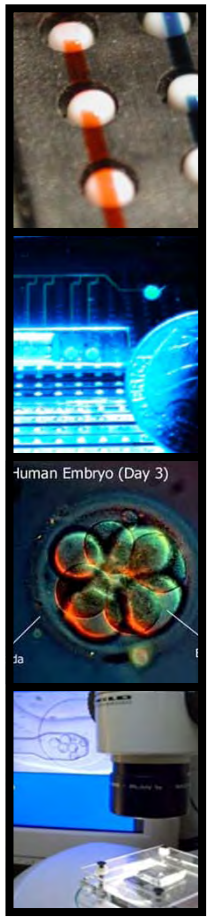
Dutch Assoc. of Clinical Embryologists, Nijmegen, Netherlands, 1/12/2012

Microfluidic-Generated Dynamic Microenvironment For Gametes and Embryos



Gary D. Smith, Ph.D.

Professor
Director of Reproductive Sciences Program

Departs of OB/GYN, Physiology, and Urology
smithgd@umich.edu

Outline

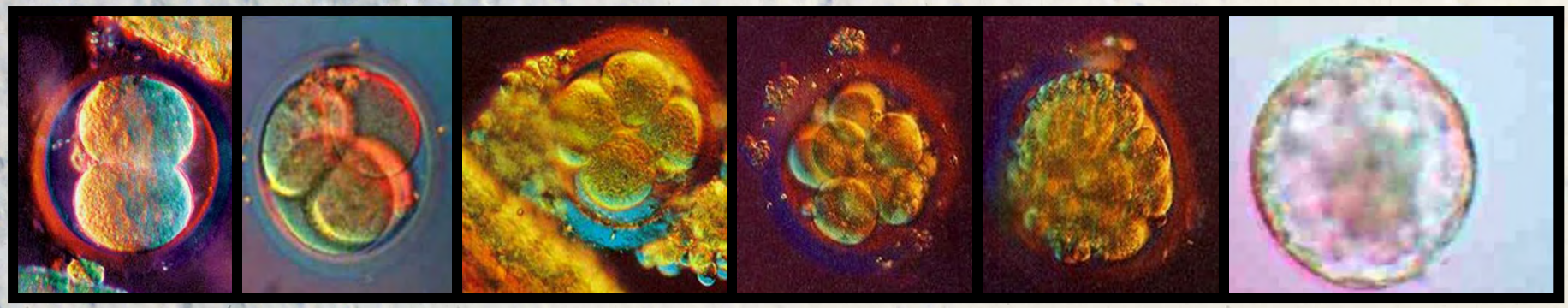
1. Microfluidics for sperm isolation
2. Microfluidics for embryo culture
3. Microfluidics for embryo analysis
4. Microfluidics for cryopreservation
5. Conclusions and questions

In Vitro Fertilization: A Micro-Process

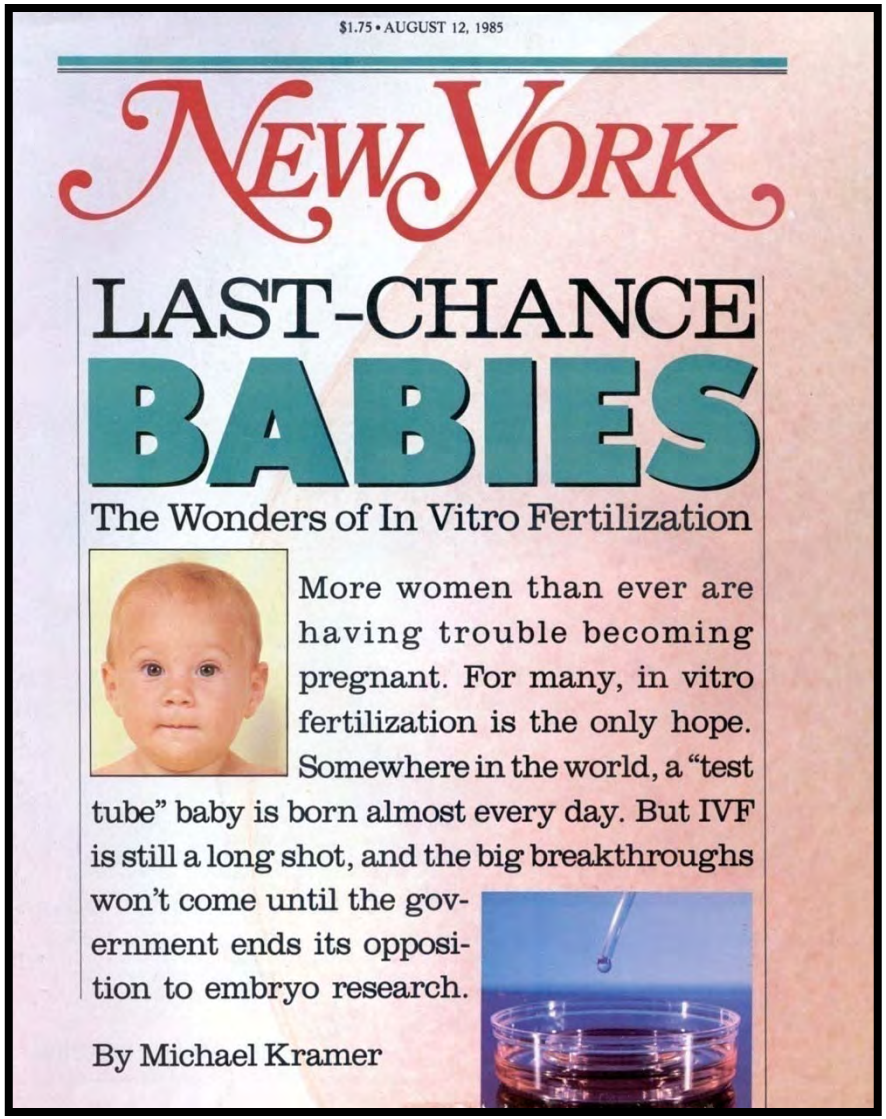
Sperm (head 3 x 7 μm)

Oocyte (135 μm)

Embryo Development (135 μm to 170 μm)



In Vitro Fertilization of the Past and Present



$1.75 • AUGUST 12, 1985

NEW YORK

LAST-CHANCE BABIES

The Wonders of In Vitro Fertilization

More women than ever are having trouble becoming pregnant. For many, in vitro fertilization is the only hope. Somewhere in the world, a "test tube" baby is born almost every day. But IVF is still a long shot, and the big breakthroughs won't come until the government ends its opposition to embryo research.

By Michael Kramer

***In Vitro* Fertilization and Embryo Culture:**

- Media have changed substantially

- Processes have changed minimally (ICSI / extended culture)

- Hardware / related environments remain the same

Microfluidics

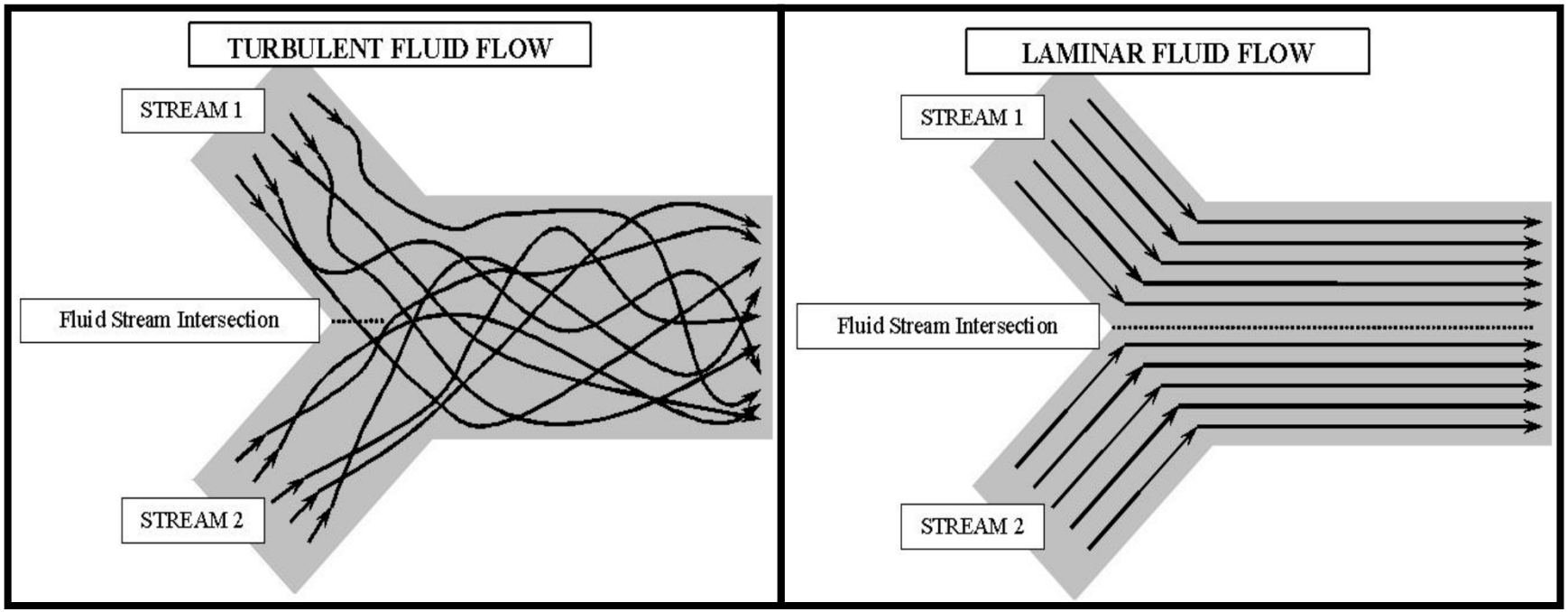
- study of physical principles of fluid behavior in a microenvironment and its application to chemistry, molecular biology, and cell biology

1) **Size / Mechanical Advantages**
2) **Microenvironment / Physiological Advantages**

Turbulent Versus Laminar Flow

Fluid at the microscale exhibits laminar flow

Laminar flow is streamline and predictable

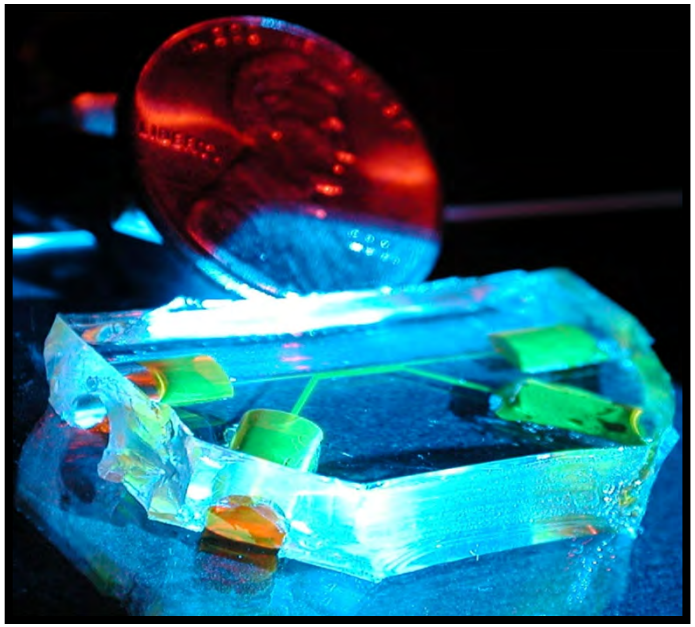


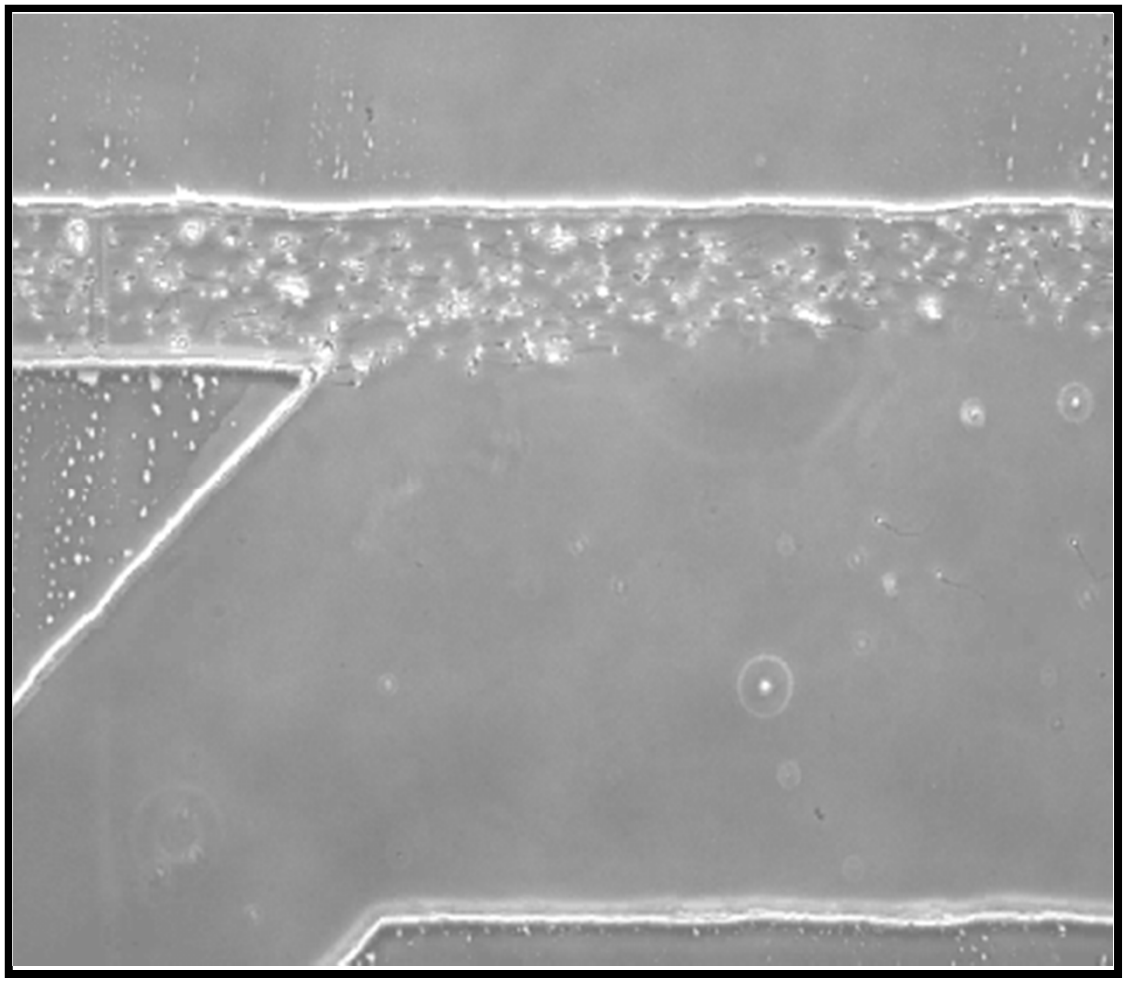
Could Microfluidics Be Useful In Isolation of Motile Sperm?

Theory: In a microfluidic device, motile sperm would be able to deviate from their initial stream-of-flow, cross the inter-streamline, and be isolated and enriched.

Initial stream-of-flow

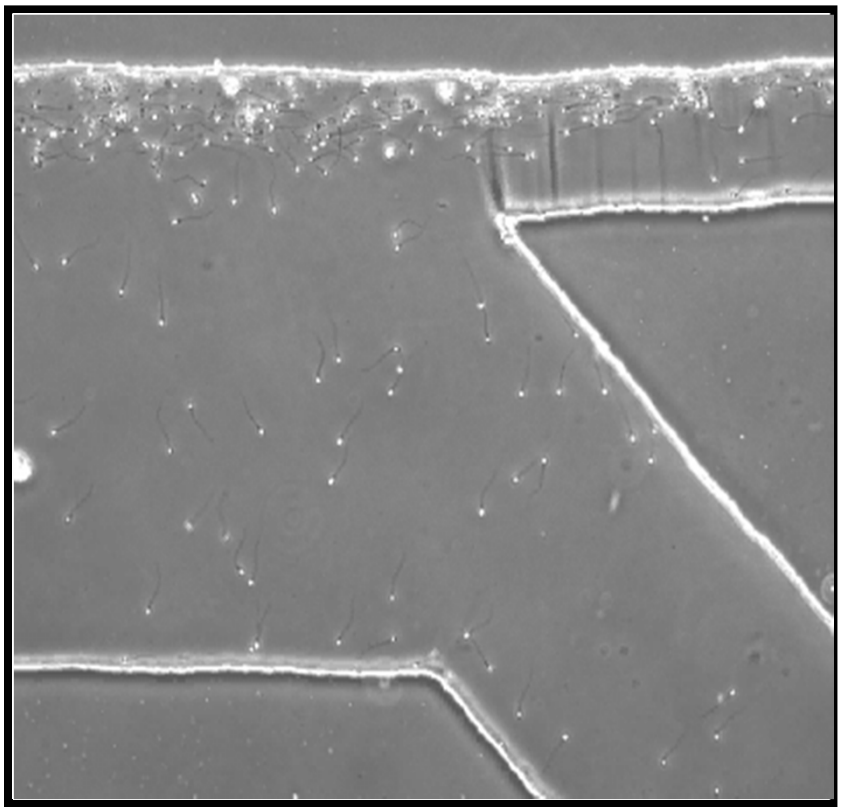
Microfluidic Sperm Separation

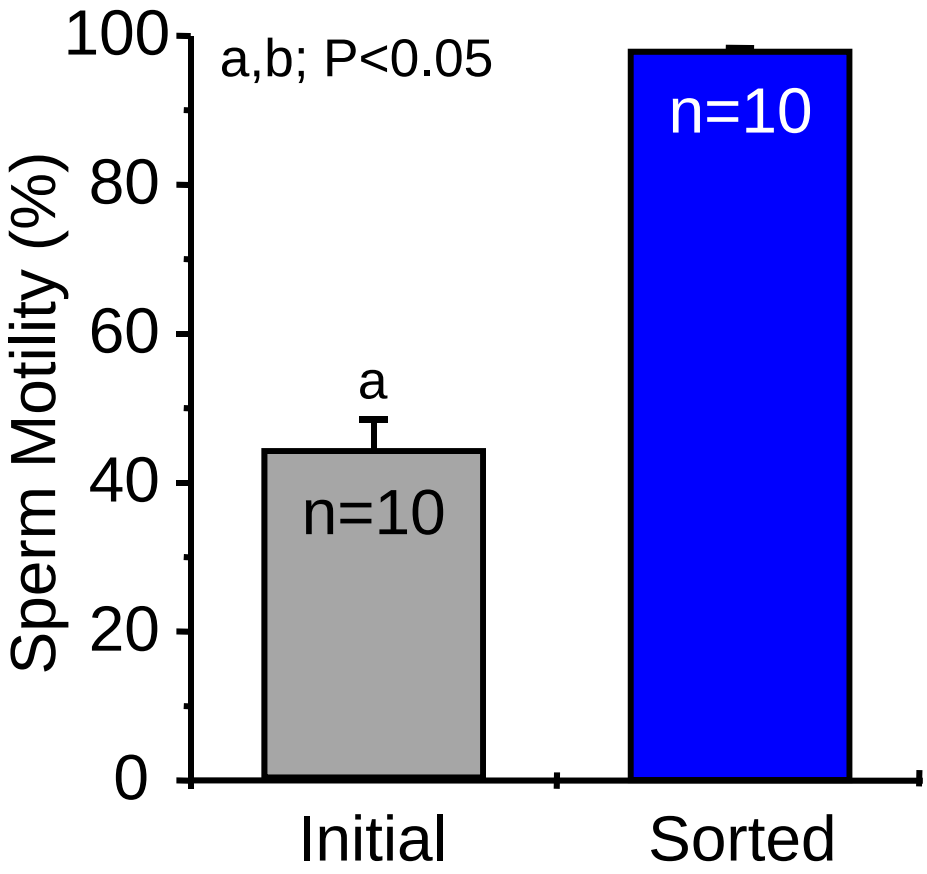




Cho et al., ***Anal Chem;* 2003**
Schuster et al., ***Reprod Biomed Online;* 2003**

Microfluidic Motile Sperm Isolation

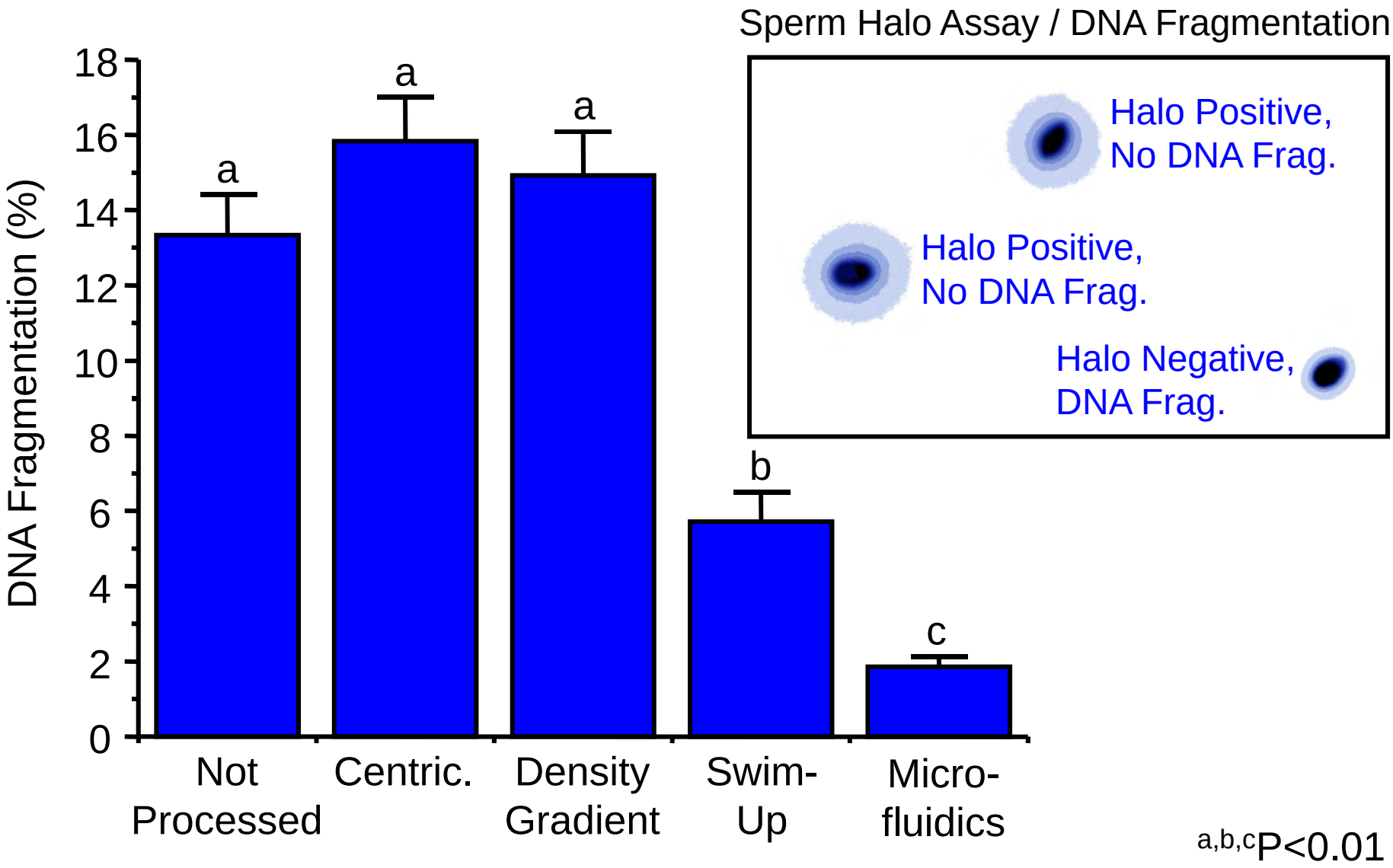




Schuster et al., ***Reprod Biomed Online;* 2003**

Cho et al., ***Anal Chem;* 2003**

Microfluidic Sperm Isolation Reduces DNA Fragmentation

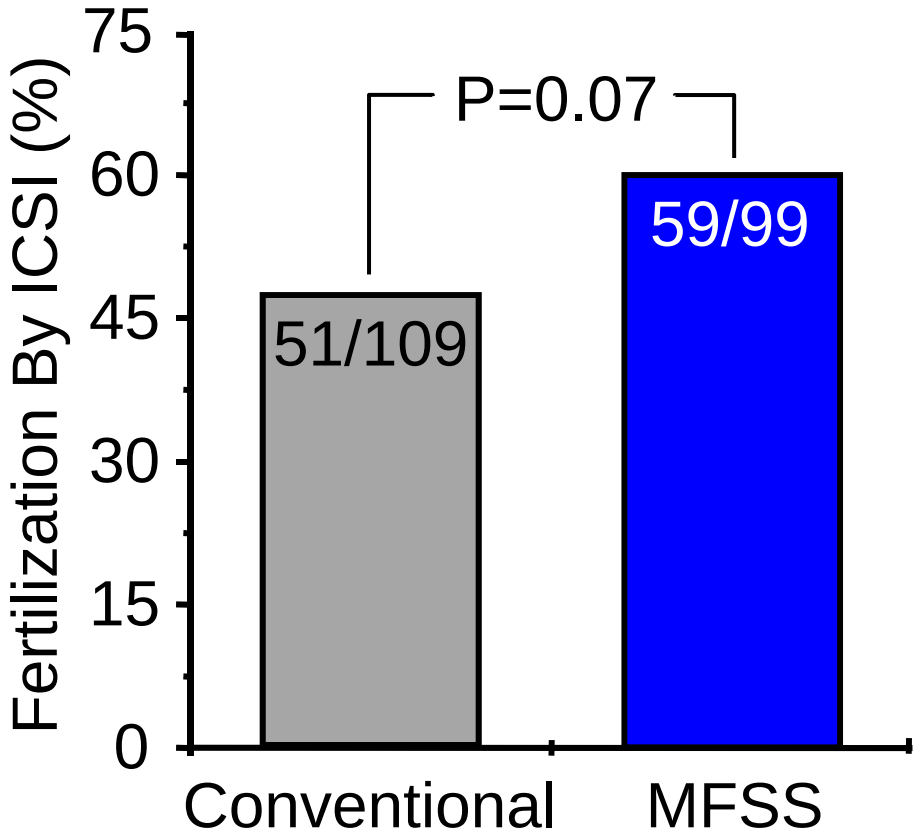


$^{a,b,c}P<0.01$

Microfluidic Sperm Sorter (MFSS) : Clinical Trial Ongoing (Japan)

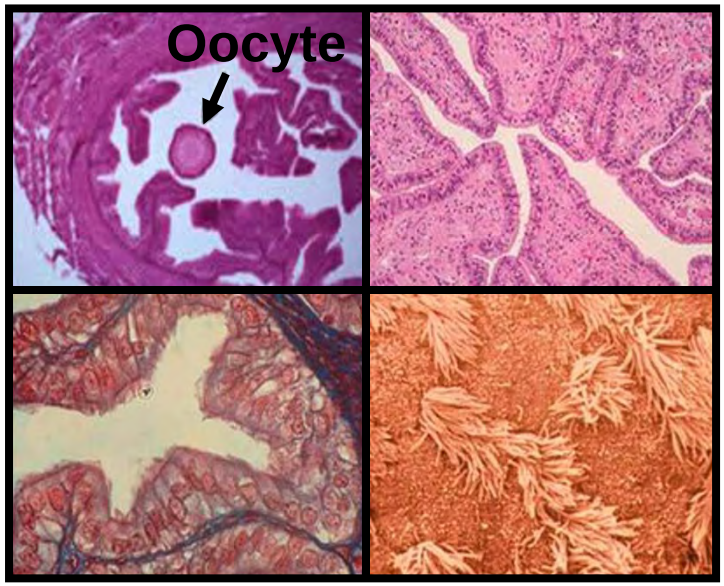
Nagoya University Hospital

- IRB approved / 40 couples
- inseminate 4 oocytes/cycle with MFSS isolated sperm



Embryo Development Environment

In Vivo



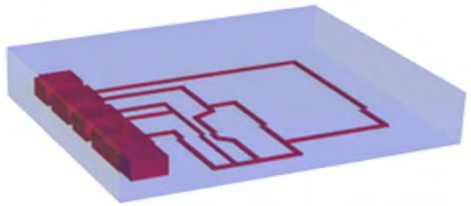
1) Moist
2) Moving
3) Chemically dynamic
4) Surfaces glycoprotein rich
5) Micro-environment

In Vitro

1) Fluid
2) Stagnant
3) Static
4) Inert
5) Macro-environment

Bioengineering and Embryo Culture

smART Flo Chips



- Single use

PinFlo Device

- Compatible with lab equipment
- Easy to use

PinFlo Software

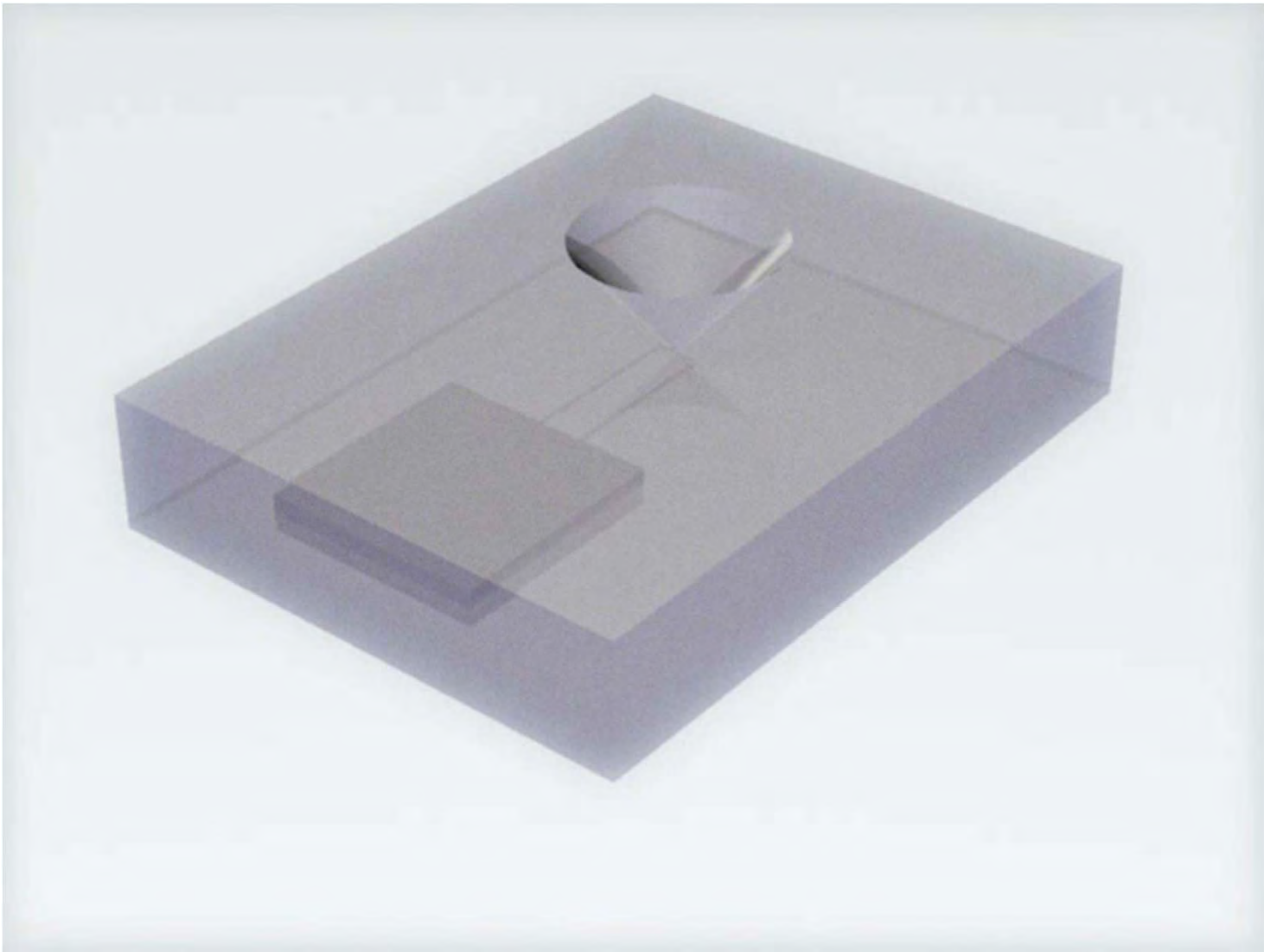
- Software operating system
- Programmable

Artificial Living System for Cell Culture

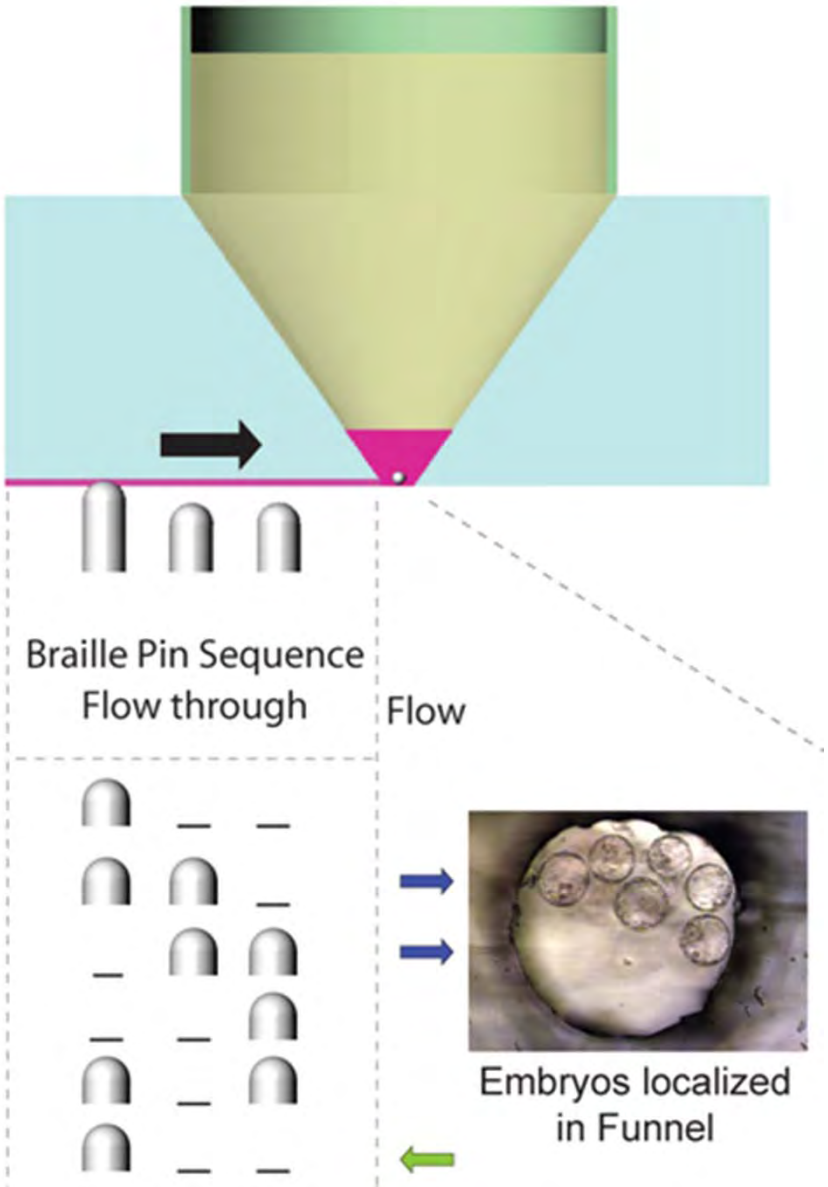
Shuichi Takayama
Professor
Bioengineering

Gu et al., ***PNAS*; 2004**
Heo et al., ***Anal Chem*; 2007**
Heo et al., ***Hum Reprod*; 2010**

Microfluidic / Braille Actuated Dynamic Culture System

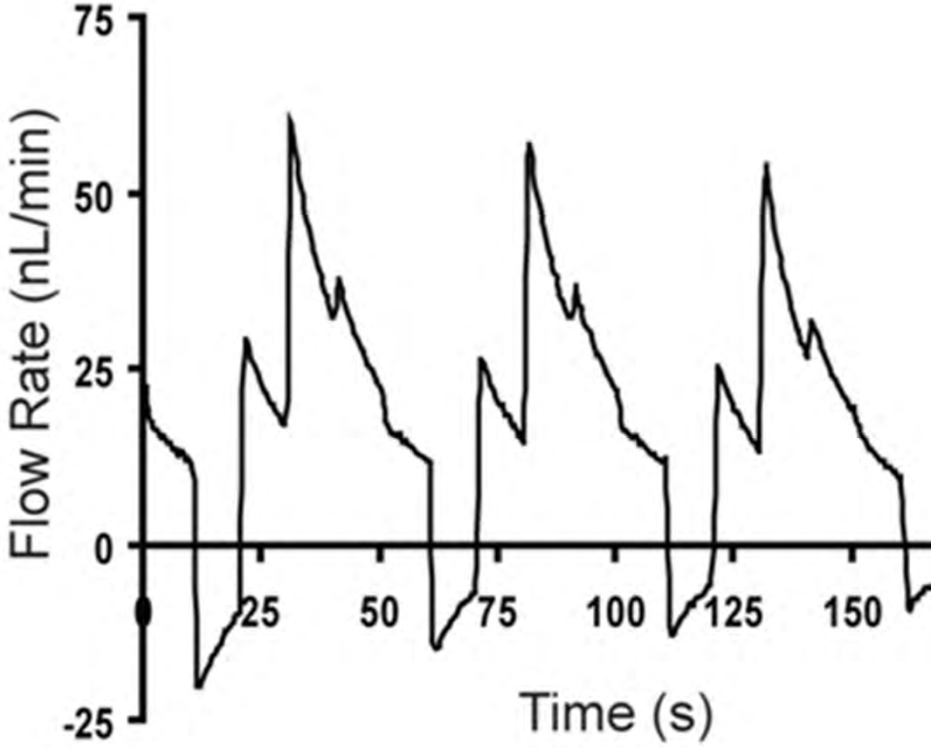


Pumping Cycles and Flow Rates



- Pumping cycle = 0.1 Hz
  - 0.14 Hz in rabbit oviduct
  - 0.06 Hz in human uterus

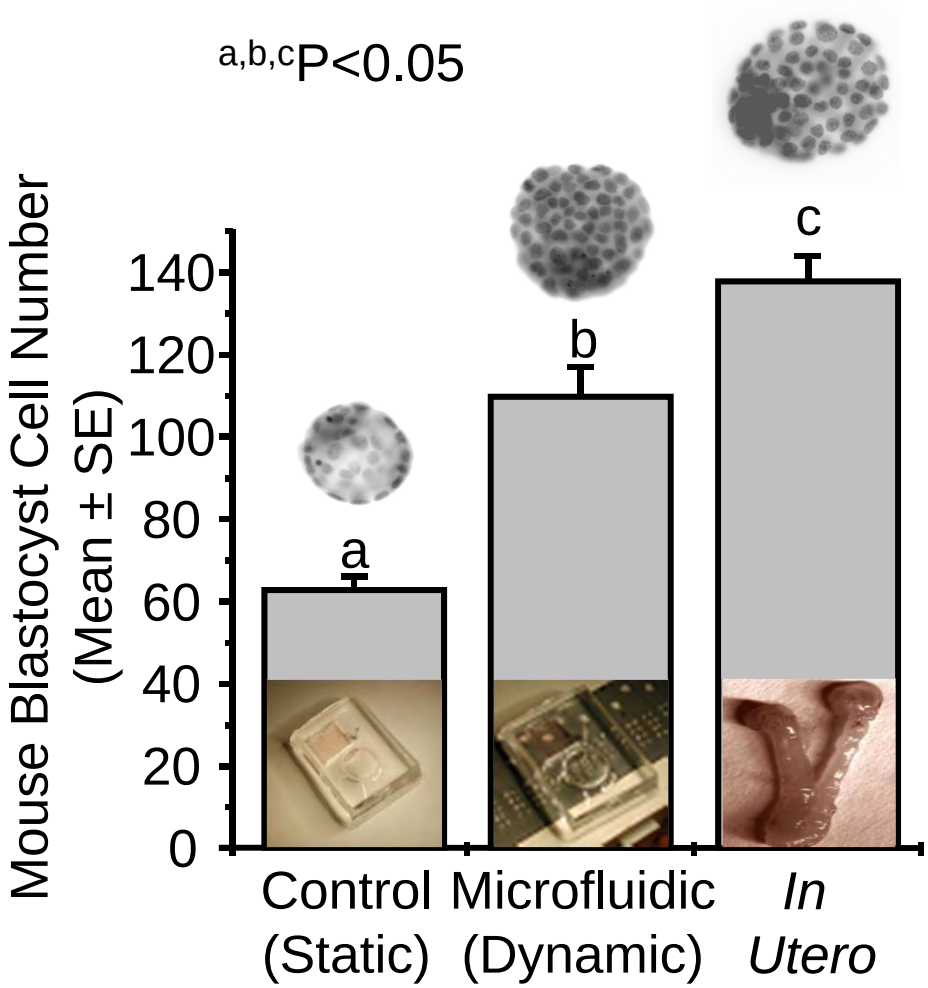
- Average flow rate 20nl/min



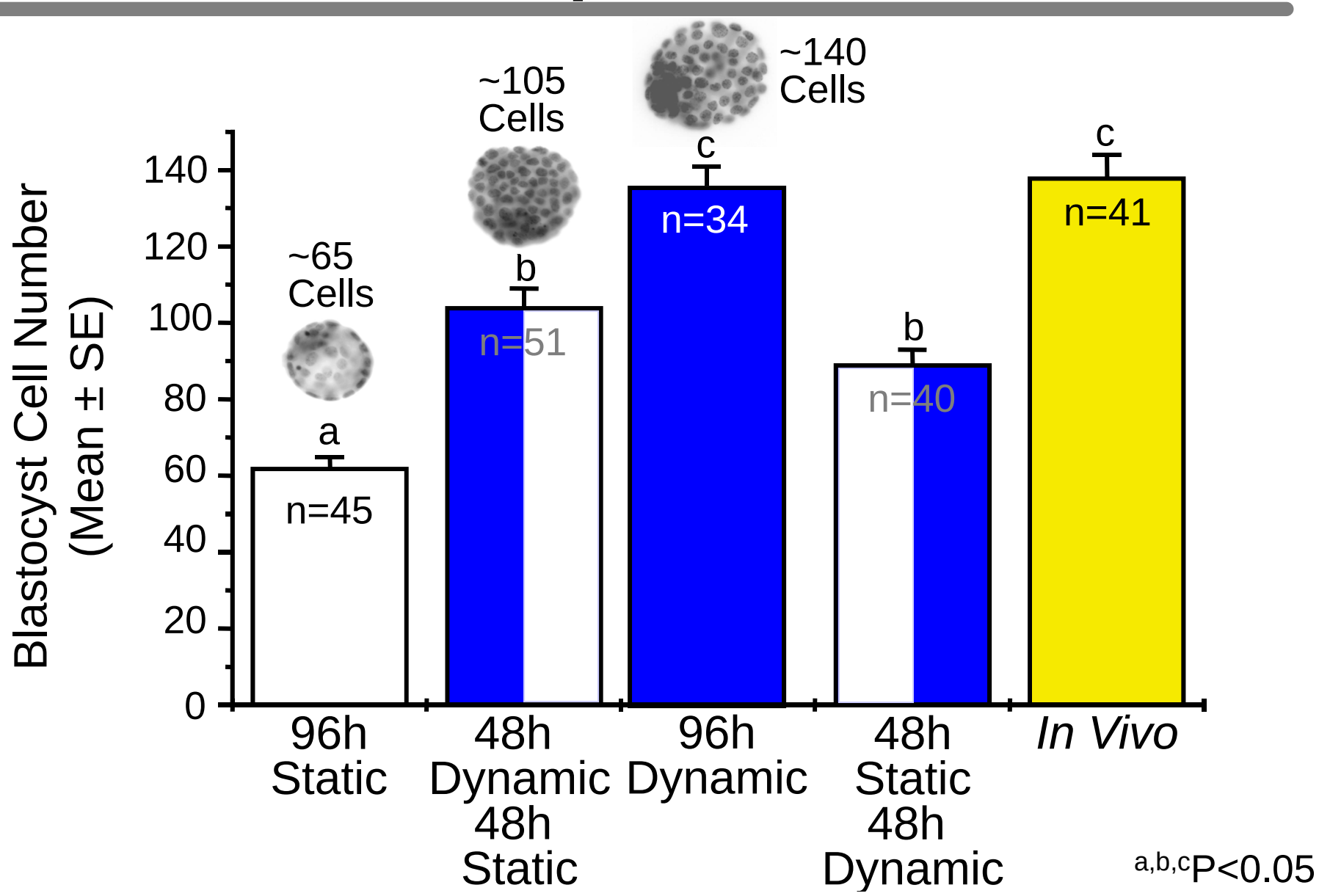
Blastocyst Quality and Dynamic Culture

- Compared to static culture, 96hr microfluidic dynamic culture improves mouse blastocyst development rate and quality *(Heo et al., 2010)*

- Compared to static culture, 144hr microfluidic dynamic culture improves bovine blastocyst quality *(Bormann et al., submitted)*

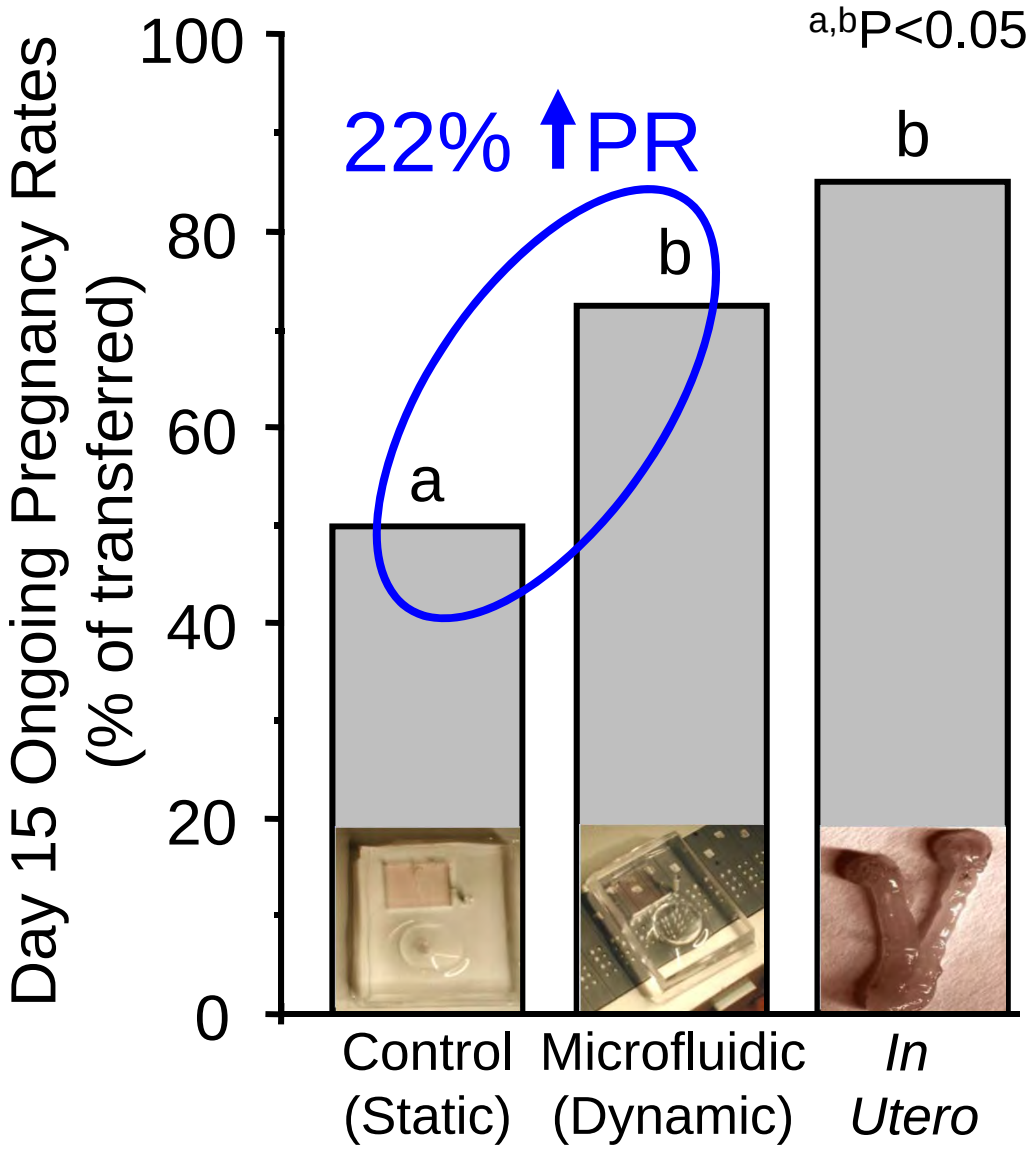


Benefit: Time-Dependent, Stage-Independent



Pregnancy Rate and Dynamic Culture

- Compared to static culture, 96hr microfluidic dynamic culture improves mouse embryo implantation and ongoing pregnancy rate *(Heo et al., 2010)*

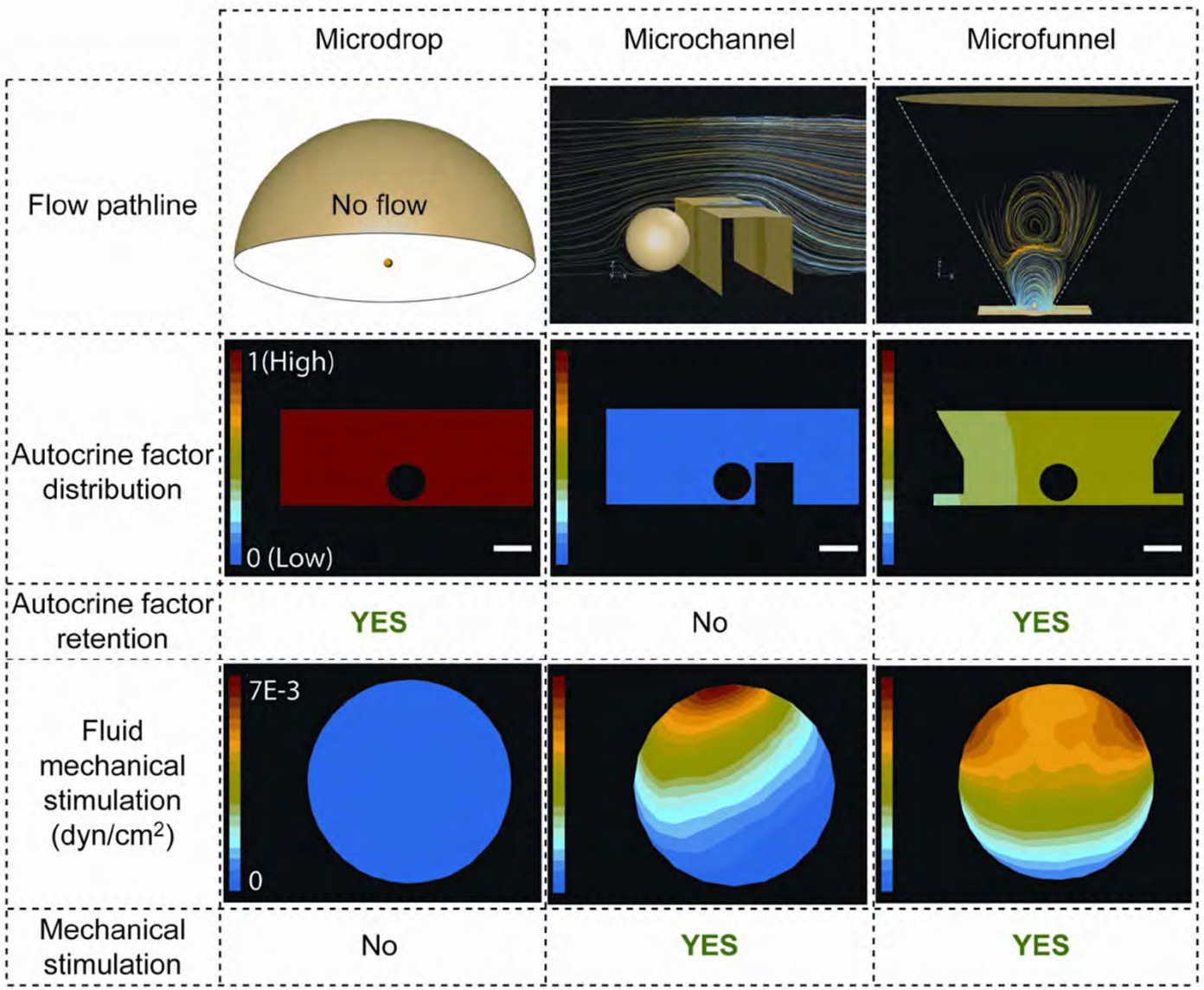


Preimplantation Environment and Placental Gene Expression

	Relative Placental Gene Expression	
	H19	***Igf2***
***In Utero* Grown** (n=29)	1.0±0.2	1.0±0.3
Static Control (n=23)	0.2±0.2**	0.3±0.2*
Dynamic Microfluidic (n=36)	0.3±0.2**	0.5±0.2

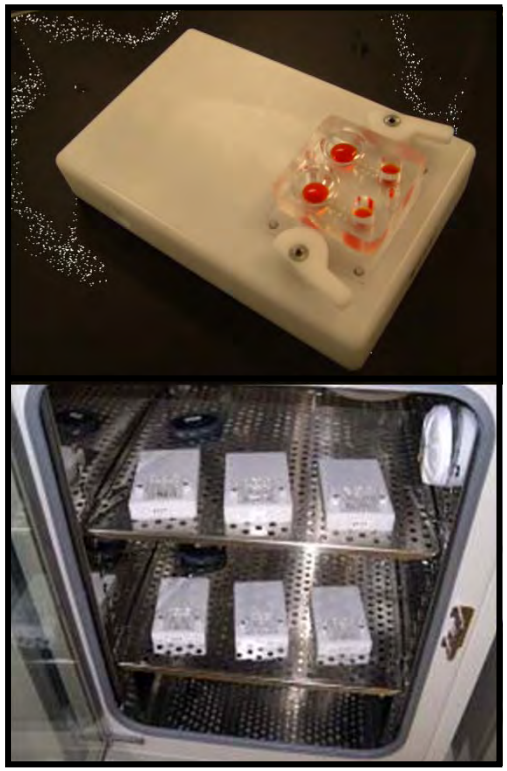
*P<0.05; **P<0.01 compared to *in utero*

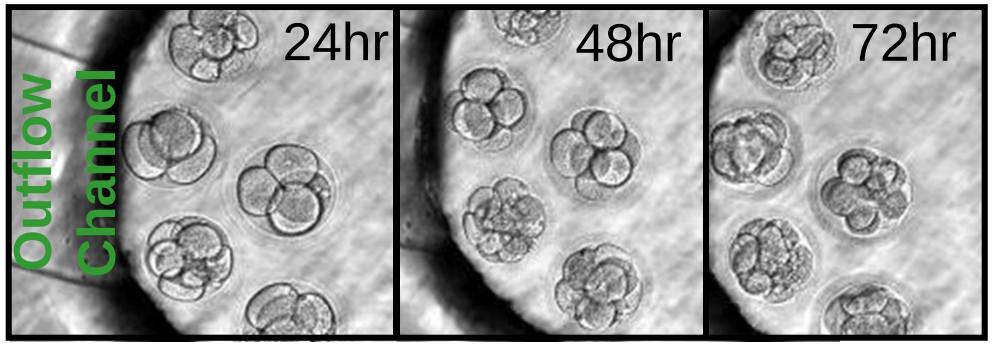
Dynamic Microfluidic Culture: Fluid Mechanical Stimulation with Retention of Autocrine Factors

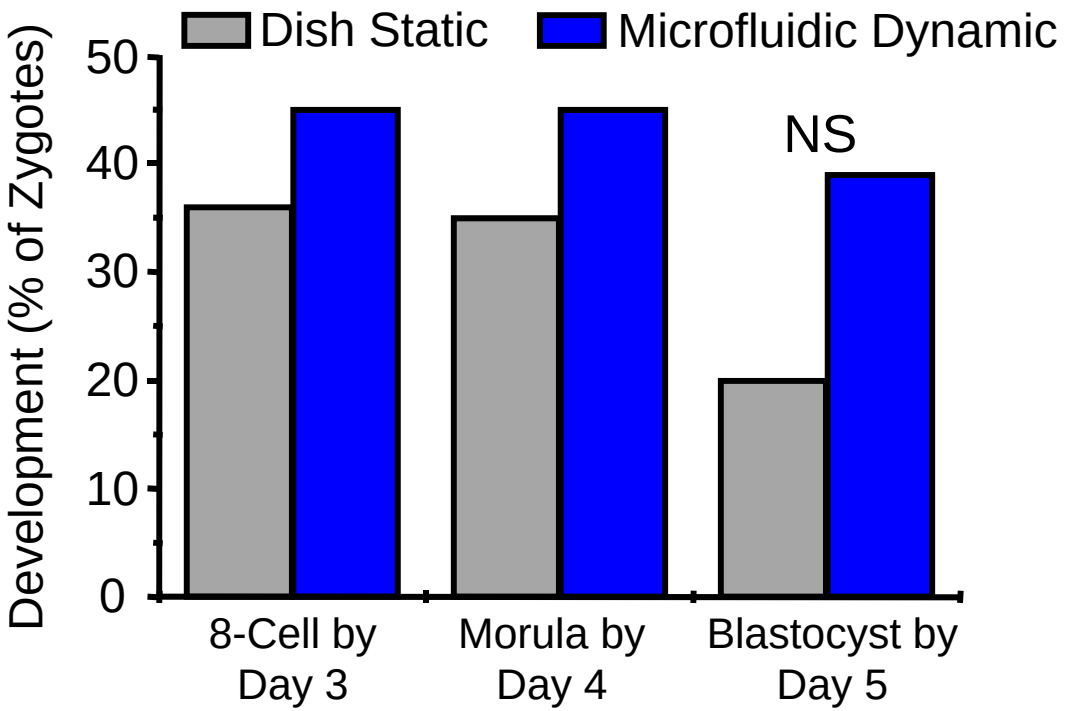


Human Embryo Development: Frozen Zygotes

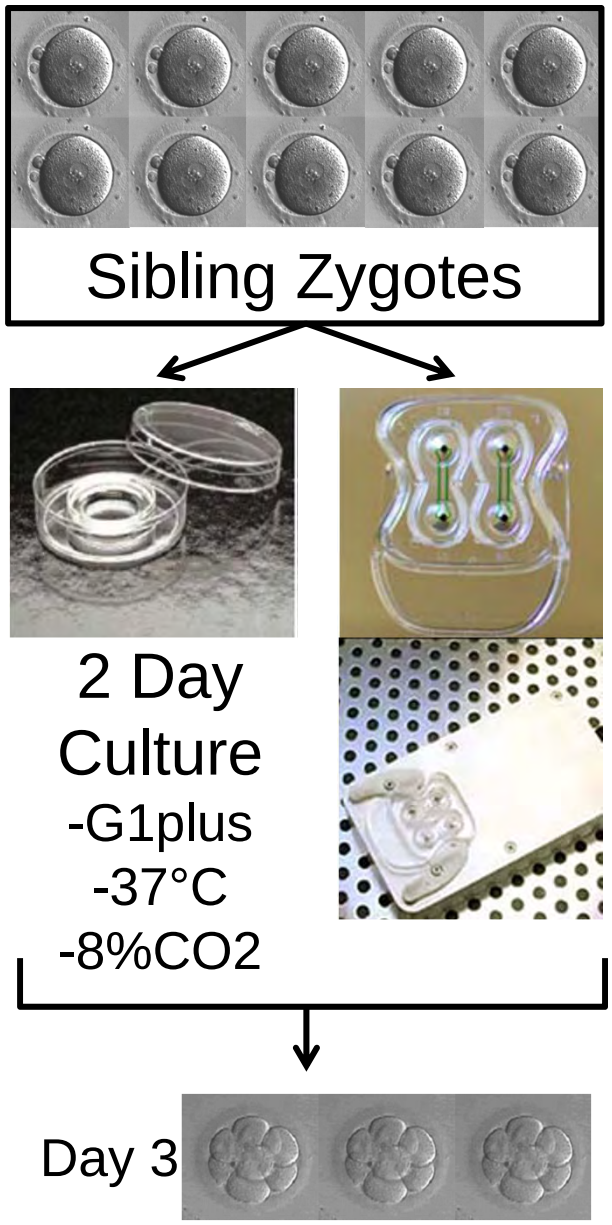
- IRB-approved study using <u>frozen/thawed</u> human PN-stage zygotes donated for research. Used 96 zygotes / 20 patients).



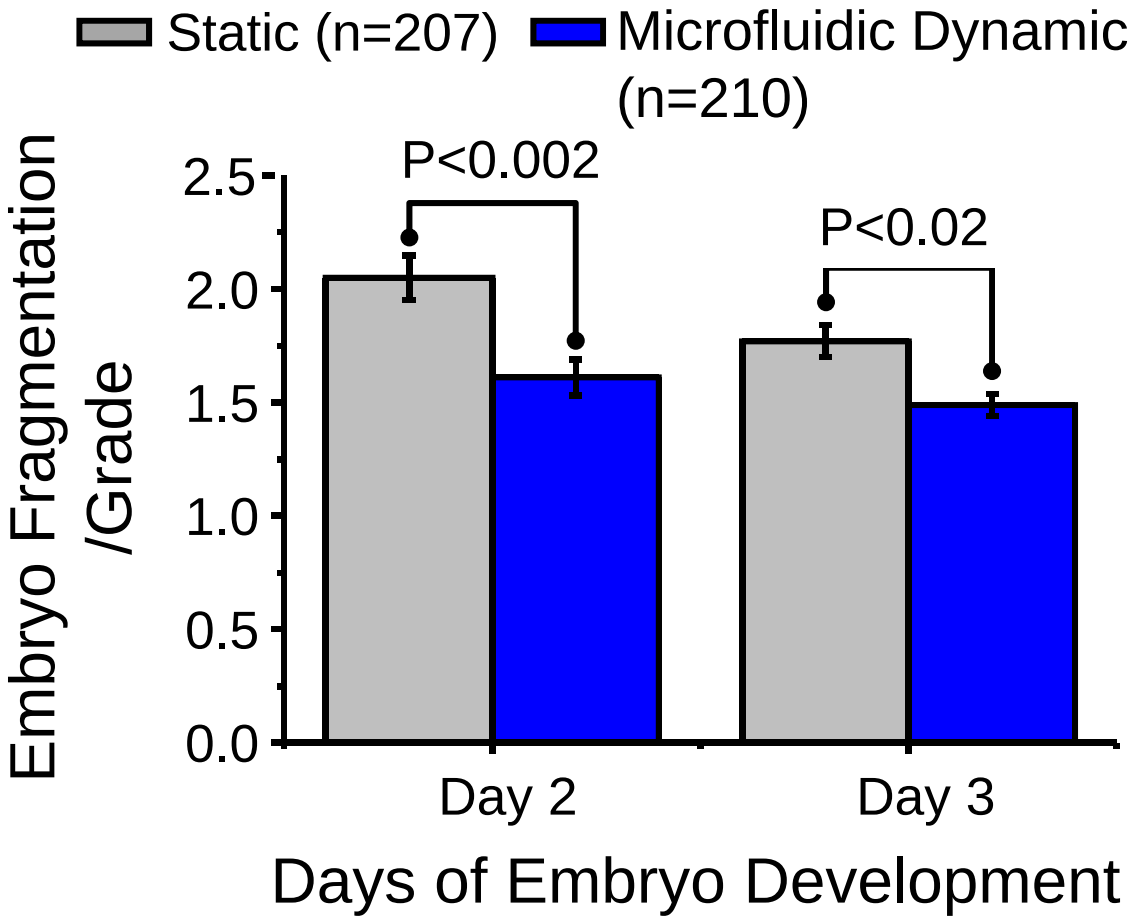




Clinical Trial: Dynamic Embryo Culture



- No significant difference in cell number



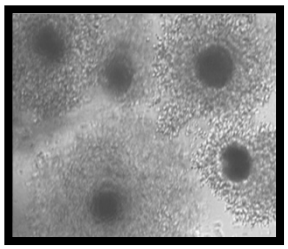
Dynamic Culture of Human Embryos and Day 3 Quality

- Collective embryo quality (cell # and grade)
  - Top Quality Embryos = 8-9 cell / grade 1
  - Good Quality Embryos = 6-9 cell / grade 1&2
  - Poor Quality Embryos ≤ 4 cell and/or ≥ grade 3

Day 3 Embryo Quality	Static Culture	Dynamic Culture
Top Quality	28%[a]	39%[b]
Good Quality	38%[a]	50%[b]
Poor Quality	29%[c]	16%[d]

[a,b]P<0.03; [c,d]P<0.002

Dynamic IVM and Embryo Developmental Competence



Immature Bovine Oocytes

TCM-199 + FCS, FSH, LH, EGF; 22hr

Microdrop IVM

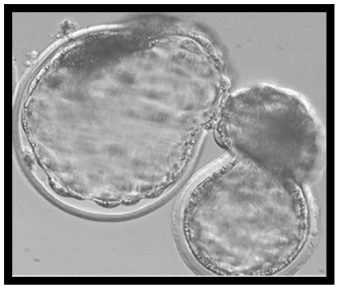
Dynamic IVM

Co-incubated with bovine sperm at $1x10^6$/ml in IVF-TALP for 12h at 39°C in 5% CO2 and 100% humidity.

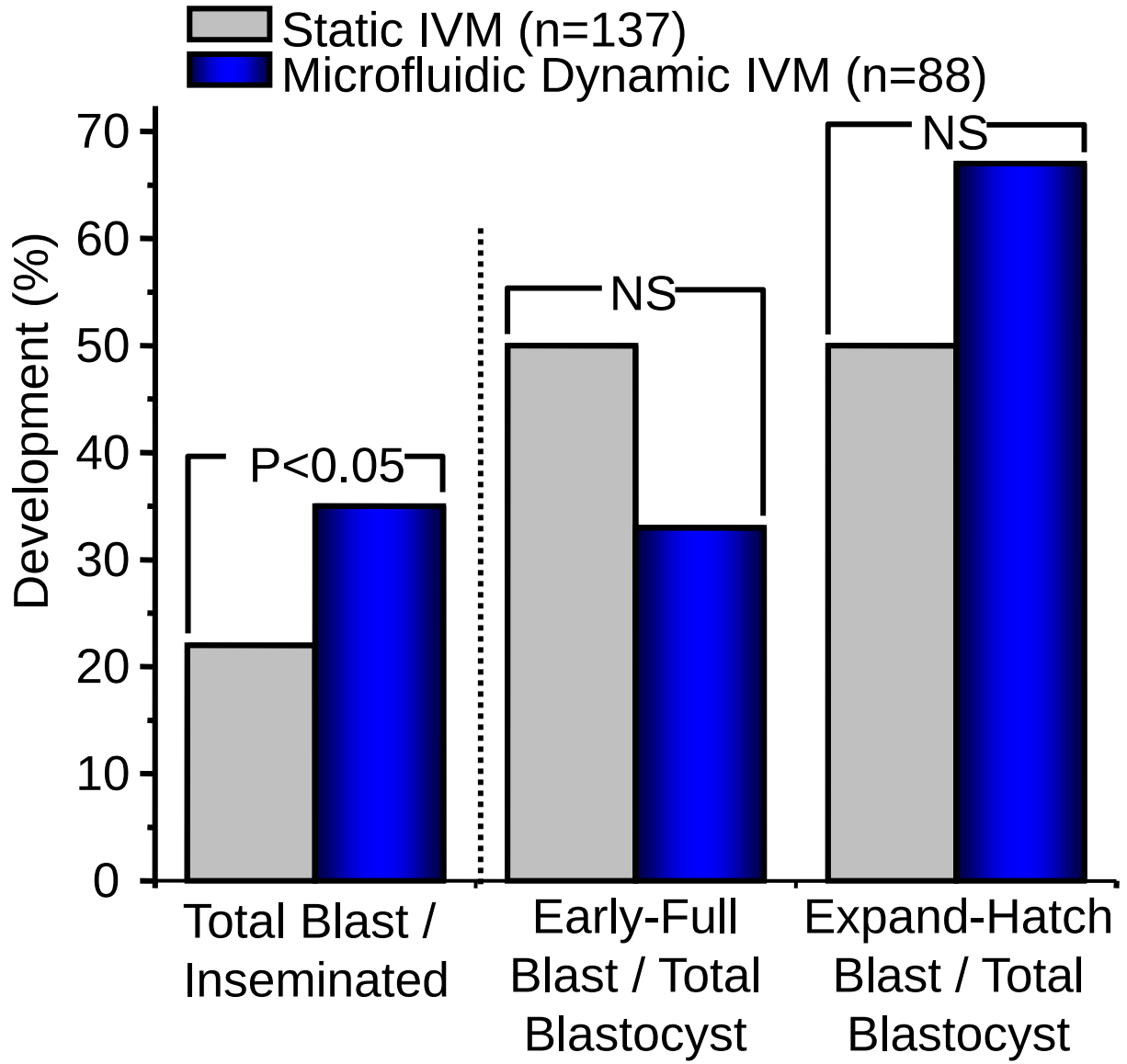
Microdrop Embryo Culture

144hr

Morphology Assessment

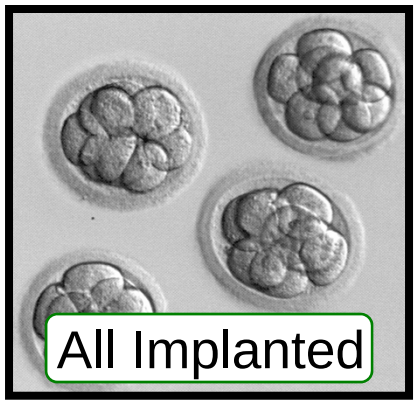


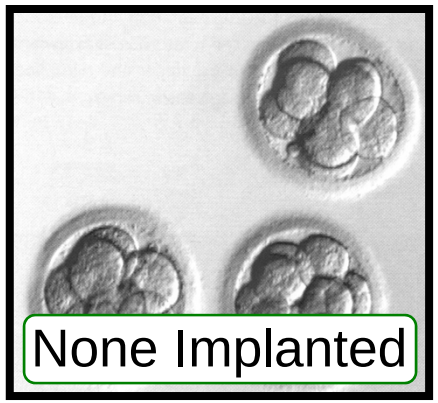
Dynamic Microfluidic IVM Improves Embryonic Developmental Competence

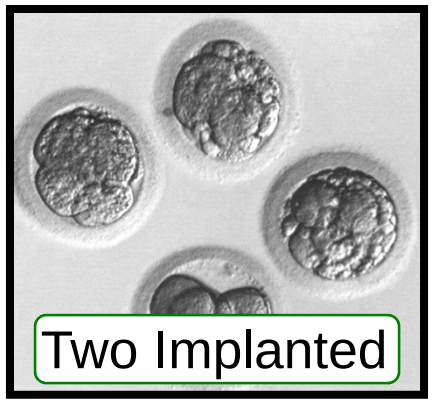


Embryo Implantation (Health) Prediction

- Today embryos are selected for transfer based on microscopic observations of morphology



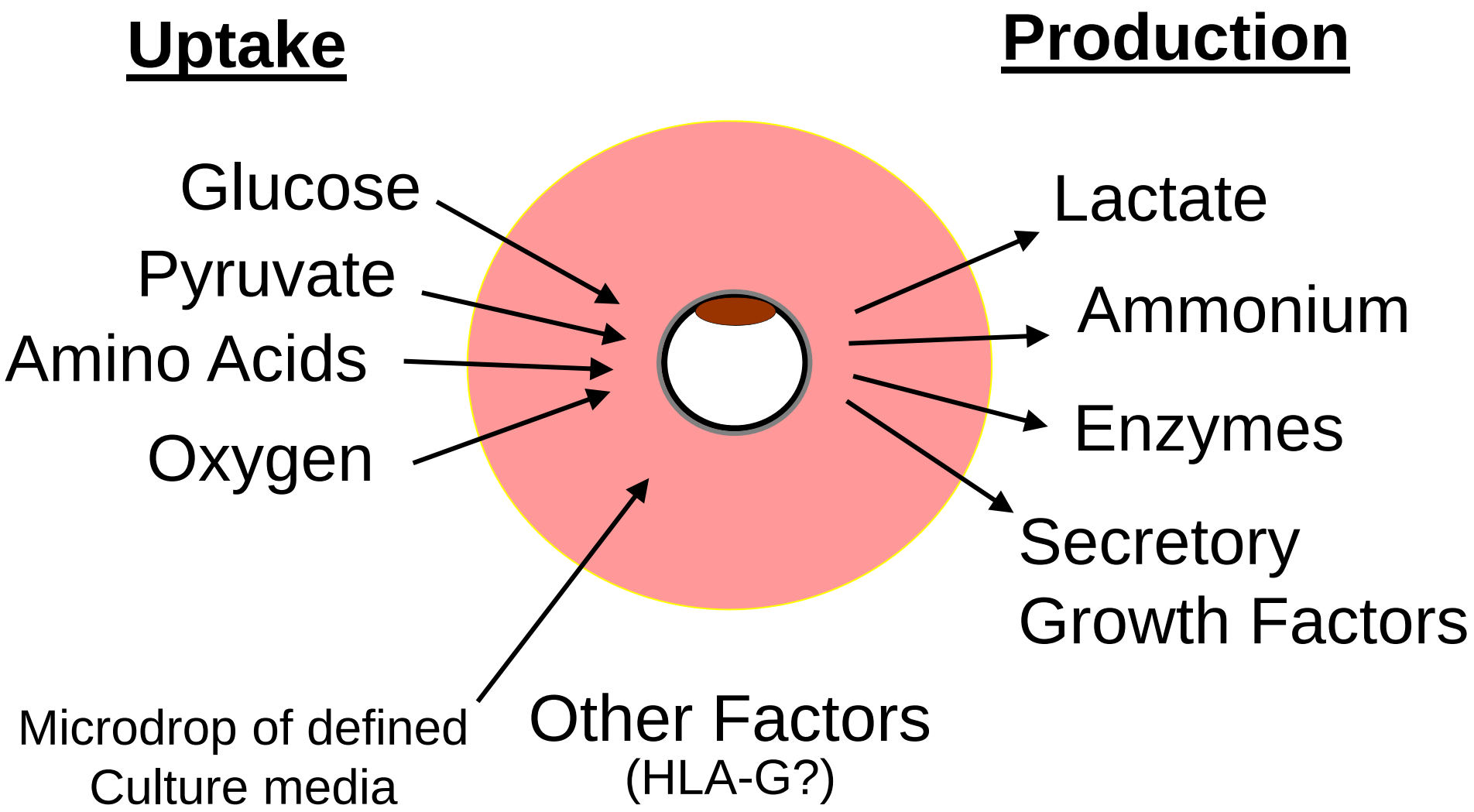




- Subjective and lacks between program consistency
- Lacks predictive value

Need Measurable Biomarker(s) of Embryo Health

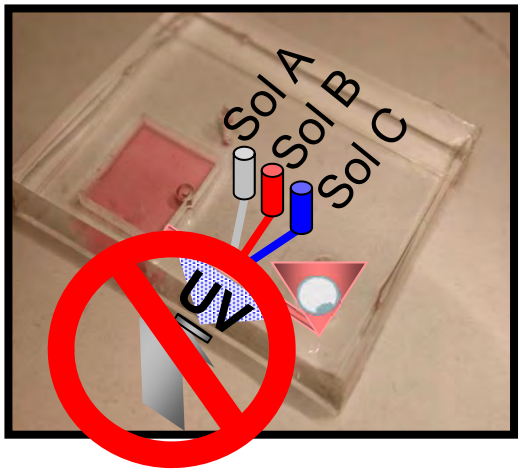
What Can Be Assessed?



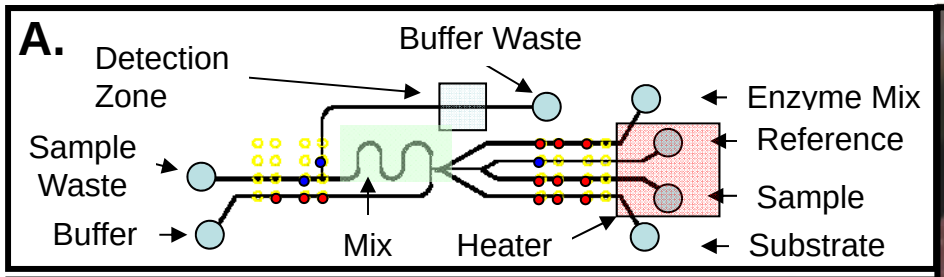
Development of Microfluidic In-Line Embryo Analysis

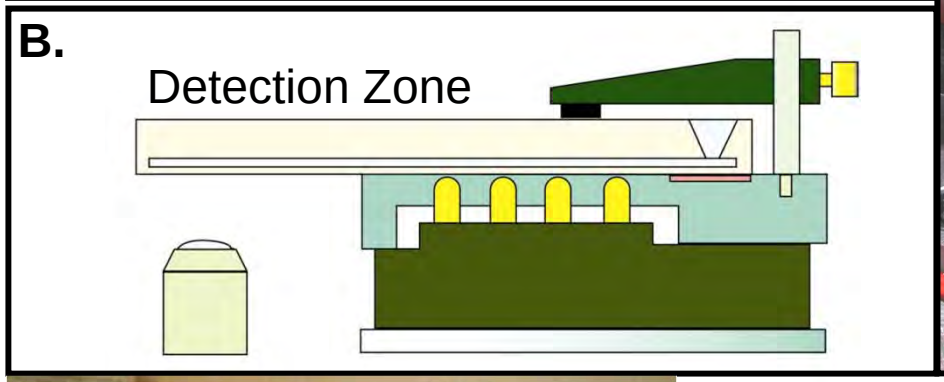
- Real-time, on-chip, multiparametric assays
  - precise automated nano-liter volume sampling (no dilution)
  - on-chip controls, reagents, segmented flow, mixing, and detection
  - computer controlled, no embryo manipulation, anytime (day or night)
  - on-chip assay with a **Non-UV** detection

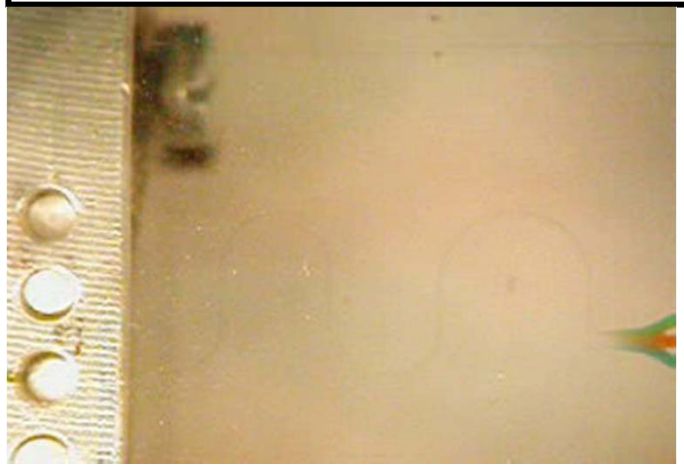
- Glucose consumption
- Lactate production
- IGF-1 secretion
- HLA-G



Real-Time, On-Chip Analysis of Embryo Metabolism

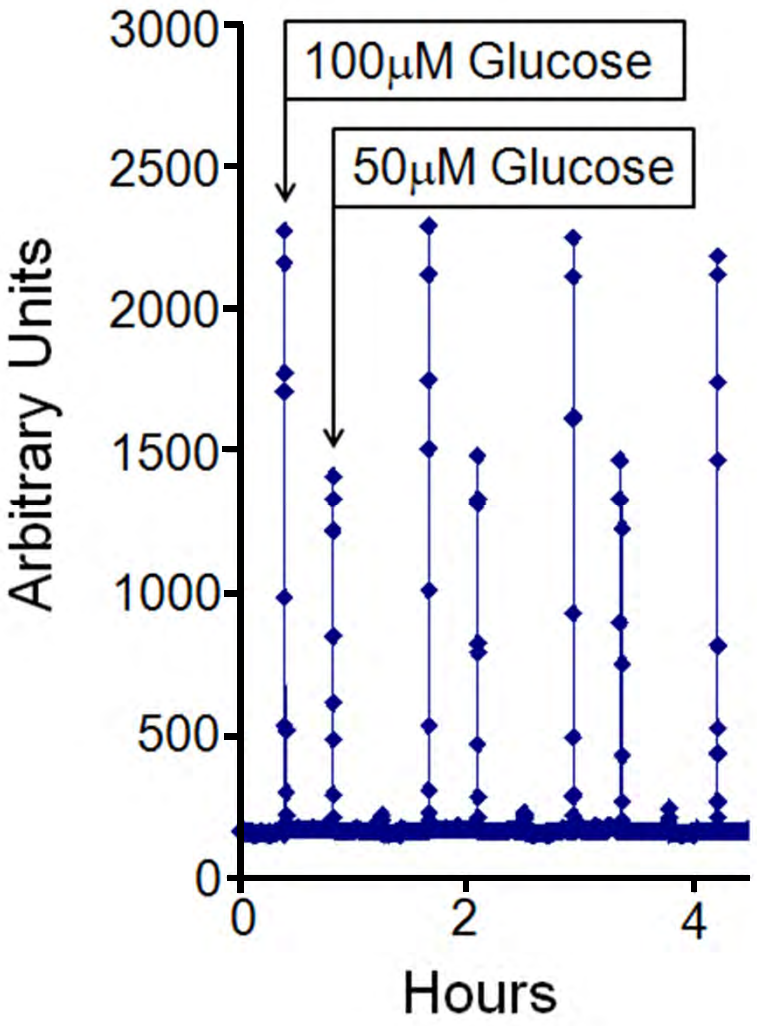


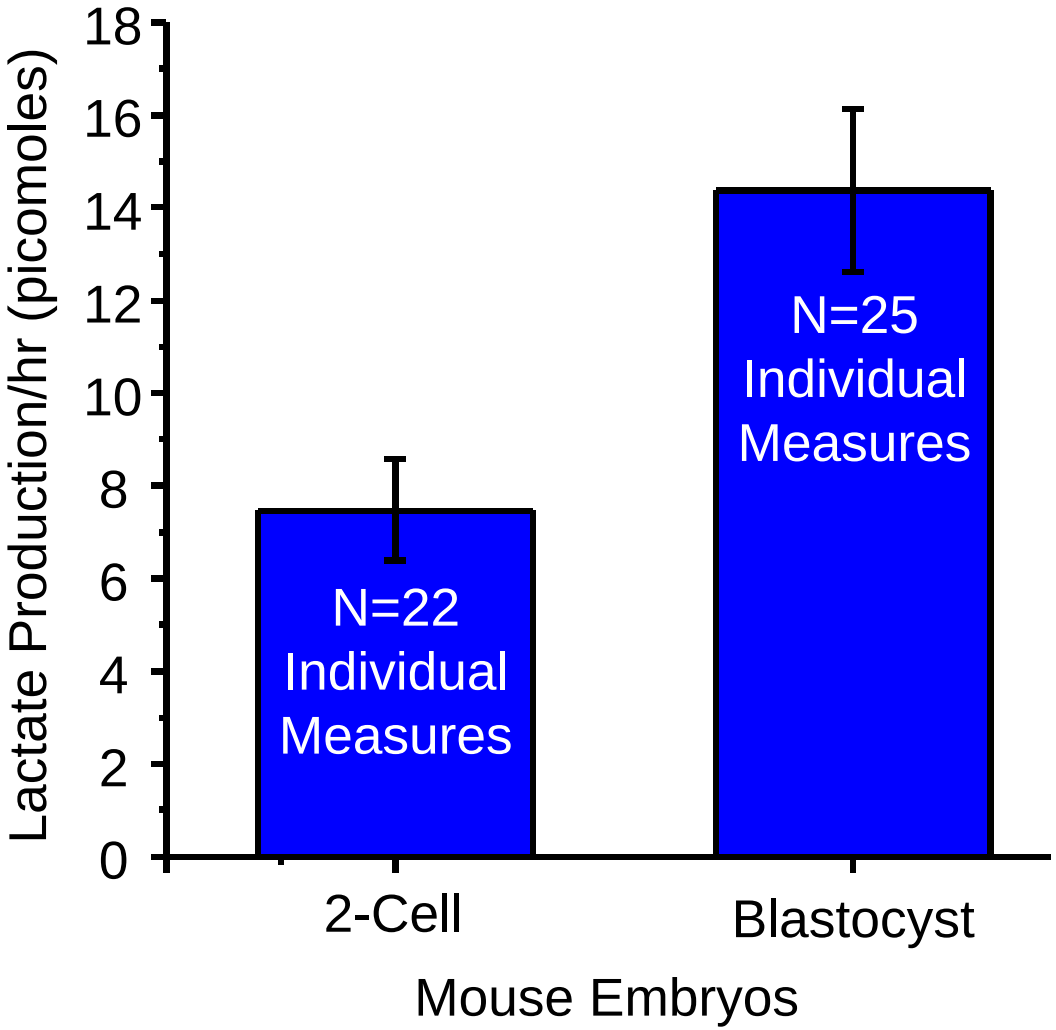




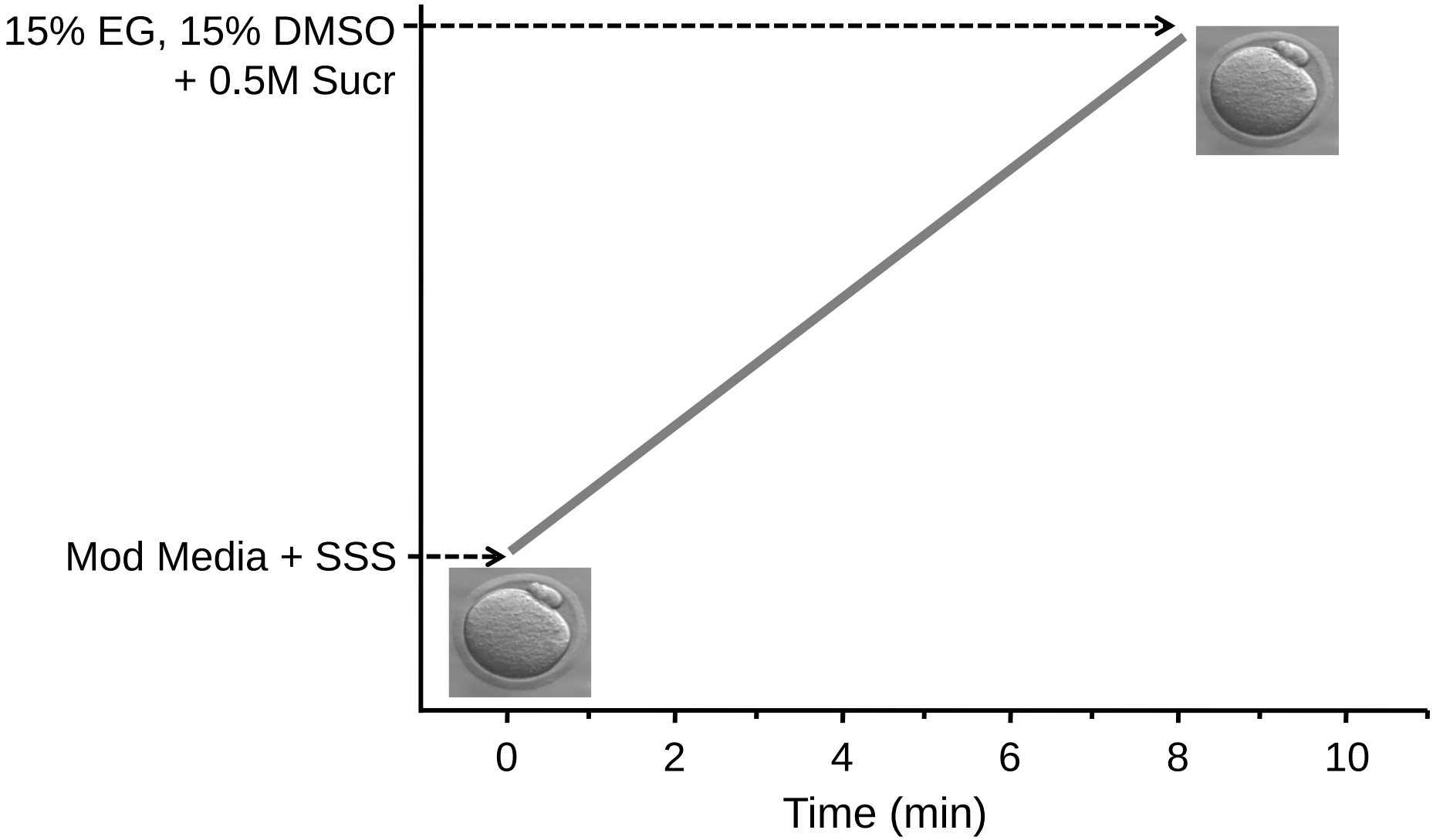
1. Sample loading and mixing
2. Enzyme reaction
3. Detection
4. Real-time analysis (day or night)

Real-Time, On-Chip Analysis of Embryo Metabolism





Microfluidics For Vitrification: Moving Solutions Over Cells, Not Cells Through Solutions



Why Might One Use Microfluidics in the Future?

1) Does something we cannot do today.

2) Does something we do today, but better.

3) Does something as well as we do today, yet less expensive.

4) Does something as well as we do today, yet less work.

5) Does something we do today, but safer.

Acknowledgements

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