**ESHRE Cryobiology Mtg – Athens, Greece 9/26/09**

# Vitrification of Oocytes: Biological Lessons Learned From Mice, Applied to Women


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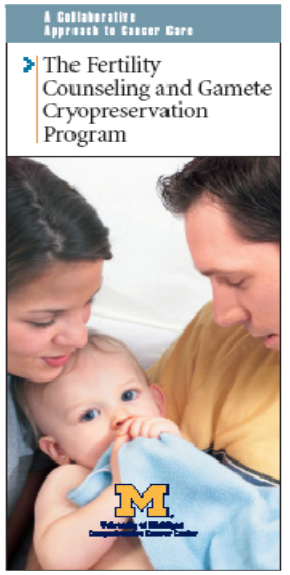


# Utility of Oocyte Cryopreservation

1) Preserving fertility in cancer patients and/or women undergoing oophorectomy.

2) Ethical / moral / social concerns with embryo cryopreservation.

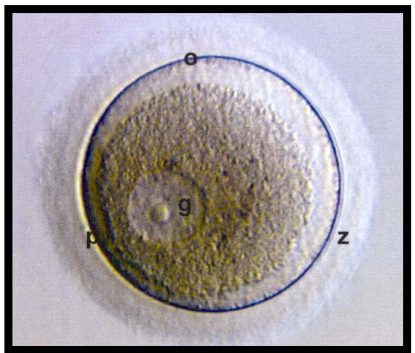
3) Salvaging interrupted IVF cycles.

4) Oocyte banking in anticipation of reproduction at an advanced maternal age.

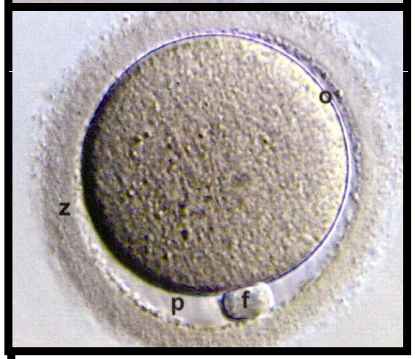
- Slow-rate freezing

- Vitrification

# Cryo-Damage and Subsequent Oocyte Function



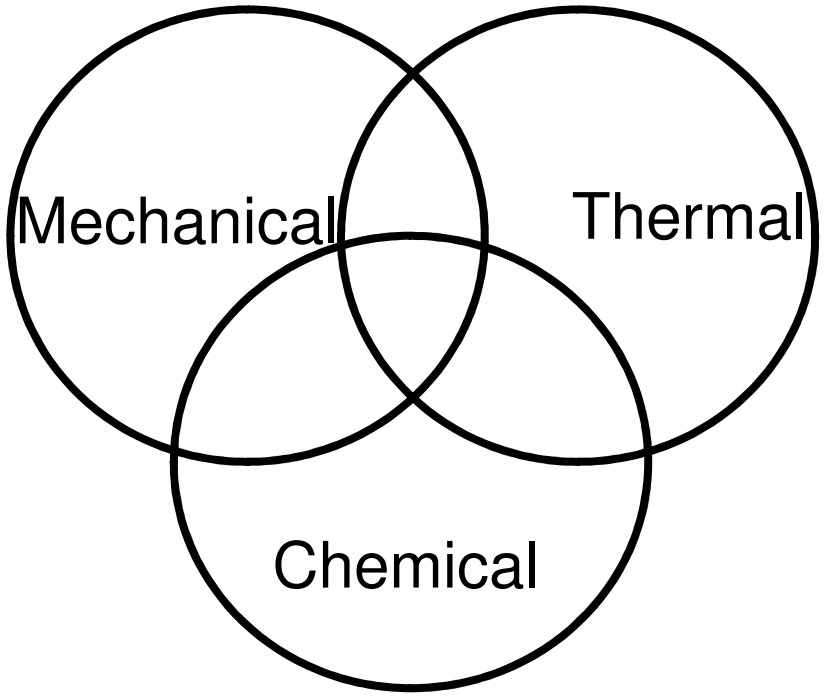

Germinal vesicle-intact (immature)




Metaphase II (mature)

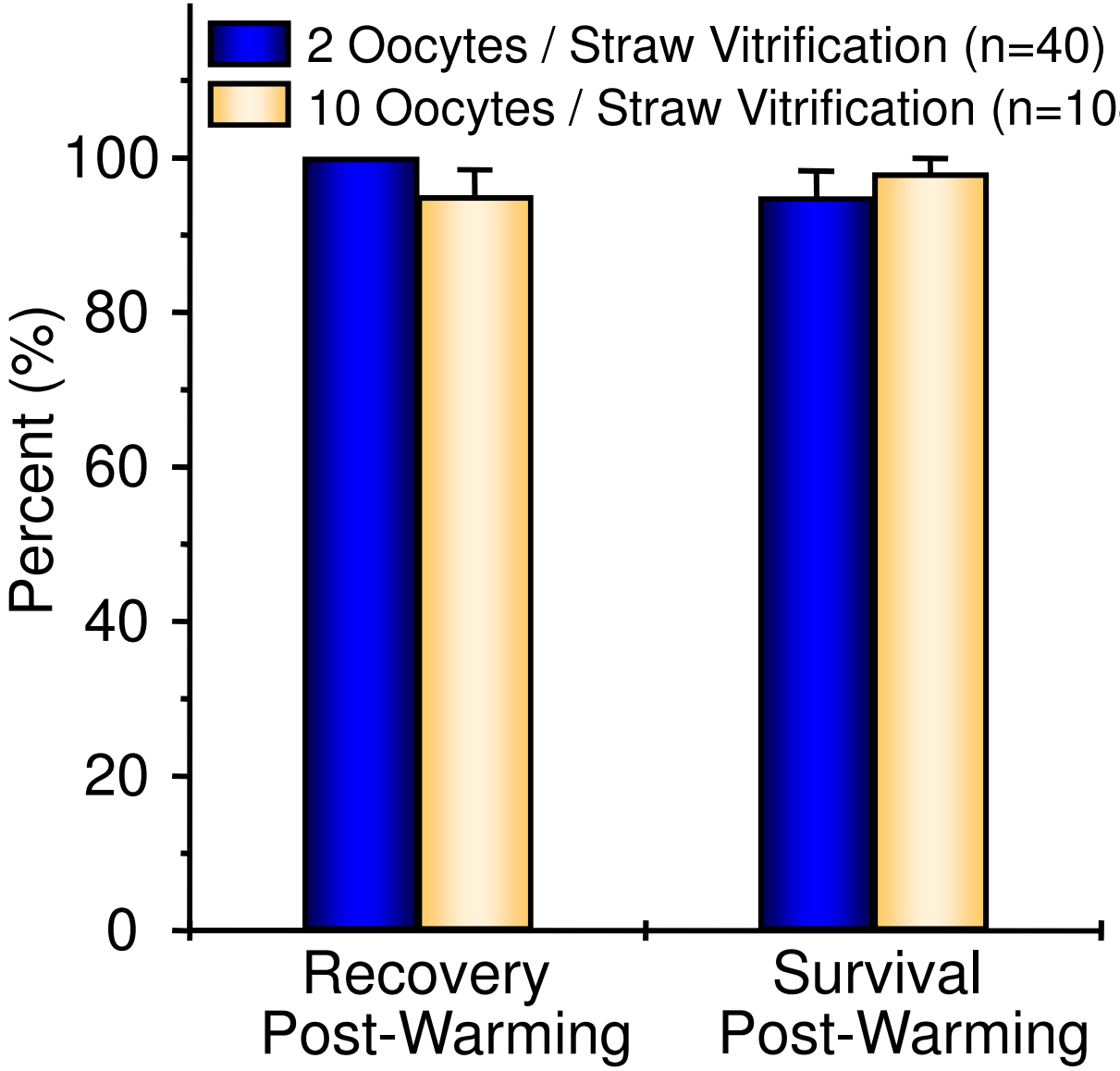
Veeck, 1999

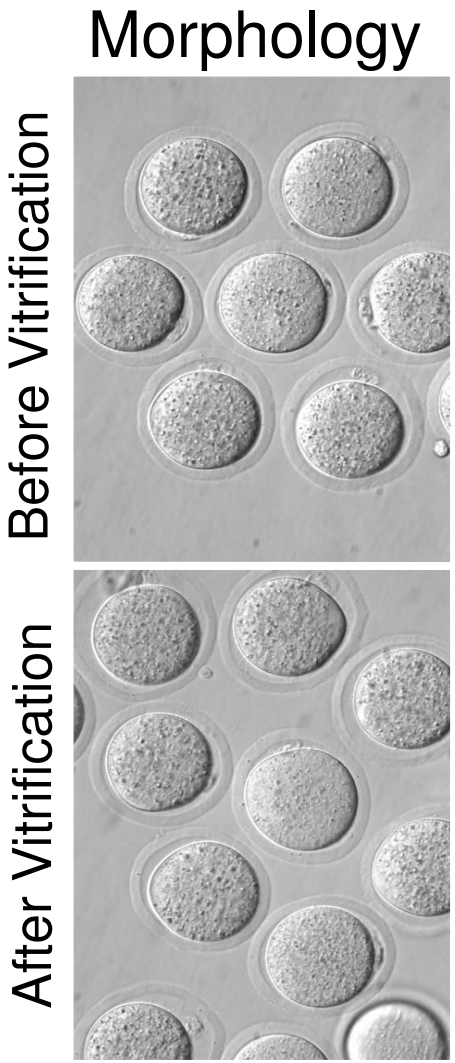
BIG CELLS
Lot of responsibilities



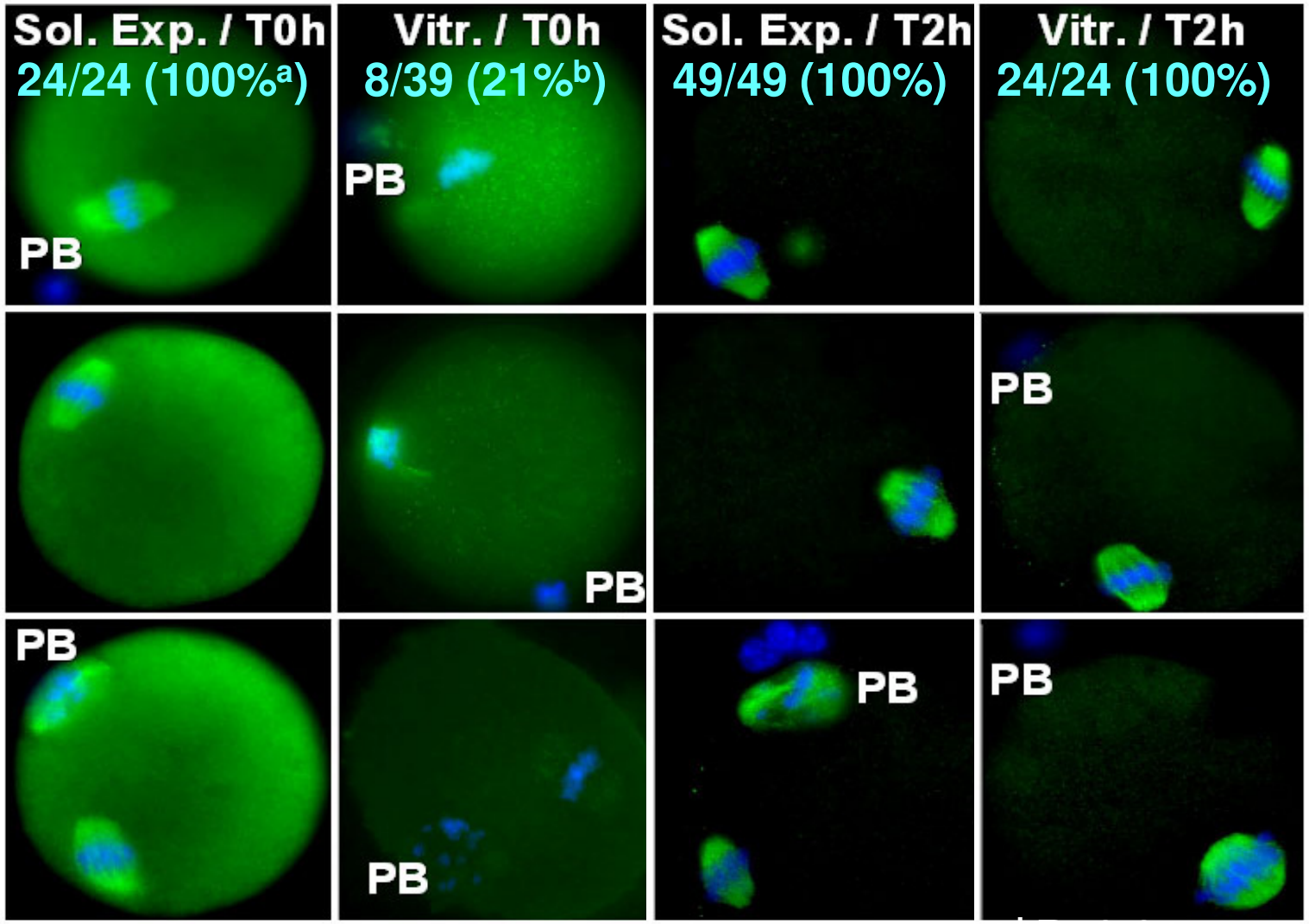

1) Spindle Formation / Function
2) Microfilament Function
3) Zona Pellucida

# Recovery / Survival of Vitrified Mouse MII Oocytes



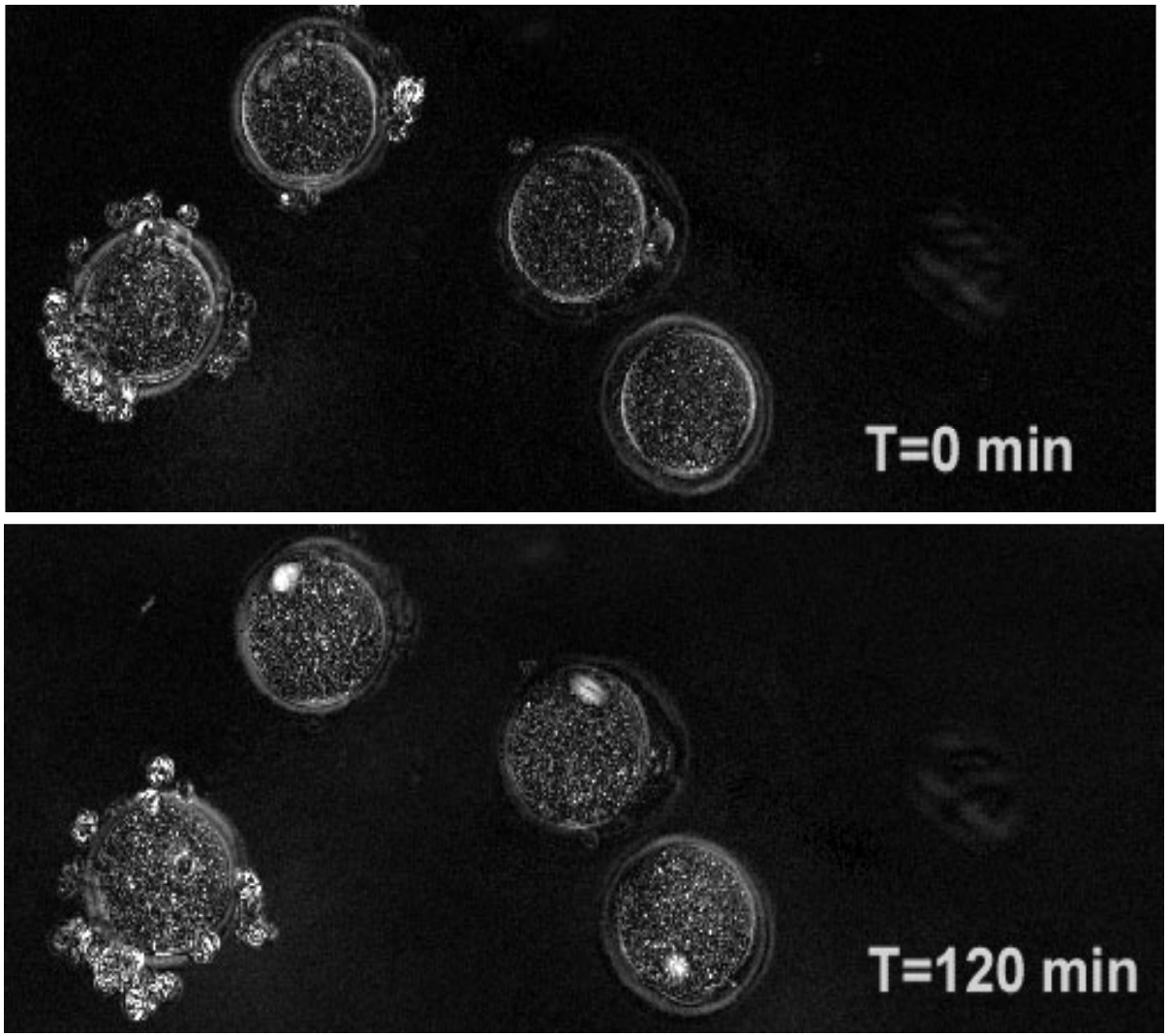



# Influence of Vitrification and Time on Metaphase II Spindle Morphology




Gomes et al., *Fertil & Steril*, 2008

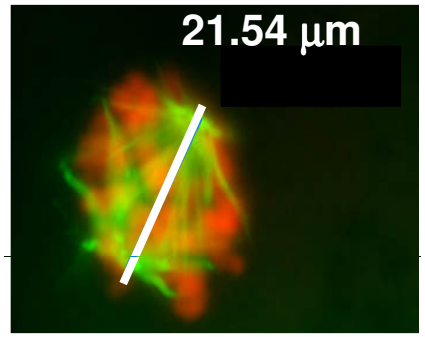
# Polscope® View of Spindle Assembly After Vitrification / Warming



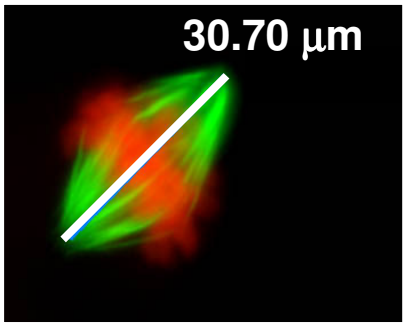

Gomes et al., *Fertil & Steril*, 2008

# Does Vitrification Affect Spindle Morphology (Length)?

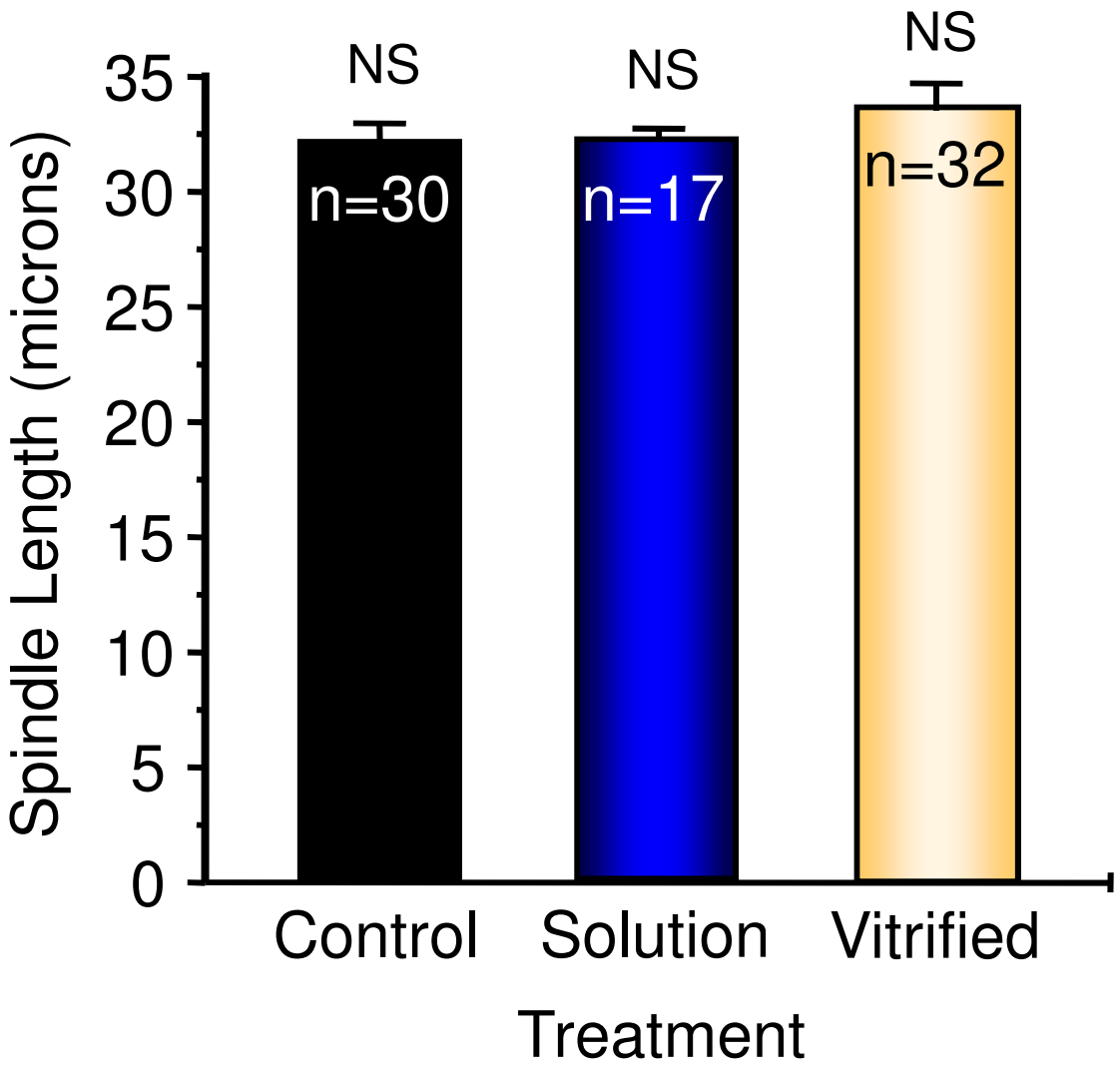
**Strict Criteria:**



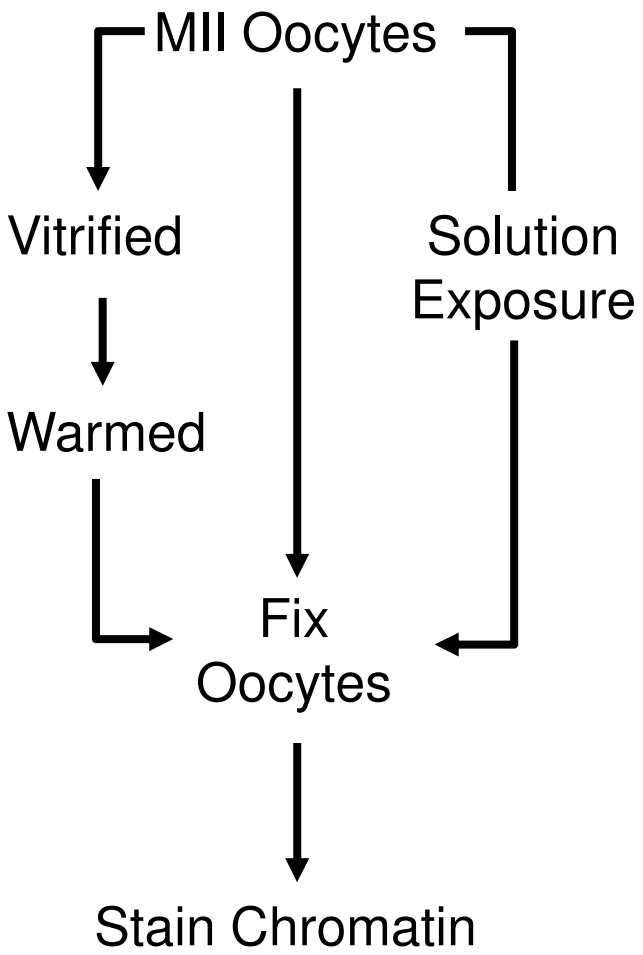

No measurement

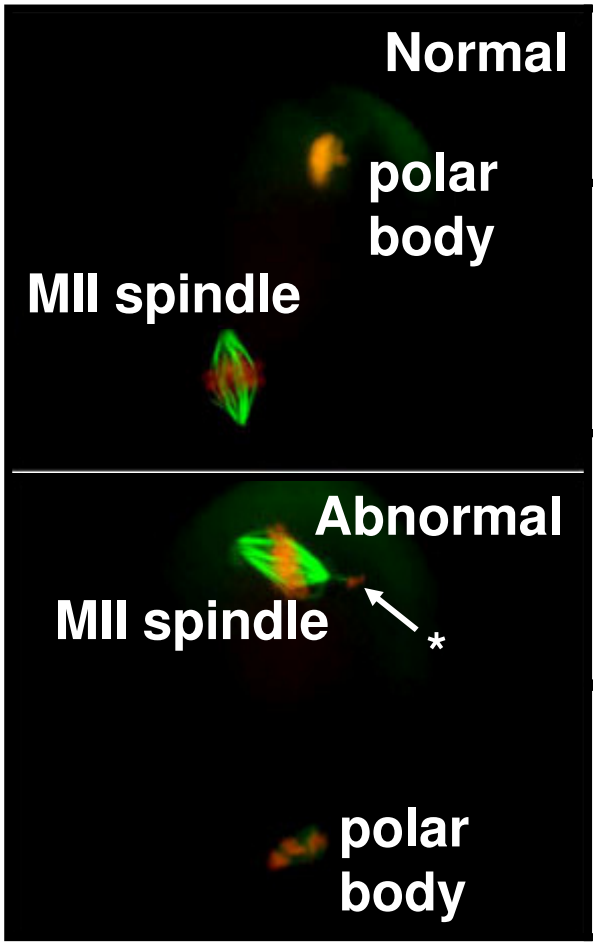



Measurement



# Does Vitrification Cause Aberrant Spindle Chromatin Alignment?




| Abnormal Chromatin Alignment |
|---|
| Control (no vitrification) 1/37 = 2.7%[a] |
| Solution Exposure Only 1/47 = 2.1%[a] |
| Vitrification 3/49 = 6.1%[a] [a]P=0.63 |

Gomes et al., *Fertil & Steril*, 2008

# Spindles and Cryopreservation

The Bottom Line:

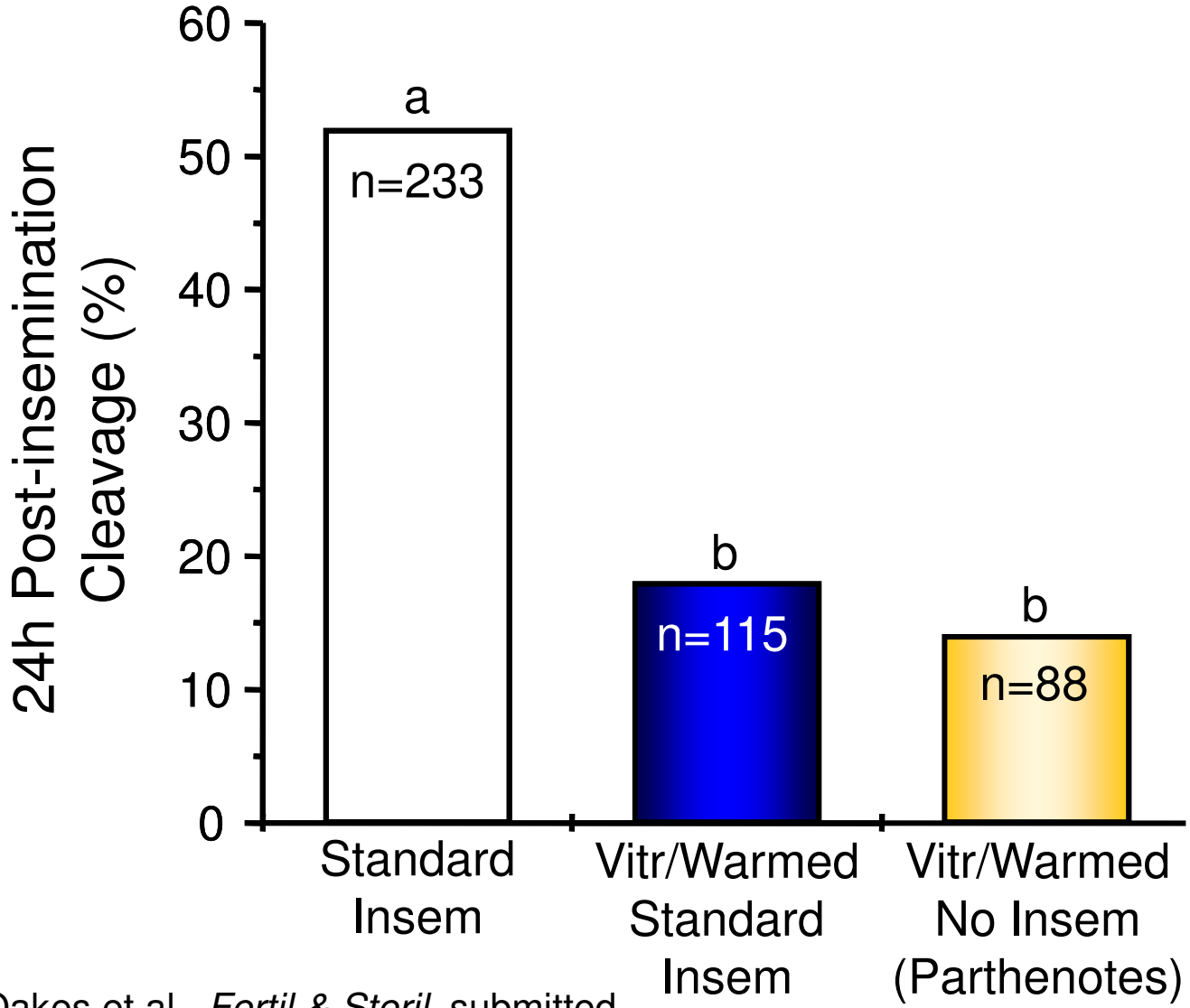
1) All three forces are detrimental
   - Mechanical (Ice crystals)
   - Thermal (Microtubule depolymerization)
   - Chemical (DMSO & PROP)

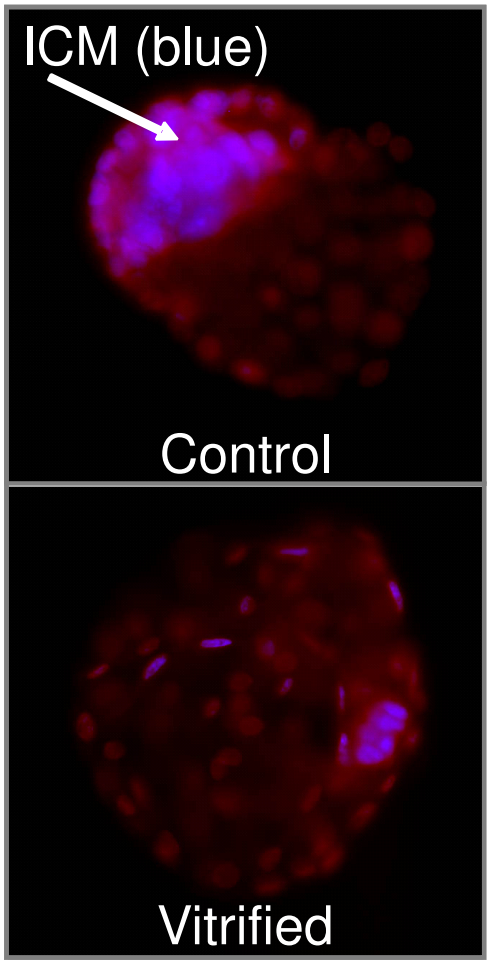
2) Debate on which meiotic stage most susceptible to damage

3) Remember microtubules are in dynamic flux
   - polymerization / depolymerization

Today: greatest success is cryopreservation of MII oocytes

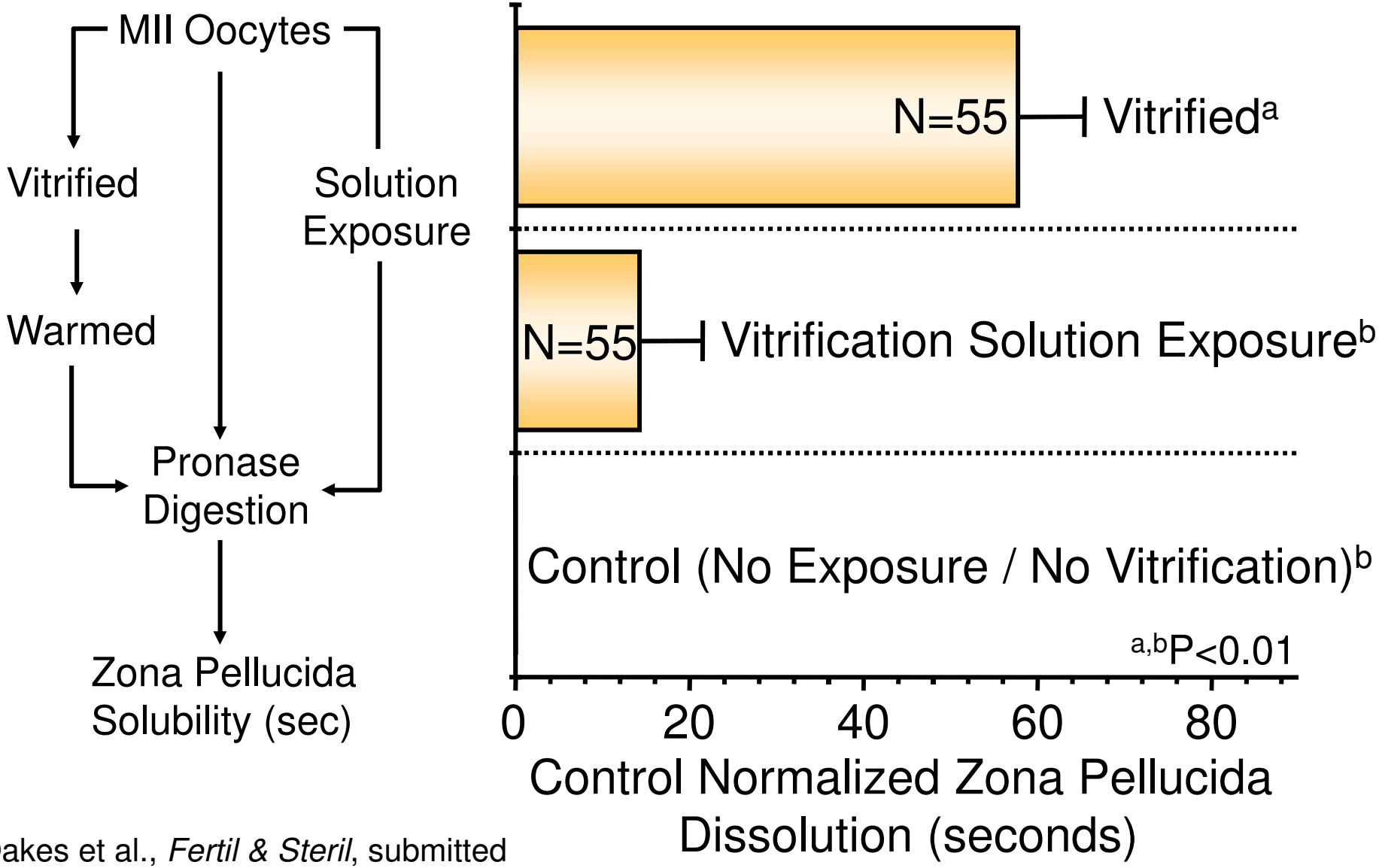
# Oocyte Vitrification and Fertilization By Standard Insemination



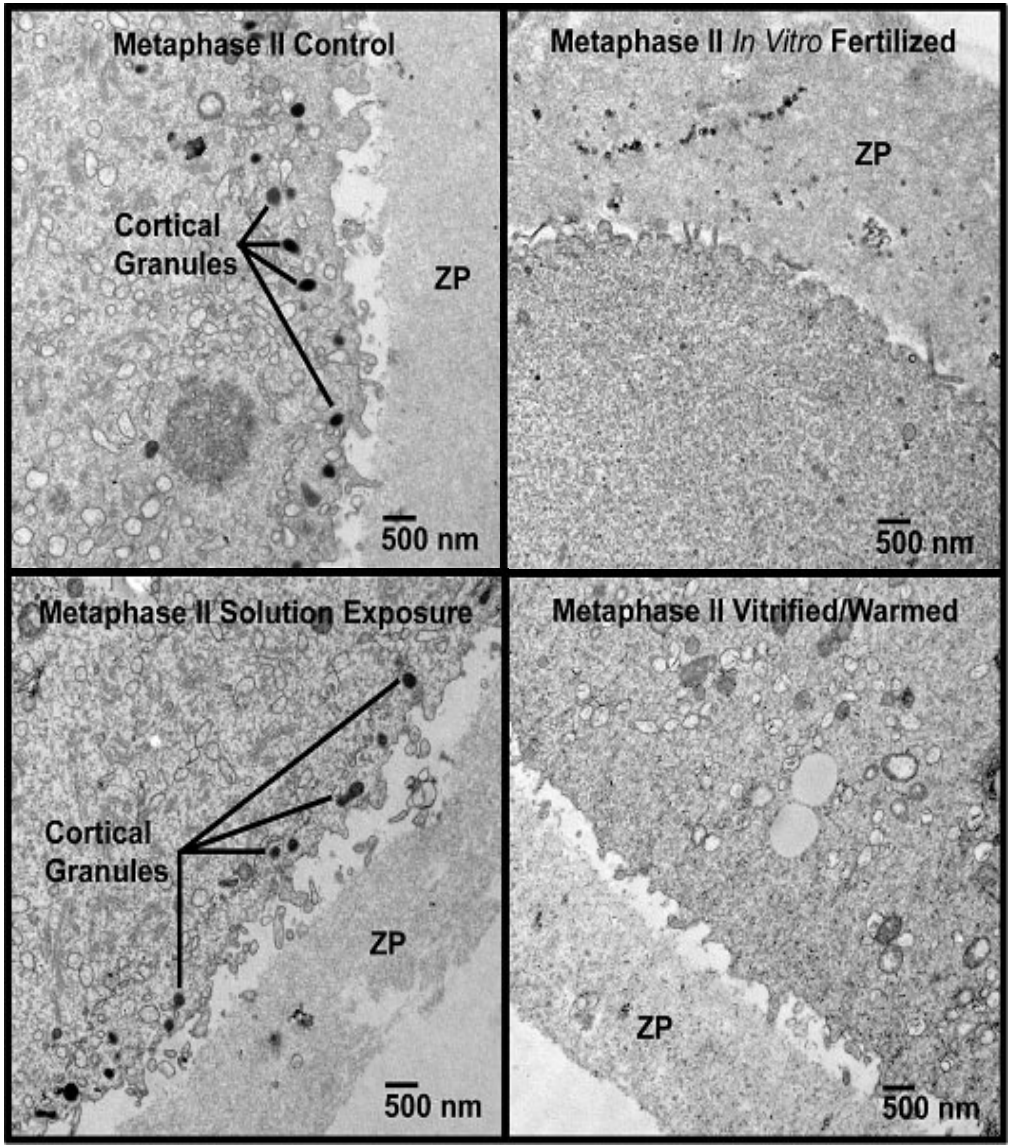

Oakes et al., *Fertil & Steril*, submitted

# Why Do Vitrified Oocytes Not Fertilize?

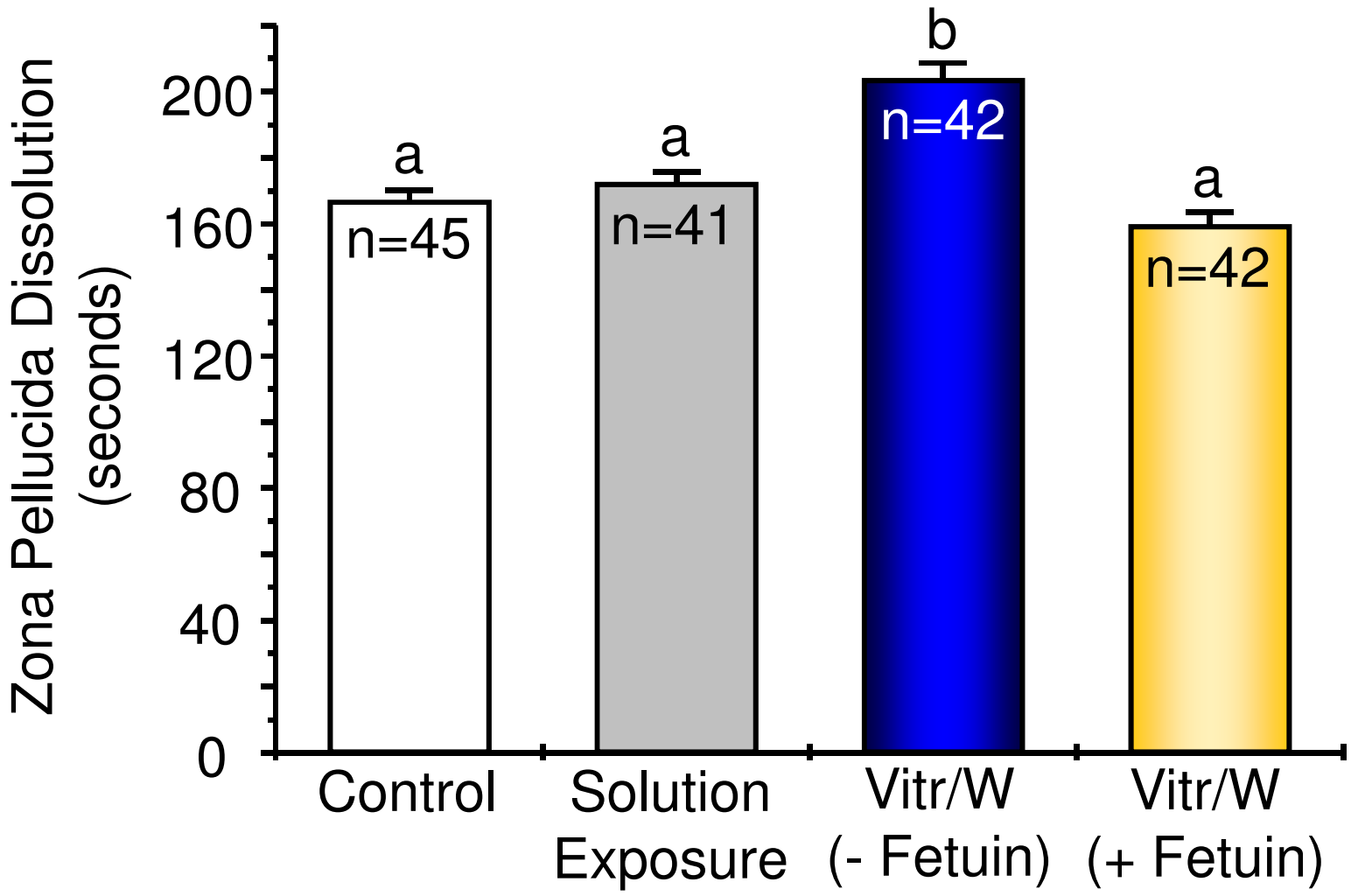



Oakes et al., *Fertil & Steril*, submitted

# Does Vitrification Induce Premature Cortical Granule Release?



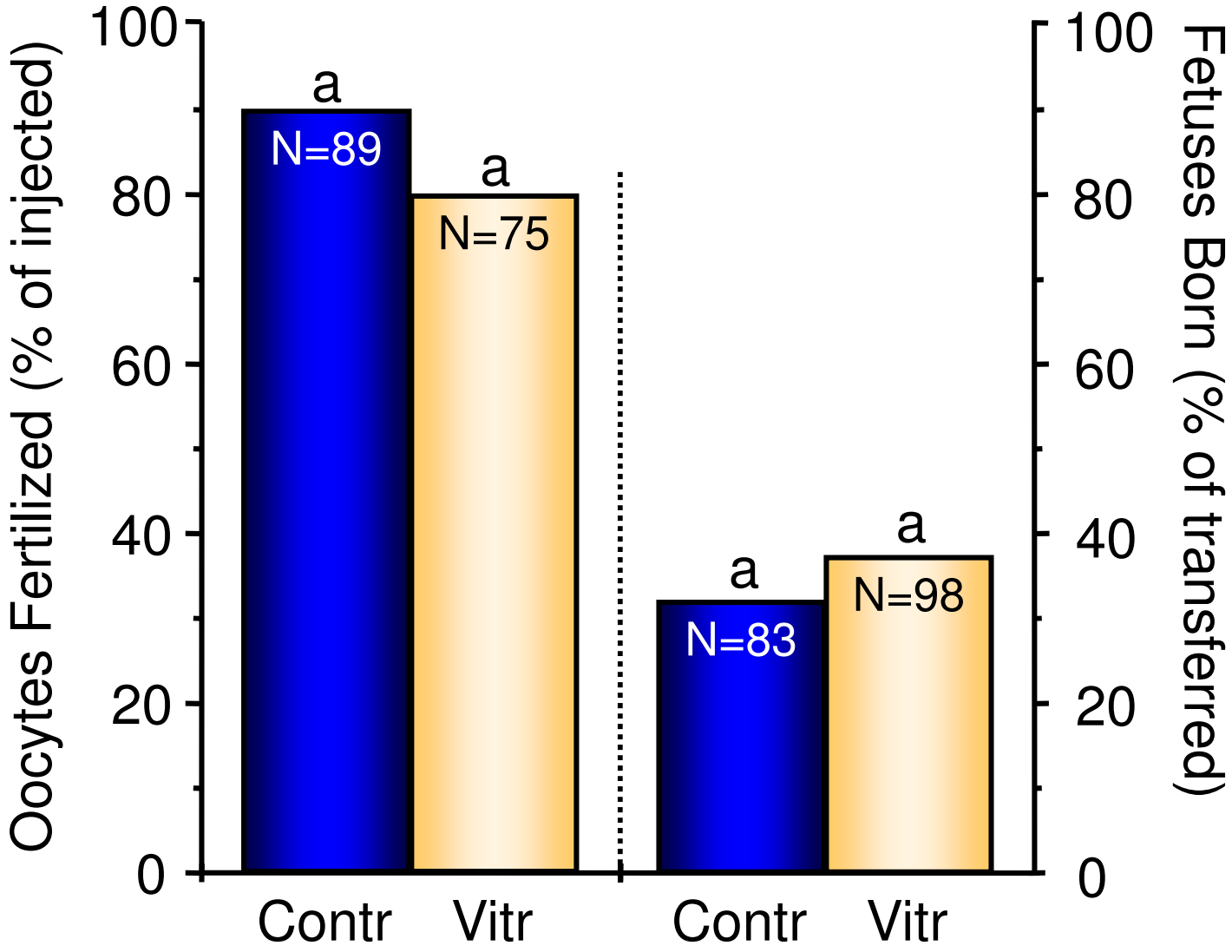
# Can Fetuin in Cryo-Media Influence Zona Pellucida Modifications?




Oakes et al., *Fertil & Steril*, submitted

$^{a,b}P<0.05$

# Mouse Oocyte Vitrification, ICSI, Fertilization, and Live-Births




Oakes et al., *Fertil & Steril*, submitted

[a,b]P<0.05

# Oocyte Cryopreservation

1) Slow-rate freezing can work

- 50 to 76% survival
~13 to 35% pregnancy rate/transfer

(Porcu *et al*., 2000, *Mol & Cell Endo*)

(Boldt *et al.,* 2006, *Reprod Biomed Online*)

(Bianchi *et al.,* 2006, *Reprod Biomed Online*)

2) Vitrification current area of focus

- two primary factors to consider for success

a - vitrification means (container and coolant)

b - solutions

# Vitrification Containers

**What makes a good vitrification container?**

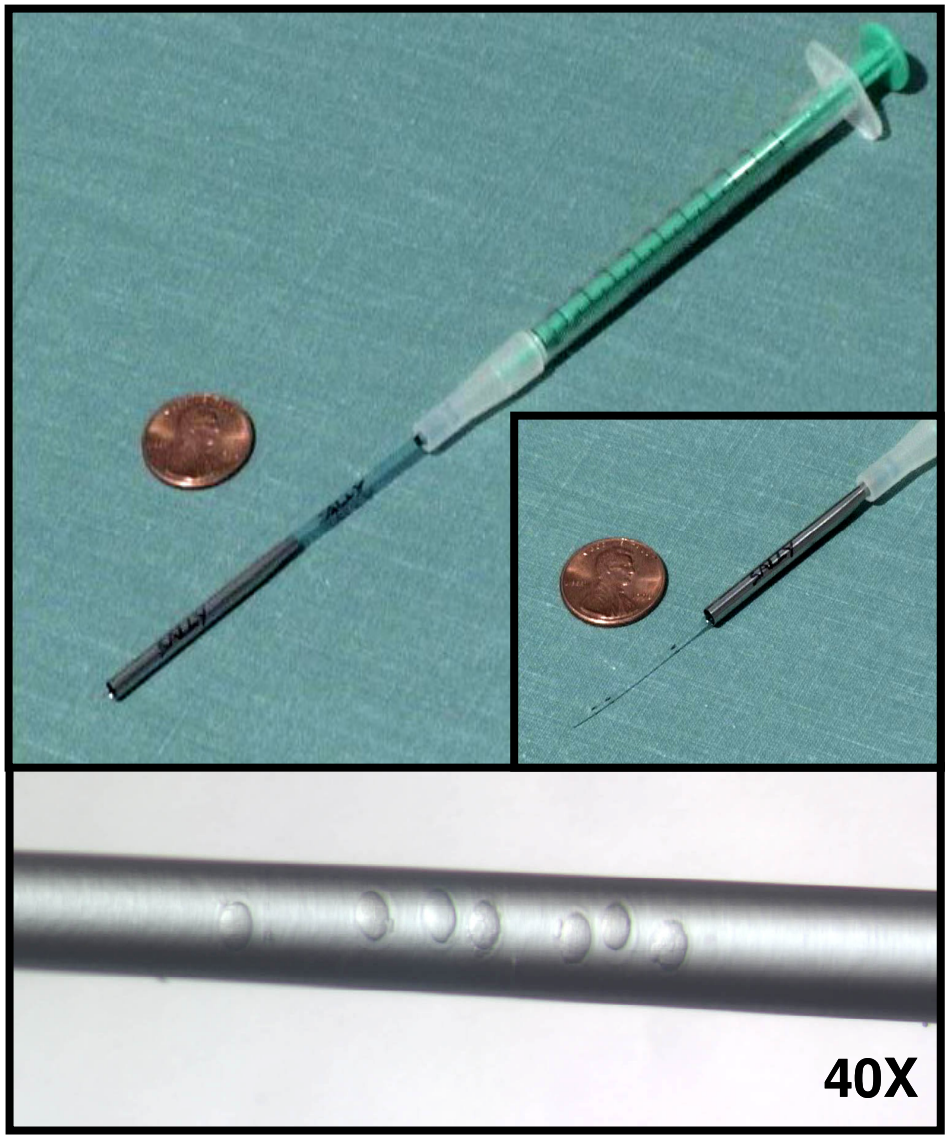
1) Low volume of cryo-solution
   - rapid heat transfer
   - fastest cryopreservation / warming
   - no ice crystal formation

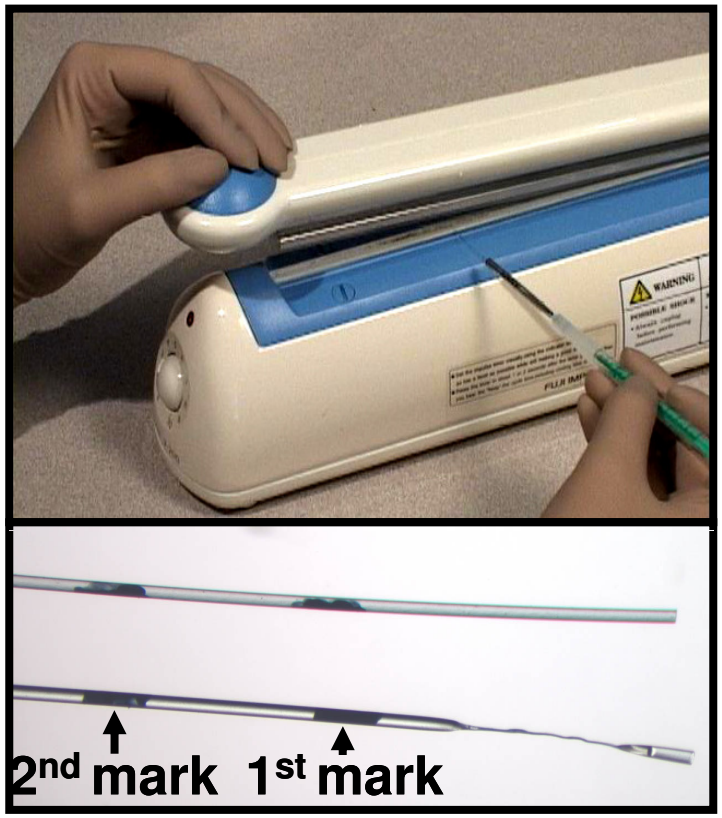
2) User friendly

3) Security

EM Grids - Martino et al., 1996, *Biol Reprod,* Vol 54
Open Pulled Straws (OPS) - Vajta et al., 1998, *Mol Reprod & Dev,* Vol 51
Cryoloops® - Lane et al., 1999, *Fertil & Steril*, Vol 72
Cryotop® - Katayama et al., 2003, *Fertil & Steril*, Vol 80
CryoTip® - Kuwayama and Smith

# Closed Pulled Straw System




- low volume ~ 0.4 μl (inner diam. = 200 μm)
- secure and protected (heat-sealed at both ends)

# Vitrification / Warming Solutions

## Vitrification Solutions

7.5% ethylene glycol
7.5% DMSO
20% Protein
in M199-H

→ Equilibration Solution

15% ethylene glycol
15% DMSO
0.5 M sucrose
20% Protein
in M199-H

→ Vitrification Solution

## Warming Solutions

**Initial Warming Solution (IWS)**
- M199-H
- 1.0 M Sucrose
- 20% Protein

**Dilution Solution (DS)**
- M199-H
- 0.5 M Sucrose
- 20% Protein

**Wash Solution (WS)**
- M199-H
- 20% Protein

# Objective

Compare human metaphase II oocyte cryopreservation by slow-rate freezing and vitrification in a prospective, randomized, controlled clinical study.

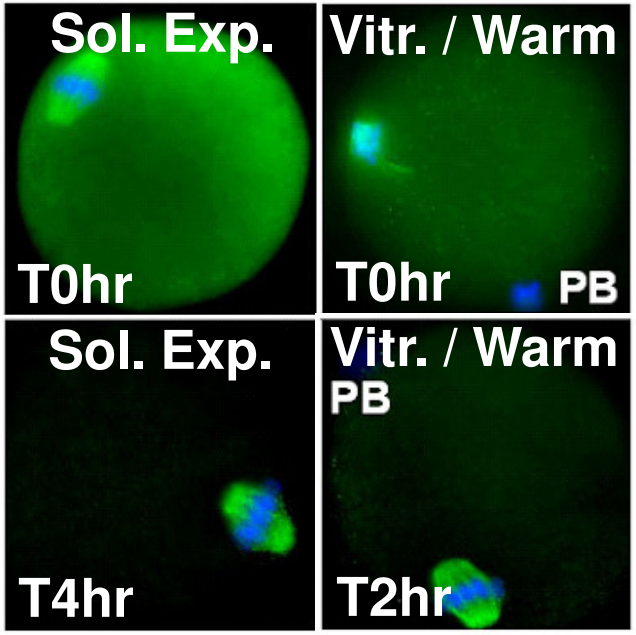
**Joyce Fioravanti, BSc**

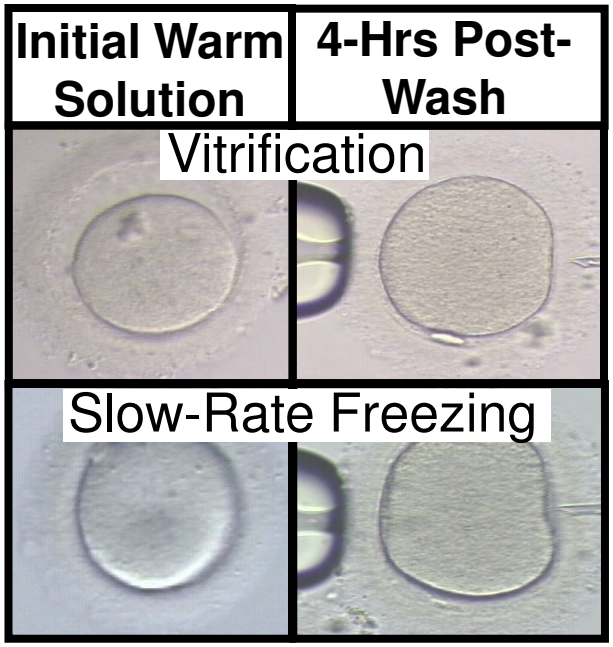
2004 Technical Training:

1) Oocyte slow-rate freezing
   - Dr. Porcu, M.D.
   - Bologna, Italy
2) Oocyte vitrification
   - University of Michigan

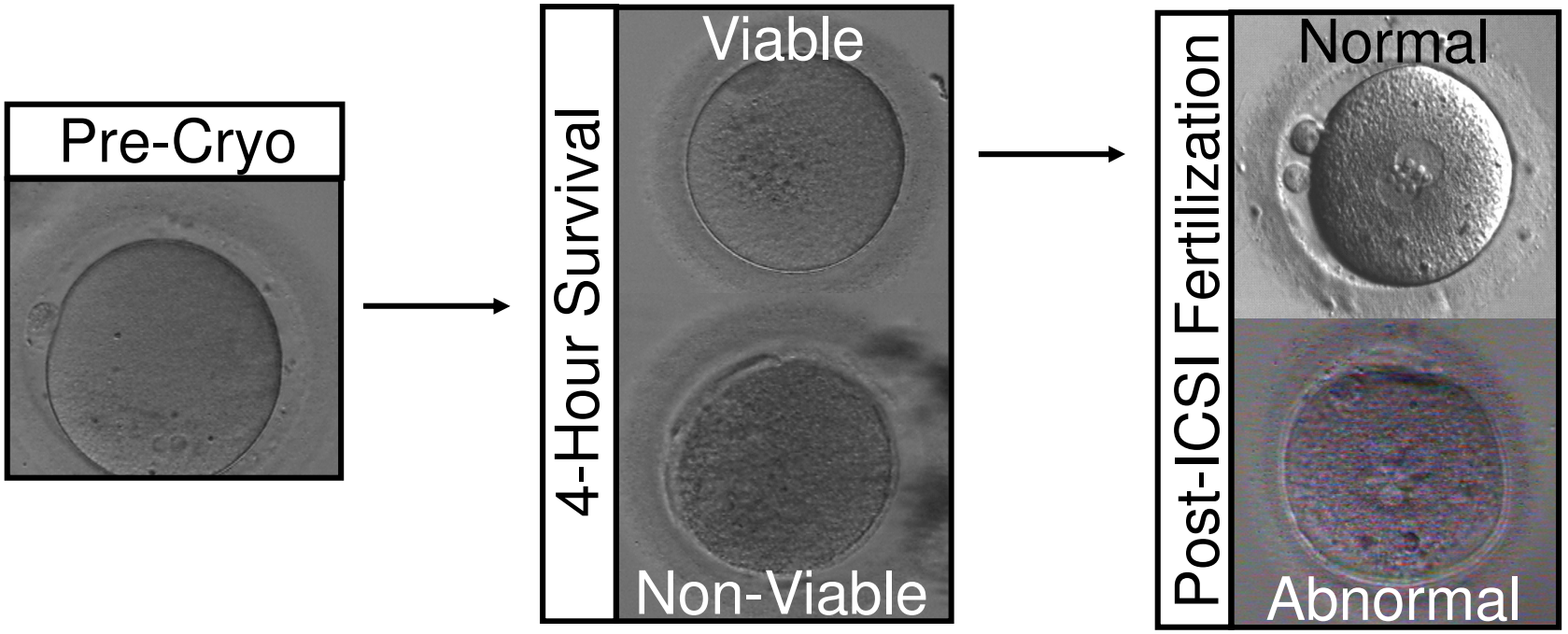
# Prospective Randomized Controlled Study

- IRB approval and written informed consent
- Offered to women > 9 oocytes collected in fresh cycles
  Randomized to slow-rate freezing (Porcu) vs. vitrification
- Began January, 2005
- As of Dec 2008:  230 cases cryopreserved, 78 thaws/warmings
- Four hours incubation before intracytoplasmic sperm injection



# Survival and Fertilization

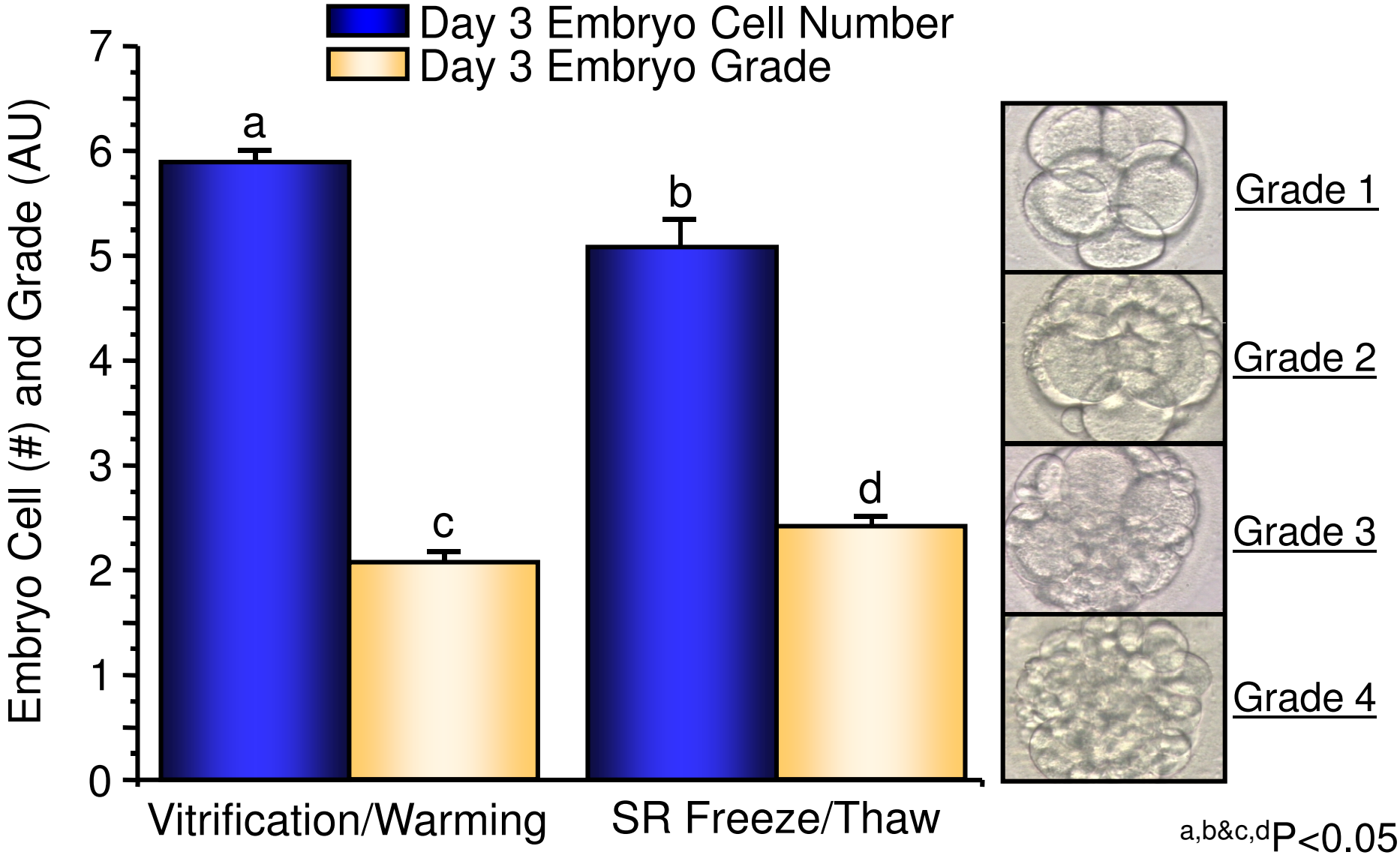



| | Age | Thaws/ Warming | Survival | Normal Fertilization | Cleavage |
|---|---|---|---|---|---|
| Slow-Rate Freezing | 31±1 | 30 | 67%[a] | 67%[c] | 70%[a] |
| Vitrification | 32±1 | 48 | 81%[b] | 77%[d] | 91%[b] |

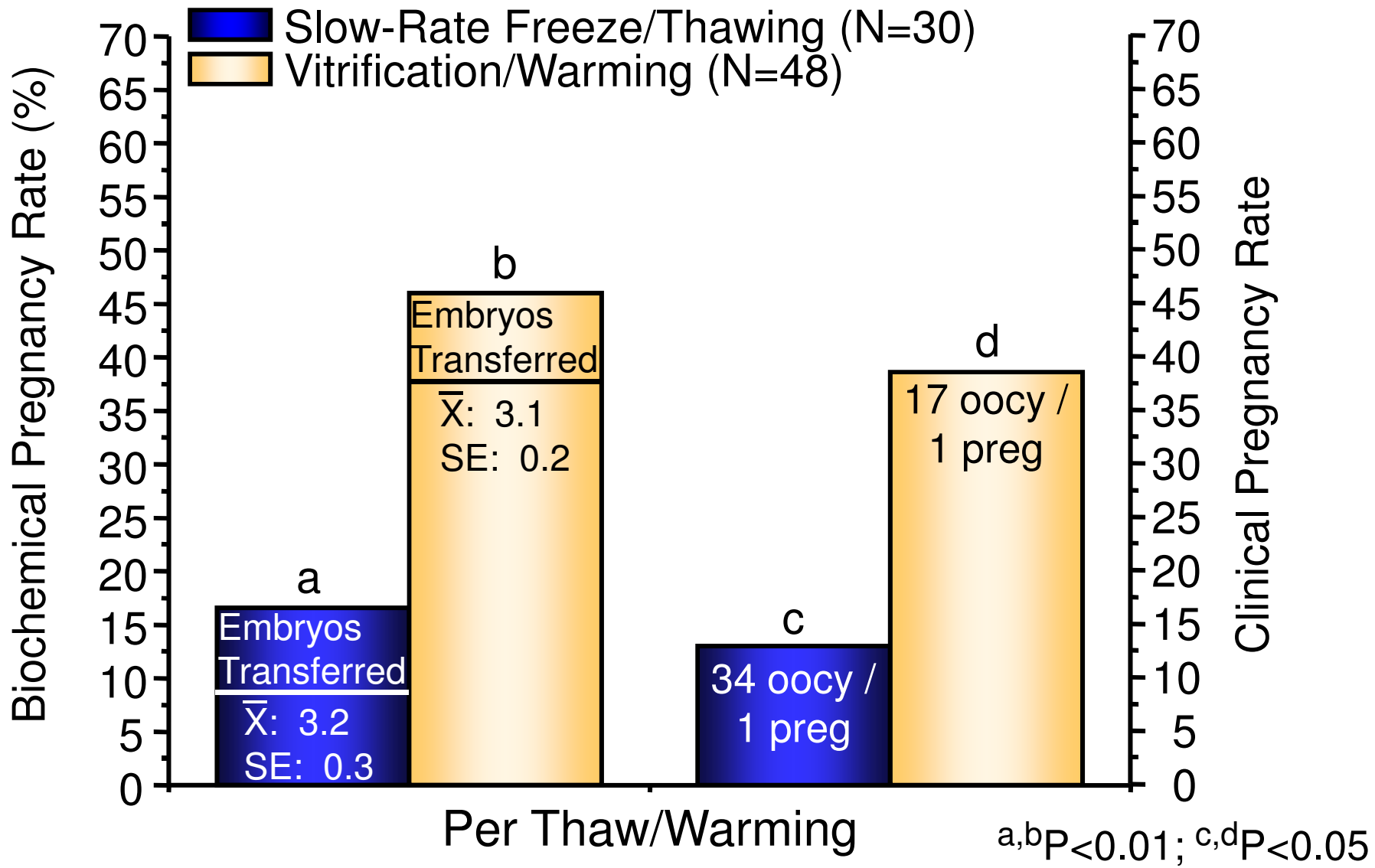
Smith et al., 2009, submitted Fert & Stert

[a,b]$P<0.01$; [c,d]$P<0.05$

# Day 3 Embryo Development Following Oocyte Cryopreservation



# Prospective Randomized Controlled Study: Pregnancy Rates



# Oocyte Cryopreservation Meta-Analysis

| | Vitrification | | Slow-Rate Freezing | |
|---|---|---|---|---|
| Age | 32 | 32 | 34 | 31 |
| Fertilization Rate | 74% | 77% | 65% | 67% |
| Clinical PR Per Oocytes Thawed/Warmed | 4.5% | 5.2% | 2.3% | 1.7% |
| Clinical PR Per Thawing/Warming | ? | 38% | ? | 13% |
| Clinical PR/ Transfer | 46% | 38% | 21% | 21% |

Oktay et al., *Fertil & Steril,* 2006

Meta-Analysis

Prospective-Randomized

Meta-Analysis

Prospective-Randomized

# Conclusions

1) Oocyte cryopreservation by slow-rate freezing or vitrification are experimental procedures to be performed under IRB and informed consent.

2) Long-term follow-up of offspring obtained through oocyte cryopreservation is wanting (human and model systems).

3) Vitrification is not difficult.

4) Vitrification requires practice, can carry a technical signature, and can be “unforgiving” to technical mistakes.

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# Thank You

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