



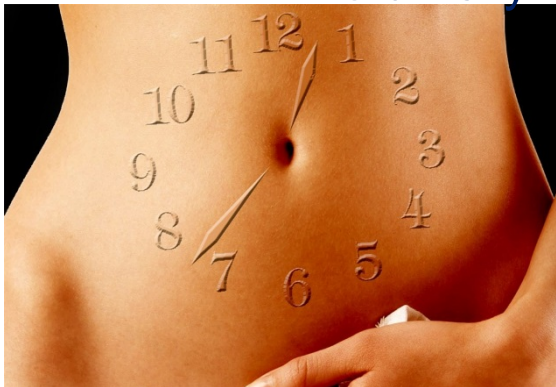
# The Biologic Clock: Timepieces and Time Machines

Steven J Ory, MD

IVF Florida, Reproductive Associates

Professor, Obstetrics and Gynecology, FIU

Voluntary Associate Professor, Obstetrics and Gynecology, University of Miami



# Disclosures

- No conflicts of interest to disclose

# Learning Objectives

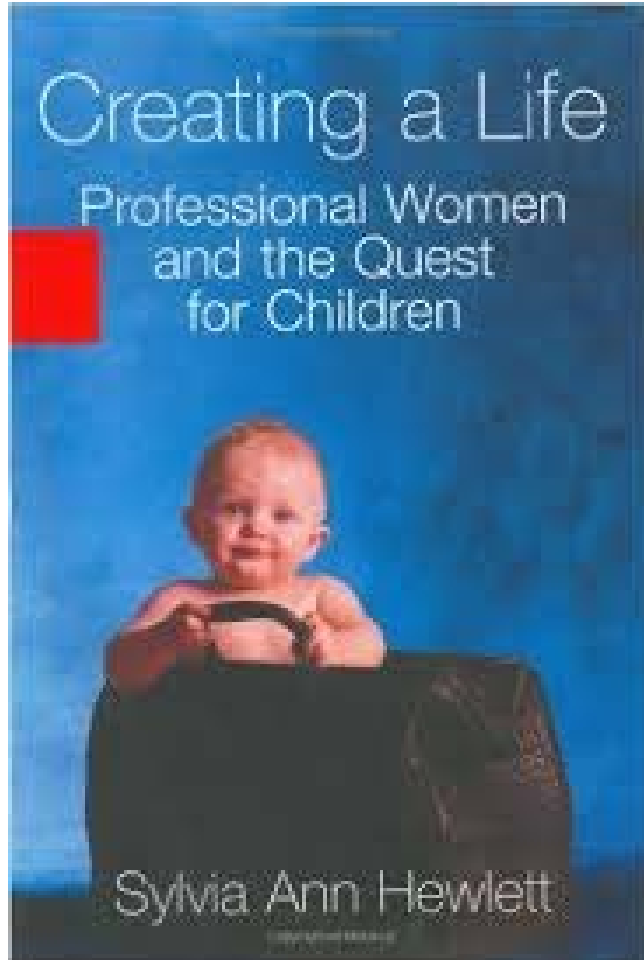
1. Recognize the process of the normal physiologic depletion of oocytes and its effect on reproductive potential
2. Describe various tests to assess ovarian reserve and their inherent limitations
3. Understand the advantages and appreciate the limitations of options for preserving fertility potential, specifically oocyte cryopreservation

# WHY NOW?

## Timeline for Egg Freezing

- 1986: 1<sup>st</sup> pregnancy using cryopreserved oocytes, Chen
- 1999 1<sup>st</sup> 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

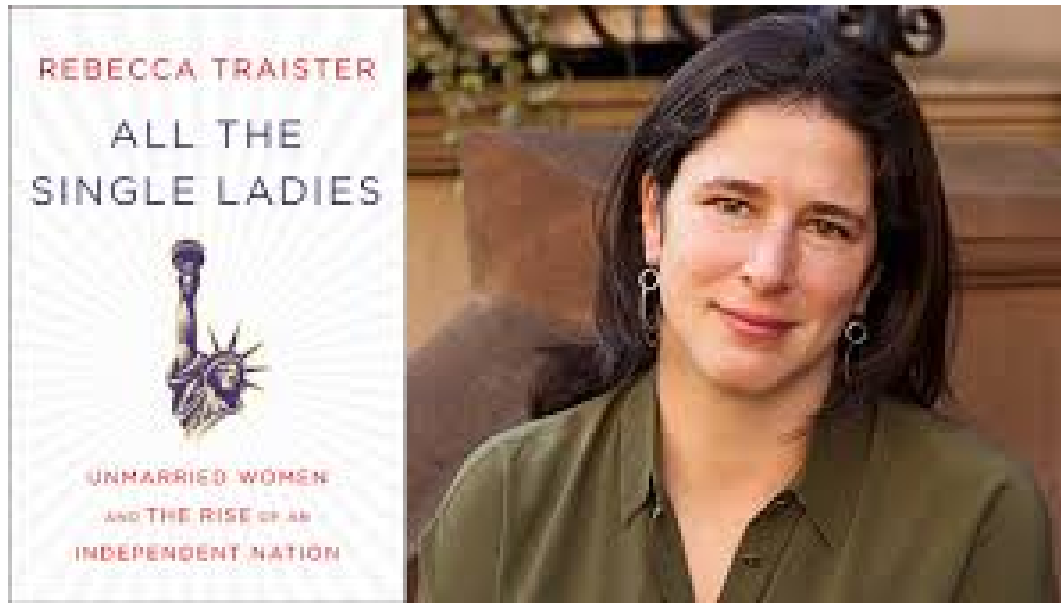


# Saturday Night Live Sketch

Video Not Provided

# All the Single Ladies

## Unmarried Women & the Rise of an Independent Nation

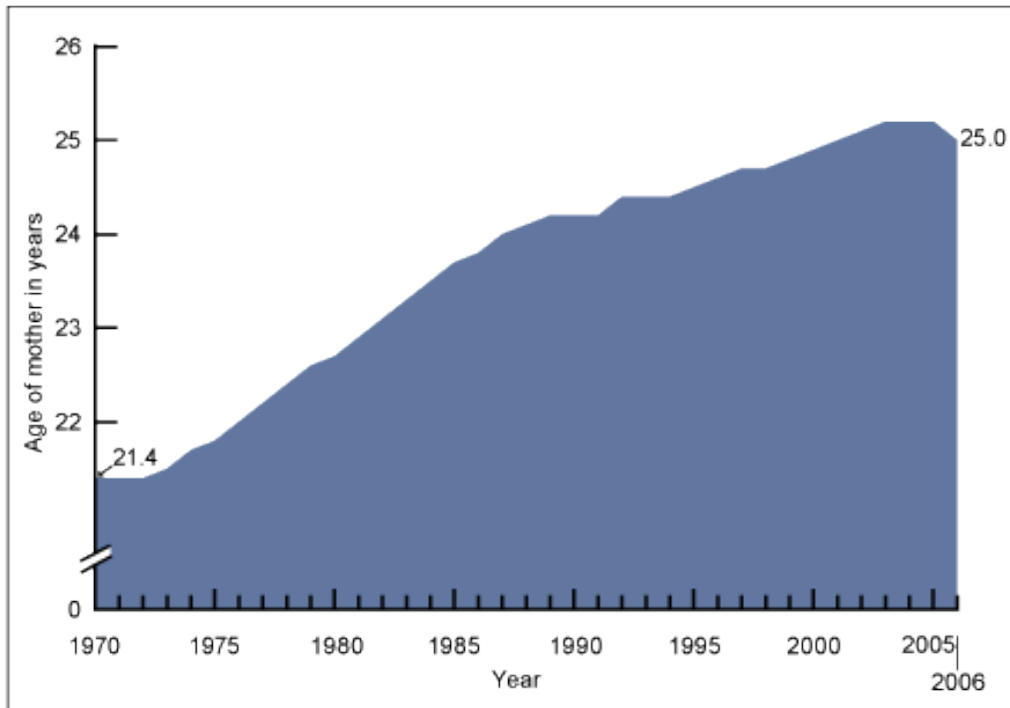


- 1960: 60% of Americans wed by 29 y.o.
- 2016: 20% wed by 29 y.o.
- 2013: >10,000 had frozen eggs; <1,500 had used them

# Why now?

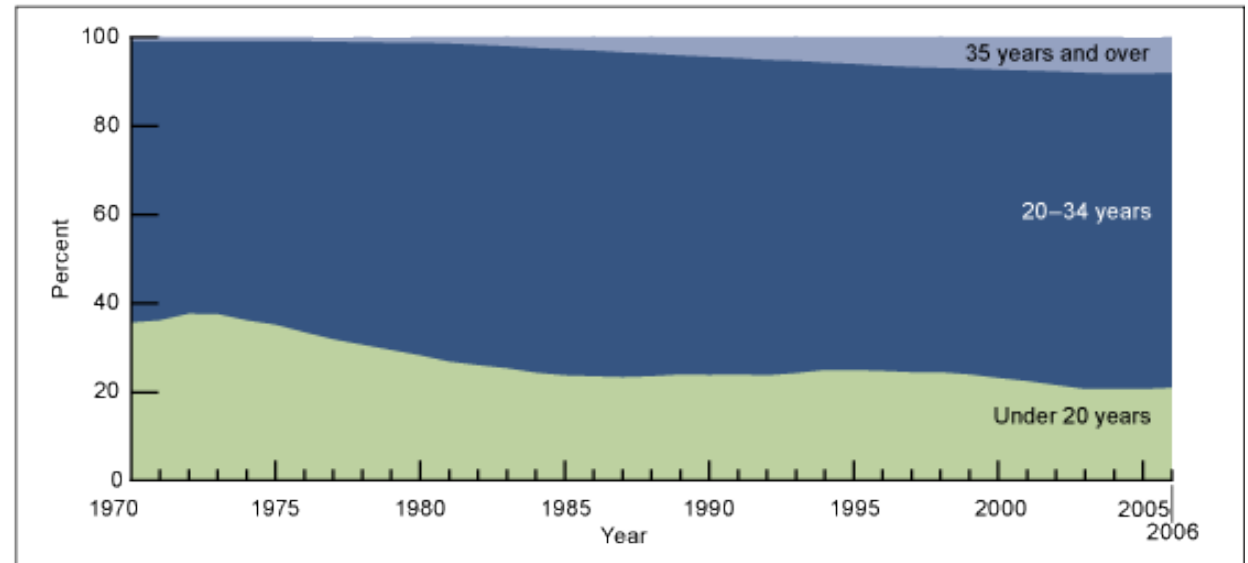
## What are the demographic changes that have made this an urgent issue now?

Average age of mother at 1<sup>st</sup> birth: US 1970-2006



SOURCE: CDC/NCHS, National Vital Statistics System.

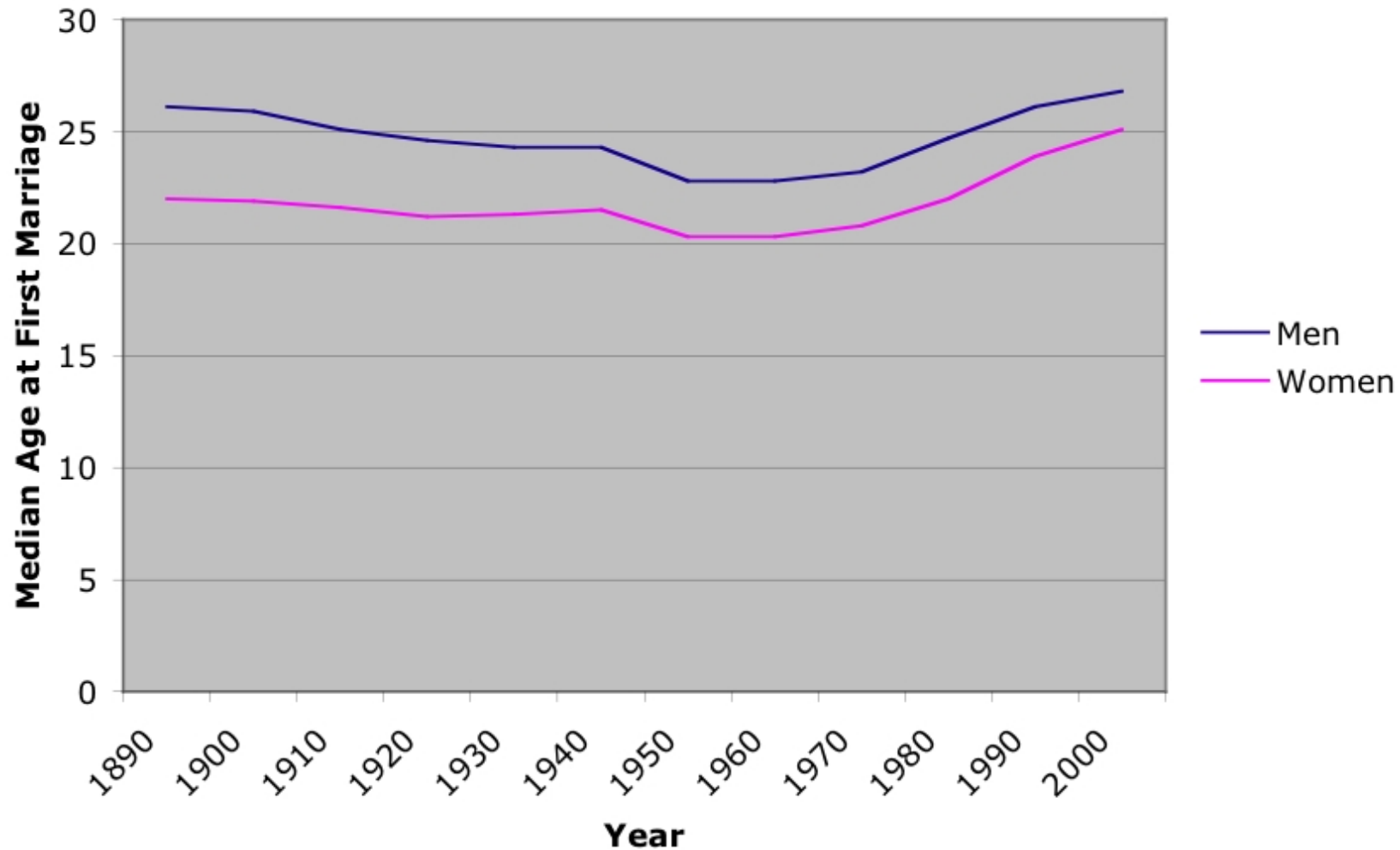
Percentage of 1<sup>st</sup> births by age of mother: US 1970-2006



SOURCE: CDC/NCHS, National Vital Statistics System.

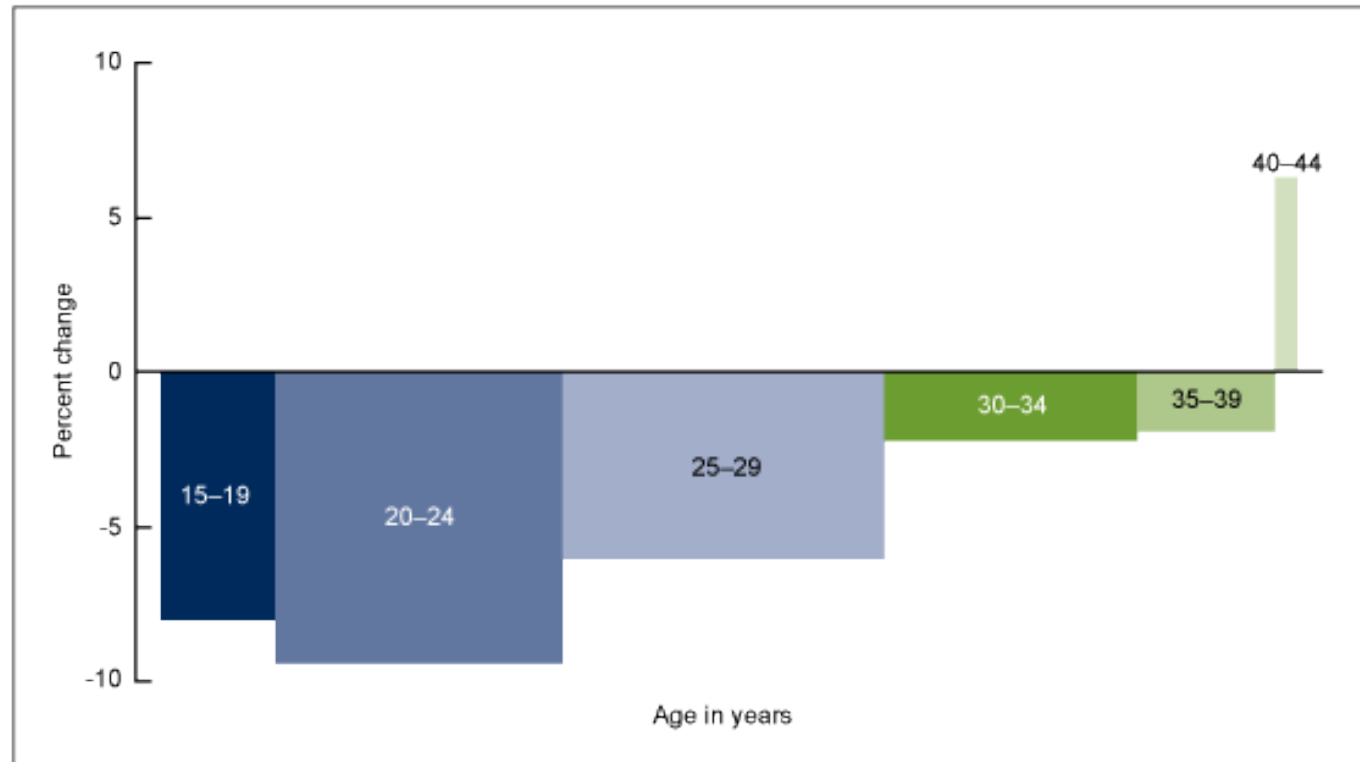


# Age at 1<sup>st</sup> Marriage 1890 - 2000



Age data from the U. S. Bureau of the Census, Current Population Reports (2000), "Estimated Age at First Marriage"

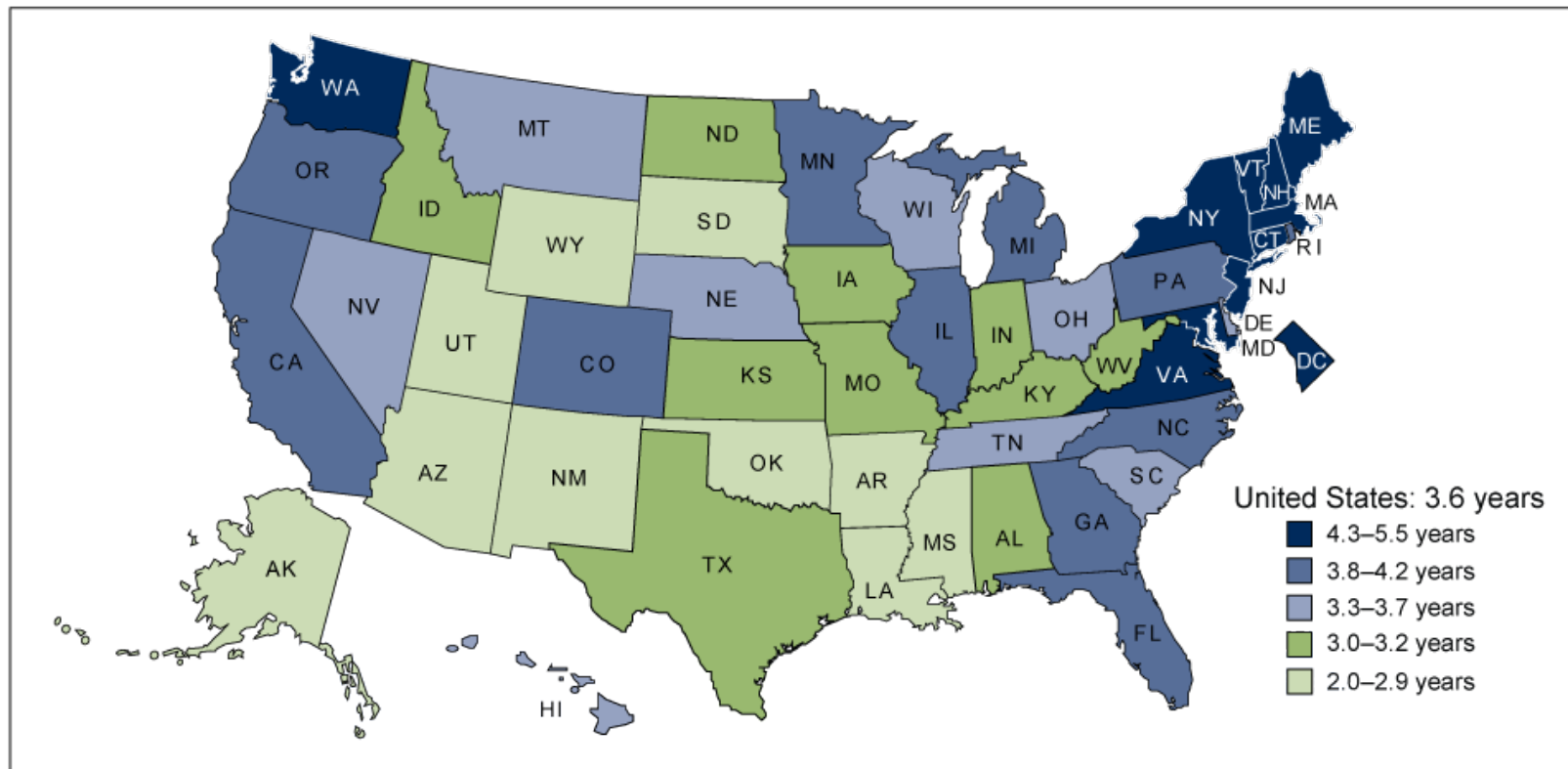
# Changes in US Birth Rates by Selected Age of Mother 2007-2009



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.

SOURCE: CDC/NCHS, National Vital Statistics System.

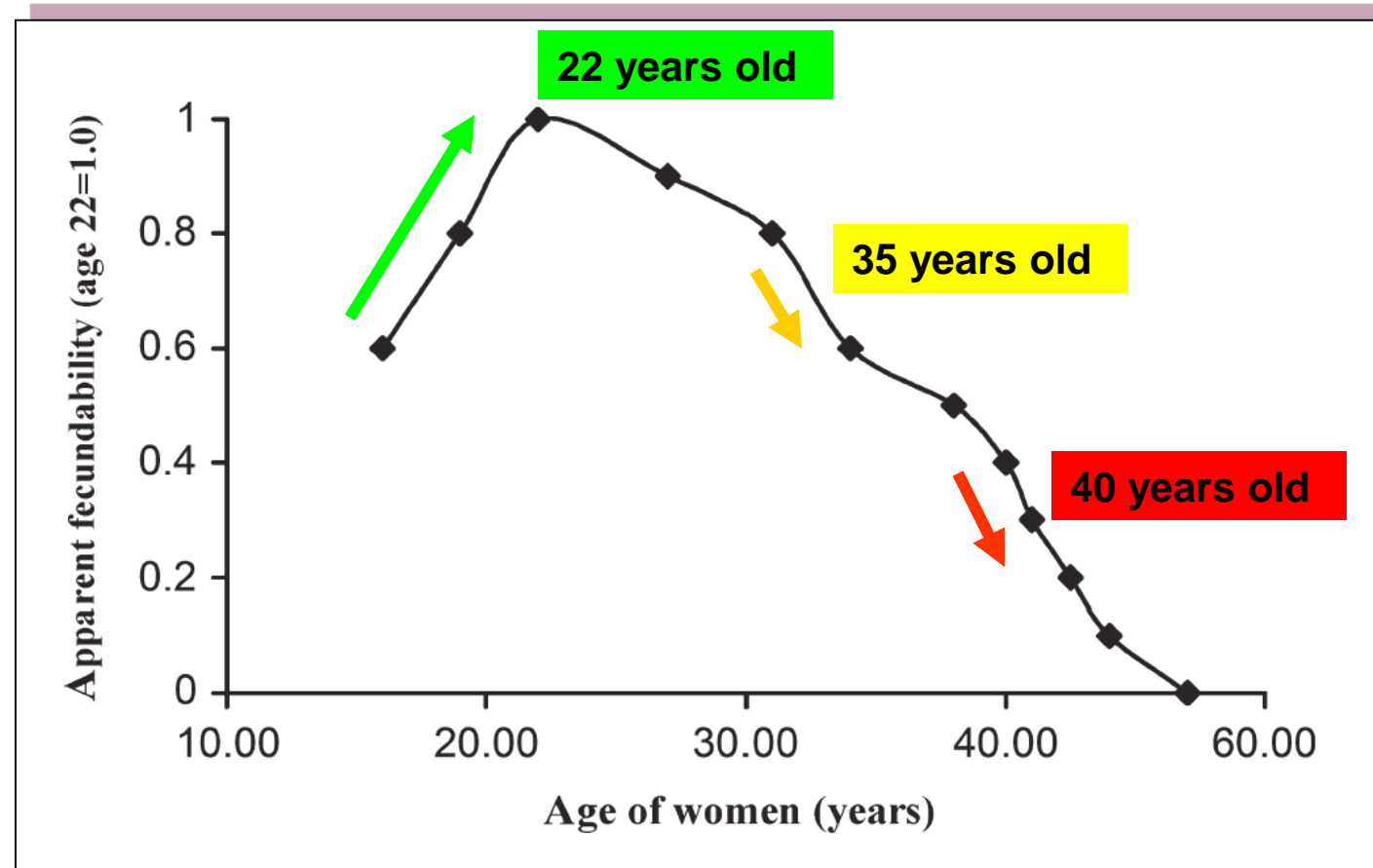
# Absolute Increase in Mother's Average Age at 1<sup>st</sup> Birth by State 1970 -2006



SOURCE: CDC/NCHS, National Vital Statistics System.

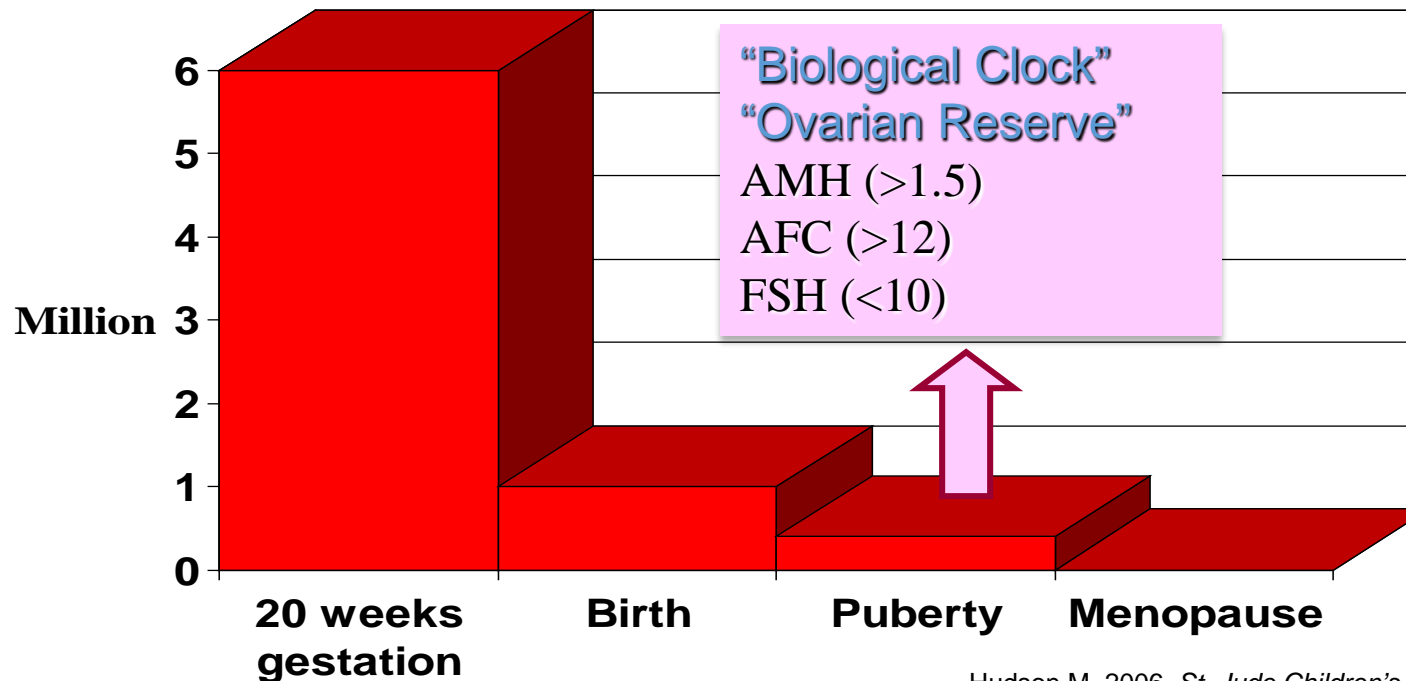
# Probability of Conceiving (Fecundability)

**Peak: Age 22 = 1.0**



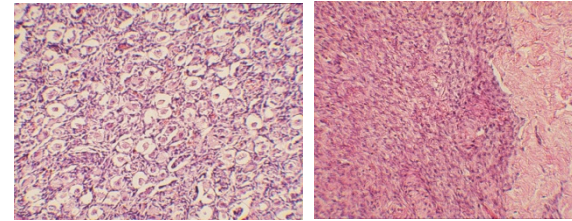
# “Biological Clock” (Natural Oocyte Decline)

- 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**

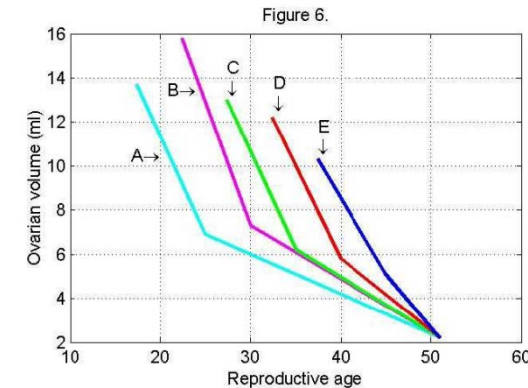


# Indicators of Oocyte Depletion

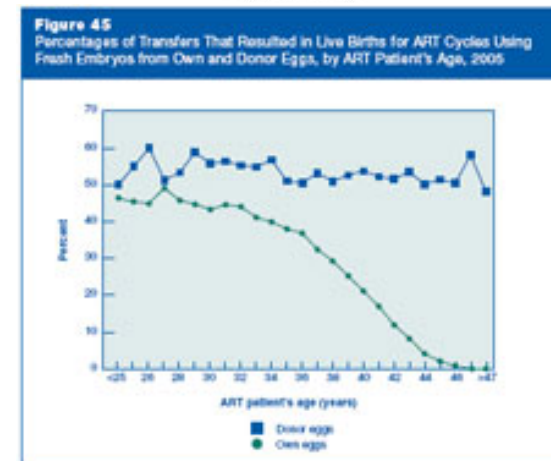
Remaining follicles  
pre and post menopause



Ovarian volume

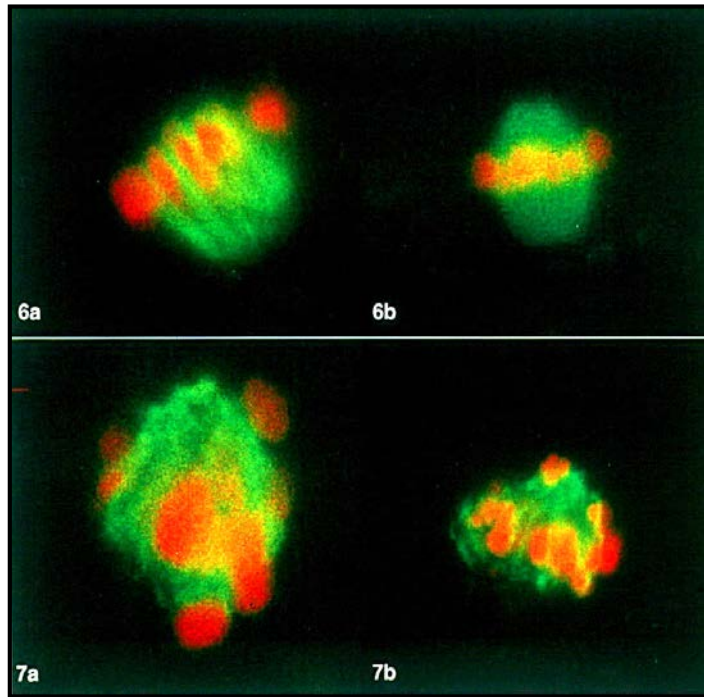


Pregnancy and Live  
Birth rates



# Decline in Oocyte Quality

Oocyte meiotic spindle abnormalities

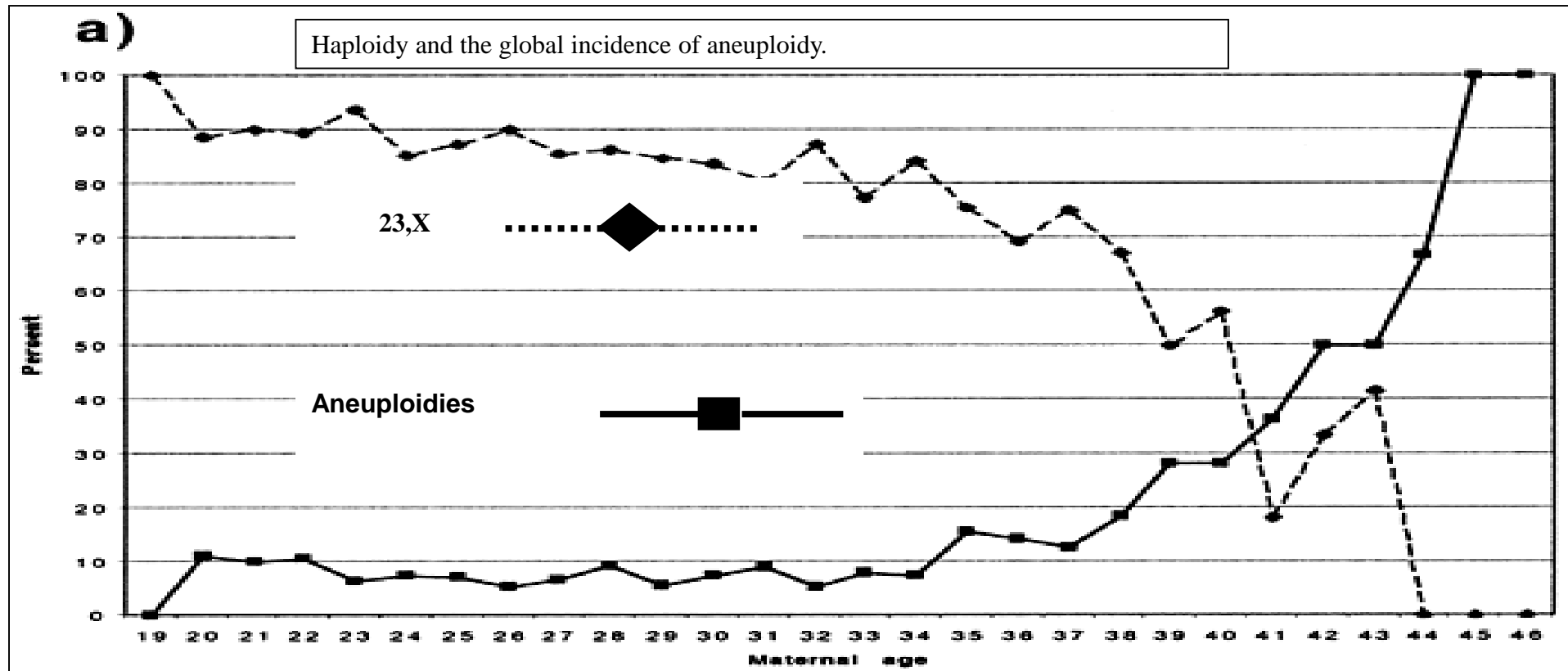


Aneuploidy Risk Increases with Age



# Aneuploidy in Unfertilized Human Oocytes

- 3,042 unfertilized human oocytes analyzed
- 792 patients





# 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)

- 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

Best predictor of oocyte Quality: **AGE**

# Testing & interpreting measures of ovarian reserve: A Committee Opinion

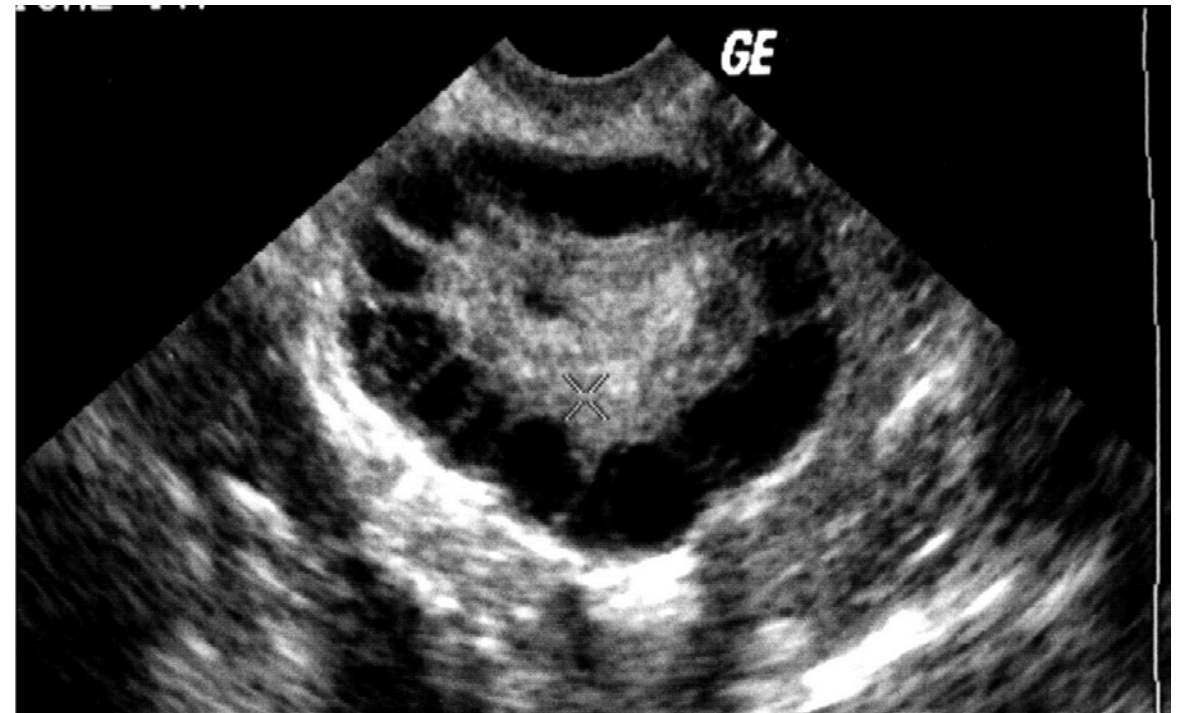
- Currently, there is no uniformly accepted definition of DOR as the term may relate to 3 related but distinctly different outcomes:
  - **Oocyte Quality**
  - **Oocyte quantity**
  - **Reproductive Potential**
- The use of a screening test for DOR in a low risk population will yield a larger # of false + 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

# 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.

# 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

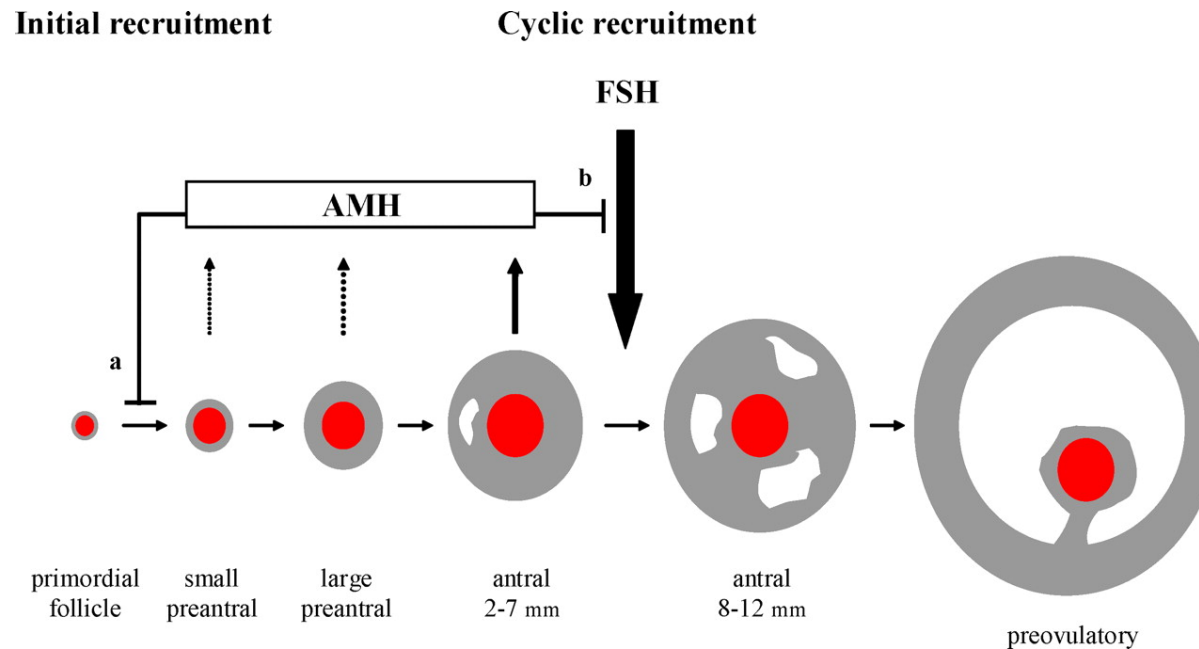


# AMH

- 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!)

# Anti Mullerian Hormone (AMH)

- Dimeric glycoprotein of TGF-  $\beta$  family
- Exclusive expression in granulosa cells
- Greatest production in preantral and small antral follicles  $\leq 2 - 6$  mm





# AMH Assay Variability

## Sources of AMH assays over past 3 years

1. Manual: Immunotech (Europe/limited US)
2. Manual: DSL Gen I
3. Manual: Beckman "Gen II"
4. Manual: Beckman, Fix 1 (dilution method)
5. Manual: Beckman, Fix 2 (premix)
6. Manual: Ansh 1
7. Manual: Ansh 2 "pico"
8. Automated: Roche (Europe)
- Soon...
9. Automated: Beckman Access
10. Automated: Ansh

# Inter-Lab AMH Variation

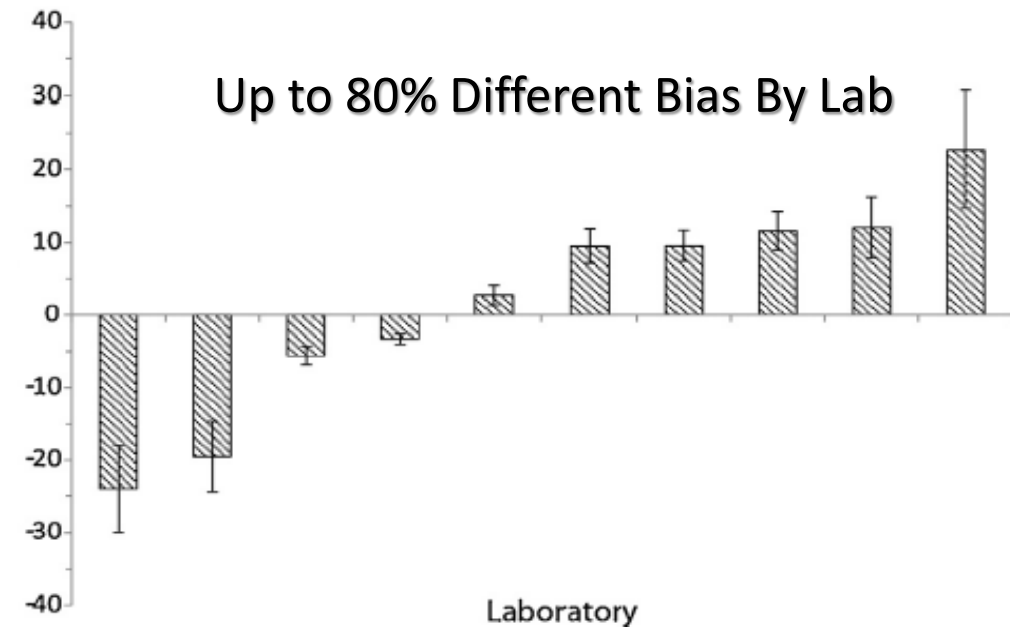
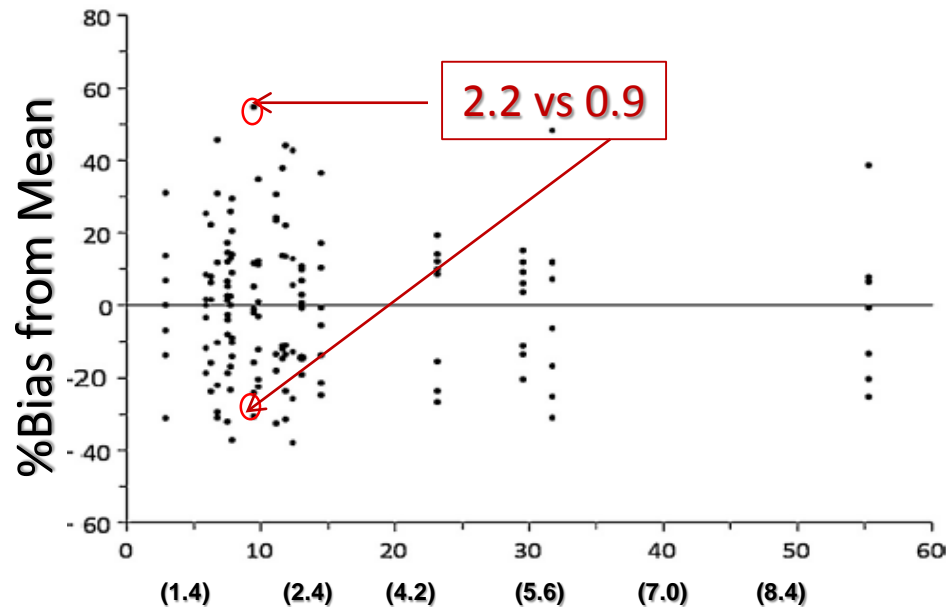
**CAP 2014**  
45 Labs,  
Same two  
samples

Method	No. Labs	Mean	S.D.	C.V.	Median	Low Value	High Value
All Methods	44	1.535	0.337	21.9	1.50	0.83	2.37
All Methods	45	3.944	0.905	22.9	4.00	1.88	5.76

**Even with the same AMH Kit, large differences**

Zuvela, et al *Reprod Biol* 2013; 13(3): 255-7.

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

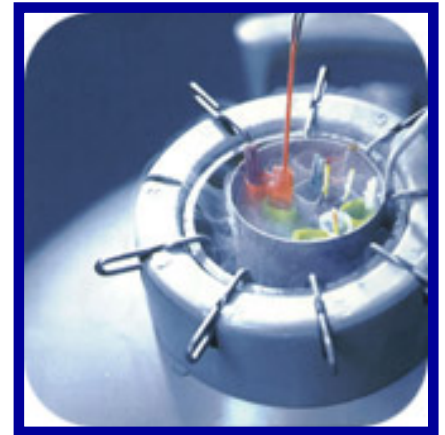
- Age is most important predictor of reserve
- Ovarian reserve testing predicts the number of oocytes that can be retrieved
- Abnormal testing demonstrates a smaller resting follicular pool and is correlated with worse outcomes

# Interpretation of Ovarian Reserve Testing

	Diminished Ovarian Reserve	Good Ovarian Reserve	Exceptional Ovarian Reserve
FSH	> 10-12 IU/L	<8 IU/L	NA
AMH (ng/ml)	<.8	>1.0	> 1.6
Antral Follicle Count	< 8-10	10-20	> 20

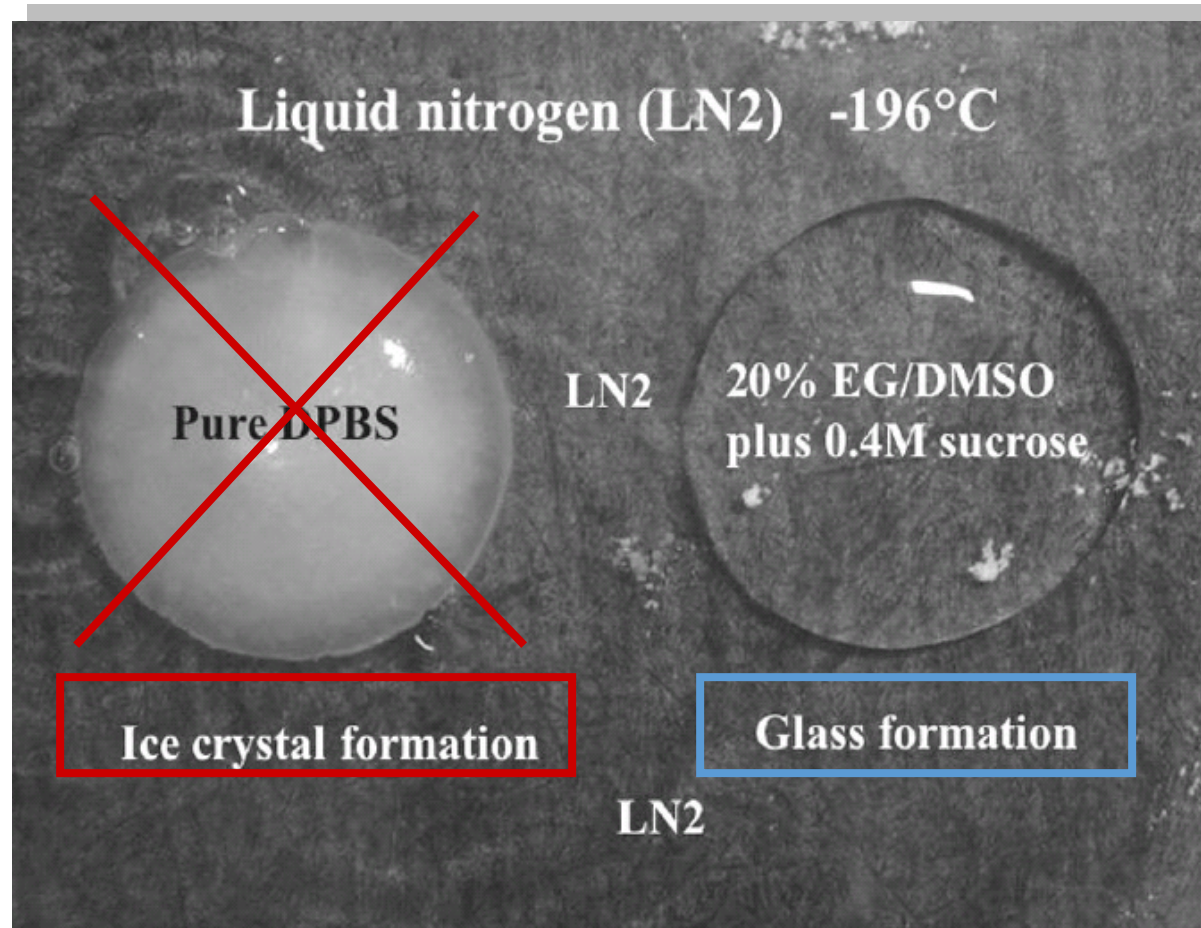
# Oocyte Cryopreservation

- 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 H<sub>2</sub>O 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

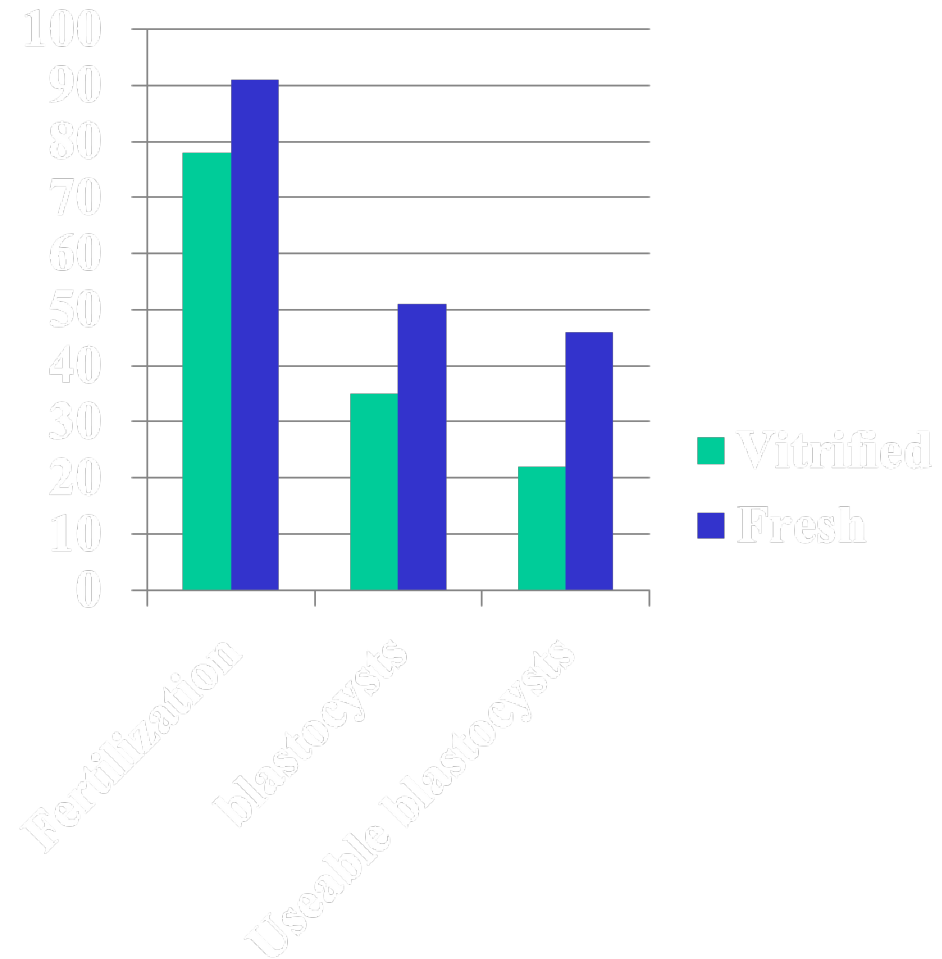
	Vitrified	Fresh
# oocytes	186	204
Survival	148 (79.6%)	
Fertilized	124 (66.6%)	153 (75%)
# good quality day 3 embryos	90 (48.4%)	101 (49.5%)
# blastocysts	59 (55.1%)	65 (53.2%)

# RCT's: Vitrification vs. Fresh

	Cobo 2008 Donors	Cobo 2010 donors	Rienzi 2010 Extra oocytes	Parmegiani 2011 Extra oocytes >5
<b># patients</b>	<b>60</b>	<b>584</b>	<b>80</b>	<b>62</b>
<b>Age</b>	<b>26</b>	<b>26</b>	<b>35</b>	<b>35</b>
<b>Survived</b>	<b>96.9%</b>	<b>92.5%</b>	<b>96.8%</b>	<b>89.9%</b>
<b>% Fertilized Vit</b>	<b>76.3%</b>	<b>74%</b>	<b>79.2%</b>	<b>71%</b>
<b>Fresh</b>	<b>82.2%</b>	<b>73%</b>	<b>83.3%</b>	<b>72.6%</b>
<b># ET Vit</b>	<b>3.8</b>	<b>1.7</b>	<b>2.3</b>	<b>2.5</b>
<b>Fresh</b>	<b>3.9</b>	<b>1.7</b>	<b>2.5</b>	<b>2.6</b>
<b>IR Vit</b>	<b>40.8%</b>	<b>55.4%</b>	<b>38.5%</b>	<b>35.5%</b>
<b>Fresh</b>	<b>100% (1 pt)</b>	<b>55.6%</b>	<b>43.5%</b>	<b>13.3%</b>
<b>CPR/warmed oocyte</b>	<b>6.1%</b>	<b>4.5%</b>	<b>12%</b>	<b>6.5%</b>

# 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 1<sup>st</sup> 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”

# So what is the downside?

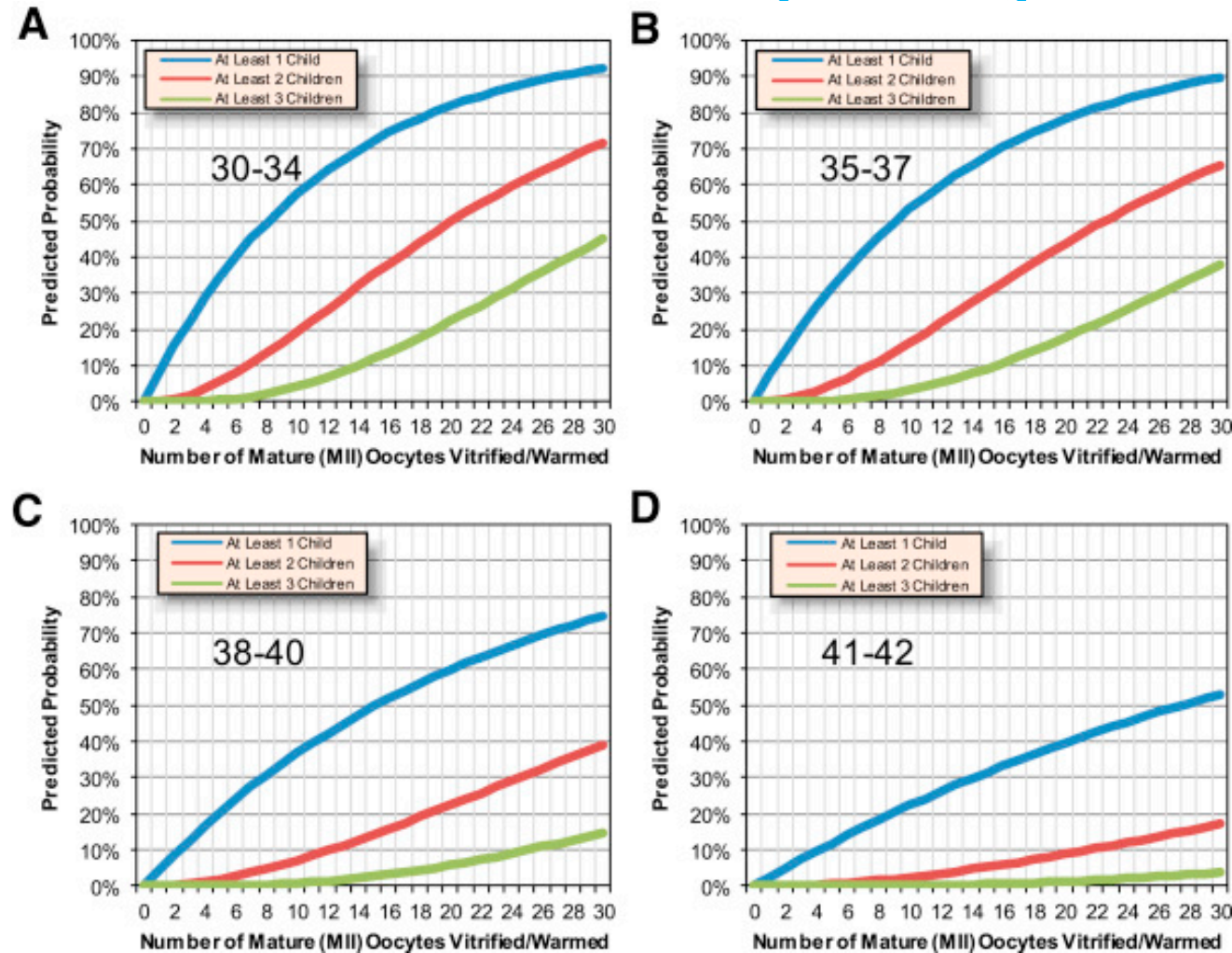
- Data on efficacy of oocyte freezing for deferring childbearing sparse
- Data on safety, efficacy, cost-effectiveness, and emotional risks of elective oocyte cryopreservation are insufficient to recommend routine elective oocyte cryopreservation
- Requires more interventions to generate embryos (freezing, 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.**”

# 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