



# SIERR update

In collaboration with:



Π.Ε.Κ.Ε.  
Πανελλήνια Ένωση  
Κλινικών Εμβρυολόγων



GR.A.C.E  
Greek Association  
of Clinical Embryologists



## Insemination and embryo culture

# Chemical and physical parameters affecting embryo developmental competence

Albert Martínez-Casado  
Co-head of QC Center, Embryotools S.L.  
[albert.martinez@embryotools.com](mailto:albert.martinez@embryotools.com)



# Successful IVF lab

- The primary aim of any IVF program is to attain the highest possible implantation and live birth rates.
- IVF success is dependent, in part, on the culture system, which is shaped by the chemical and physical parameters that influence embryo developmental competence.
- Improper culture conditions may lead to embryo development arrest.
- Careful selection and screening of each component of the culture system is essential for optimal results and consistency. This is possible if we have a good QC and QA program, which should guarantee a stable culture environment and quick identification of sub-optimal conditions.

# Culture system

## Culture medium:

- Sequential or single step medium
- HSA source | Purification | components
- Shipment | Storage | Aliquots
- Osmolality
- Dishes preparation (underlay or overlay method)
- pH (equilibration time | pCO<sub>2</sub> | altitude)
- Temperature
- Size | position of droplets

## Human resources:

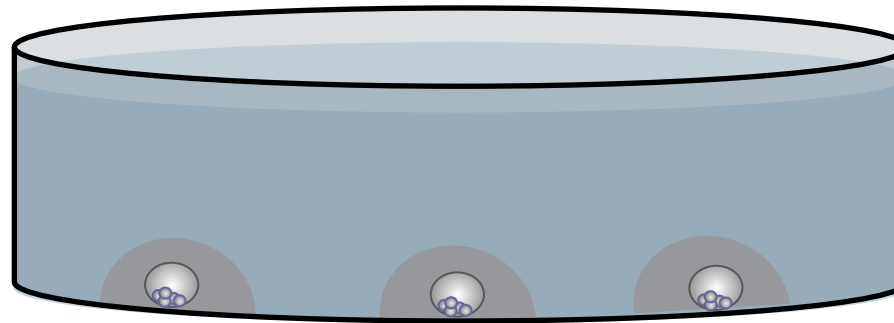
- Number of personnel
- Experience
- Shifts
- Team work

## Air quality in the Lab:

- AHU (Air handling unit)
- Filters (Hepa; chemical air filtration)
- Particles
- VOC's
- Room temperature
- Humidity
- Soaps | disinfectants

## Heated stages:

- Temperature
  - Heating stages microscopes
  - Heating stages flow hoods
  - Heating blocks
- Laminar flow



## Incubator environment:

- Type (big box, benchtop)
- pH
- Temperature
- VOC's
- Gas supplies (CO<sub>2</sub> | low O<sub>2</sub> tension)
- % and pCO<sub>2</sub> | % and pO<sub>2</sub> tension)
- Humidity

## Plastic ware:

- Design
- Medical grade plastic
- MEA tested
- Production
- Sterilization process
- Packaging
- Outgassed or not

## Oil:

- Type
- Handling
- Storage
- Volume
- Equilibration time

## Control of lots and QC tasks recording:

- Register all daily QC measurements in the IVF lab
- Culture medium, manipulation medium, PVP, Hyaluronidase, flushing, vitrification and thawing media; oils, plasticwares


# Temperature

- Critical parameter for embryo developmental competence.


- Ideal temperature  **37°C.**

- Oocytes, followed by cleavage-stage embryos, reportedly displaying greater signs of temperature-sensitivity, with thermotolerance increasing after compaction<sup>1</sup>.

## ❖ Temperature increase ( $\approx 2^{\circ}\text{C}$ ):

- Metaphase II oocytes' meiotic spindle disassembles and may not fully reassemble even returning to  $37^{\circ}\text{C}$ <sup>2</sup>.
- Stress response genes expression  loss of developmental competence<sup>3</sup>.

## ❖ Temperature decrease:

- Mild and drastic temperature drops linked to meiotic spindle instability<sup>4,5</sup>.
- 
- Reduced fertilization rates, delayed embryo development, and decreased clinical pregnancy rates<sup>5</sup>.

<sup>1</sup>Wale and Gardner, 2016

<sup>2</sup>Sun et al., 2004

<sup>3</sup>Hansen, 2007

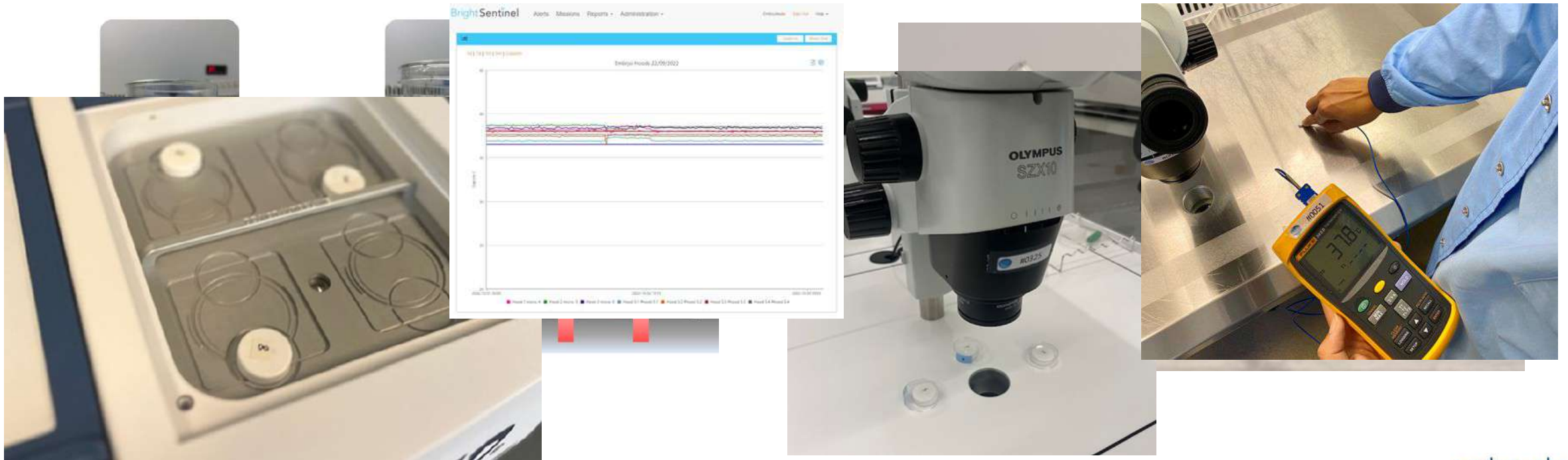
<sup>4</sup>Zenzes et al.,  
2001

<sup>5</sup>Wang et al., 2002

# Temperature

Maintaining a constant 37°C temperature in culture medium, along with continuous **monitoring**, is critical to ensuring an optimal embryonic development.

- Incubators, cabinets and microscope stages should be kept at 37°C.
- Awareness of potential temperature fluctuations in incubators and heated stages is mandatory.



# pH

- Well-established influence on embryonic developmental competence.
- Optimal pH range for embryo culture → **7.2 – 7.4**.
- Enzymatic activity, cell division, differentiation, membrane transport, protein synthesis, cell communication, mitochondrial and actin cytoskeletal elements' location, chromosomal organization<sup>1</sup>.



pH-conditioned

- Extracellular pH alterations imply an increased cellular resource expenditure that may negatively impact embryonic development<sup>1</sup>.

pH regulation commonly based on a **bicarbonate/CO<sub>2</sub> buffering system**.

→ **5% – 9% CO<sub>2</sub>**



# pH

Control of the culture medium pH can be achieved by **monitoring and measuring the CO<sub>2</sub>** levels inside incubators or through direct pH measurements.



*Infra-red gas analyzers*



*External CO<sub>2</sub> Analyzers/Monitors*



*Built-in CO<sub>2</sub> Sensors*

# pH

Control of the culture medium pH can be achieved by monitoring and measuring the CO<sub>2</sub> levels inside incubators or through **direct pH measurements**.

No exact and uniform CO<sub>2</sub>-pH correlation exists:

- Different altitude-driven effective CO<sub>2</sub> partial result in pH variability at equivalent incubator CO<sub>2</sub> settings.
- Amino acids, proteins and monocarboxylic acids influence culture medium pH<sup>1,2</sup>.



**Direct pH measurements**



**Blood-gas analyzers**



**pH-meters**

# Mineral oil overlay: overview

- Petroleum-derived component widely used in IVF<sup>1</sup> → air – culture medium **barrier**.
- Different kinds of mineral oils can be currently found in the market, differing in:
  - ❖ Viscosity and density (g/ml): **light** vs **heavy** oil.
- Potential source of embryo toxicity:
  - ❖ Heterogeneous.
  - ❖ Different within-lot toxicity levels.
  - ❖ Peroxides → most common source of toxicity → peroxidation reduced through **avoiding high-temperature and UV light oil-exposures**<sup>2</sup>.



<sup>1</sup>Brinster, 1963

<sup>2</sup>Otsuki et al., 2007, 2009

# Mineral oil overlay: benefits

- Potential mineral oil toxicity oils can be addressed through the implementation of **appropriate QC testing**.



## Mouse Embryo Assay (MEA)

- Mineral oil **washing procedures** have been shown as detoxifying agents and to reduce toxic contamination<sup>1</sup>.
- Moreover, oil provides a wide range of benefits for embryo developmental competence:
  - ❖ **Protection** from contamination<sup>2</sup>.
  - ❖ Removes accumulated lipophilic toxic substances from the medium<sup>3</sup>.
  - ❖ Acts as a stabilizer.

<sup>1</sup>Morbeck et al., 2010

<sup>2</sup>Ainsworth et al., 2017

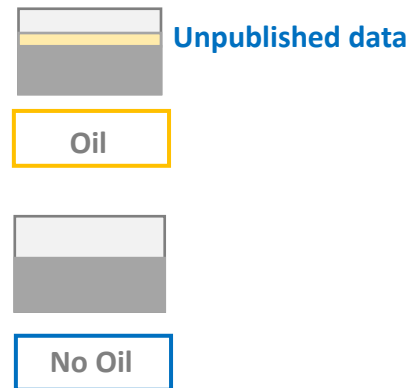
<sup>3</sup>Van Soom et al., 2018

# Mineral oil overlay: stabilizer agent

## Osmolality

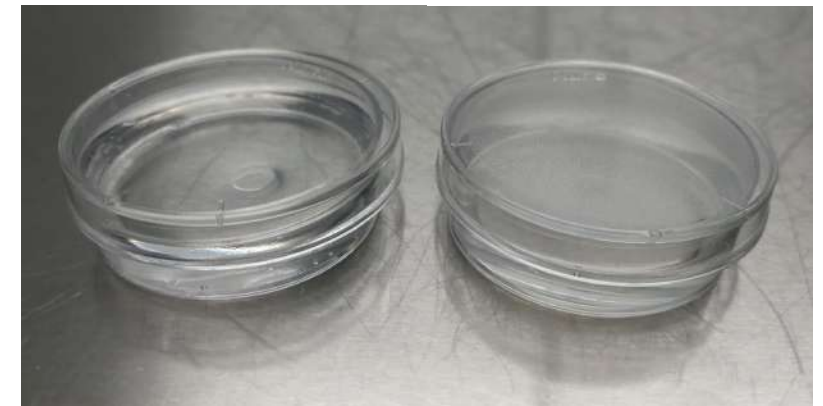
- Concentration of all solutes in a given weight of solvent (mOsm/kg).
- Optimal osmolality for human embryo culture still unknown, but media in the **255 – 265 mOsm/kg starting range** are advised<sup>1</sup>.
- High osmolality linked to developmental arrest at 2-cell stage, altered gene expression and epigenetic modifications<sup>2</sup>.
- Mouse embryo development inhibited above 310 mOsm/kg<sup>3</sup>.

	pH		Osmolality	
	t 0 min	t 10 min	t 0 min	t 10 min
OIL	7,292	7,323	286	289
NO OIL	7,371	7,759	356	368



Media covered with oil

Media without oil



Mineral oil overlays **limit evaporation** and **stabilize osmolality**, reducing osmotic stress-induced adverse metabolic effects.

<sup>1</sup>Swain, 2019

<sup>2</sup>Wang et al., 2011

<sup>3</sup>Swain, 2012

# Mineral oil overlay: stabilizer agent

## Osmolality

Yumoto et al., 2019

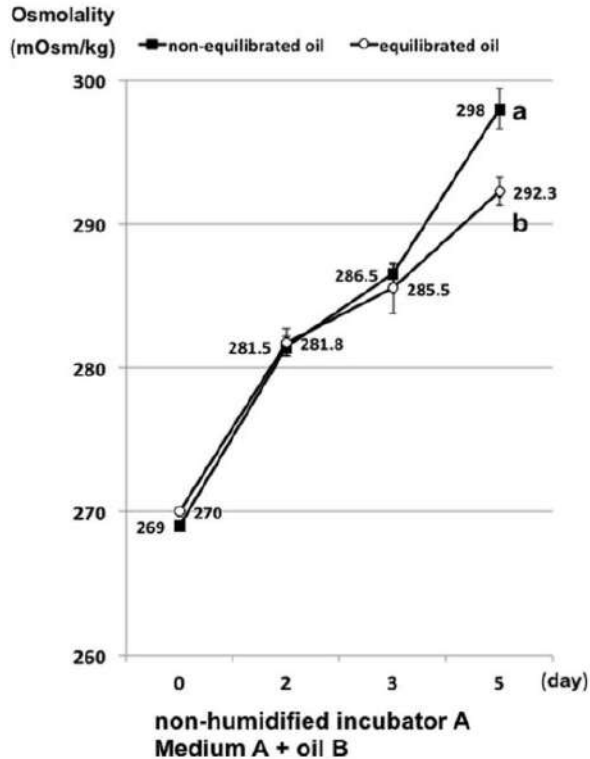
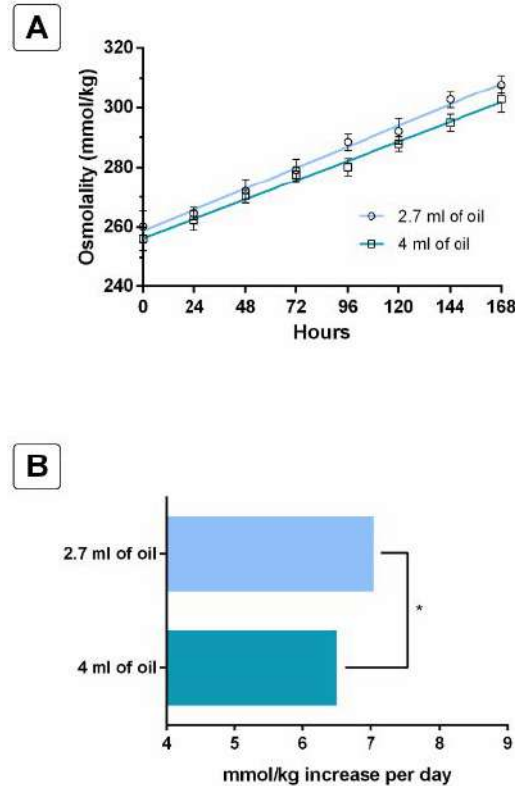


Fig. 4 Osmolality of microdrops covered with non-equilibrated or equilibrated (humidified) oil and then incubated in non-humidified incubator A. a vs. b  $P < 0.05$  according to Student's  $t$  test

Mestres et al., 2021



Swain, 2019

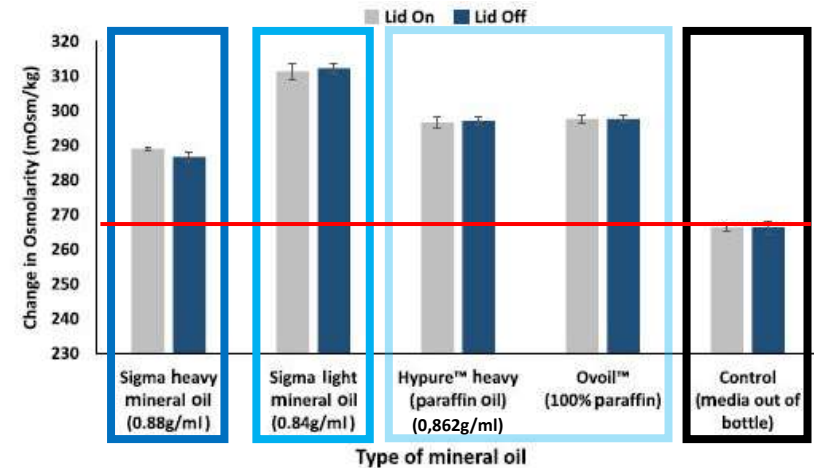


FIGURE 3 Type of mineral oil can affect rate of media evaporation. Data show media osmolality after incubation over time, presented as the mean  $\pm$  SEM. Oils with higher density result in lower rates of media evaporation compared with lighter oils (data from Swain, 2018) (conditions presented were 25  $\mu$ l drops under 3.5 ml of paraffin mineral oil in a 35 mm culture dish in a dry incubator for 144 h). Presence or absence of lid had no effect on rate of evaporation under these conditions.

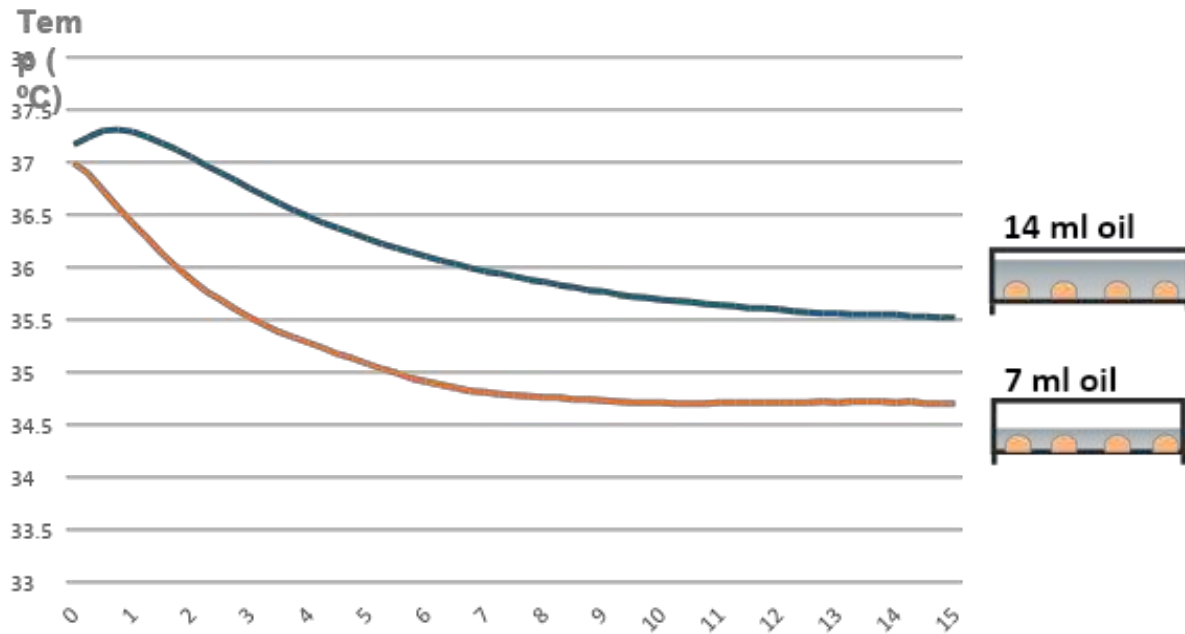
Mineral oil humidification results in a significant reduction in the osmolality increase.

Higher oil volumes significantly decrease evaporation.

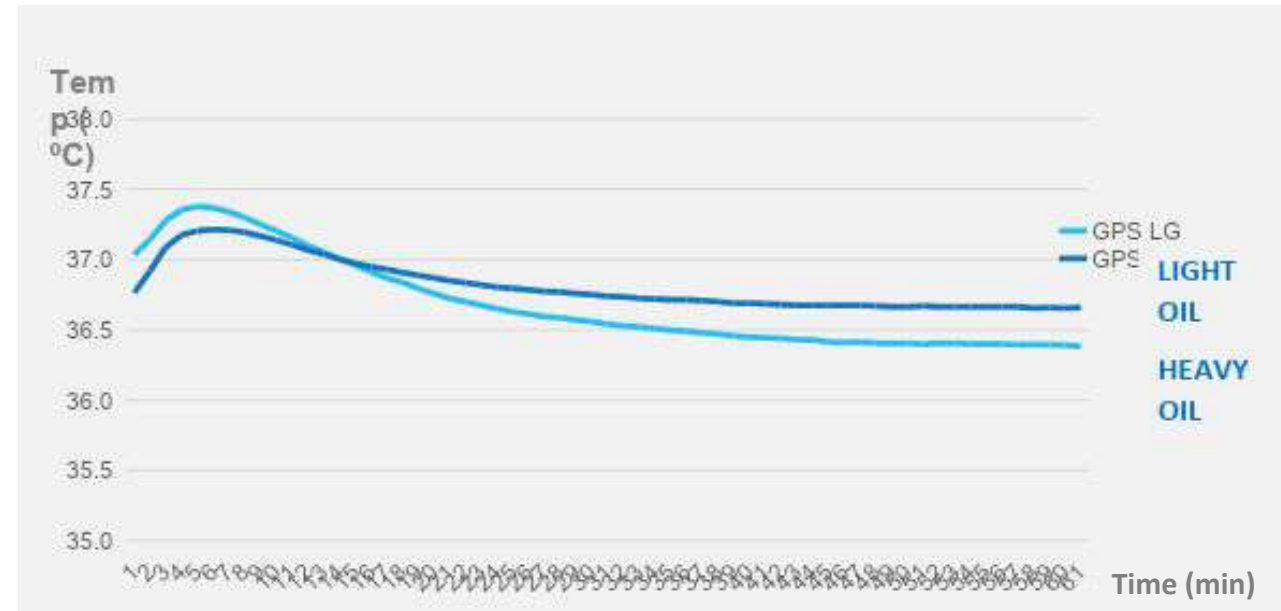
Heavy oils result in lower rates of media evaporation compared to lighter oils.

# Mineral oil overlay: stabilizer agent

## Temperature



Effect of mineral oil viscosity in temperature dynamics in a culture dish in a heated stage



Unpublished data

Effect of volume of mineral oil on temperature dynamics:

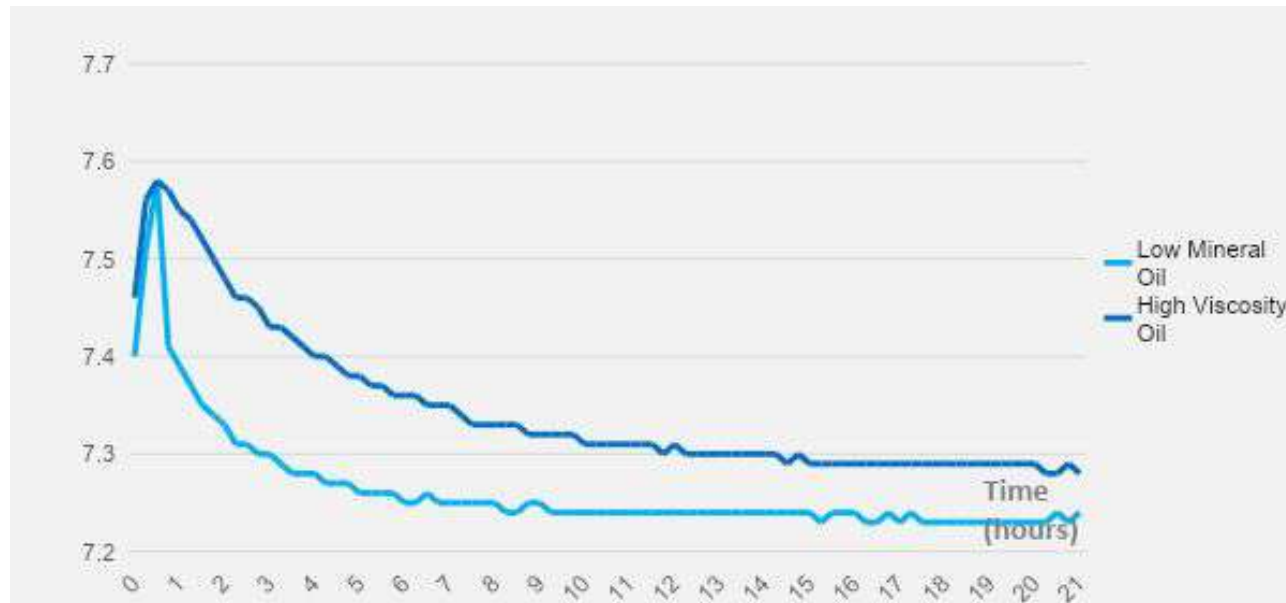
- A thinner mineral oil leads to a faster cooling rate.
- A thinner mineral oil leads to a greater temperature decrease.

Heavy oils have been shown to maintain a greater culture temperature stability compared to light oils.

# Mineral oil overlay: stabilizer agent

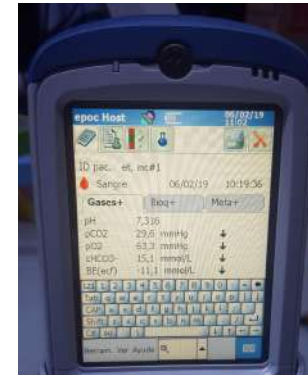
pH

Typical pH dynamics O/N at 37°C in humidified incubator with % CO<sub>2</sub> - low O<sub>2</sub>

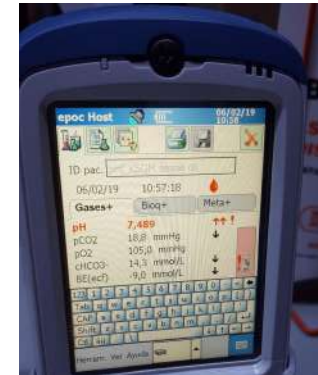


Unpublished data

Heavy oil takes more time to equilibrate than light oil but maintains variables more stable.



Media covered with oil



Media without oil

# Oxygen

- Transition from atmospheric (20%) to physiological (5-7%) O<sub>2</sub> levels in incubators → biological rationale overcomes technical limitations.
- Physiological O<sub>2</sub> levels reportedly improve embryonic development:
  - ❖ Detrimental Reactive Oxygen Species (ROS) linked to higher oxygen levels found to increase methylation patterns<sup>1</sup>.
  - ❖ Less global pattern of gene expression perturbations under physiological oxygen levels<sup>2</sup>.
  - ❖ Protein profiles from embryos cultured at 20% O<sub>2</sub> display divergent proteomes<sup>3</sup>.
  - ❖ Reduced O<sub>2</sub> culture (5%) increases implantation and live birth rates<sup>4</sup>.
  - ❖ 20% O<sub>2</sub> impairs embryo gene expression, metabolism, and glucose uptake<sup>5,6</sup>.
  - ❖ Embryos in 20% O<sub>2</sub> show delayed cleavage, fewer cells, and compromised viability<sup>7</sup>.
- The optimal O<sub>2</sub> level for human embryo development remains under investigation<sup>8</sup>.

<sup>1</sup>Li, 2014

<sup>2</sup>Rinaudo et al., 2006

<sup>3</sup>Katz-Jaffe et al., 2005

<sup>4</sup>Meintjes et al., 2009

<sup>5</sup>Forristal et al., 2013

<sup>6</sup>Harvey et al., 2014

<sup>7</sup>Wale and Gardner, 2010

<sup>8</sup>Wale and Gardner, 2016

# Oxygen

Technical advancements have enabled working with physiological O<sub>2</sub> levels inside incubators, which should nevertheless be **routinely measured** and **constantly monitored**.



*Infra-red gas analyzers*



*External O<sub>2</sub> Analyzers/Monitors*



*Built-in O<sub>2</sub> sensors*

# Humidity

- Dry vs **humid** incubators.
- Incubators with humidified atmosphere → **80-100%**.
- Humidification supplied via **evaporation of a water reservoir**.



## Different humidification systems

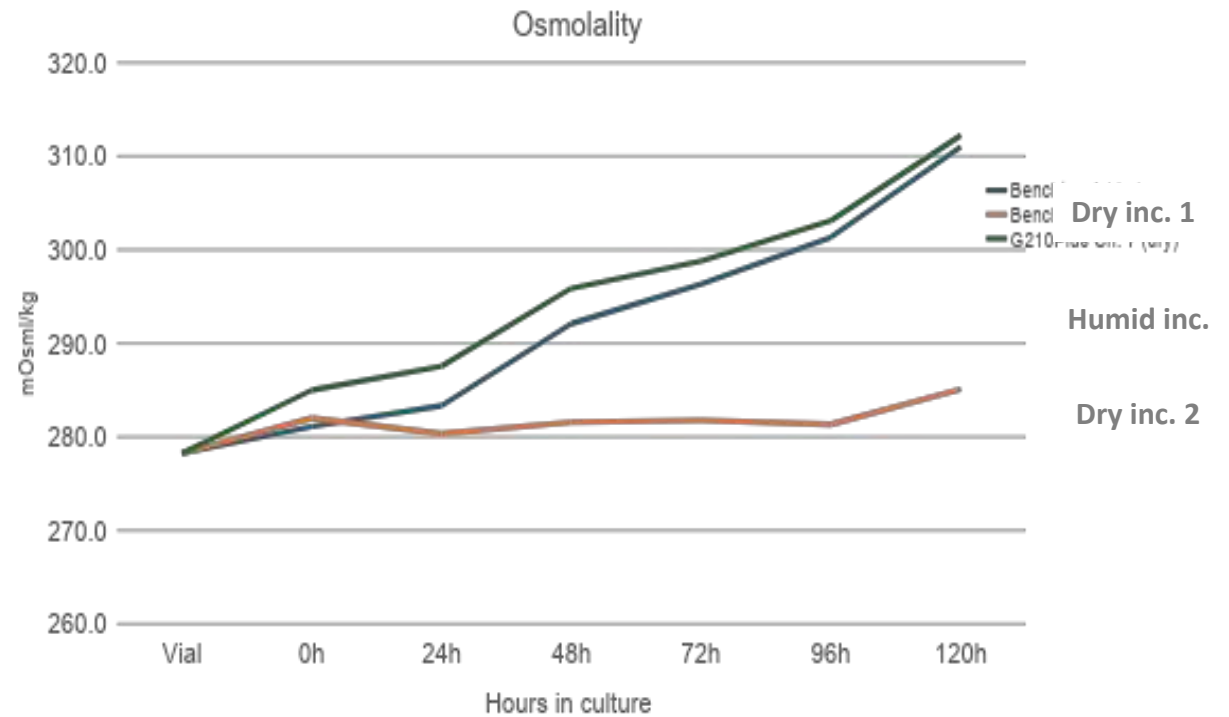
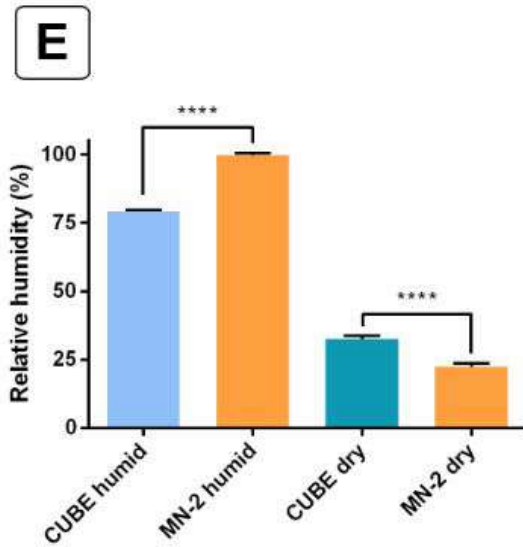
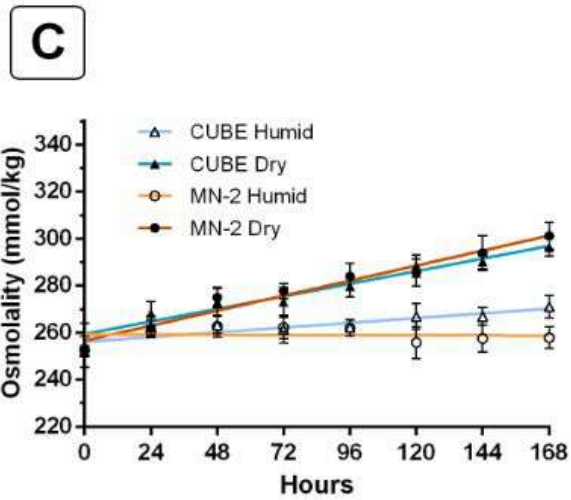
- Culture of human embryos under humid conditions has been linked to significantly higher embryo quality, compaction, and blastocyst formation, as well as higher clinical and ongoing pregnancy rates compared to dry culture<sup>1</sup>.



<sup>1</sup>Matsumoto et al., 2017

# Humidity

- Humidity prevents media evaporation during culture, and, therefore avoids harmful rises in medium osmolality.



Human Reproduction, Vol.00, No.0, pp. 1-9, 2021  
doi:10.1093/humrep/dhac373

human reproduction ORIGINAL ARTICLE Embryology

## Factors of the human embryo culture system that may affect media evaporation and osmolality

E. Mestres<sup>1</sup>, M. García-Jiménez<sup>1</sup>, A. Casals<sup>1</sup>, J. Cohen<sup>2</sup>, M. Acacio<sup>1</sup>, A. Villamar<sup>1</sup>, Q. Matia-Algué<sup>1</sup>, G. Calderón<sup>1</sup>, and N. Costa-Borges<sup>1,\*</sup>

<sup>1</sup>Embryotools R&D Center, Parc Científic de Barcelona, Barcelona, Spain <sup>2</sup>FART Institute of Washington, Hudson, NY, USA

\*Correspondence address: Embryotools R&D Center, Parc Científic de Barcelona, Avinguda Doctor Marañón, 8, 08028 Barcelona, Spain. Tel: +34 93 4 497 198; E-mail: nuno.borges@embryotools.com

Submitted on November 4, 2020; resubmitted on December 14, 2020; editorial decision on December 18, 2020

Mestres et al., 2021

Unpublished data

# Humidity

Given its role in reducing culture medium evaporation and preventing osmolality shifts, **maintaining and monitoring** of optimal relative humidity environments should be considered.

Humidification via water reservoirs at the incubator base poses a contamination risk that can be minimized with **regular monitoring and water replacement**.



**Built-in humidity sensors**



**External humidity sensors /  
hygrometers**

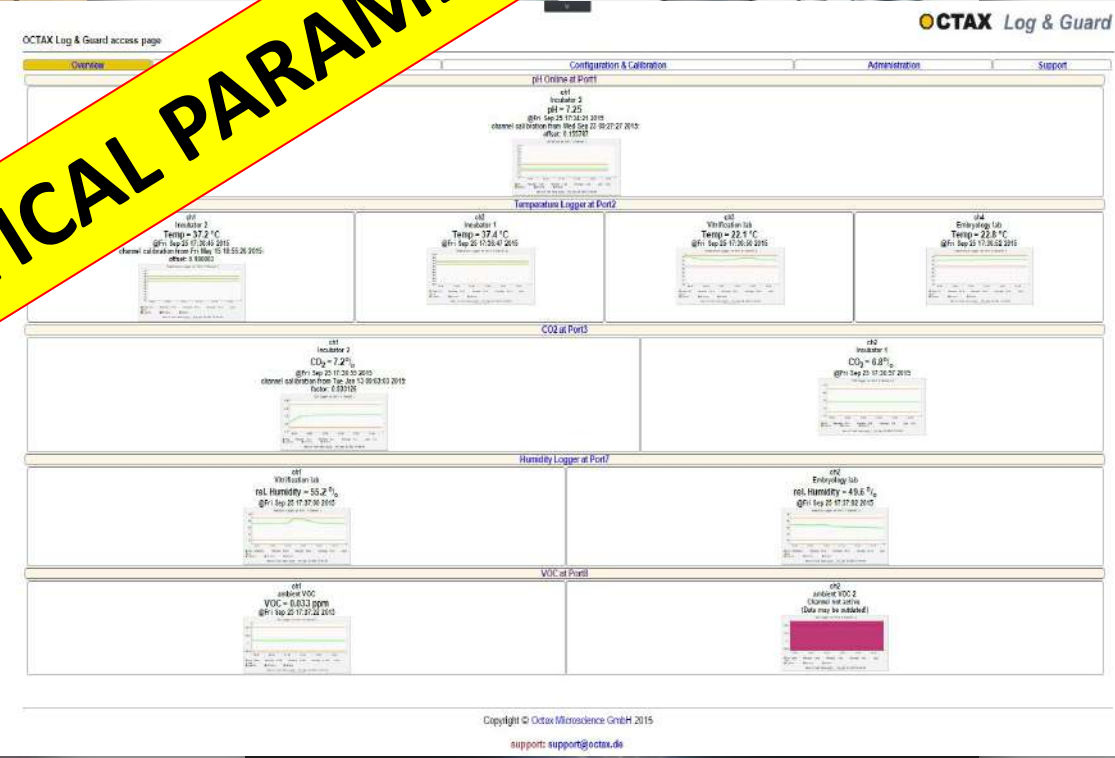


**Osmometers: osmolality as an  
evaporation indicator**

# Additional monitoring factors

ME 00 ISSUE 0 2019

**MONITORING SYSTEM FOR CRITICAL PARAMETERS!**



# Additional information factors

ME 00 ISSUE 0 2019

STERS!

O-092: High-viscosity mineral oils provide enhanced protection to embryo culture systems against volatile organic compounds-induced embryotoxicity

Session details

## SESSION TITLE

Session 26: Fine-tuning ART: small details, big impacts

## SESSION TYPE

Selected oral communications



PRESENTED BY:

**Mrs Queralt Matia Algué**

Embryotools  
Spain

## BIOGRAPHY

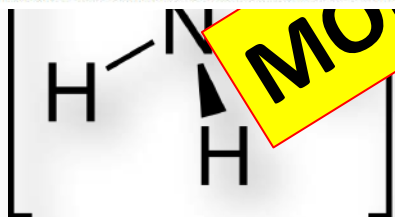
Queralt Matia-Algué is an embryologist who finished her degree in Biology at Autonomous University of Barcelona in 2016. Afterwards, she specialized in the embryology field with the Master's Cytogenetics and Reproductive Biology and later she deepened in assisted reproduction with the postgraduate course Updates in Assisted Reproduction Techniques by Dexeus Hospital and the Master's in Assisted Human Reproduction by Complutense University of Madrid. She finally joined the Embryotools team in 2020, where she works in the research and development department.

## ABSTRACT TEXT

Q. Matia Algué<sup>1</sup>, M. Acacio<sup>1</sup>, E. Mestres<sup>1</sup>, A. Flores-Saiffe<sup>2</sup>, A. Martínez Casado<sup>1</sup>, A. Villamar<sup>1</sup>, A. Franco-Roig<sup>1</sup>, A. Casals<sup>1</sup>, C. Castelló<sup>1</sup>, L. Romera<sup>1</sup>, J. Cohen<sup>2</sup>, N. Costa-Borges<sup>1</sup>.

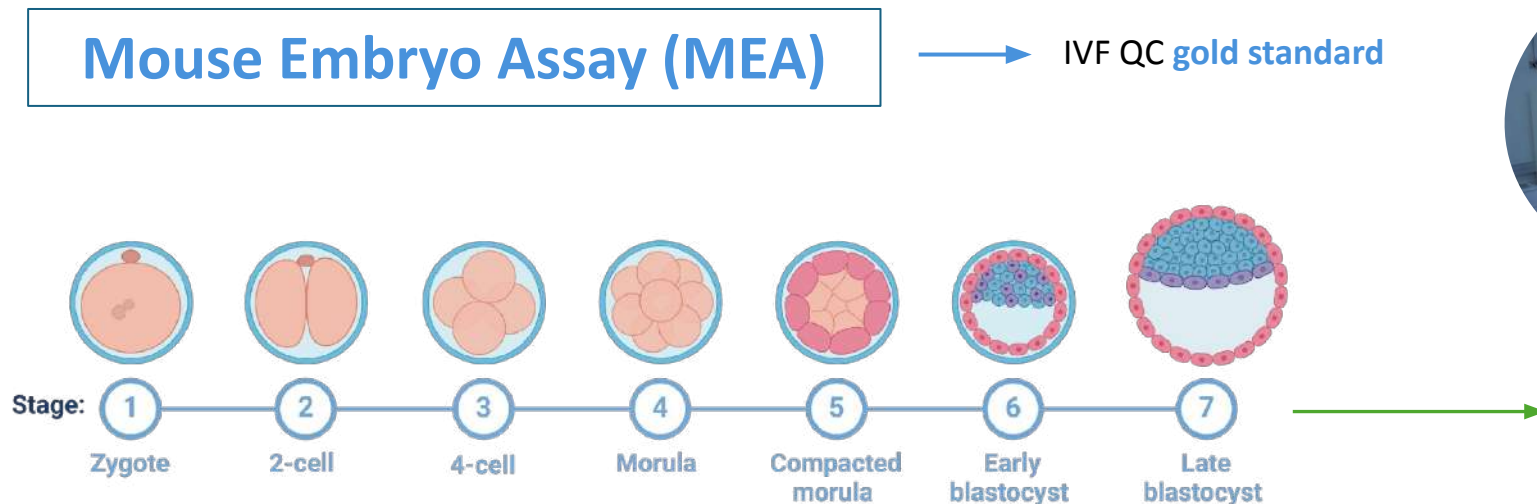
<sup>1</sup>Embryotools, Research&Development, Barcelona, Spain.

<sup>2</sup>Conceivable Life Sciences, Research, Guadalajara, Mexico.



# Consumables: Mouse Embryo Assay (MEA)

- **Strict and continuous monitoring** of laboratory and culture conditions is essential, as even minimal fluctuations can significantly compromise IVF success rates, making it critical to **consistently** work under **optimal conditions**.
- However, all these efforts can be rendered useless if any embryotoxic consumable is inadvertently introduced into the system.
- A reliable **QC program** must be implemented to avoid such extremes and achieve IVF's greatest chances of success.



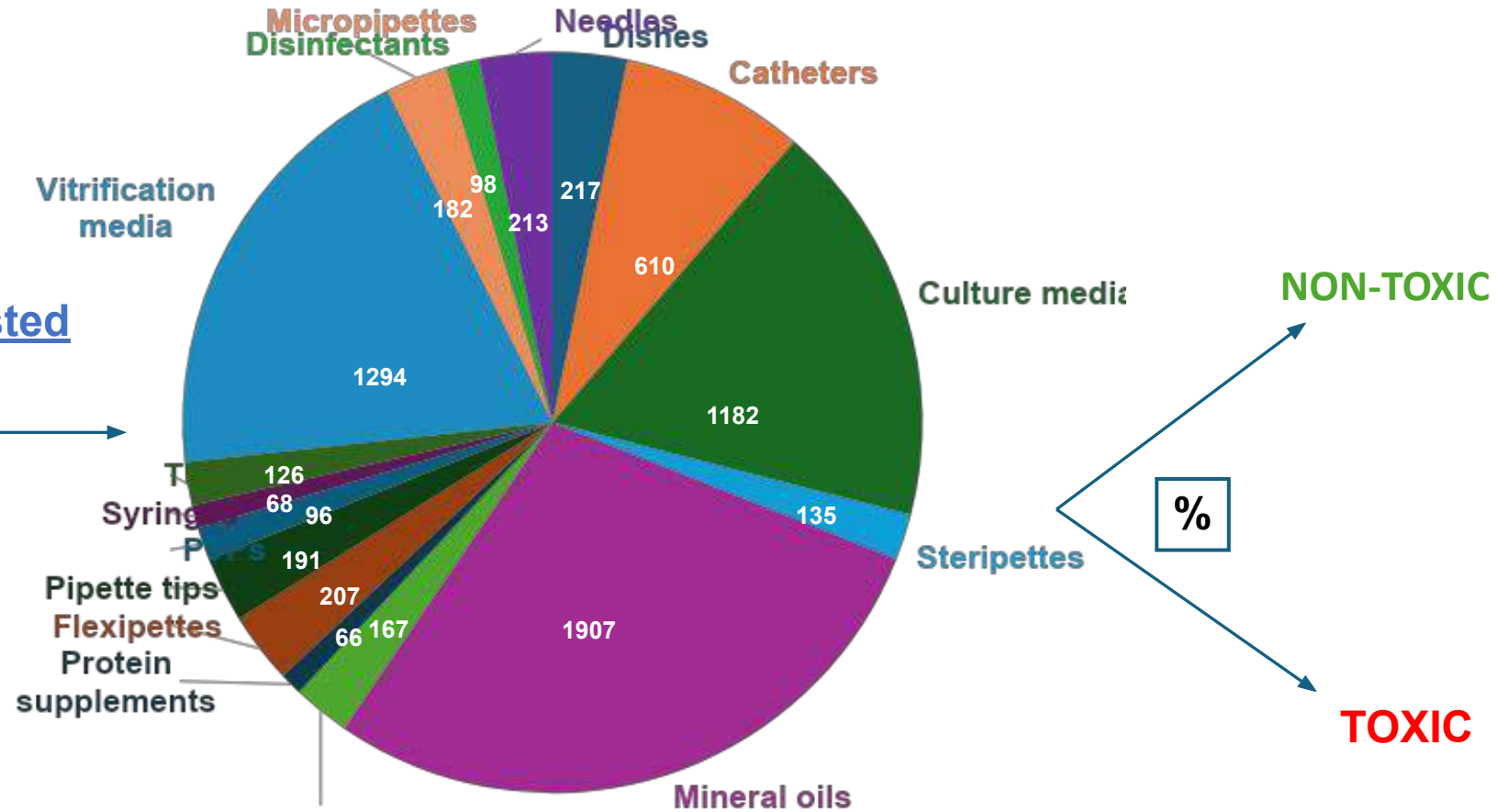
*Embryotools S.L. QC center*  
**ISO accredited**

# Consumables: Mouse Embryo Assay (MEA)



6759 MEA-tested samples

5 years



NON-TOXIC

TOXIC

%

- Extreme toxicity
- Mild toxicity
- Low toxicity

- 0 – 30% EBFR
- 30 – 70% EBFR
- 70 – 80% EBFR

# Consumables: Mouse Embryo Assay (MEA)

## TOP TOXIC-SAMPLES RATE CATEGORIES

EXTREME  
EMBRYOTOXICITY  
(0-30% EBFR)

**20.75% EBFR**

MILD  
EMBRYOTOXICITY  
(30-70% EBFR)

**43.60% EBFR**

EXTREME  
EMBRYOTOXICITY  
(0-30% EBFR)

**16.23% EBFR**

### CATHETERS

EXTREME  
EMBRYOTOXICITY  
(0-30% EBFR)

**10.31% EBFR**

### STERIPETTES

EXTREME  
EMBRYOTOXICITY  
(0-30% EBFR)

**10.79% EBFR**

# Consumables: Mouse Embryo Assay (MEA)

MINERAL OIL & CULTURE MEDIUM

Lengthy contact time with embryos

Mineral oils

Toxic

NEARLY-EXTREME EMBRYOTOXICITY  
(≈30% EBFR)

35.1% EBFR

CULTURE MEDIA

Toxic

NEARLY-EXTREME EMBRYOTOXICITY  
(≈30% EBFR)

31.7% EBFR

Despite not being among the top toxic-simples rate groups, a nearly-extreme embryotoxicity was reported within the samples assessed as toxic for both categories.

# Conclusions

- Embryo developmental competence is linked to multiple chemical and physical parameters which should be optimized to achieve the highest IVF success rate.
- Maintenance of consistent optimal culture conditions should be ensured by constantly monitoring key parameters and performing routine checks.
- Strict and rigorous control of each culture system component facilitates the prompt and unmistakable identification of any sub-optimal condition that, even if minimal, might impair embryo developmental competence, ensuring a quick and effective corrective response.
- The control, monitoring and optimization of key parameters may be rendered ineffective without the implementation of rigorous and reliable quality control systems, such as the Mouse Embryo Assay, ensuring that no toxic consumables are employed during the IVF process.



# SIERR update

In collaboration with:



Π.Ε.Κ.Ε.  
Πανελλήνια Ένωση  
Κλινικών Εμβρυολόγων



GR.A.C.E  
Greek Association  
of Clinical Embryologists



## Acknowledgements:

Nuno Costa-Borges

Queralt Matia

Enric Mestres

Aina Franco

Carolina Castelló

Lydia Romera

Alba Casals

Sonia Gago

Andrea Villamar

Pau Soler

Mònica Acacio

Carlota Gutiérrez



# SIERR update

In collaboration with:



Π.Ε.Κ.Ε.  
Πανελλήνια Ένωση  
Κλινικών Εμβρυολόγων



GR.A.C.E  
Greek Association  
of Clinical Embryologists



# Thank you for your attention



Contact: [albert.martinez@embryotools.com](mailto:albert.martinez@embryotools.com)

