

«ICSI Procedure and advanced techniques  
in medically-assisted procreation»

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# MAIN TOPICS

**-HUMAN EMBRYO CULTURE: TIPS AND TRICKS**

**- QUALITY CONTROL INSIDE A.R.T. LABORATORY**

**THEROICAL LESSON**

**- MEET THE EXPERT**

**VIRTUAL EDUCATIONAL  
AND  
SCIENTIFIC ACTIVITIES**



# WHAT IS EMBRYO CULTURE MEDIA?

- ✓ Embryo culture is a component of in vitro fertilization;
- ✓ Embryos are cultured in an artificial medium consisting of glucose, pyruvate and other energy-providing components;
- ✓ The addition of amino acids, nucleotides, vitamins, and cholesterol may also improve the performance of embryonic growth and development;
- ✓ Substances like antioxidants, antibiotics, macromolecules, hormones and growth factors can be added;
- ✓ Optimization of the culture media is essential by optimizing all other components of the culture system, including the environment and equipment.

# WHY CULTURE MEDIA IS SO IMPORTANT?

Culture media is artificial but supports key functions:

- ✓ Sperm function (motility, capacitation, acrosome reaction..)
- ✓ Cleavage stage
- ✓ Genomic activation
- ✓ Early differentiation of cell types

# HUMAN EMBRYO CULTURE



**1957: calcio lattato  
in soluzione salina**



**1968: calcio lattato e acido  
piruvico in soluzione complessa**

**Whitten, W. K.** (1957). Culture of tubal ova. *Nature*, **179**:1081-1082.

**Whitten, W.K., Biggers, J.D.** (1968). Complete development of in vitro of the pre-implantations stages of the mouse in a simple chemically defined medium. *J. Reprod. Fertil.* **17**:399-401.

# HUMAN EMBRYO CULTURE



1998: “sequential” embryo culture system

**Gardner DK.** 1998 Changes in requirements and utilization of nutrients during mammalian preimplantation embryo development and their significance in embryo culture. *Theriogenology* 49:83-102



2004: “single-step” embryo culture system

**Reed ML, et al.,** 2010 Challenging traditional embryo culture techniques with a simplified, continuous single medium protocol. *J. Clin. Embryol* 13:33-41

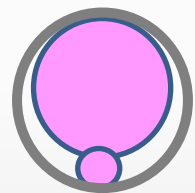
# HUMAN EMBRYO CULTURE

## Sequential Culture System: “back to nature”

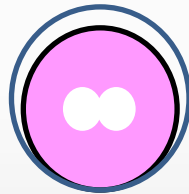
**Step 1: HTF**  
Non essential aminoacids  
glucose  
EDTA  
Pyruvate  
Lactate

**Step 2: CLEAVAGE**  
Non essential aminoacids  
Low glucose  
EDTA  
Pyruvate  
Lactate

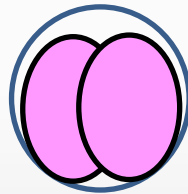
**Step 3: BLATOCYST**  
Non essential aminoacids  
Essential aminoacids  
High glucose  
Pyruvate  
Lactate



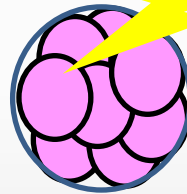
DAY 0



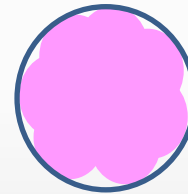
DAY 1



DAY 2



DAY 3



DAY 4



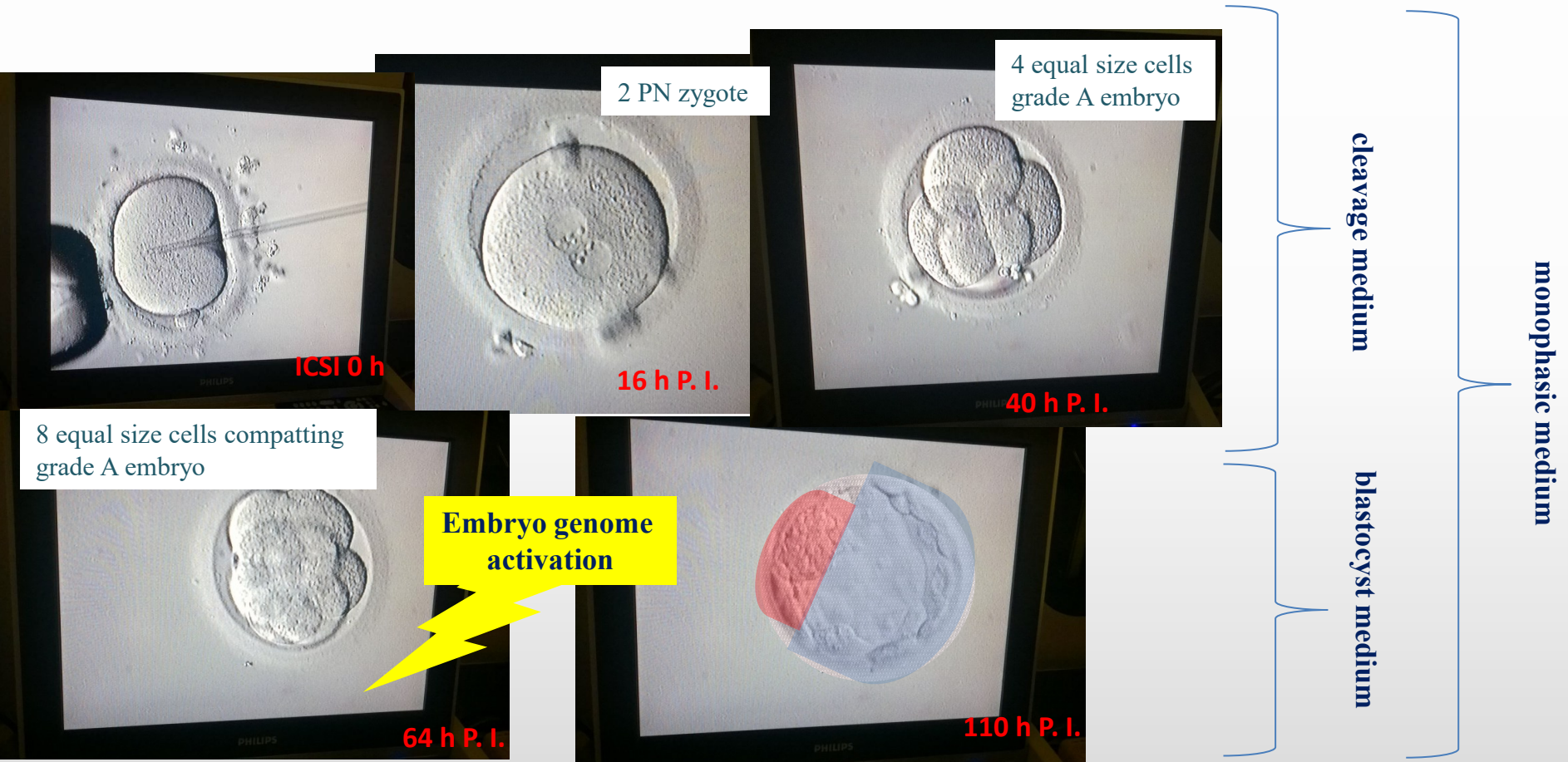
DAY 5

**Embryo genome  
activation**

## Single-Step Culture System: “let the embryo choose”

**Step 1**  
Non essential aminoacids  
Essential aminoacids  
Low glucose  
EDTA  
Pyruvate  
Lactate

# HUMAN EMBRYO CULTURE





# HUMAN EMBRYO CULTURE

Sequential media		Single-step media	
<i>pros</i>	<i>cons</i>	<i>pros</i>	<i>cons</i>
mimic the natural environment	uneconomic culture system	easy to use	EDTA at blast
nutrients replenishment	risk of loosing	economic	ammonium accumulation
oil refreshment	busy management	little burocracy	nutrients depletion
little to no EDTA at blast	culture milieu disruption	autocrine and paracrine factors accumulation	catabolism accumulation
No ammonium accumulation		risk of loosing	oil oxidation
		pH fluctuation	pH fluctuation

# HUMAN EMBRYO CULTURE

## CULTURE STRATEGY

### INDIVIDUAL EMBRYO CULTURE

**In small volume of culture medium** (10-20  $\mu$ l drop)

- *Human is a monovulatory specie*
- *A bad embryo can be detrimental to the others*
- *Real traceability of every single embryo during culture*

### GROUP EMBRYO CULTURE

**In small volume of culture medium** (25-50  $\mu$ l drop)

- *In-vitro conditions are different from the in vivo ones*
- *Positive influence of paracrine factors*
- *Faster management of the entire embryo culture*

**In large volume of culture medium** (500  $\mu$ l well)

- *Human is a monovulatory specie*
- *A bad embryo can be detrimental to the others*
- *With large volume, the osmolarity increase slowly*

**In large volume of culture medium** (500-1000  $\mu$ l well)

- *In-vitro conditions are different from the in vivo ones*
- *Positive influence of paracrine factors*
- *Faster management of the entire embryo culture*
- *No need for medium replenishment*

# HUMAN EMBRYO CULTURE

## CULTURE STRATEGY

Small volume of culture medium



Large volume of culture medium



# OPTIMIZING EMBRYO CULTURE SYSTEM



MEDIA AND  
CONSUMABLES



MEDIA PH AND CO2  
CONCENTRATION



OIL AND  
OSMOLARITY

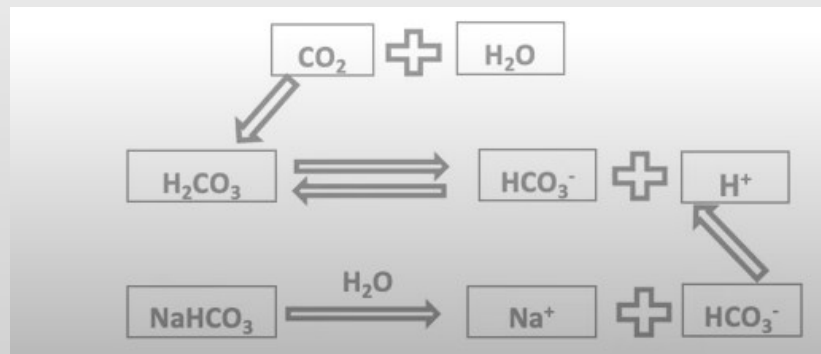
# pH

## pH PROFOUNDLY IMPACTS OOCYTES AND EMBRYOS

- ✓ Oocyte mitochondrial localization and development competence, oocyte metabolism (correlated to maturational and developmental status).  
Denuded oocytes cannot regulate pH.
- ✓ Embryo metabolic activity, intracellular organization, and overall embryo development;
- ✓ Rate of blastomere division;
- ✓ Fragmentation rates;
- ✓ Overall embryo morphology.

# pH SET BY %CO<sub>2</sub> + [HCO<sub>3</sub><sup>-</sup>] BUT TARGET UNKNOWN

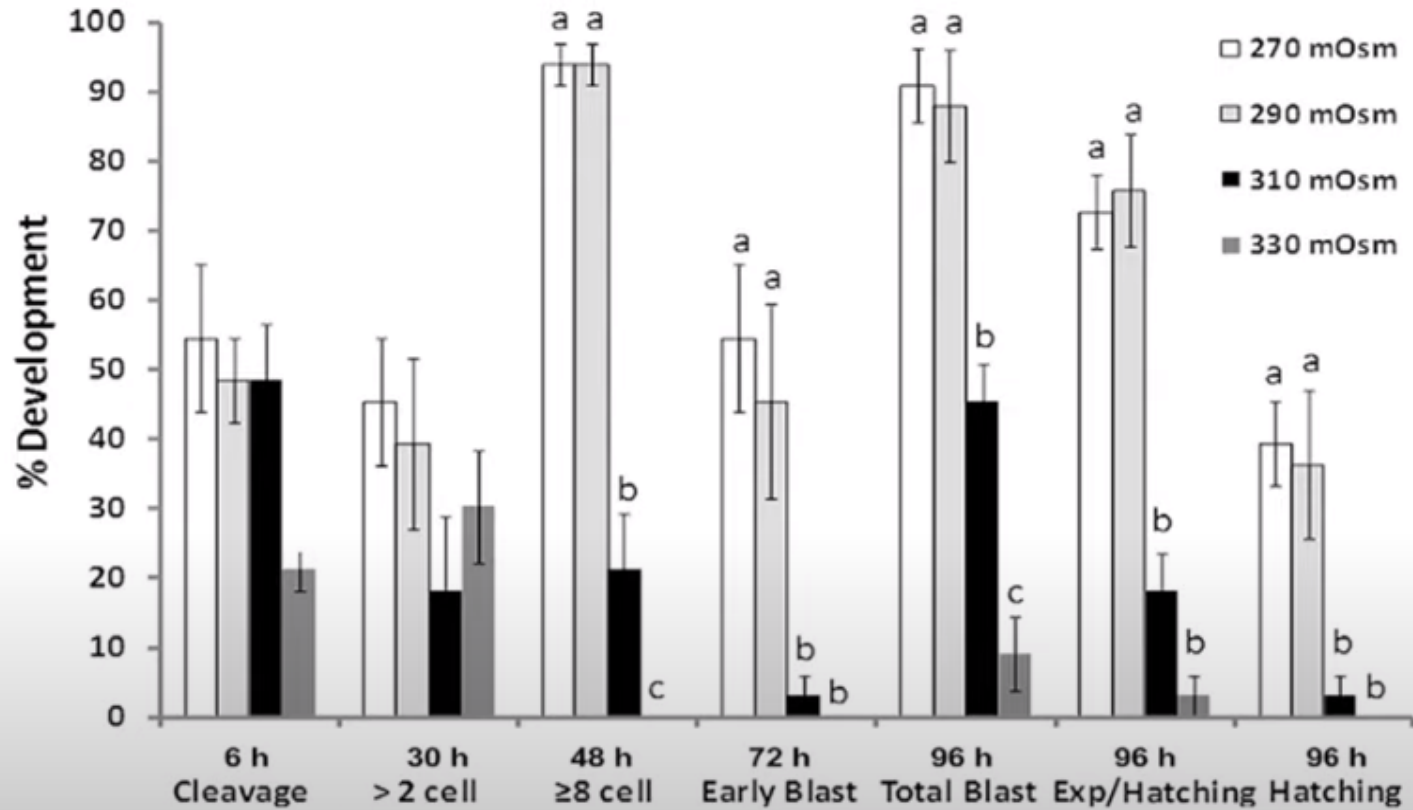
- Optimal pH<sub>0</sub> for human embryos is not yet determined
- pH<sub>i</sub> of human embryos is typically around 7.1
- To avoid acidification, the lower level is therefore usually set at 7.2
- Studies have suggested poorer embryo development at pH<sub>0</sub> of > 7.4
- Most media are used at 7.3 ± 0.1
- pH<sub>0</sub> is a function of HCO<sub>3</sub><sup>-</sup> in media
- HCO<sub>3</sub><sup>-</sup> concentration is set by the manufacturer
- %CO<sub>2</sub> can be manipulated in the lab to control pH<sub>0</sub>



# FACTORS IMPACTING pH LEVELS

- Length of equilibration period
- Frequency of incubator door openings
- Type of CO<sub>2</sub> sensors
- Temperature changes within the incubators
- Bacterial/ fungal contamination
- Altitude
- Humidity

# CONTROL OF OSMOLARITY IS CRITICAL



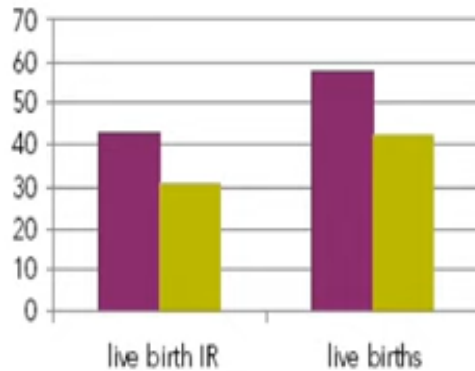
↑ osmolality → ↓ development



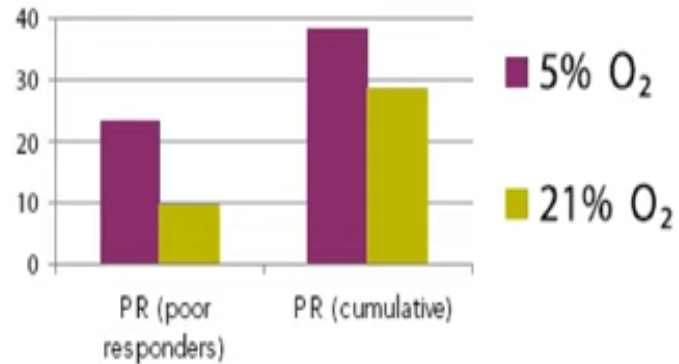
# OPTIMIZING SYSTEMS: OSMOLARITY

- ✓ Optimal volume per embryo is not yet determined;
- ✓ Some labs successfully use 2-5  $\mu\text{l}$  per embryo, more typically 20-50  $\mu\text{l}$ ;
- ✓ Drop size may affect longer term changes in osmolarity;
- ✓ More care needed during dish preparation, example...
  - One dish at a time;
  - Evaluate type of dish;
  - Airflow minimized;
  - Underlay drops;
  - Use maximum oil volume;
  - Evaluate type of oil

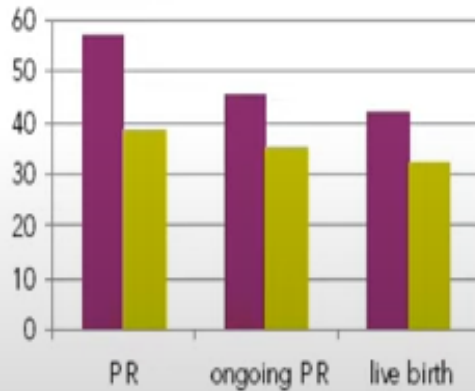
# LOW VS HIGH OXYGEN CONCENTRATIONS FOR EMBRYO CULTURE



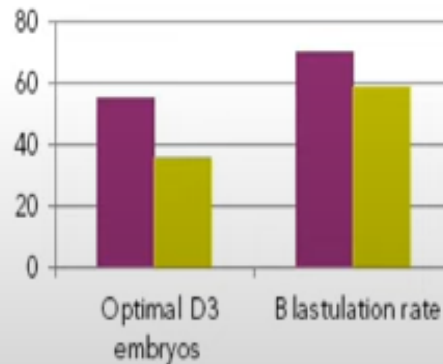
Meintjes et al 2009



Kovacic et al 2009



Waldenström et al 2008

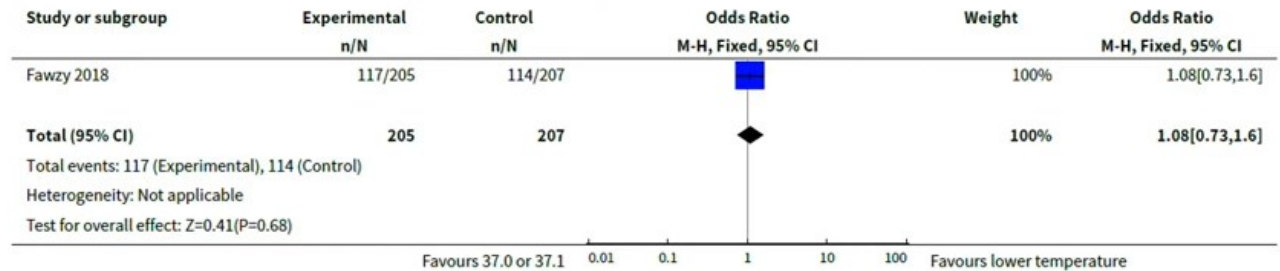


Kovacic & Vlasisvjevic 2008

# OPTIMIZING SYSTEMS: TEMPERATURE

- Suggestion that temperatures below 37°C may give better outcomes
- Temperature above 37°C NOT recommended

**Analysis 1.2. Comparison 1 37°C versus any lower temperature, Outcome 2 Clinical pregnancy.**



**Analysis 1.3. Comparison 1 37°C versus any lower temperature, Outcome 3 Ongoing pregnancy.**



# OPTIMIZING SYSTEMS: ROLES OF OIL

Oil have different roles in optimizing a culture systems:

- Minimizes pH shift;
- Prevents changes in osmolarity;
- Reduces risk of temperature fluctuation;
- Protects samples from contamination, particularly VOCs from gas supply, plastic ware and general lab environment



Use maximum amount of oil in culture dish

# MAIN TOPICS

**-HUMAN EMBRYO CULTURE: TIPS AND TRICKS**

**- QUALITY CONTROL INSIDE A.R.T. LABORATORY**

**THEROICAL LESSON**

**TARGET: DON'T DO THAT**



# TARGET: DO THAT

ABSENCE OF  
QUALITY CONTROL



PRESENCE OF  
QUALITY CONTROL

BIOLOGICAL PROCESS  
**OUT OF CONTROL**

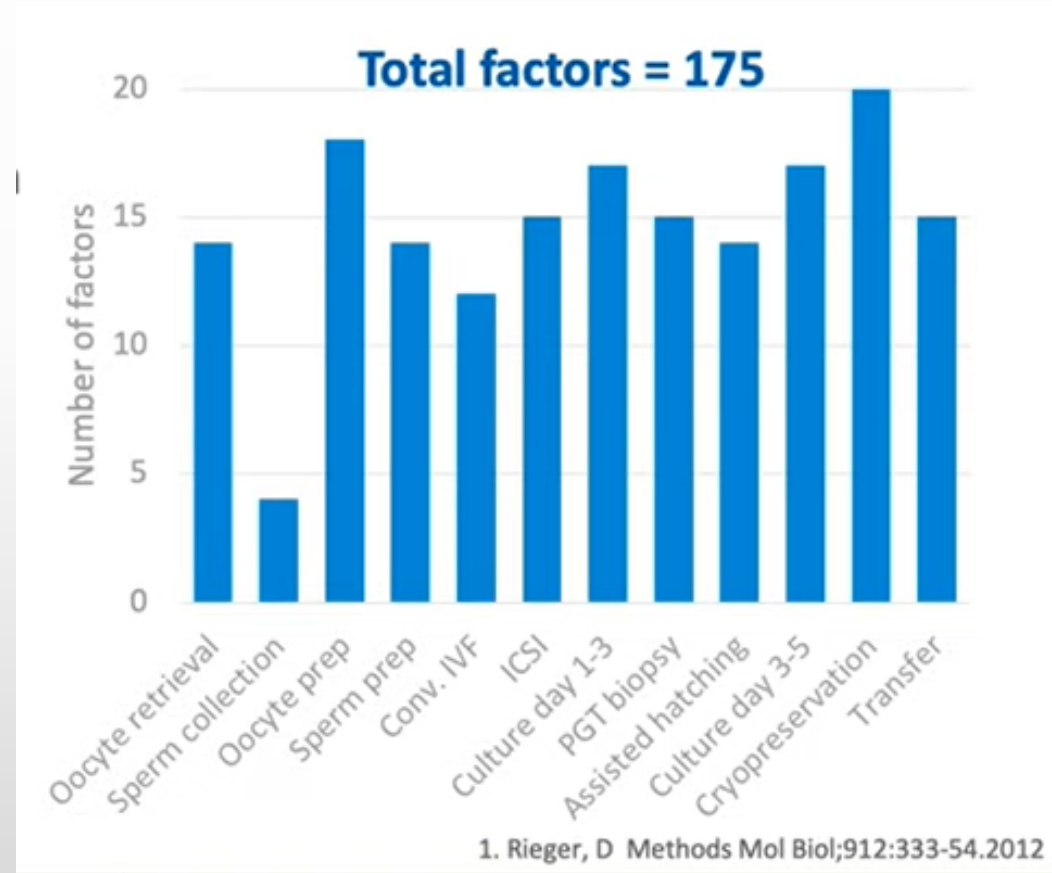
BIOLOGICAL PROCESS  
**UNDER CONTROL**

LOW RESULTS  
FLUCTUATING RESULTS  
NO SECURITY  
ECONOMIC IMBALANCE  
FRUSTRATION

HIGH RESULTS  
REPEATABLE RESULTS  
SECURITY  
SAVE MONEY  
HAPPINESS - SATISFACTION

# LABORATORY FACTORS THAT CAN AFFECT OUTCOME OF IVF

Air quality;  
 Pipettes/hyaluronidase/denudation;  
 Warm surfaces/stages;  
 Microscope light;  
 Culture medium;  
 Culture dishes;  
 Protein supplementation;  
 Culture oil;  
 Incubator gas/temperature;  
 Incubator gas partial pressure.



# QUALITY CONTROL



“sperm survival test”: donor sperm survival after 24-48 h of culture inside icubator

“VOC’s test”: inorganic volatile compounds

“LAL test”: endotoxins presence



### CERTIFICATE OF ANALYSIS

Product: <b>Quinn's Advantage<sup>®</sup> Cleavage Medium</b>		Lot No.:	<b>F264 B</b>
50 mL		Catalog No.:	<b>ART-1026</b>
Storage: <b>2°- 8° C</b>		Exp. Date:	<b>2015 11 30</b>
		Approved:	<i>T. D'Souza 06/03/15</i>
ASSAY		SPECIFICATION	RESULT
Clarity/Color		Clear, Particle-Free, Pink-Rose	Conforms
Sterility <sup>1</sup>		Pass Test	Pass
pH <sup>2</sup>		7.10 – 7.30	7.19
Osmolality <sup>3</sup>		257 – 273 mOsm/Kg	264
Endotoxin <sup>4</sup>		<1.0 EU/mL	< 0.02
Gentamicin Assay <sup>5</sup>		5 – 11 µg/mL	Conforms
Mouse Embryo Test (One Cell) <sup>6</sup>	No. of zygotes cultured		≥ 21
	% expanded to blastocysts	Control	≥ 80%
		This lot	≥ 80%

<sup>1</sup> In accordance with the USP by the membrane filtration method (SAL 10<sup>-3</sup>).

<sup>2</sup> pH @ 37° C under 5% CO<sub>2</sub>/5% O<sub>2</sub>/Balance N<sub>2</sub>.

<sup>3</sup> As measured by freezing point depression.

<sup>4</sup> Utilizes the LAL gel clot assay (sensitivity = 0.06 EU/mL), or the LAL kinetic assay (sensitivity = 0.01 EU/mL).

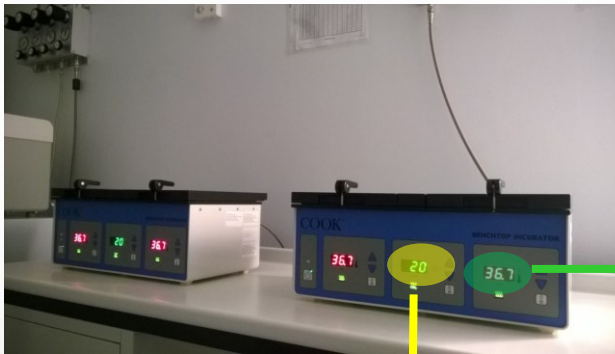
<sup>5</sup> BioLis 24i Chemistry Analyzer.

<sup>6</sup> Each lot of medium is tested for its ability to support the development of one-cell mouse embryos to the expanded blastocyst stage by the method of Quinn.

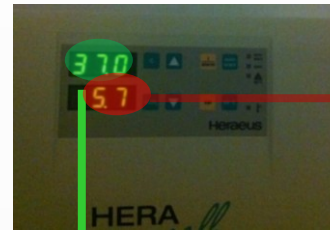
**Caution: Federal Law restricts this device to sale by or on the order of a physician (or properly licensed practitioner).**

# QUALITY CONTROL

## 1. INCUBATORS



Gas flow (CO<sub>2</sub> – O<sub>2</sub> – N<sub>2</sub>):  
15 – 25 kPA/min  
*(check 1 a year)*

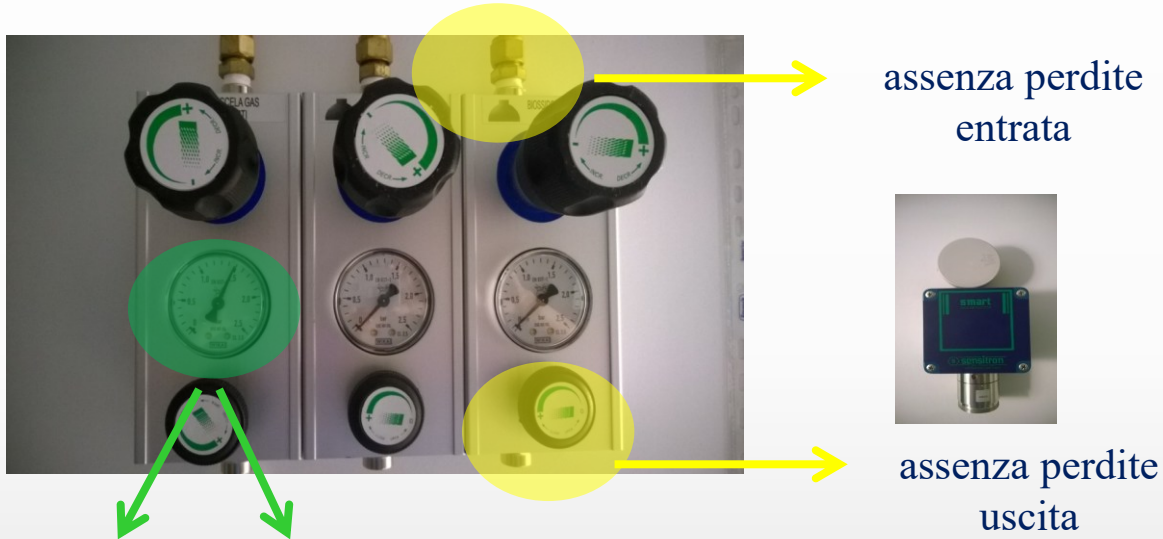


Temperature (it depends on plasticware):  
36.5 – 37.5 °C  
*(check every day)*  
*(calibrate every 2 months)*

CO<sub>2</sub>/O<sub>2</sub> (it depends on culture media)  
5% - 6%  
*(check every day)*  
*(calibrate every 2 months)*

# QUALITY CONTROL

## 2. GAS PLANT

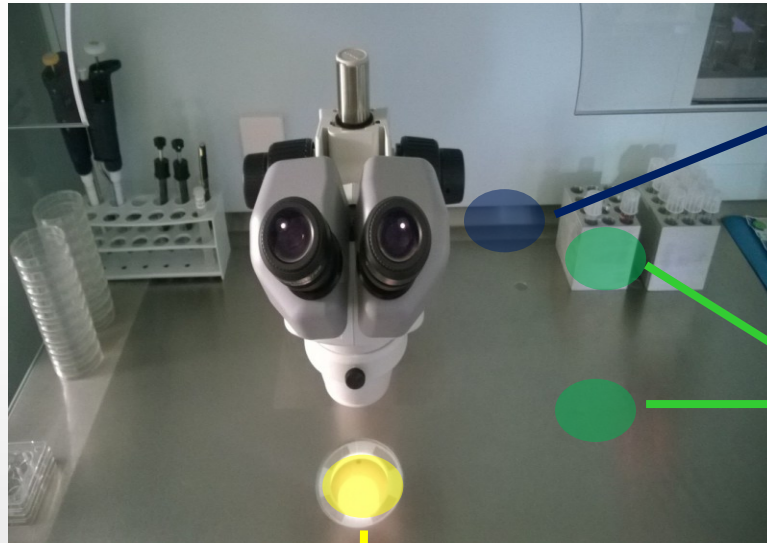


Temperature:  
36.5 – 37.5 °C

Entering pressure:  
max 1.5 bar

# QUALITY CONTROL

## 3. WORKSTATION



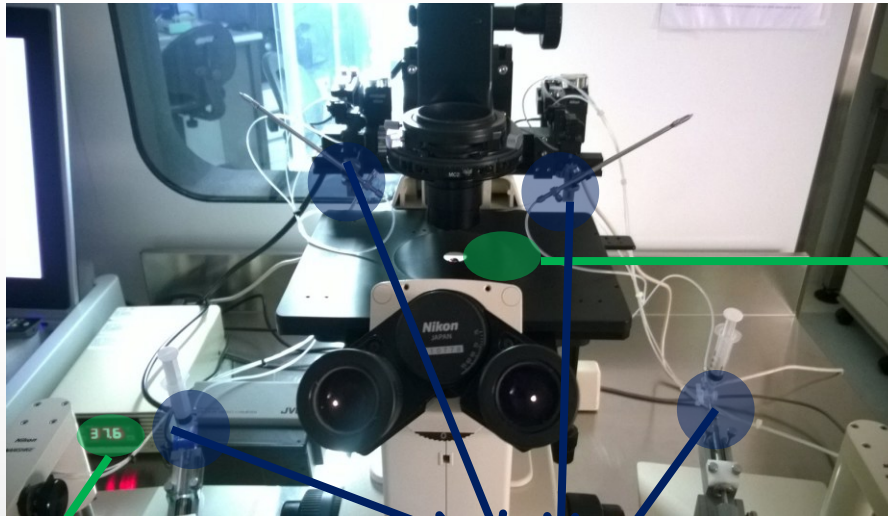
Flow intensity (*2 time a year*)  
Particles count (*2 time a year*)  
Microbic count (*2 time a year*)

Temperature: 36.5 – 37.5 °C  
(*check every day*)  
(*calibrate every 2 months*)

Light intensity  
Light beam condensation  
(*check every day*)

# QUALITY CONTROL

## 4. MICROMANIPULATION STATION

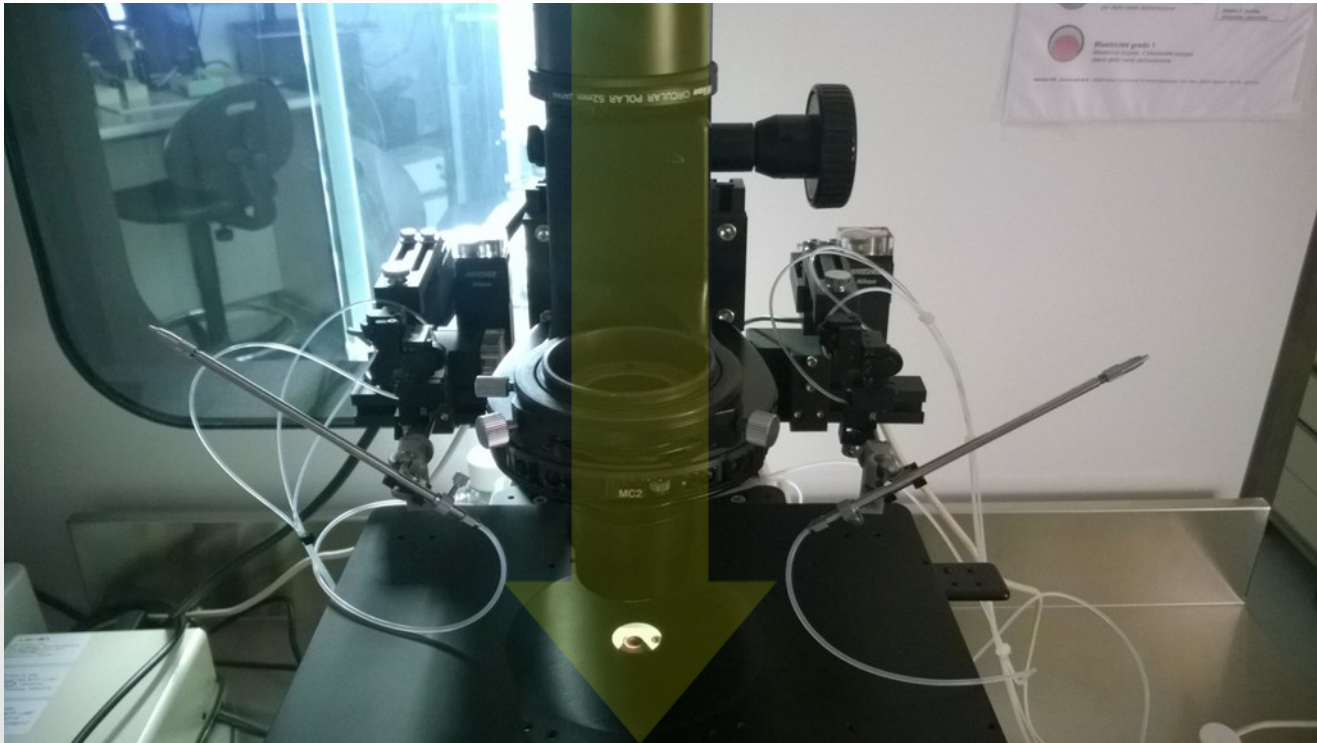


Temperature  
(it depends on plasticware):  
36.5 – 37.3 °C  
(check every day)  
(calibrate every 2 months)

integrity, accuracy,  
precision of mincoinjection system

Temperature  
(it depends on plasticware):  
36.5 – 37.3 °C  
(check every day)  
(calibrate every 2 months)

# QUALITY CONTROL



*integrity, accuracy,  
precision of minicoinjection system*

# QUALITY CONTROL: DEVELOPMENT INDICATORS



# SUMMARY

- ✓ To achieve optimal results in an IVF lab. Attention to detail, adherence to protocols and QC are vital!!!
- ✓ IVF culture media is an integral part of the embryo culture system. Its use must be optimized in every laboratory;
- ✓ pH, temperature and osmolarity must be carefully monitored and maintained;
- ✓ Choice and use of oil is critical;
- ✓ Consumables and equipment should be of the highest quality, tested regularly and maintained, where appropriate



# Meet the Expert

- ❑ All about Intra Cytoplasmic Sperm Injection (ICSI)

<https://www.youtube.com/watch?v=i4phDnDuSdY>

- ❑ Trophectoderm biopsy and tubing of cells for preimplantation genetic testing  
– part 1

[https://www.youtube.com/watch?v=FyBh\\_814jtI#action=share](https://www.youtube.com/watch?v=FyBh_814jtI#action=share)

– part 2

<https://youtu.be/19JUcdISuAM>