

# Are we there yet?

## Ultimate culture conditions in 2017

Barry Behr PhD., HCLD  
Professor and IVF Laboratory Director  
Fertility and Reproductive Health  
Division of Reproductive Endocrinology and Infertility  
Dept OB/GYN Stanford University



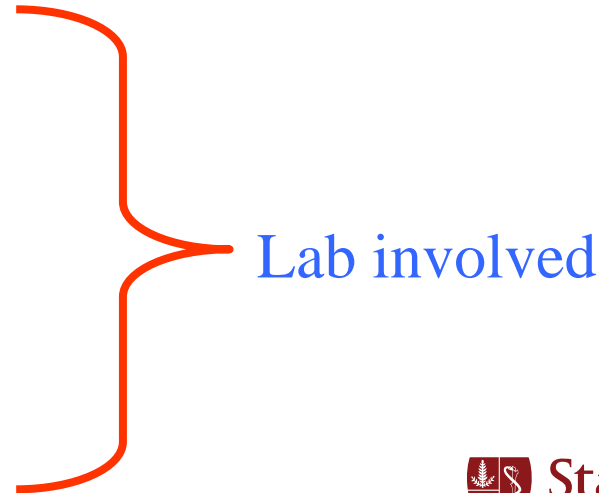
**Stanford**  
MEDICINE

---

Fertility and  
Reproductive Health

# Major steps involved in an IVF procedure:

1. Ovarian Stimulation
2. *Collection of Oocytes*
3. *Collection of Sperm*
4. *Insemination*
5. *In-vitro culture of Embryos*  
(Embryo Selection)
6. *Embryo Transfer*
7. Obstetrical Follow-up



**Stanford**  
MEDICINE

Fertility and  
Reproductive Health

“Culture conditions” span from follicular flush media to embryo transfer or vitrification media



**Stanford**  
MEDICINE

---

Fertility and  
Reproductive Health

# Personalized Medicine?

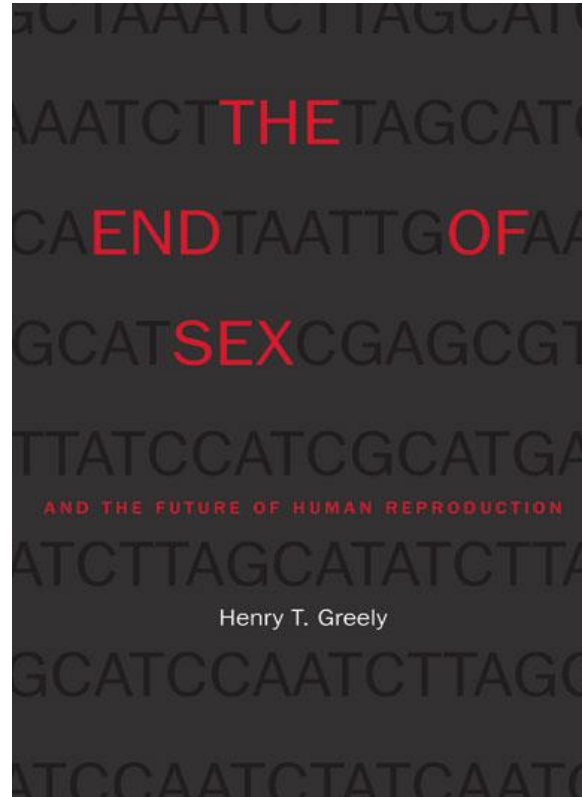
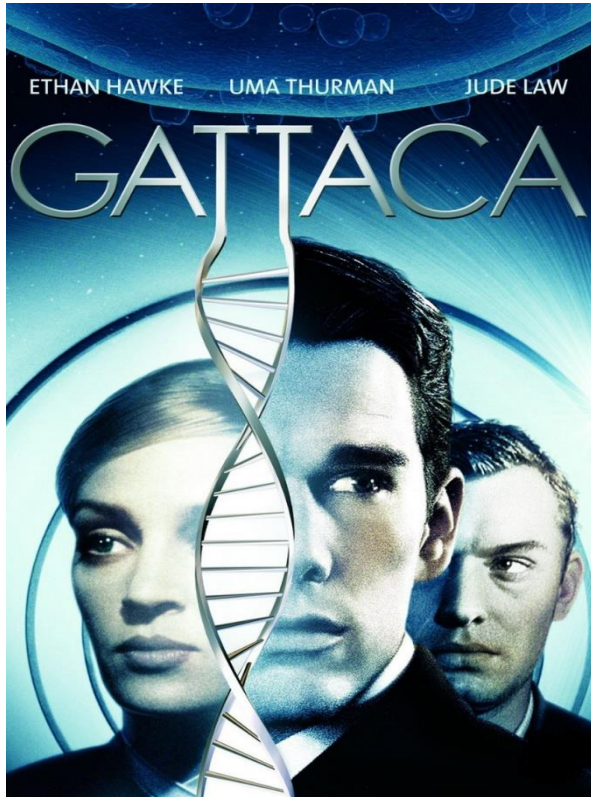
- “Precision medicine”
  - Chemotherapy
- What about tailoring the culture conditions to the pt or cycle?
- Patient-specific culture requirements
- Do they exist?



---

Fertility and  
Reproductive Health

# 30-40 years.....and now?



## UK approves three-person babies

By James Gallagher  
Health editor, BBC News website

24 February 2015 | Health



## Scientists get 'gene editing' go-ahead

By James Gallagher  
Health editor, BBC News website

1 February 2016 | Health



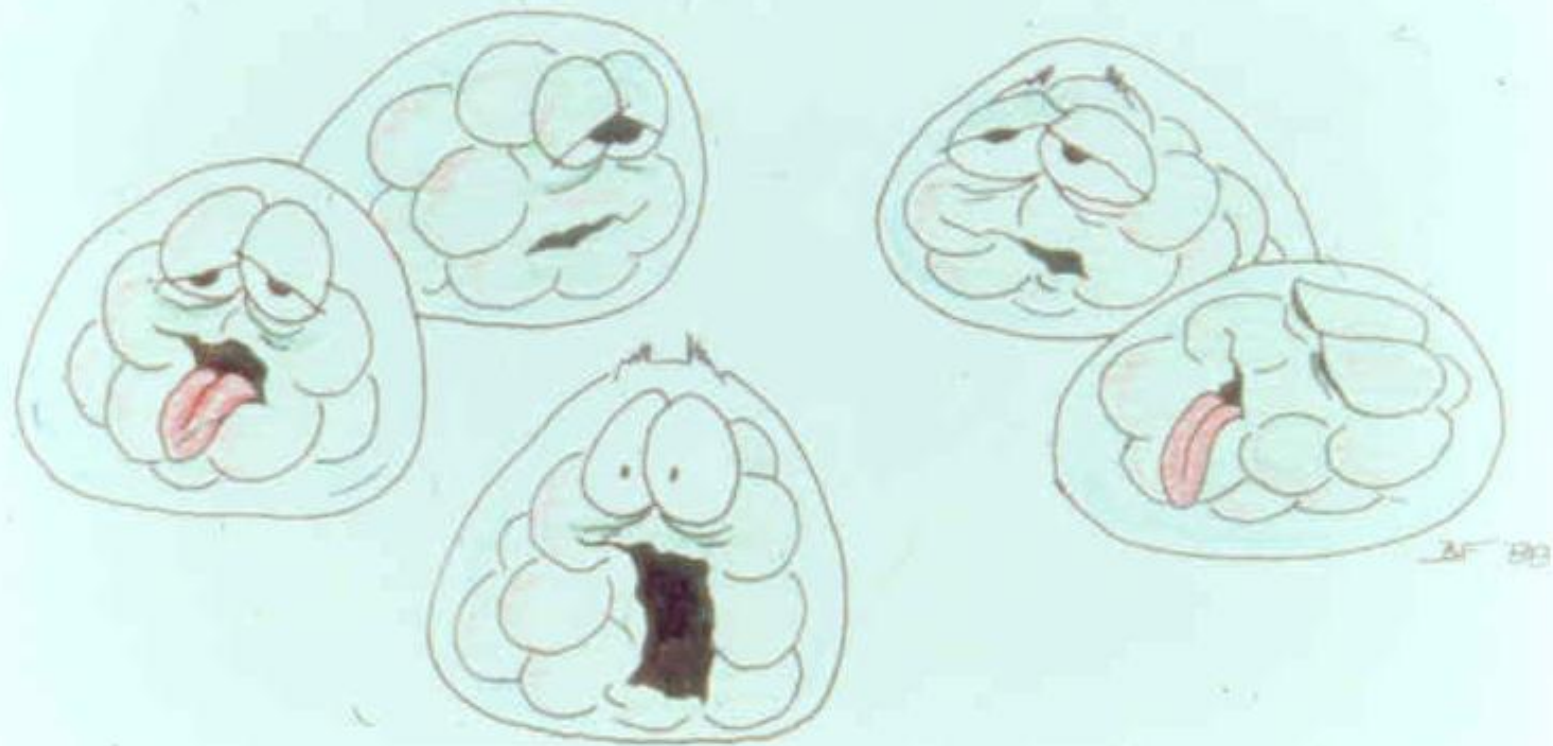
Can (suboptimal) culture conditions be used to screen out embryos with poor potential?



**Stanford**  
MEDICINE

---

Fertility and  
Reproductive Health



# Can culture conditions be too good?

- Promotes “non viable” embryos to develop
- Increased blastocyst formation rate does not always lead to increased pregnancy rate



**Stanford**  
MEDICINE

---

Fertility and  
Reproductive Health



# What are the end points/metrics?

- For determining ultimate culture conditions
    - Media pH
    - Fert rate
    - Development rate (TLM)
    - Embryo grades
    - Blast rates
    - Euploidy rates
    - Implantation rates
- Healthy Babies

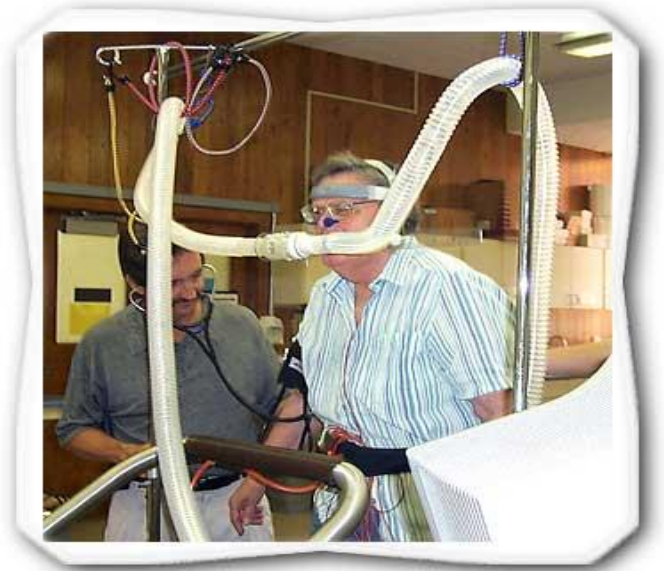


**Stanford**  
MEDICINE

Fertility and  
Reproductive Health

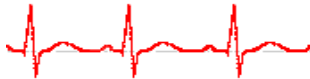
# What is the embryo equivalent?

i.e. non-invasive, quantitative assessment



=

?



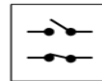
**Stanford**  
MEDICINE

Fertility and  
Reproductive Health

# Lab Environment

- Integral part of the “culture environment”
  - Must be considered
- Air quality
  - Construction materials and equipment
- Lab design and work flow
- Monitoring

# OCTAX LOG & GUARD MONITORING, DOCUMENTATION AND ALARMING SYSTEM



**Stanford**  
MEDICINE

Fertility and  
Reproductive Health





IVFtech

IVFtech



PROCEDURE  
122



IVFtech







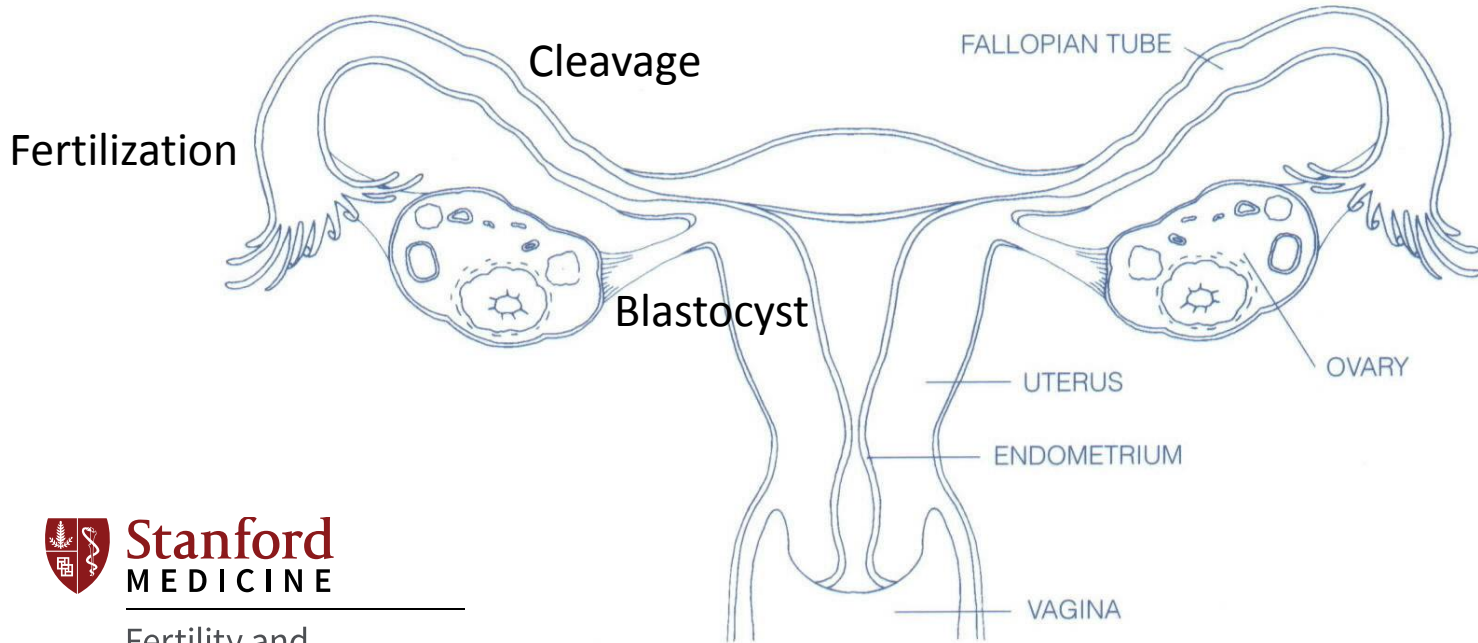






Emergency Eye Wash Station

# Critical sites to emulate, or not?



**Stanford**  
MEDICINE

Fertility and  
Reproductive Health

# Incubation Chamber

- “Big box” incubator
  - Full size vs mini
  - Recovery times vs work flow
- Bench top
  - Dry vs humidified
  - Mixed gas vs individual gas vs gas mixer



**Stanford**  
MEDICINE

---

Fertility and  
Reproductive Health

# Cryopreservation

- Vitrification
  - DMSO vs no DMSO
  - RT vs 37 degrees



**Stanford**  
MEDICINE

---

Fertility and  
Reproductive Health

Stanford Fertility and Reproductive Medicine Center  
4:51:54 PM 2008/03/26



HAMILTON THORNE

Stanford Fertility and Reproductive Medicine Center  
4:44:51 PM 2008/03/26



HAMILTON THORNE

Stanford Fertility and Reproductive Medicine Center  
3:37:55 PM 2008/03/26



HAMILTON THORNE



**Stanford**  
MEDICINE

Fertility and  
Reproductive Health

# Oocyte/Embryo Handling

- Isolette vs Heated workstation
- “Gas bubblers” vs Ambient air buffered media



**Stanford**  
MEDICINE

---

Fertility and  
Reproductive Health



# Age old questions

- Assisted hatching
  - For advanced maternal age/repeat failures/thick zonae
  - Pre trophoctoderm biopsy



**Stanford**  
MEDICINE

---

Fertility and  
Reproductive Health

# Oil Overlay

- Mineral Oil
  - Heavy or light
- Paraffin oil
- Storage
  - Light or dark
  - Rt or 2-8 C



**Stanford**  
MEDICINE

---

Fertility and  
Reproductive Health

# Oxygen Concentration

- 20% < 5% < 2%? (Racowsky lab)

Table. Stage and cell count of embryos cultured from days 3 to 5 in low (5%) vs. ultralow (2%) O<sub>2</sub>

Stage per cleaved embryo n (%)	5-5% O <sub>2</sub> Group	5-2% O <sub>2</sub> Group	OR (95% CI)
Cleavage arrest	52/89 (58.4)	34/87 (39.1)	0.38 (0.18, 0.80)
Morula	17/89 (19.1)	18/87 (20.7)	1.09 (0.47, 2.53)
Early blastocyst	11/89 (12.4)	23/87 (26.4)	2.59 (1.06, 6.32)
Full, expanded or hatching blastocyst	9/89 (10.1)	12/87 (13.8)	1.43 (0.56, 3.64)
Any blastocyst	20/89 (22.5)	35/87 (40.2)	2.55 (1.27, 5.12)
Usable blastocyst	19/89 (21.3)	32/87 (36.8)	2.30 (1.16, 4.56)



**Stanford**  
MEDICINE

Fertility and  
Reproductive Health

Table. Stage and cell count of embryos cultured from days 3 to 5 in low (5%) vs. ultralow (2%) O<sub>2</sub>

Stage per cleaved embryo n (%)	5-5% O <sub>2</sub> Group	5-2% O <sub>2</sub> Group	OR (95% CI)
Cleavage arrest	52/89 (58.4)	34/87 (39.1)	0.38 (0.18, 0.80)
Morula	17/89 (19.1)	18/87 (20.7)	1.09 (0.47, 2.53)
Early blastocyst	11/89 (12.4)	23/87 (26.4)	2.59 (1.06, 6.32)
Full, expanded or hatching blastocyst	9/89 (10.1)	12/87 (13.8)	1.43 (0.56, 3.64)
Any blastocyst	20/89 (22.5)	35/87 (40.2)	2.55 (1.27, 5.12)
Usable blastocyst	19/89 (21.3)	32/87 (36.8)	2.30 (1.16, 4.56)
Mean cell count $\pm$ SD	5-5% O <sub>2</sub> Group	5-2% O <sub>2</sub> Group	P-value
Early blastocyst	44.4 $\pm$ 2.1	35.5 $\pm$ 10.9	0.01
Full, expanded or hatching blastocyst	83.4 $\pm$ 15.9	62.0 $\pm$ 14.5	0.04
Any blastocyst	60.7 $\pm$ 22.3	43.8 $\pm$ 17.3	0.01



**Stanford**  
MEDICINE

Fertility and  
Reproductive Health

# Use of growth factors

- Not in 2017
- Not going anywhere soon
- 1 commercial product in the world
- Makes sense though



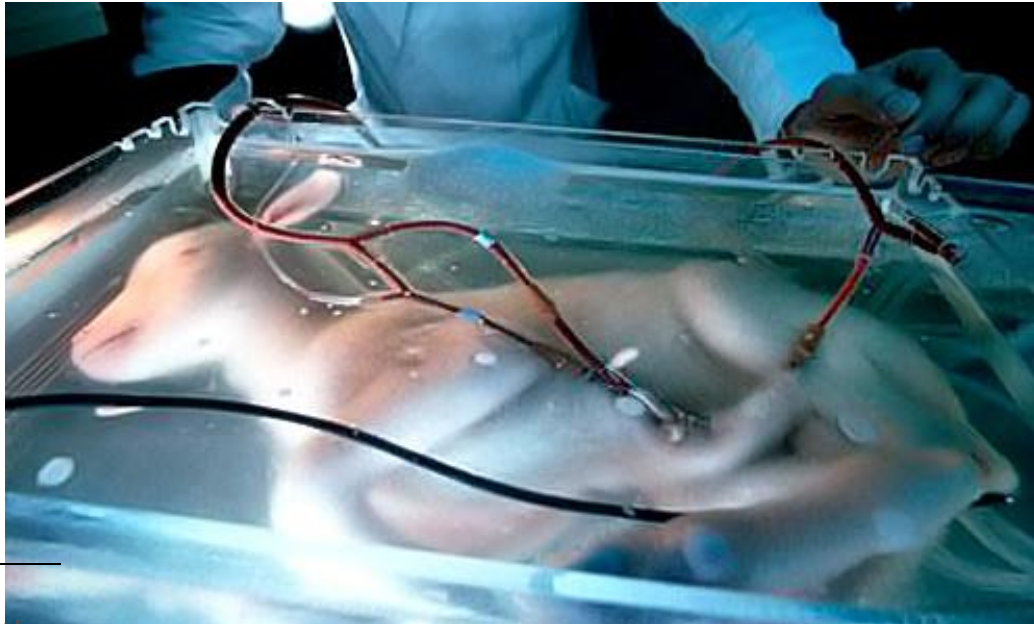
**Stanford**  
MEDICINE

---

Fertility and  
Reproductive Health

# Ectogenesis

The ultimate “culture” conditions



**Stanford**  
MEDICINE

Fertility and  
Reproductive Health

# Ectogenesis

- Not what we are doing entirely
- However.....
  - D0-D7 then 22-39 weeks, we are.



**Stanford**  
MEDICINE

---

Fertility and  
Reproductive Health

# Sequential vs Single Step

- Do single step media make compromises to stage-specific nutrient requirements? (assuming they exist)



**Stanford**  
MEDICINE

---

Fertility and  
Reproductive Health



# Specific Elements of Some “Newer Generation” Media

EDTA:

- It binds toxic heavy metals and also inhibits glycolytic enzyme phosphoglycerol kinase

(Sodium) Citrate

- It acts as a direct energy substrate, feeding into the TCA cycle. Originally found bound to albumin.



**Stanford**  
MEDICINE

---

Fertility and  
Reproductive Health

# Cation's

- Variable  $Mg^{++}$  Concentration
  - High  $Mg^{++}$  concentration decreases the uptake of exogenous  $Ca^{++}$ . Good to protect embryos from mitochondrial damage and subsequent energy metabolism. But, need  $Ca^{++}$  cascade for for normal fertilization. (capacitation and acrosome reaction.)



**Stanford**  
MEDICINE

---

Fertility and  
Reproductive Health

# Antibiotic choice

- Penicillin/Streptomycin
  - Possible pt interactions
- Gentamycin
  - More broad and stable



**Stanford**  
MEDICINE

---

Fertility and  
Reproductive Health

# Use of Zwitter ion buffers

- Embryo/oocyte handling
- HEPES
- MOPS
- HEPES/MOPS



**Stanford**  
MEDICINE

---

Fertility and  
Reproductive Health

# Ultimate Culture conditions

- Sum of many parts
- All controllable
- Requires significant oversight
- Will change over time



**Stanford**  
MEDICINE

---

Fertility and  
Reproductive Health

# Will we get here?



**Stanford**  
MEDICINE

---

Fertility and  
Reproductive Health