UNIVERSITÀ DEGLI STUDI DI TERAMO

Second-Cycle Degree Course in "REPRODUCTIVE BIOTECHNOLOGIES"

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

Teramo, 28-30 Marzo 2022

A.Y. 2021 - 2022

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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

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WHAT IS EMBRYO CULTURE MEDIA?

- Embryo culture is a component of *in vitro fertilisation*
- 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

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Culture media is artificial but supports key functions

- Artificial environment for the embryo
- cf the dynamic and complex milieu of the *in vivo* situation
- But, culture media need to support:
  - Sperm function (motility, capacitation, AR)
  - Oocyte-mediated cleavage stages
  - Genomic activation
  - Early differentiation of cell types
- Provides minimum requirements for competency

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

2004: "single-step" 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

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

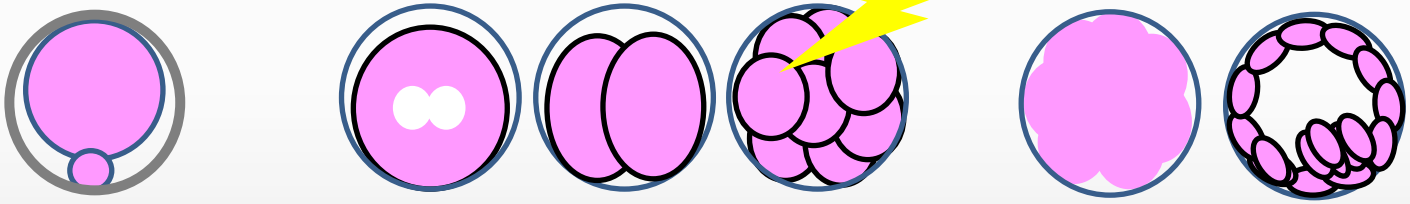
Sequential Culture System: “back to nature”

Step 1: HTF
Non essential aminoacids
glucose
EDTA
Pyruvate
Lactate

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

Embryo genome activation

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

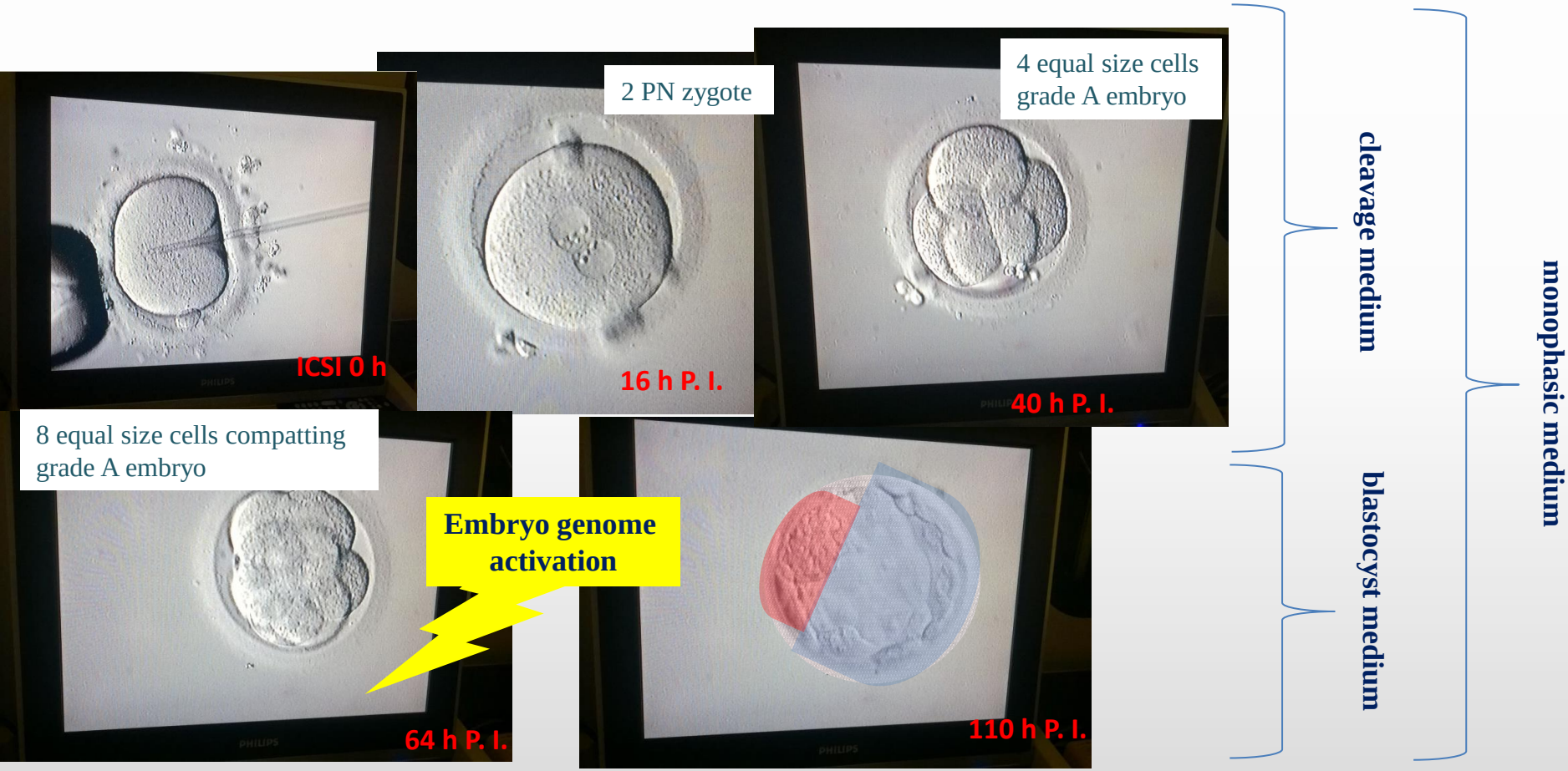


DAY 0 DAY 1 DAY 2 DAY 3 DAY 4 DAY 5

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

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

HUMAN EMBRYO CULTURE



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HUMAN EMBRYO CULTURE

Sequential media		**Single-step media**	
pros	***cons***	***pros***	***cons***
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 µl 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 µl 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 µl well)

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

In large volume of culture medium (500-1000 µl 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

SAGE

SAGE In Vitro Fertilization
A CooperSurgical Company
95 Corporate Drive
Trumbull, CT 06611
(203) 601-5200
FAX (203) 601-4737

CERTIFICATE OF ANALYSIS

Product: **Quinn's Advantage® Cleavage Medium**	Lot No.:	**F264 B**
50 mL	Catalog No.:	**ART-1026**
	Exp. Date:	**2015 11 30**
Storage: **2°- 8° C**	Approved:	T. D'Souza 06 OCT 15

ASSAY			SPECIFICATION	RESULT
Clarity/Color			Clear, Particle-Free, Pink-Rose	Conforms
Sterility[1]			Pass Test	Pass
pH[2]			7.10 – 7.30	7.19
Osmolality[3]			257 – 273 mOsm/Kg	264
Endotoxin[4]			<1.0 EU/mL	< 0.02
Gentamicin Assay[5]			5 – 11 µg/mL	Conforms
Mouse Embryo Test (One Cell)[6]	No. of zygotes cultured		≥ 21	30
	% expanded to blastocysts	Control	≥ 80%	Conforms
		This lot	≥ 80%	Conforms

[1] In accordance with the USP by the membrane filtration method (SAL 10^{-3}).
[2] pH @ 37° C under 5% CO_2/5% O_2/Balance N_2.
[3] As measured by freezing point depression.
[4] Utilizes the LAL gel clot assay (sensitivity = 0.06 EU/mL), or the LAL kinetic assay (sensitivity = 0.01 EU/mL).
[5] BioLis 24i Chemistry Analyzer.
[6] 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

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

"VOC's test": inorganic volatile compounds

"LAL test": endotoxins presence

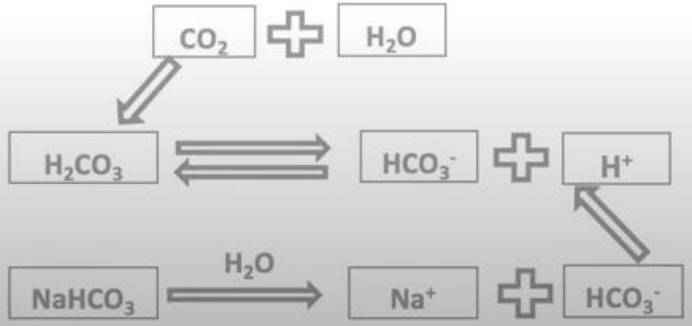
pH profoundly impacts oocytes and embryos[1-3]

- Denuded oocytes cannot regulate pH_i
- pH affects:
  - Oocyte mitochondrial localization and developmental competence, oocyte metabolism (correlated to maturational and developmental status), embryo metabolic activity, intracellular organization, and overall embryo development
  - Rate of blastomere division
  - Fragmentation rates
  - Overall embryo morphology
- Cryopreserved/thawed embryos have reduced ability to regulate pHi during a ~3-4h recovery

1. FitzHarris *et al*, Development 134: 4283-95; 2007

pH set by %CO_2+[HCO_3^-] but target unknown

- Optimal pH_o for human embryos is not yet determined
- pH_i 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_o of >7.4
- Hence most media are used at 7.3 ± 0.1 (or stipulate smaller windows in this range)



- pH_o is a function of HCO_3^- in media
- HCO_3^- concentration is set by the manufacturer
- % CO_2 can be manipulated in the lab to control pH_o

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Factors impacting pH levels

- Length of equilibration period
- Frequency of incubator door openings
- Type of CO_2 sensors
- Location of HVAC outlets
- Temperature changes within the incubators
- Bacterial/fungal contamination
- Altitude
- Humidity

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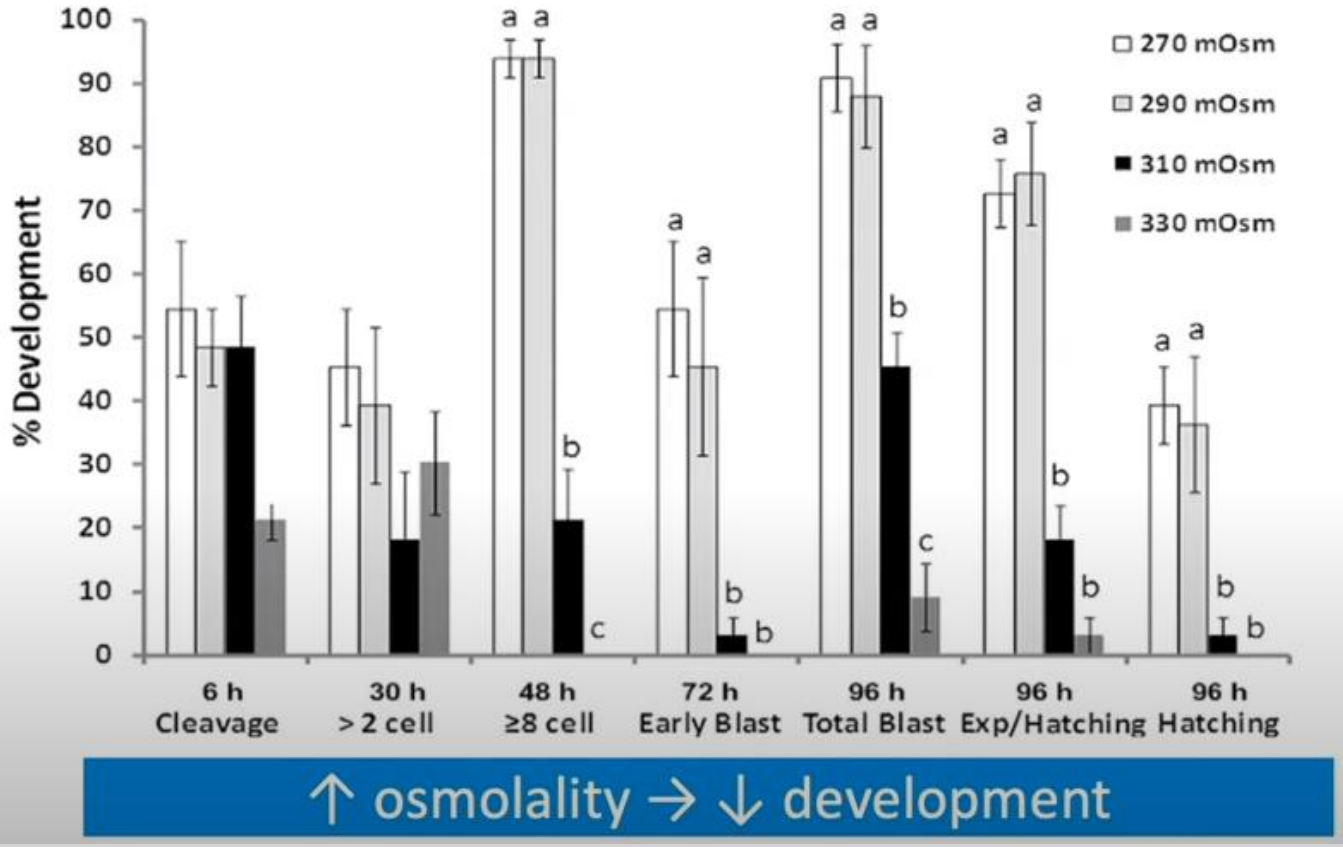
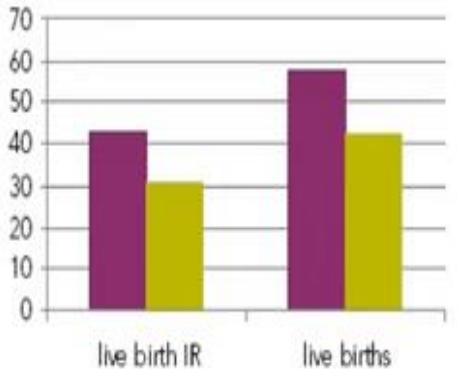
Control of osmolality is critical[1]



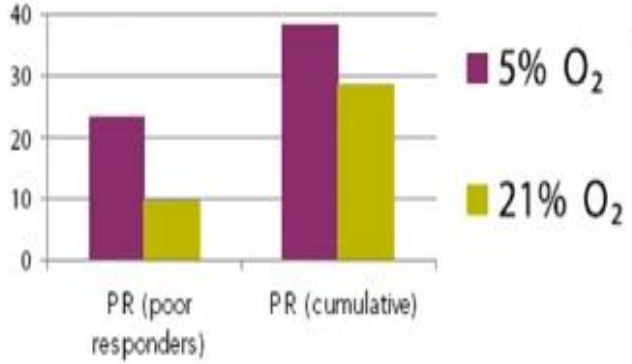
OPTIMIZING SYSTEMS: OSMOLARITY

- Optimal volume per embryo is not yet determined
- Some labs successfully use 2-5µl per embryo but may not work in all systems
- 20-50µl more typical
- Drop size may affect longer term changes in **osmolality**
- More care needed during dish preparation…
  - One dish at a time
  - Type of dish?
  - Airflow minimized
  - Unheated stage
  - Underlay drops
  - Use maximum oil volume
  - Type of oil?

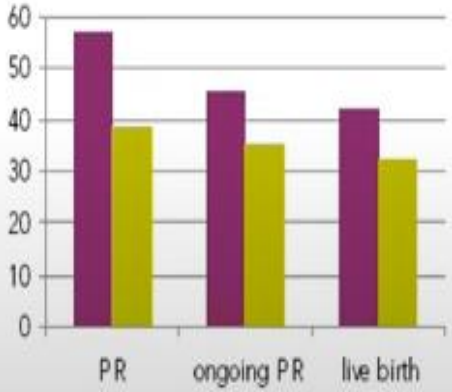
LOW VS HIGH OXYGEN CONCENTRATIONS FOR EMBRYO CULTURE



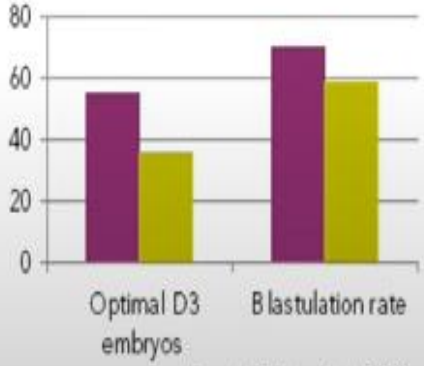
Meintjes et al 2009



Kovacic et al 2009



Waldenström et al 2008



Kovacic & Vlaisavljevic 2008

OPTIMIZING SYSTEMS: TEMPERATURE

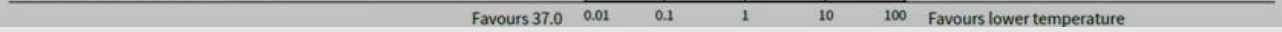
- Suggestion that temperatures below 37°C may give better outcomes
- Not supported by recent Cochrane Review[1]
- Temperatures above 37°C NOT recommended

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

Study or subgroup	Experimental n/N	Control n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
Fawzy 2018	117/205	114/207		100%	1.08[0.73,1.6]
Total (95% CI)	**205**	**207**		**100%**	**1.08[0.73,1.6]**
Total events: 117 (Experimental), 114 (Control)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.41(P=0.68)					

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

Study or subgroup	Experimental n/N	Control n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
Fawzy 2018	101/205	97/207		100%	1.1[0.75,1.62]
Total (95% CI)	**205**	**207**		**100%**	**1.1[0.75,1.62]**
Total events: 101 (Experimental), 97 (Control)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.49(P=0.62)					



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OPTIMIZING SYSTEMS: ROLES OF OIL

- Important but often being ignored
- Various roles:
  - Minimizes pH shift
  - Prevents changes in osmolality
  - 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

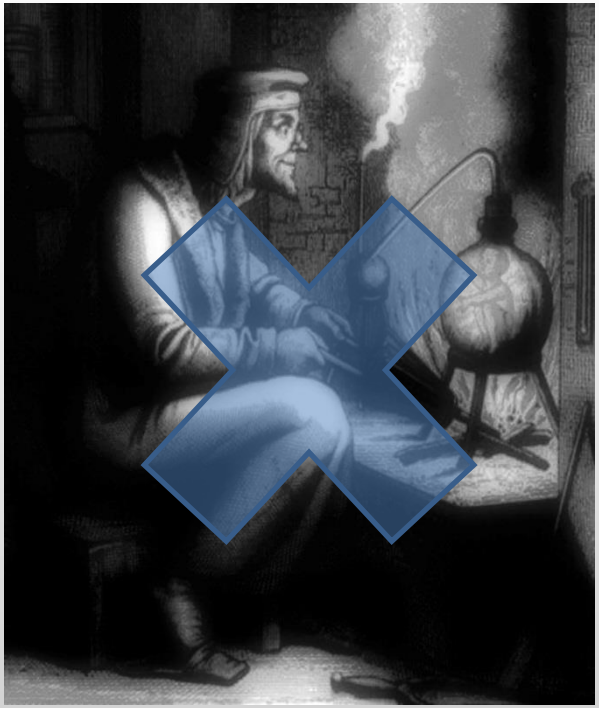
MAIN TOPICS

-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

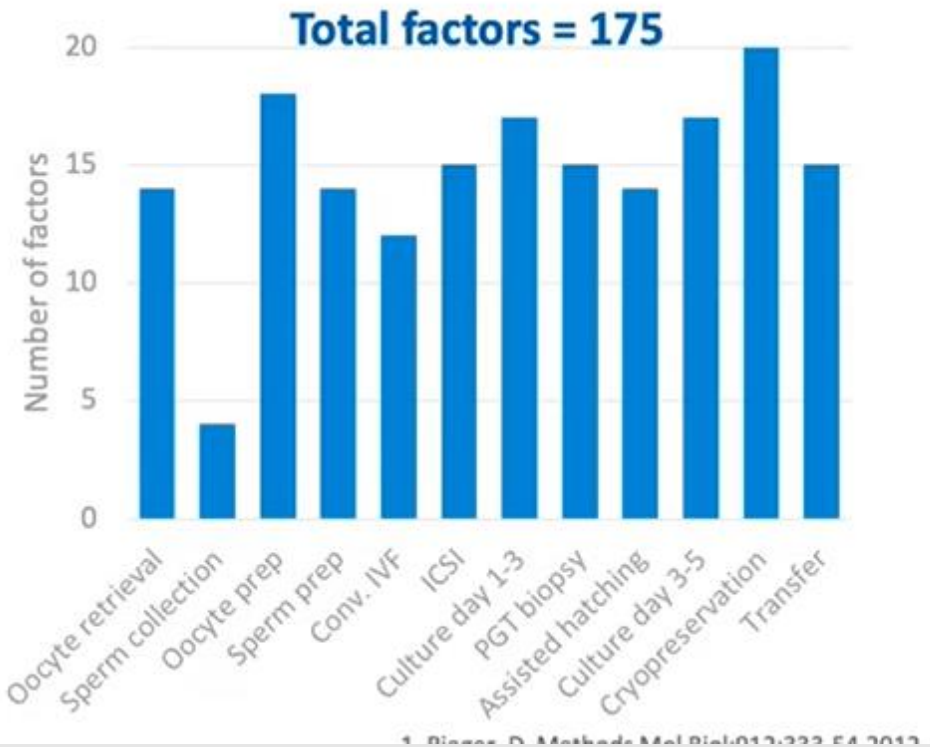
LOW RESULTS
FLUCTUATING RESULTS
NO SECURITY
ECONOMIC IMBALANCE
FRUSTRATION

BIOLOGICAL PROCESS
UNDER CONTROL

HIGH RESULTS
REPEATABLE RESULTS
SECURITY
SAVE MONEY
HAPPINESS - SATISFACTION

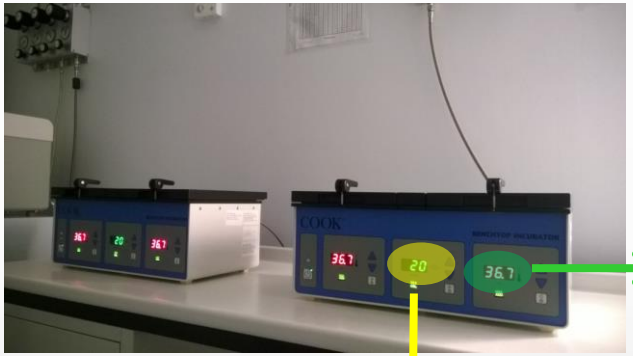
LABORATORY FACTORS THAT CAN AFFECT OUTCOME OF IVF

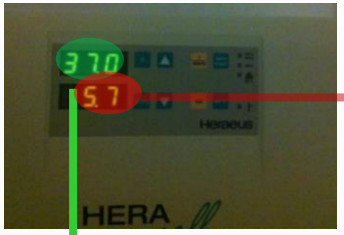
- Air quality
- Pipettes/hyaluronidase/denudation
- Warm surfaces/stages
- Microscope light
- Culture medium
- Culture dishes
- Protein supplement
- Culture oil
- Incubator gas/temperature
- Incubator CO_2 partial pressure



QUALITY CONTROL

1. INCUBATORS





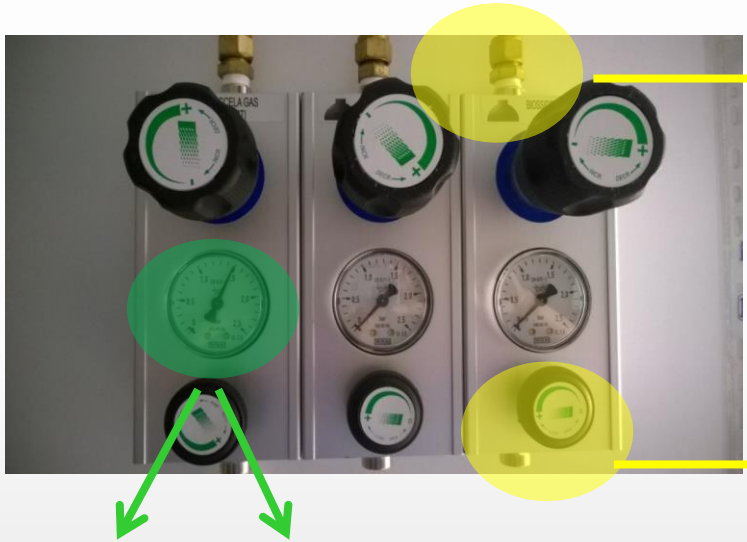
Temperature (it depends on plasticware):
36.5 – 37.5 °C
(check every day)
(calibrate every 2 months)

Gas flow (CO_2 – O_2 – N_2):
15 – 25 kPA/min
(check 1 a year)

CO_2/O_2 (it depends on culture media)
5% - 6%
(check every day)
(calibrate every 2 months)

QUALITY CONTROL

2. GAS PLANT



assenza perdite
entrata

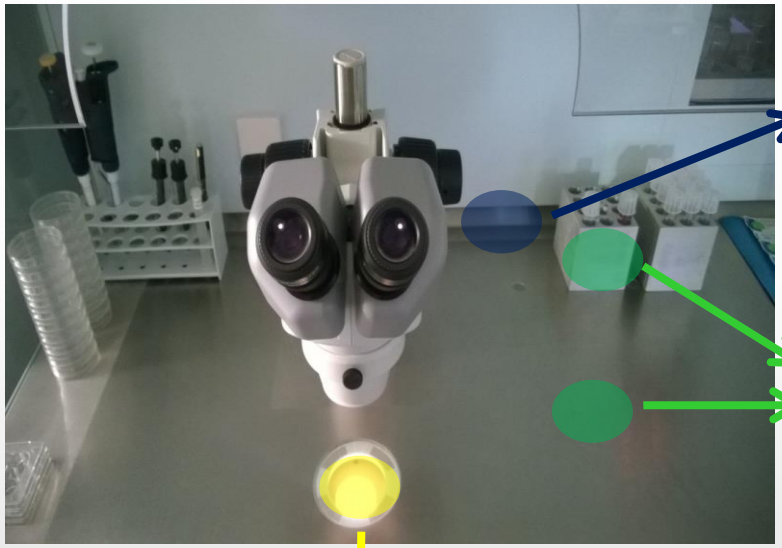
assenza perdite
uscita

Temperature:
36.5 – 37.5 °C

Entering pressure:
max 1.5 bar

QUALITY CONTROL

3. WORKSTATION



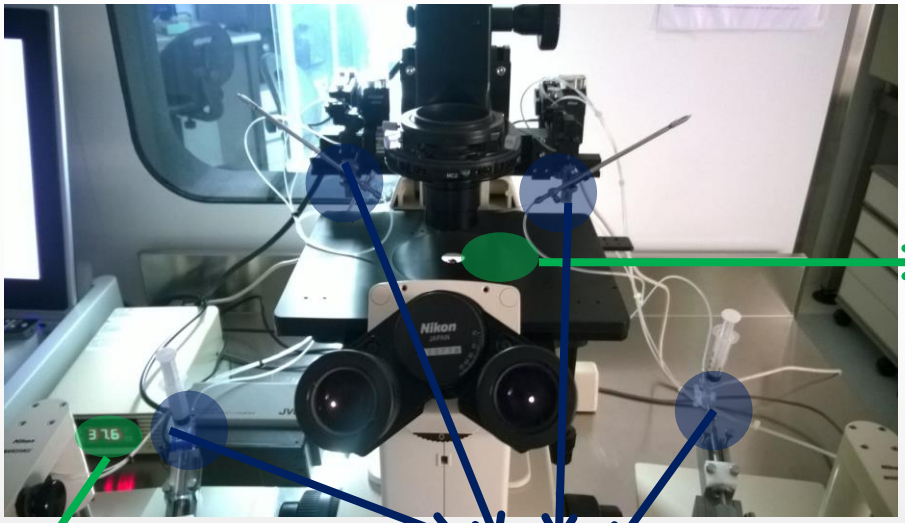
Flow intensity *(2 time a year)*
Particles count *(2 time a year)*
Microbic count *(2 time a year)*

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

Light intensity
Light beam condensation
(check every day)

QUALITY CONTROL

4. **MICROMANIPOLATION STATION**

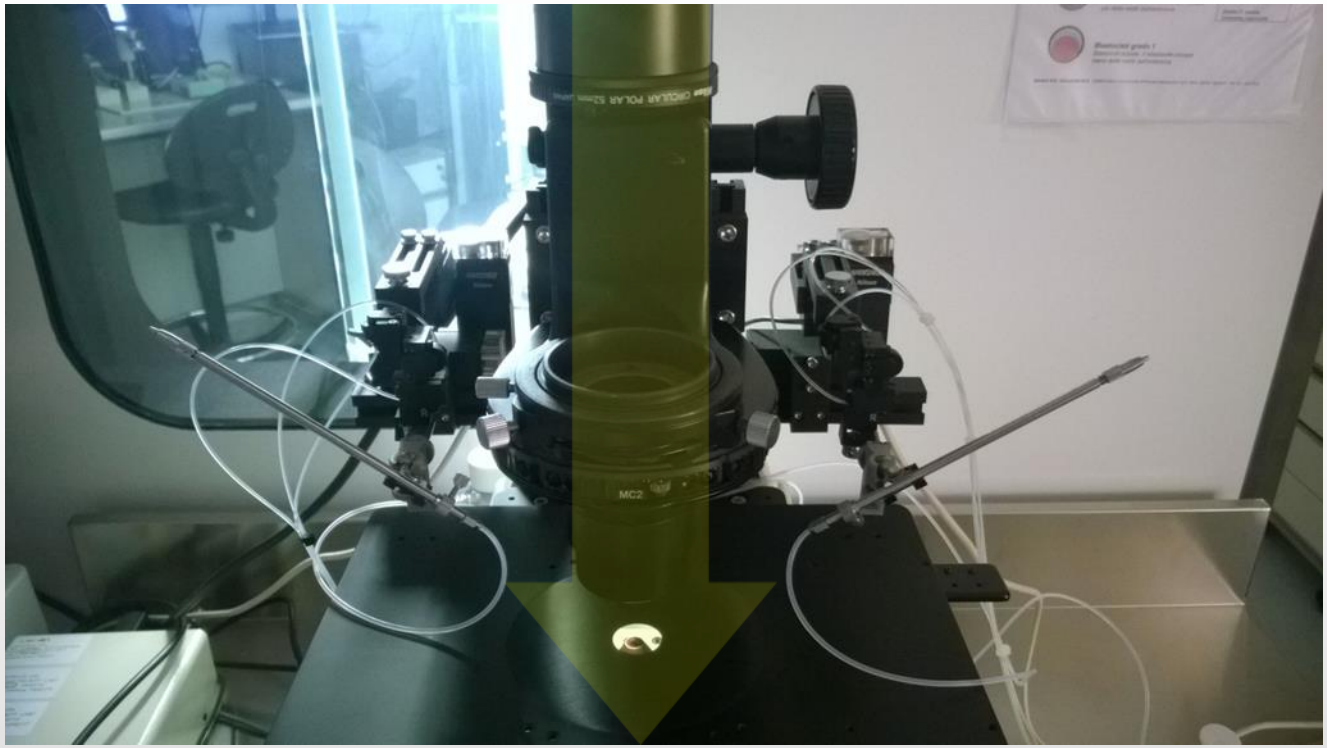


Temperature
(it depends on plasticware):
36.5 – 37.3 °C
(check every day)
(calibrate every 2 months)

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 mincoinjection system

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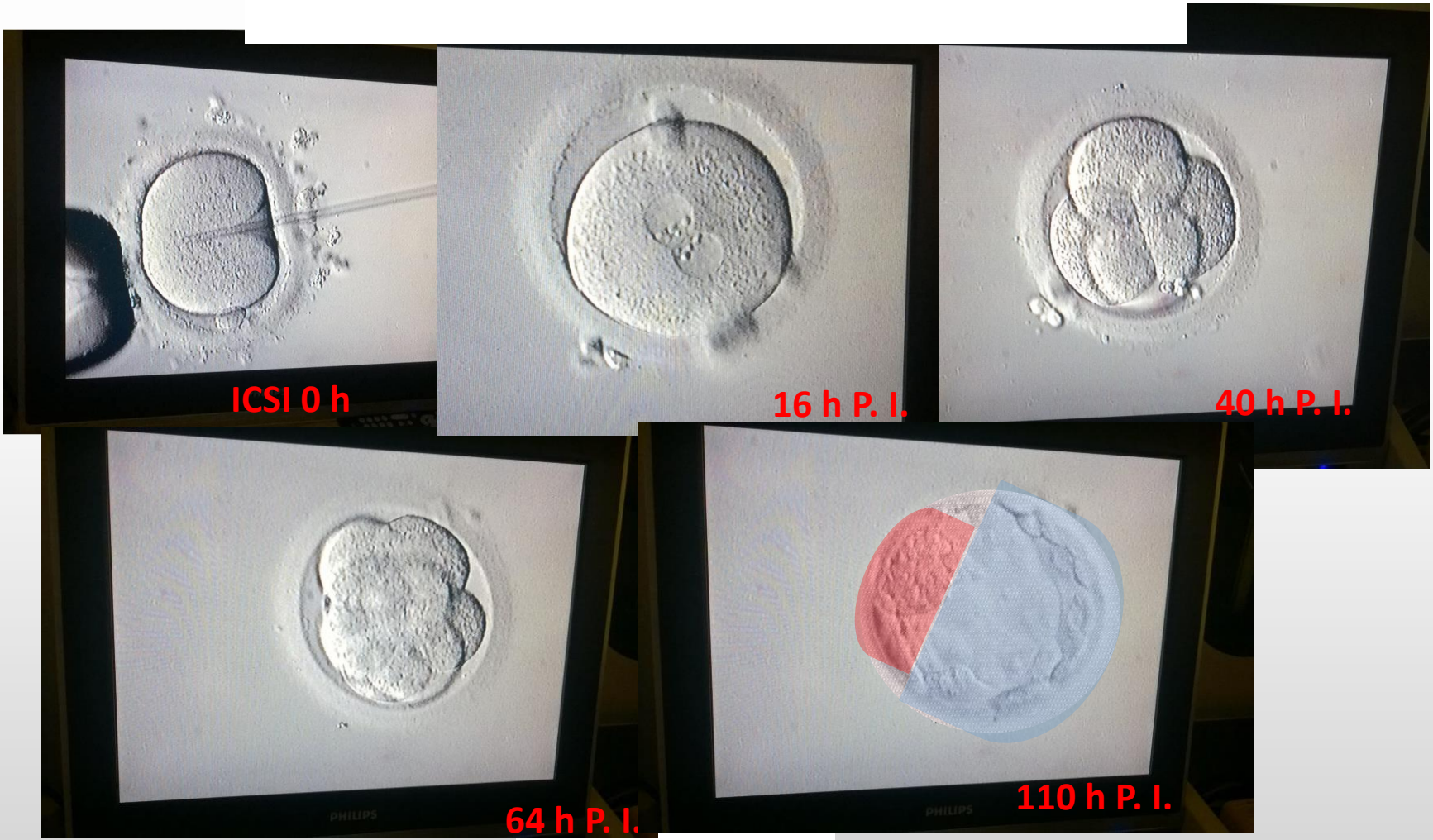
QUALITY CONTROL: DEVELOPMENT INDICATORS



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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, however, be optimized in every laboratory
- pH, temperature and osmolality 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

– part 2

https://youtu.be/19JUcdISuAM