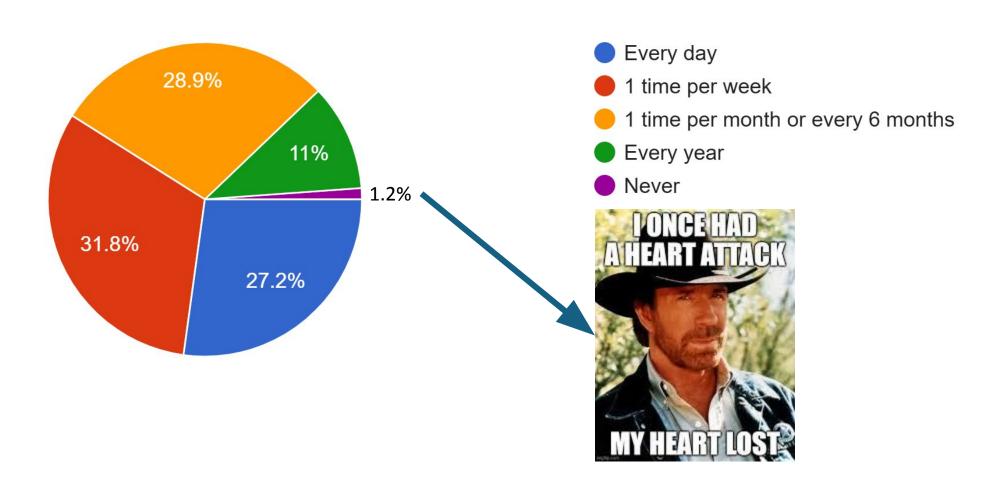
SIERR Survey Presentation Insemination and embryo culture: best practices or best countries? Miguel Gallardo Ph.D.

For a stable embryo culture, we need to guarantee the optimum temperature for gametes and embryos. To achieve this, how often do you monito... laboratory (microscopes and air flow chambers)? 173 responses



For a stable embryo culture, we need to guarantee the optimum temperature for gametes and embryos. To achieve this, how often do you monito... laboratory (microscopes and air flow chambers)?

What does it mean to monitor?

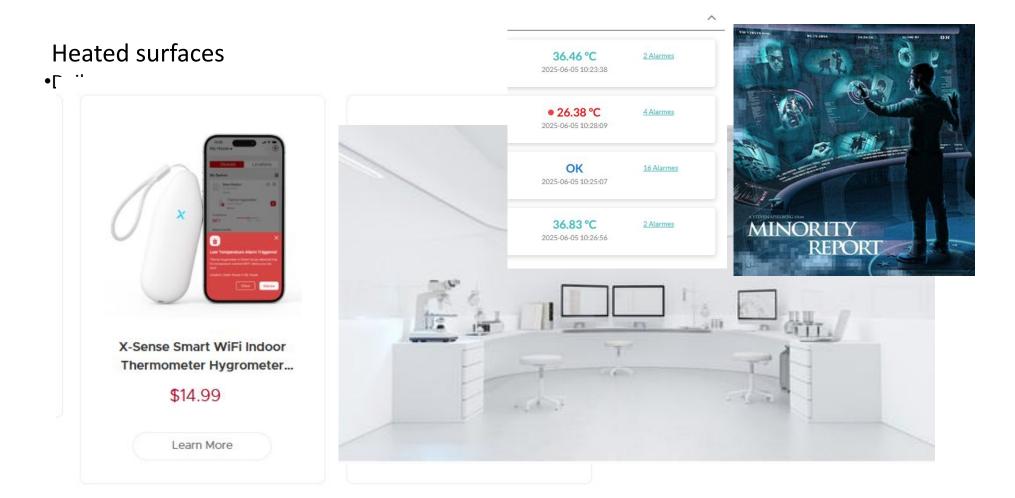
Do whatever it takes to **guarantee** the optimum temperature for gametes and embryos

☐The only answer is **every day, and also weekly, and also monthly, and also biyearly or yearly.**

Heated surfaces

- Daily
- Check the display setpoint
- Check a continuous monitoring independent temperature probe
- Goal: Detect sudden fluctuations and failures that can impact daily activity





Heated surfaces

- Weekly
- Independent, calibrated temperature probe.
- Direct measurement in the surface.
- The goal is to get readings similar to setpoint, to detect significant variations / drifting





Heated surfaces

- Monthly-every six months-yearly
 - Independent, calibrated temperature probe(s?)
 - every time you change an element of your culture system (dishes, oil, temperature setting, etc.)
 - Simulation of working conditions, measure inside a drop inside a dish with oil overlay
 - Do not take a static measurement, but register the temperature evolution
 - You want to answer real world questions: how long do you make the ICSI dishes until you use them?
 How long do you use them with oocytes inside
 - The goal is to adjust working philosophy to the reality of the temperature, and adjust the setpoints for the culture system

Incubators are a whole different problem.

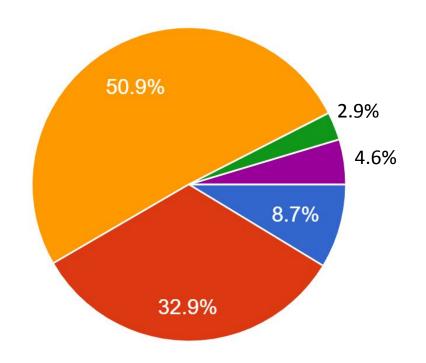
- •10 chambers
- •2 areas per chamber (top and bottom)
- •1 temperature setting display
- •We assume they are designed to achieve 37 in the petri dish when they are at setpoint 37°C
- •Continuous measurement with alarm to detect failures in a single point
- •Weekly-monthly direct to surface measurement to assess each chamber and detect drift.



ICSI Timing - SPMR

The time interval between oocyte pick-up and ICSI varies greatly between ART centres. Which time interval best suits your clinical practice?

173 responses



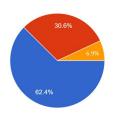
- Less than 2 hours
- Up to 2 hours
- Up to 4 hours
- Up to 6 hours
- It depends on the lab work flow

ICSI Timing - SPMR

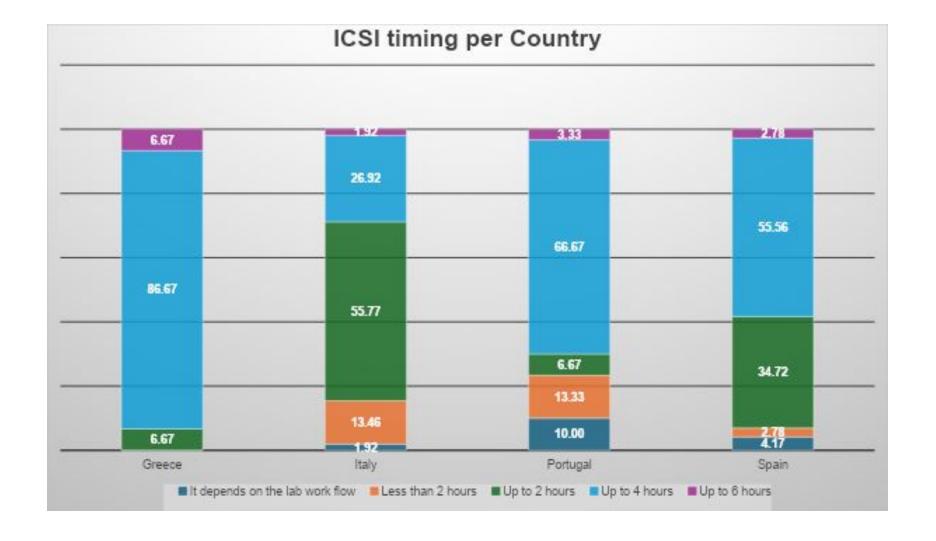
Answers differ only by Country (p<0.001)

Very small effect by public-privat e

Is your clinic private, public or both?
173 responses



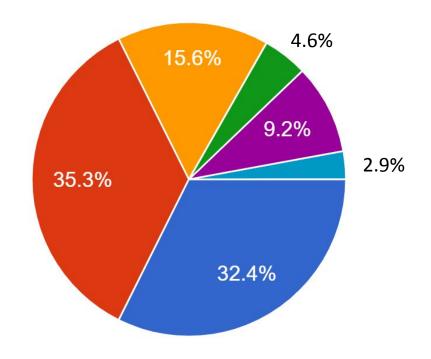




ICSI vs FIV - SPMR

Today, ICSI has taken on a key role in fertilisation techniques. Which is the proportion of ICSI and conventional IVF in your clinic?

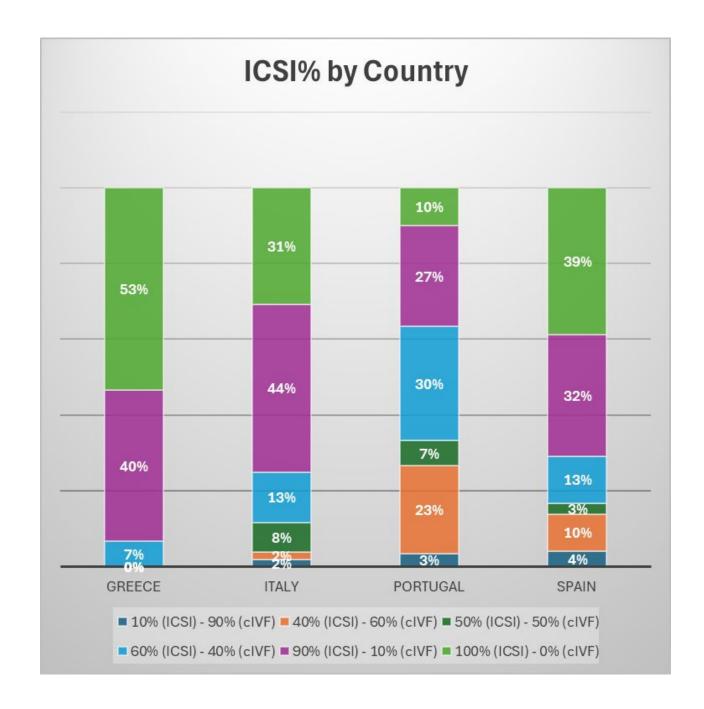
173 responses



- 100% (ICSI) 0% (cIVF)
- 90% (ICSI) 10% (cIVF)
- 60% (ICSI) 40% (cIVF)
- 50% (ICSI) 50% (cIVF)
- 40% (ICSI) 60% (cIVF)
- 10% (ICSI) 90% (cIVF)

ICSI vs FIV - SPMR

Answers differ only by Country (p=0.012)



ICSI vs FIV - SPMR

The main drawback for cIVF is the risk of Total Fertilization Failure, which is hard to explain/digest to patients in a private setting



Article

https://doi.org/10.1038/s41591-025-03621-x

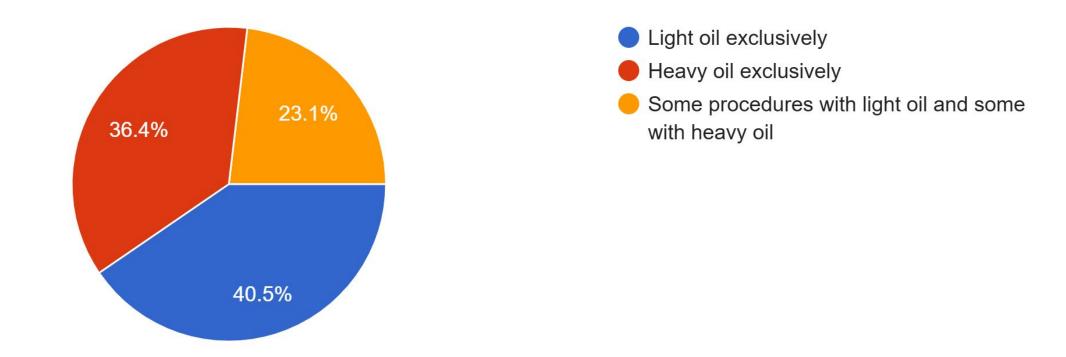
Table 4 | Fertility outcomes (ITT analysis)

Parameters	ICSI (n=414)	c-IVF (n=408)	Risk ratio (95% CI)	P value ^a
Number of retrieved oocytes	3,628	3,412	-	-
Median (IQR)	7 (5.0–11.0)	8 (4.0-11.0)	~	0.82
Missing data	1 ^b /414 (0.2%)	1 ^b /408 (0.2%)		-
Number of 2PN oocytes	1940°	1983 ^d	-	-
Median (IQR)	4 (2.0-6.0)	4 (2.0-7.0)	_	0.30
Missing data	0	0).=	-
Fertilization rate per oocyte retrieved (2PN rate)	1,940/3,628 (53.5%)	1,983/3,412 (58.1%)	0.92 (0.88-0.96)	≤0.001
Number of MII oocytes	2914°	-	-	-
Median (IQR)	6 (4.0-9.0)	-	-	-
Unavailable data	3°	7	-	=
Missing data	0	-	2	=
Fertilization rate per inseminated MII (2PN rate)	1,940/2,914 (66.6%)	-	(=	-
TFF	20/414 (4.8%)	15/408 (3.7%)	1.29 (0.68-2.54)	0.44

Oil type - SPMR

The choice of the oil is not easy nowadays. What kind of oil do you use for your embryo culture?

173 responses



Choice of oil is unrelated to variables: Country, Nº Cycles, ICSI-IVFpreference, TransferDay, ICSI time

Comparing oils used for human embryo culture

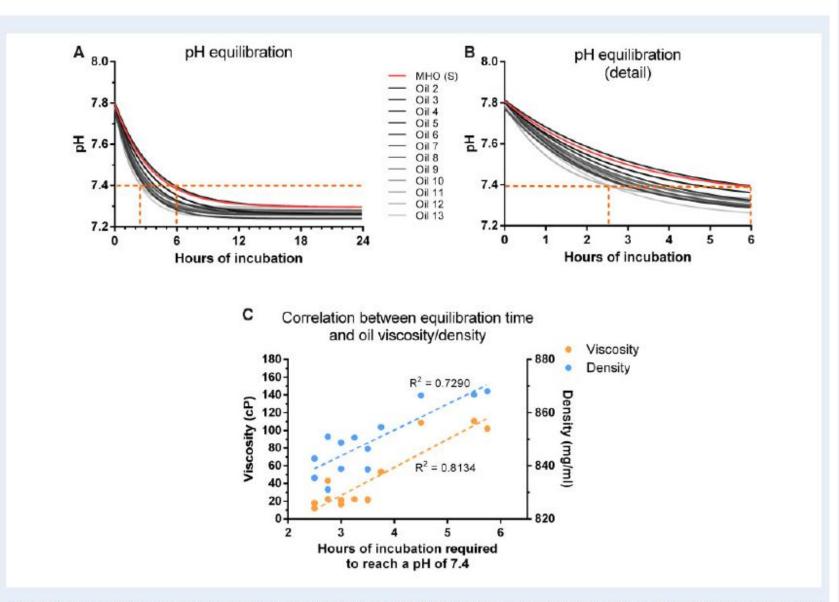
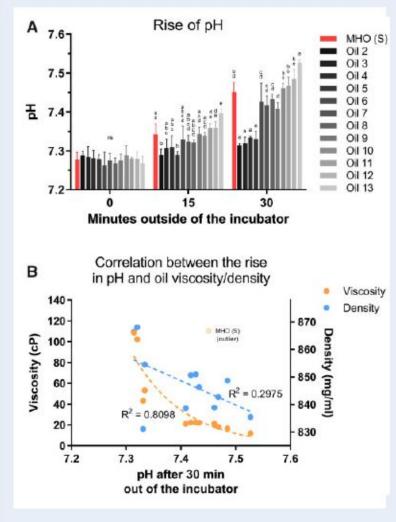


Figure 2. Media pH equilibration and its relation to the viscosity/density of the oil. The pH in a four-well dish was monitored during 24 h after preparing the dish and placing it in an incubator. Some differences were noted in the required time to achieve pH equilibration depending on the used oil, particularly during the first hours of incubation (A, B). Whilst both the oils' viscosity and density seemed to be somewhat related to the equilibration speed, viscosity proved to be a better fit after applying a linear regression model (C). MHO (S), Mineral Heavy Oil (Sigma).



217

Figure 3. Stability of the pH outside of the incubator, related to the viscosity/density of the oil. The capacity for each oil to maintain a stable pH after taking the dish out of the incubator was assessed at time 15 and 30 min (A); a different superscript within the same time-point indicates a statistically significant difference between groups (the complete intergroup comparisons at both time-points can be found in the Supplementary Tables SI and SII). The increased pH after 30 min followed a non-linear correlation with the oils' viscosity, but did not seem to be related to their density (B). MHO (S), Mineral Heavy Oil (Sigma); ns, not significant.

Oil type - SPMR

Is there a best oil? Oil is just a part of the culture system.

It needs to be assessed in conjunction with other factors: media renewal, dish configuration, incubator humidity.

In our setting, we use 60mm dishes with 8x30 uL culture drops (+6 washing drops) with 10 mL of light oil overlay.

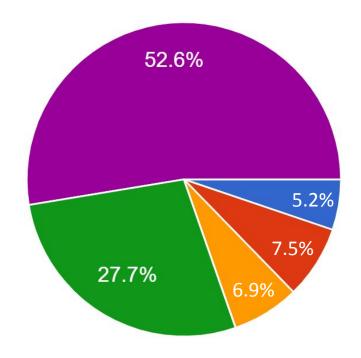
Culture in one-step media, no renewal, for up to 7 days, on *humidified* incubators

Heavy oil takes more time to equilibrate upon exposure to CO2, and reduces evaporation but...

How different are temperature dynamics of heavy oil vs. light oil? Is it a good candidate for ICSI dishes?

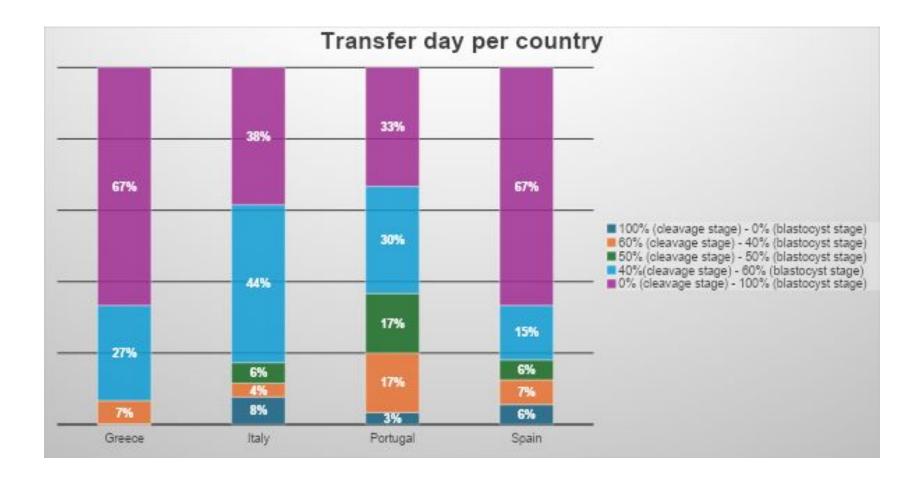
Today, most recommendations suggest transferring embryos on day 5. However, this is not always the case. Which is the proportion of ET at the cleavage stage and blastocyst stage?

173 responses

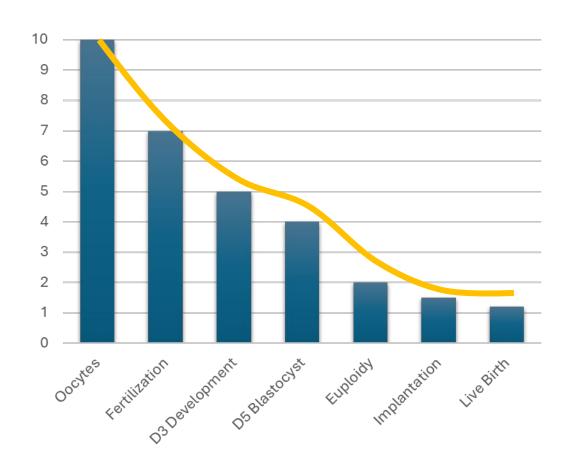


- 100% (cleavage stage) 0% (blastocyst stage)
- 60% (cleavage stage) 40% (blastocyst stage)
- 50% (cleavage stage) 50% (blastocyst stage)
- 40%(cleavage stage) 60% (blastocyst stage)
- 0% (cleavage stage) 100% (blastocyst stage)

Answersag ain differ only by Country (p<0.05)

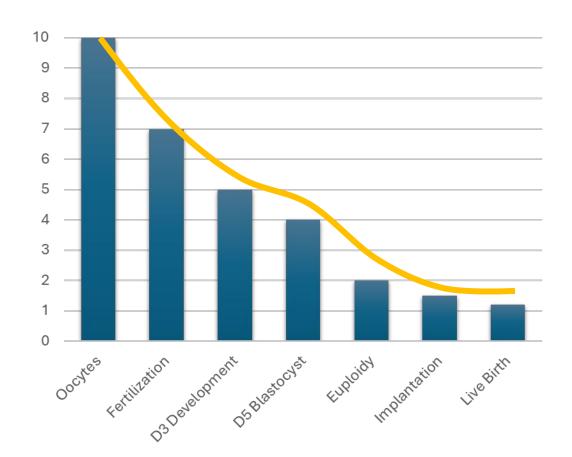


- When are we going to carry out the embryo transfer
- We could transfer sequentially all 2PN zygotes and maximize cumulative rates
- Short Time To Pregnancy reduces patient treatment burden (negative results, biochemical pregnancies, miscarriages) and increases cost-effectiveness
- We want a pregnancy in the lowest possible number of attempts



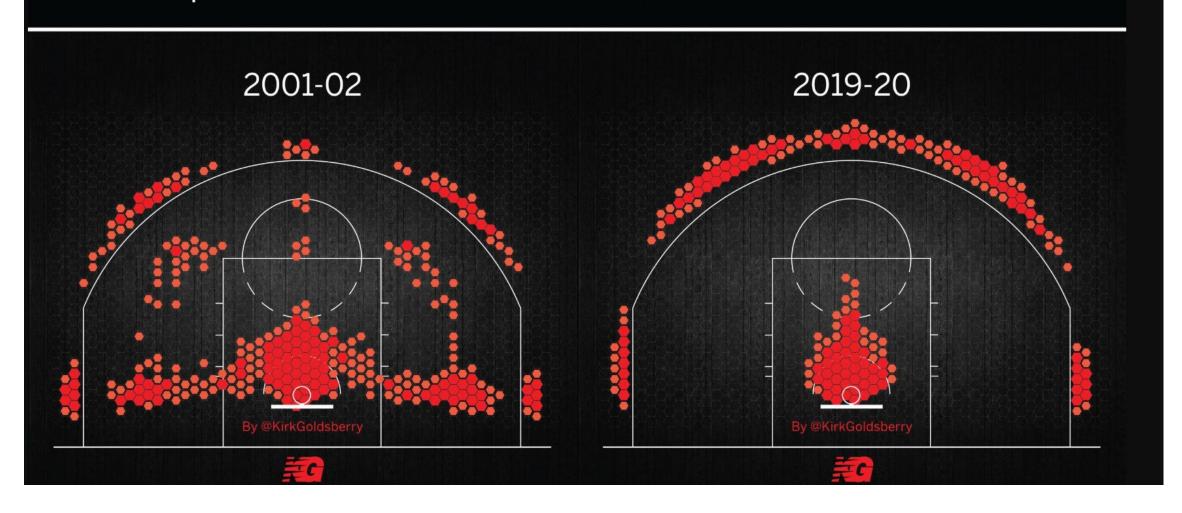
 We are afraid of impacting the cumulative outcomes when we increase embryo de-selection by blastocyst culture and by PGT-A...

But should we be afraid?



THE GAME HAS CHANGED

Top 200 shot locations in the NBA, 2001-02 versus 2019-20



Are we evidence based? No

• How is it possible that "Country" is the variable with more correlation to embryology laboratory practices.

Do we work based on evidence or on culture?

• Thank you very much!