</tbody>
</table>

## RCT’s: Vitrification vs. Fresh

<table>
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<th></th>
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<tbody>
<tr>
<td><strong># patients</strong></td>
<td>60</td>
<td>584</td>
<td>80</td>
<td>62</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>26</td>
<td>26</td>
<td>35</td>
<td>35</td>
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<tr>
<td><strong>Survived</strong></td>
<td>96.9%</td>
<td>92.5%</td>
<td>96.8%</td>
<td>89.9%</td>
</tr>
<tr>
<td><strong>% Fertilized Vit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>76.3%</td>
<td>74%</td>
<td>79.2%</td>
<td>71%</td>
</tr>
<tr>
<td></td>
<td>82.2%</td>
<td>73%</td>
<td>83.3%</td>
<td>72.6%</td>
</tr>
<tr>
<td><strong># ET Vit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>3.8</td>
<td>1.7</td>
<td>2.3</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>3.9</td>
<td>1.7</td>
<td>2.5</td>
<td>2.6</td>
</tr>
<tr>
<td><strong>IR Vit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>40.8%</td>
<td>55.4%</td>
<td>38.5%</td>
<td>35.5%</td>
</tr>
<tr>
<td></td>
<td>100% (1 pt)</td>
<td>55.6%</td>
<td>43.5%</td>
<td>13.3%</td>
</tr>
<tr>
<td><strong>CPR/warmed oocyte</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.1%</td>
<td>4.5%</td>
<td>12%</td>
<td>6.5%</td>
</tr>
</tbody>
</table>
Does Vitrification Affect Embryo Development?

- 44 patients
- Mean age 29.9
- Oocytes randomized to be vitrified for 15 minutes or fertilized
- PGS on all blastocysts
- No difference in aneuploidy rate

Oocyte vitrification does NOT increase aneuploidy

• Sibling oocyte study
  • Pts < 35y/o undergoing 1st IVF cycle
  • Normal ovarian reserve testing
  • Comprehensive Chromosome Screening of blastocysts
  • Double embryo transfer, one from each group
  • Gender determination or DNA fingerprinting in singletons to learn which embryo implanted
  • No differences in aneuploidy rates, implantation rates, pregnancy rates, live birth rates between groups

Infant Outcomes

• Noyes 2009 → systematic review

• Over 900 babies born without an apparent increase in congenital anomalies
  • Overall anomaly rate was 1.3%

• To date >1500 babies born without an increase in anomalies noted (out of ? est. over 300,000 total)
  • “Registries are needed”

Noyes N. et al., Reprod Biomed Online 2009
So what is the downside?

- Data on efficacy of oocyte freezing for deferring childbearing sparse
- Data on safety, efficacy, cost-effectiveness, and emotional risks of elective oocyte cryopreservation are insufficient to recommend routine elective oocyte cryopreservation
- Requires more interventions to generate embryos (freezing, storage, ICSI, and hatching) leading to more attrition
- Cited success rates are clinic specific and cannot be generalized and is technically much more challenging than embryo cryopreservation (unforgiving process)
- Marketing efforts for the purpose of delaying childbearing may give false hope and encourage delay of childbearing
So how do I counsel my patients?

- Still a new technology with unproven long-term efficacy & outcomes
- Safe and probably works well for young women with average to above average egg reserves. Ideal age: < 35 y.o.
- May offer peace of mind but still fraught with many uncertainties
- Which is worse: to potentially lose opportunity to have a child or go through expensive, unpleasant therapy that might not be necessary?
Predicted probabilities of having at least 1, 2 & 3 live-born children by oocytes retrieved
ASRM and ACOG Positions

• **ASRM 2013 Guideline**: “fertilization and pregnancy rates are similar to IVF/ICSI with fresh oocytes when vitrified/warmed oocytes are used as part of IVF/ICSI for young women” “Evidence indicates that oocyte vitrification and warming should no longer be considered experimental.”

• **ACOG 2014 Committee Opinion**: “The American College of Obstetricians and Gynecologists’ Committee on Gynecologic Practice endorses the joint document and encourages its use by Fellows. There are not yet sufficient data to recommend oocyte cryopreservation for the sole purpose of circumventing reproductive aging in healthy women.”

http://dx.doi.org/10.1016/j.fertnstert.2012.09.028
Conclusions

• Decline in fertility potential is a natural consequence of ageing in women
• Current interest in fertility preserving strategies is a consequence of recent demographic trends and advances in technology permitting oocyte and embryo cryopreservation
• There are no reliable tests to predict infertility resulting from ovarian ageing
• Oocyte cryopreservation represents a safe, effective option for preserving fertility potential in younger women