Equipo Ron Ginecología

Hospital quirónsalud A Coruña

"Maximizing Outcomes in the IVF Laboratory"

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“the ability to achieve better results”

“the ability to achieve results with minimal resources”

“optimal balance between achievement of results and use of resources”

How to choose a culture media?

- Sequential and continuous media systems

  (Back to nature vs Let the embryo choose)

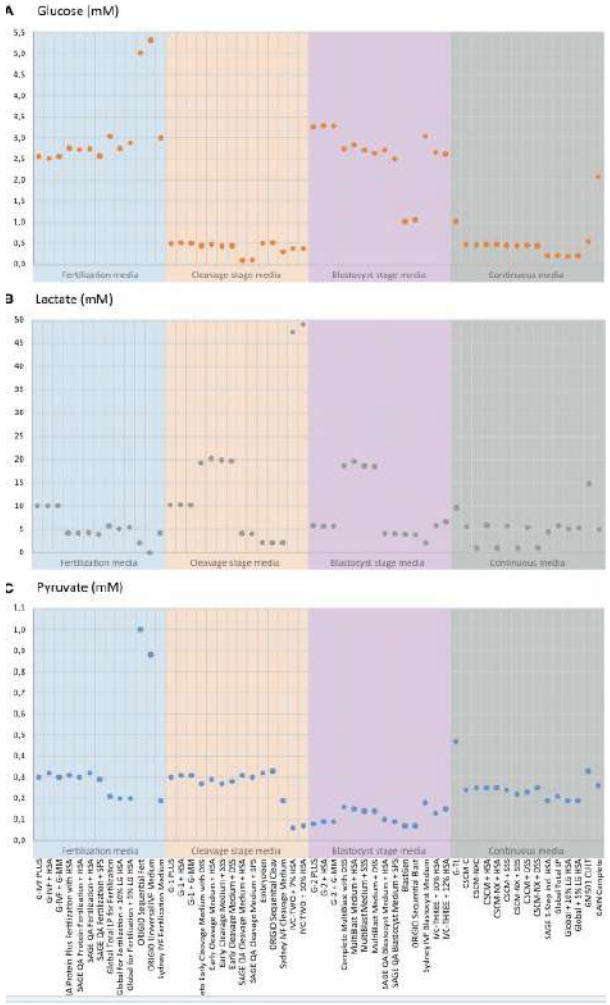
- Storage, handling and use

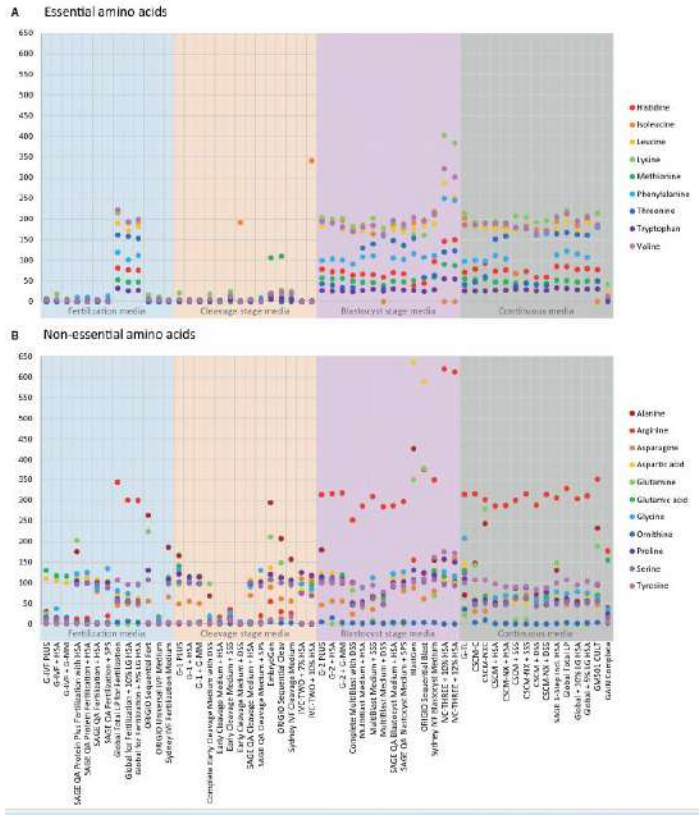
- Culture individually or in groups?

- O_2 and pH

- **Composition**

“47 different human embryo culture media”





OXFORD human reproduction

Human Reproduction, 2025, **40(1)**, 30–40
https://doi.org/10.1093/humrep/dead248
Advance Access Publication Date: November 25, 2024
Original Article

Embryology

The composition of commercially available human embryo culture media

M.S. Zagers[1,2], M. Laverde[1,2], M. Goddijn[1], J.J. de Groot[3], F.A.P. Schrauwen[3], F.M. Vaz[4,5,6], and S. Mastenbroek[1,2,*]

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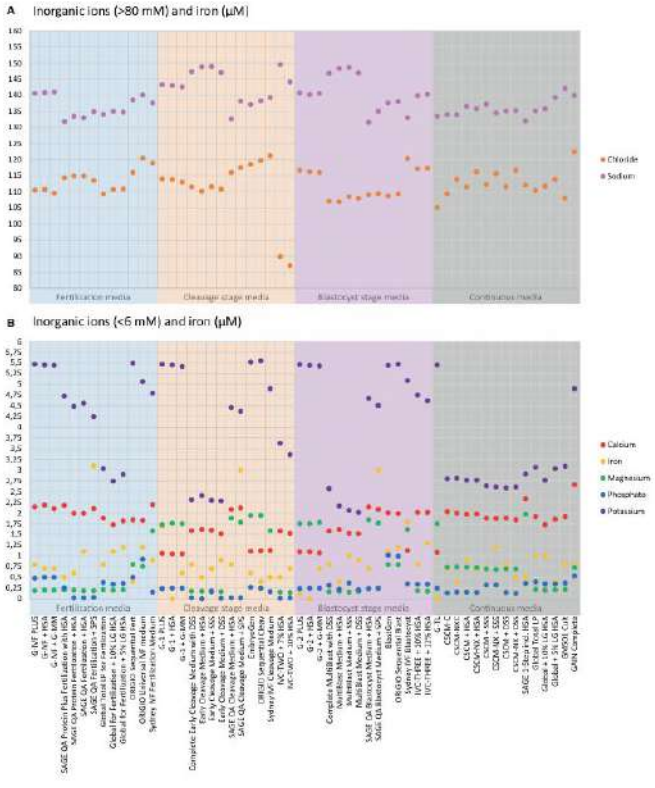

The role of lactate concentration in culture media

- Embryos cultured in low lactate media (1mM) showed improved blastocyst utilization rates compared to standard media (5-10mM) (Pastor Leary et al., 2024; Watson et al., 2022).

- Low lactate media also resulted in more euploid blastocysts (Whitney et al., 2022) and increased availability of usable blastocysts on day 5 versus day 6 (Watson et al., 2022).

- Early introduction to low lactate media was associated with reduced miscarriage rates and abnormal fertilization rates (Brewer, 2023).

Some findings suggest that excessive lactate in culture media may negatively influence embryo metabolism but the specific mechanisms are not fully understood

OXFORD human reproduction

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Original Article

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M.S. Zagers, M. Lavende, M. Goddijn, J.J. de Groot, F.A.P. Schrauwen, F.M. Vaz, and S. Mastenbroek

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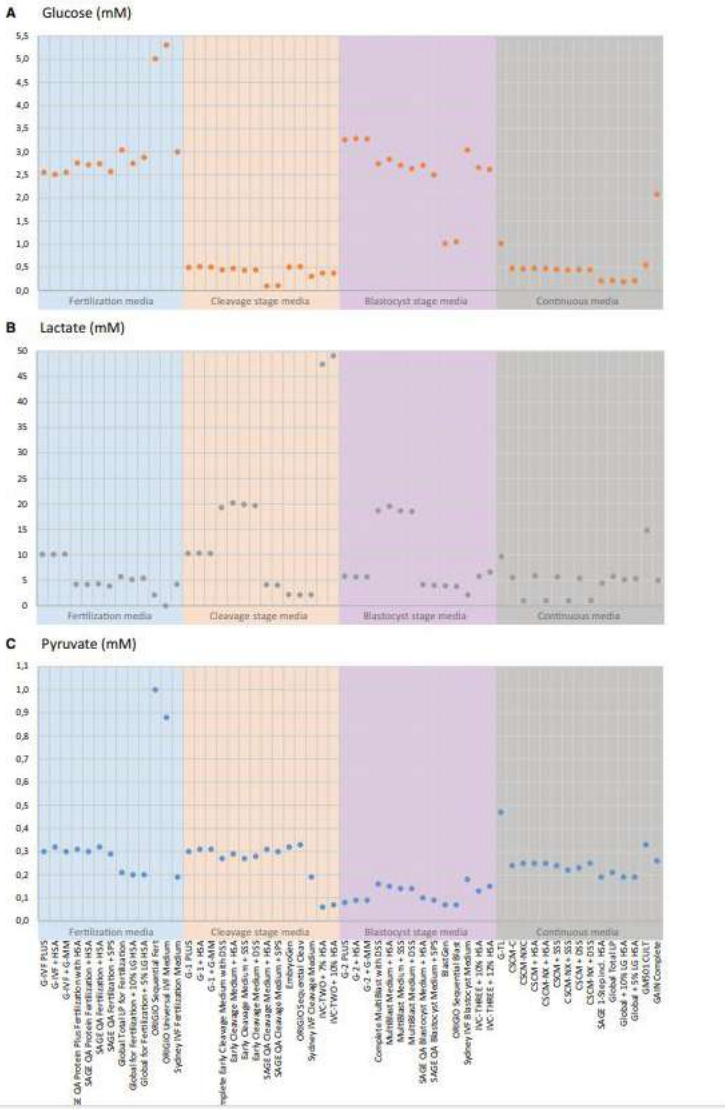
	H - Lact	CSCM-NX
Glucose	1 mM	0,5 mM
Lactate	10 mM	1 mM
Pyruvate	0,48 mM	0,25 mM



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ORIGINAL ARTICLE

Reproductive Medicine and Biology WILEY

Fertilization, embryo culture, and clinical results using low lactate embryo culture medium for pre-culture, insemination, and beyond

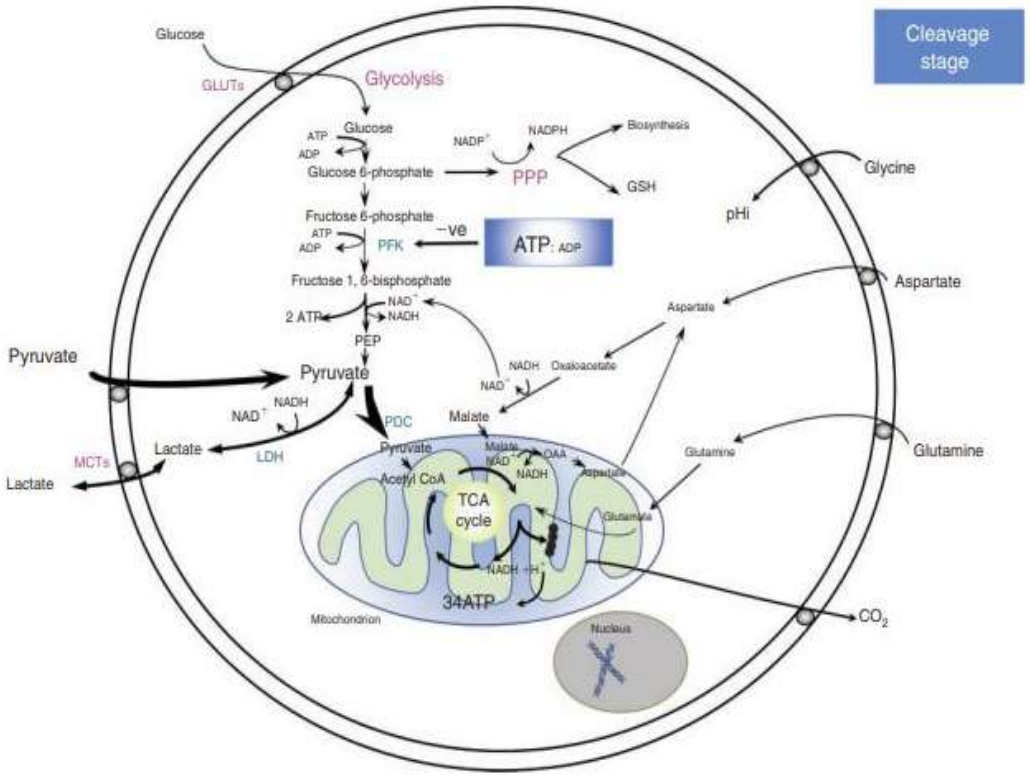
Masato Kobanawa

Early Development Metabolism

In early cleavage-stage embryos, **efficient pyruvate utilization** is crucial for mitochondrial oxidative phosphorylation and **energy production**.

Lowering lactate in the medium (rather than increasing pyruvate concentration) **increases pyruvate uptake** and its utilization, **supporting cell division and development**.

Maintaining an optimal **pyruvate/lactate ratio balances the optimal redox state**.



David K. Gardner A,B and Alexandra J. Harvey A

Reproduction, Fertility and Development, 2015, **27**, 638–654
http://dx.doi.org/10.1071/RD14421

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Blastocyst metabolism

David K. Gardner[A,B] *and Alexandra J. Harvey*[A]

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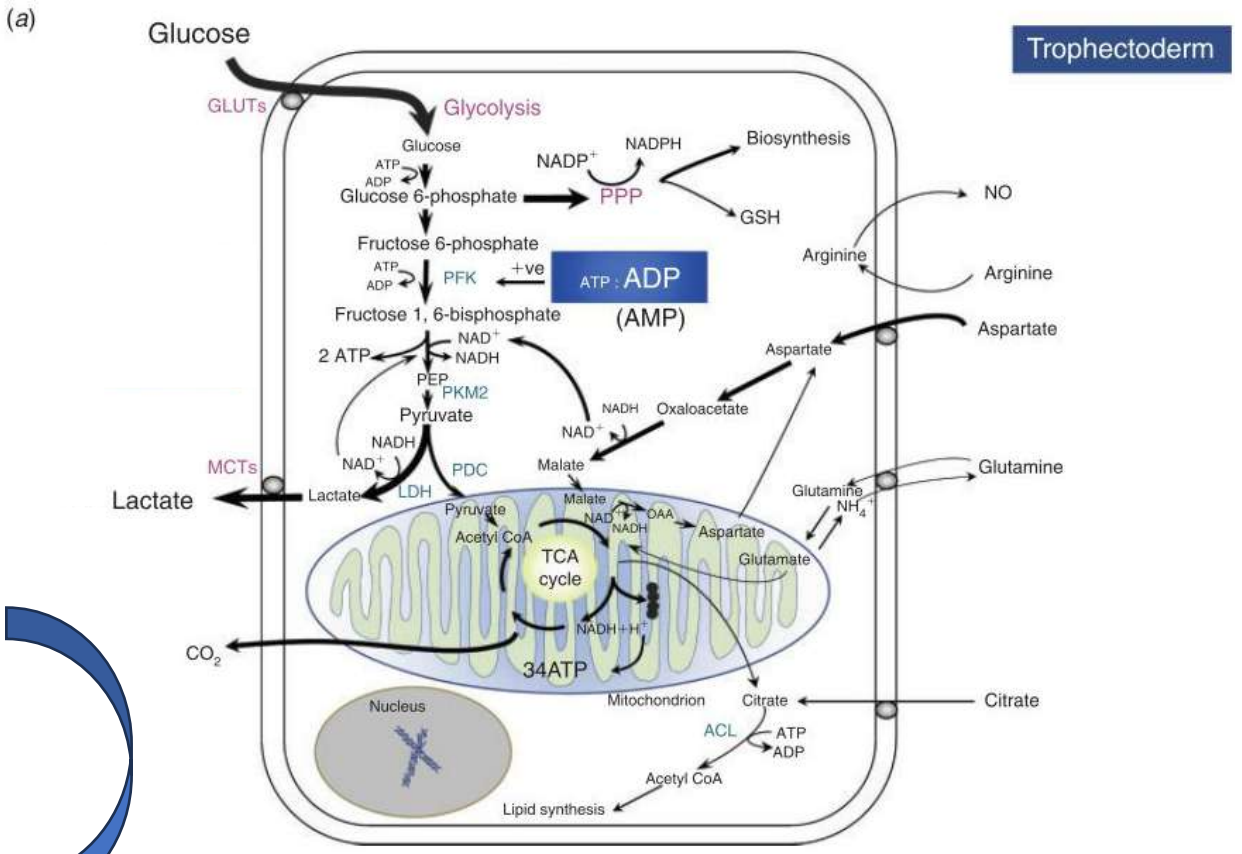
[B]Corresponding author. Email: david.gardner@unimelb.edu.au

Blastocyst Metabolism

After compaction, the embryo increases oxygen consumption and the ability to use **glucose as an energy source**

Instead of oxidizing all the glucose consumed, the blastocyst shows high levels of **aerobic glycolysis**. **Pyruvate is converted to lactate** instead of entering the TCA cycle, which helps in biomass production and redox regulation.



Warburg Effect

Warburg Effect

The Warburg effect is a metabolic strategy in which **proliferative cells** (such as tumor cells, stem cells and developing embryos) **priorize aerobic glycolysis** to oxidative phosphorylation (OXPHOS) **even in the presence of oxygen.**

Why does the blastocyst use the Warburg effect?

Rapid energy for proliferation and biomass production

Although glycolysis is less ATP efficient, it provides essential intermediate metabolites (via PPP) for the synthesis of nucleotides, lipids and amino acids necessary for rapid cell proliferation in the trophoectoderm and supports the production of (reduced) glutathione, a key intracellular antioxidant.

More glucose is consumed

More lactate is generated

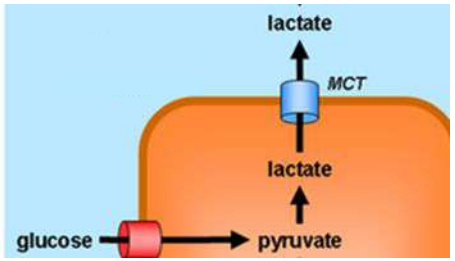
Less ATP production per glucose molecule (2 ATP vs. 36 ATP in OXPHOS)

Essential metabolites for cell growth are generated

A key cellular antioxidant is generated

Effect of lactate concentration (in culture media) on embryonic metabolism

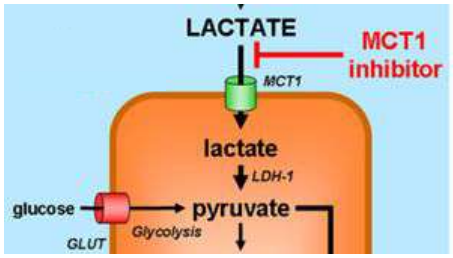
Low concentrations (1 -5 mM) in culture media



Favour the export of lactate from the blastocyt to the outside

- Enhance normal embryo development by supporting cell differentiation and implantation.

High concentrations (10 mM) in culture media



Hinder the export of lactate from the blastocyst to the outside

- May cause intracellular acidosis, disrupting pH homeostasis.
- Induce metabolic stress, impairing the embryo's ability to efficiently generate ATP.

biomolecules

MDPI

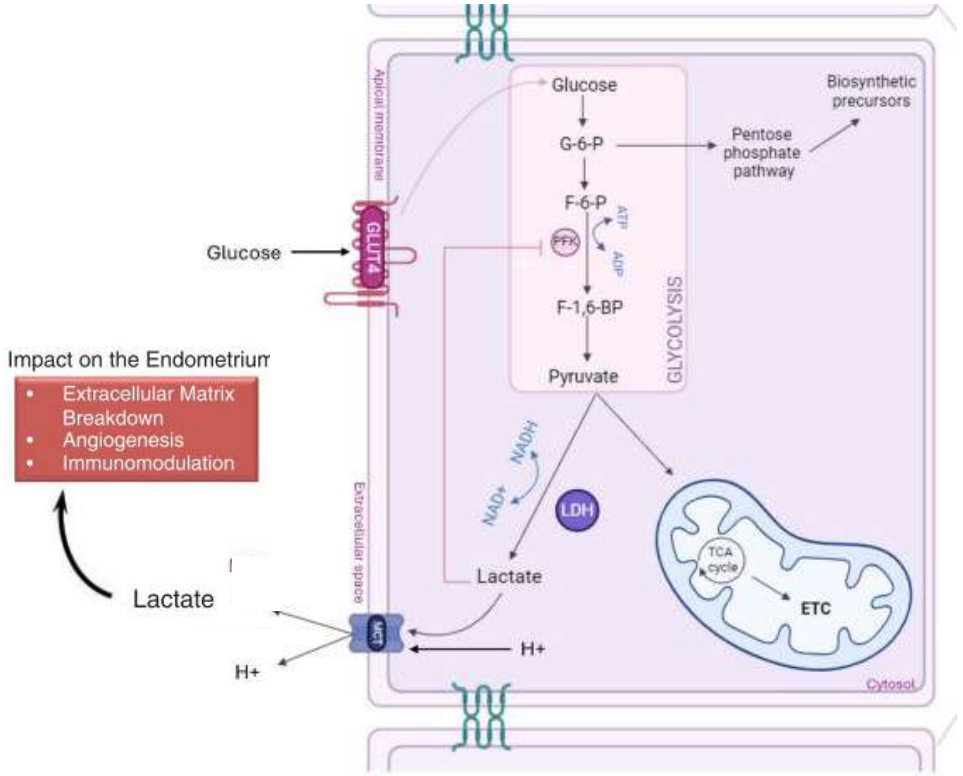
Review

Blastocyst-Derived Lactate as a Key Facilitator of Implantation

Kathryn H. Gurner [1] and David K. Gardner [1,2,*]

Lactate secreted by the blastocyst plays an important role in creating a microenvironment (high lactate levels and reduced pH) that **facilitates implantation.**

Lactate secreted by the blastocyst may contribute to modulate the maternal immune system, favoring immune tolerance to **avoid embryo rejection.**

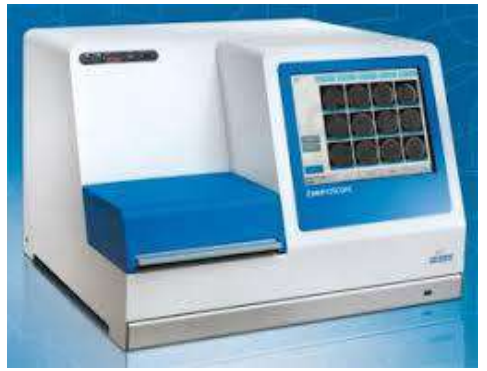


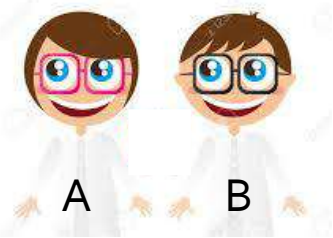
"Lactate can be considered a key factor in maternal-fetal communication to promote implantation and establishment of pregnancy. In fact, given its small size and high permeability, it could be the first embryonic signal that the endometrium receives from the embryo."

Culture Media Comparison

?

DATA	(596 eggs)
68 Patients	(8,8 eggs/pac)
48 Egg donors	(198 H-Lact) (194 IRV)
20 Autologous (34,1 – 43,6)	(99 H-Lact) (103 IRV)



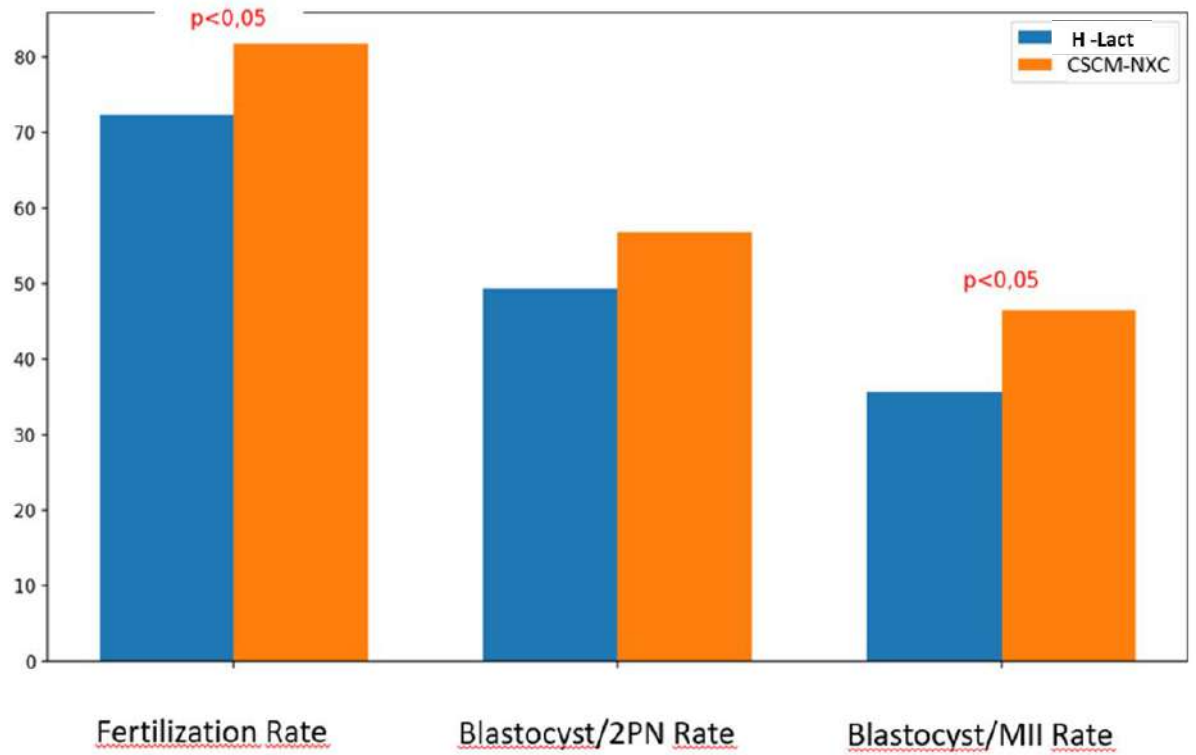


A	A	B	B					H - Lact
A	A	B	B					IRV

A	A	B	B	H - Lact
A	A	B	B	IRV

BIASES EVALUATION* (data vs control)						
	Fertilization	**OR (IC)**	**BL/2PN**	**OR (IC)**	**BL/MII**	**OR (IC)**
CONTROL	**77,1**		**53,3**		**41,1**	
EE2	78,6	1,09 (0,77-1,55)	53,4	1,04 (0,75-1,45)	42,7	1,07 (0,8-1,44)
EE3	75,9	0,94 (0,68-1,28)	52,4	0,96 (0,71-1,31)	39,7	0,95 (0,72-1,25)

EMBRYO CULTURE RESULTS						
CULTURE MEDIA	FERTILIZATION RATE	p value	BLAST/2PN	p value	BLAST/MII	p value
H - Lact	72,4	< 0,05	49,3	> 0,05	35,7	< 0,05
CSCM-NXC	81,8		56,8		46,5	



FROZEN EGG
DONATION

EGG DONORS (8 MII/patient)
H - Lact: 2,85 blastocyst/patient
IRV: 3,72 blastocyst/patient

EFFICIENCY (Means)

better results

Ongoing PR

(aprox. 50%)

2 blastocyst / delivery
(1,5 deliveries / patient)

main goal

EFFECTIVENESS

EFFICACY (Ends)

less resources

2 blastocyst / delivery
(2 deliveries / patient)

ULTRA-FAST WARMING

How important are warming process in our labs?

65.000 warming process/year (Registro SEF´22)

10 – 15´ average warming time

Flow hood cold and heated (in most of the protocols)

Important to FACILITATE process

2022

Day 5/6/7 Blastocysts Outcome (positive beta; cPR & oPR) per age after a one-step rapid-warming protocol of 1min in 1M TS

Table 1: Day 5/6/7 Blastocysts Outcome per age after a one-step warming protocol of 1min in 1M TS

	<35	35-37	38-40	>40	Total
n	65	40	33	38	176
pos.	53	33	21	25	132
%	81.5	82.5	63.6	65.8	75.0
cPR	50	31	19	18	118
%	76.9	77.5	57.6	47.4	67.0
oPR	49	31	17	17	114
%	75.4	77.5	51.5	44.7	64.8

* con permiso del autor

The Ultra-fast Warming (UFW) Protocol Offers Several Benefits

1. Greater efficiency in laboratory workflow

– UFW significantly reduces warming time (1' vs 11'). Liebermann et al. (2023)

2. Comparable reproductive outcomes

– Survival, re-expansion, and hatching rates between UFW and the conventional protocol show no significant differences. Shiolla et al. (2024)

– Clinical pregnancy rates are also comparable between both protocols. Jiang et al. (2023)

– UFW does not appear to induce additional embryonic stress or negatively impact implantation potential. Chaplia et al. (2023)

3. Decreased spontaneous miscarriage rates

– UFW has been associated with lower rates of spontaneous miscarriage. Liebermann et al. (2023)

4. Reduced handling-induced stress

– Simplifying the warming process minimizes embryo exposure to potentially harmful conditions.

– Fewer handling steps reduce the risk of temperature fluctuations and operator errors, which may negatively affect embryo viability.

Oocyte donation (bank) cases. No PGT

Single Freeze Blastocyst Transfers → Same proportion of each morphological category in both groups

Collapsed blastocysts before vitrification (PZD)

Assisted hatching before transfer (PZD)

Both warming protocols carried out in microdroplets → TS 300ul
DS 150ul / WS 150ul
(Vit Kit-Thaw – Irvine Scientific)

Protocol	FETs	Clinical PR	Miscarriage Rate	Ongoing PR
Conventional	149	76 (51.0%)	17 (22.4%)	59 (39.6%)
Ultra-fast Warming	79	48 (60.8%)	5 (10.4%)	43 (54.4%)

•**Clinical Pregnancy Rate:**

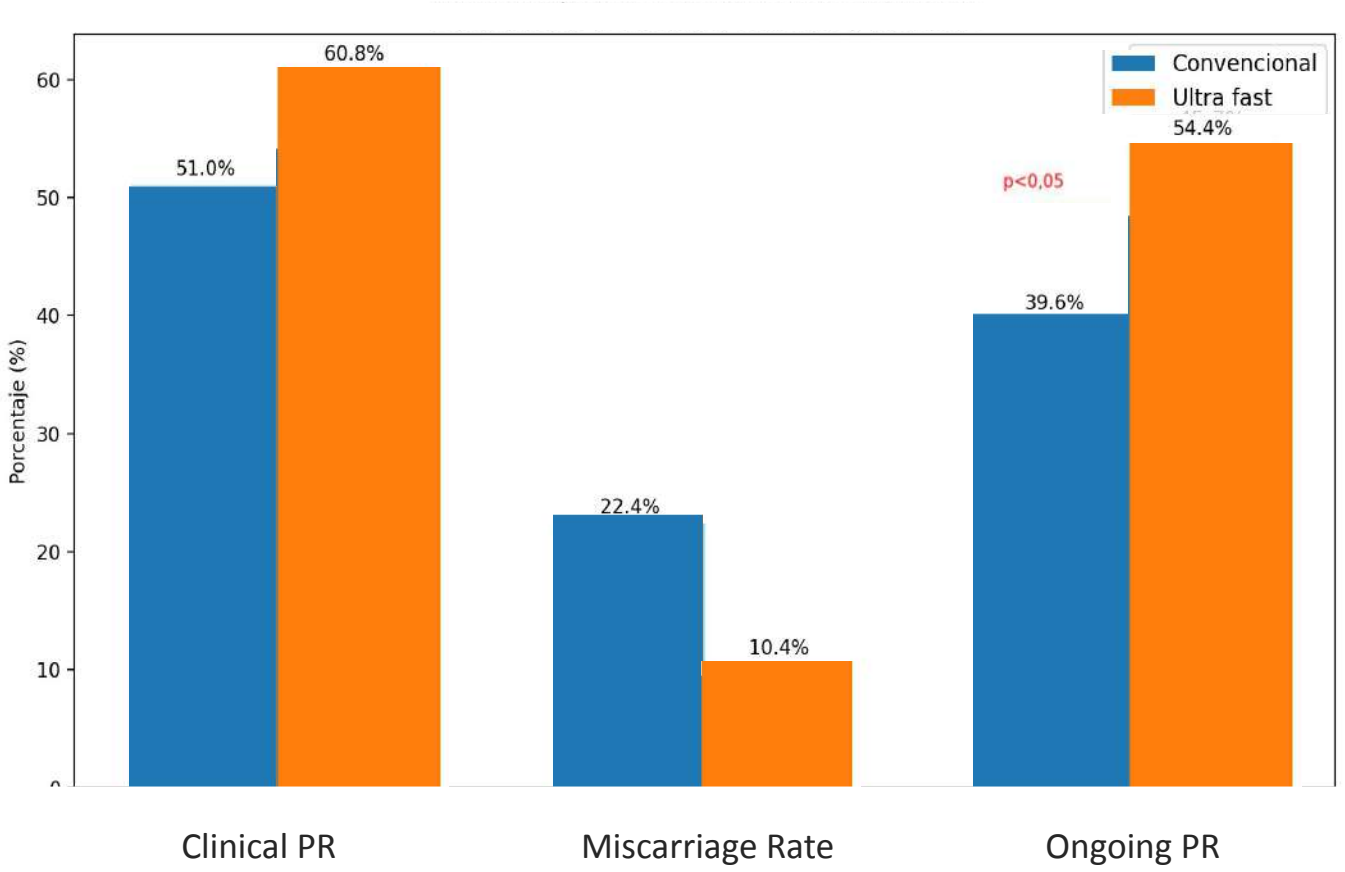
- Conventional: 51.01%
- Ultra – fast: 60.76% **(+ 9,75)**

•**Miscarriage Rate:**

- Conventional: 22.37%
- Ultra – fast: 10.42% **(-11,95)**

•**Ongoing Pregnancy Rate:**

- Conventional: 39.60%
- Ultra-fast: 54.43% **(+ 14,83)**



1. Ongoing PR:

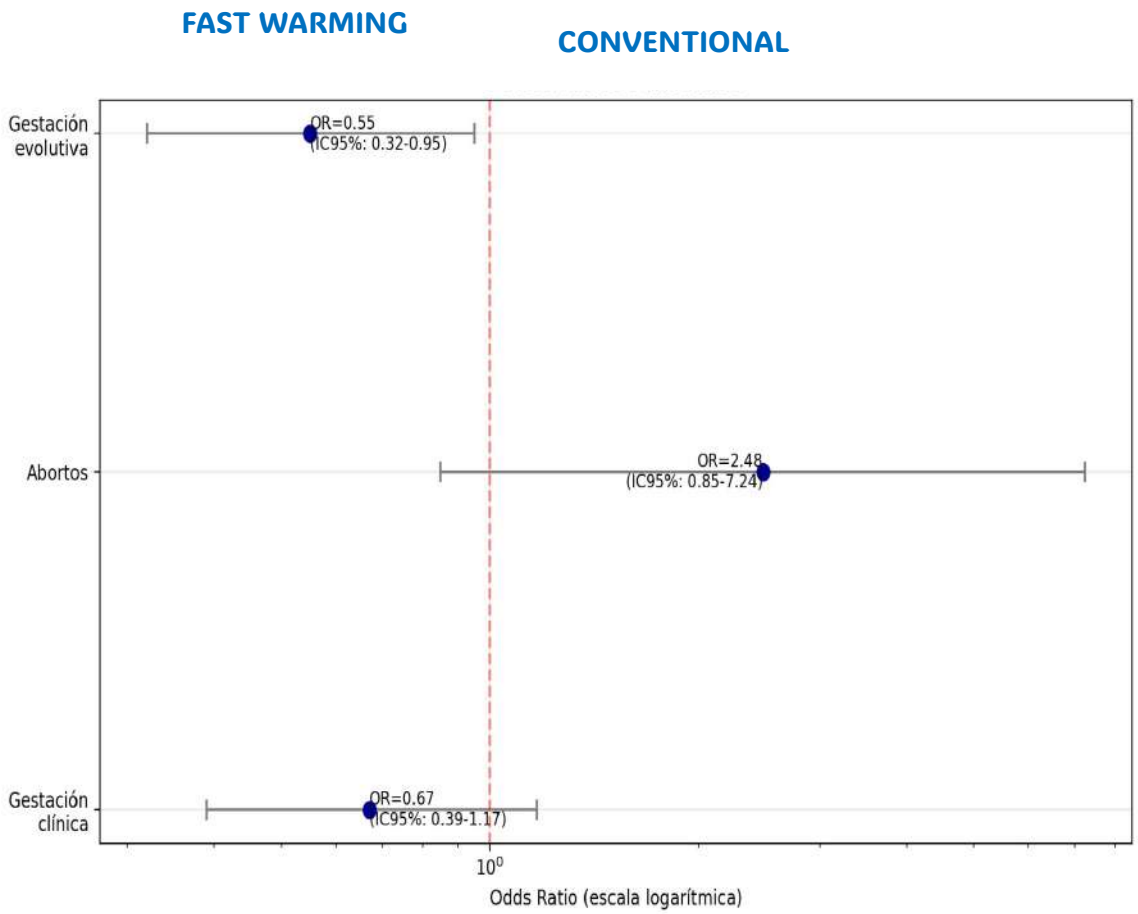
OR = 0.55 (IC 95%: 0.32 - 0.95)
Valor p = 0.0363

2.Miscarriage Rate:

OR = 2.48 (IC 95%: 0.85 - 7.24)
Valor p = 0.0986

3. Clinical PR:

OR = 0.67 (IC 95%: 0.39 - 1.17)
Valor p = 0.1658



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Vit Kit-Thaw Irvine Scientific

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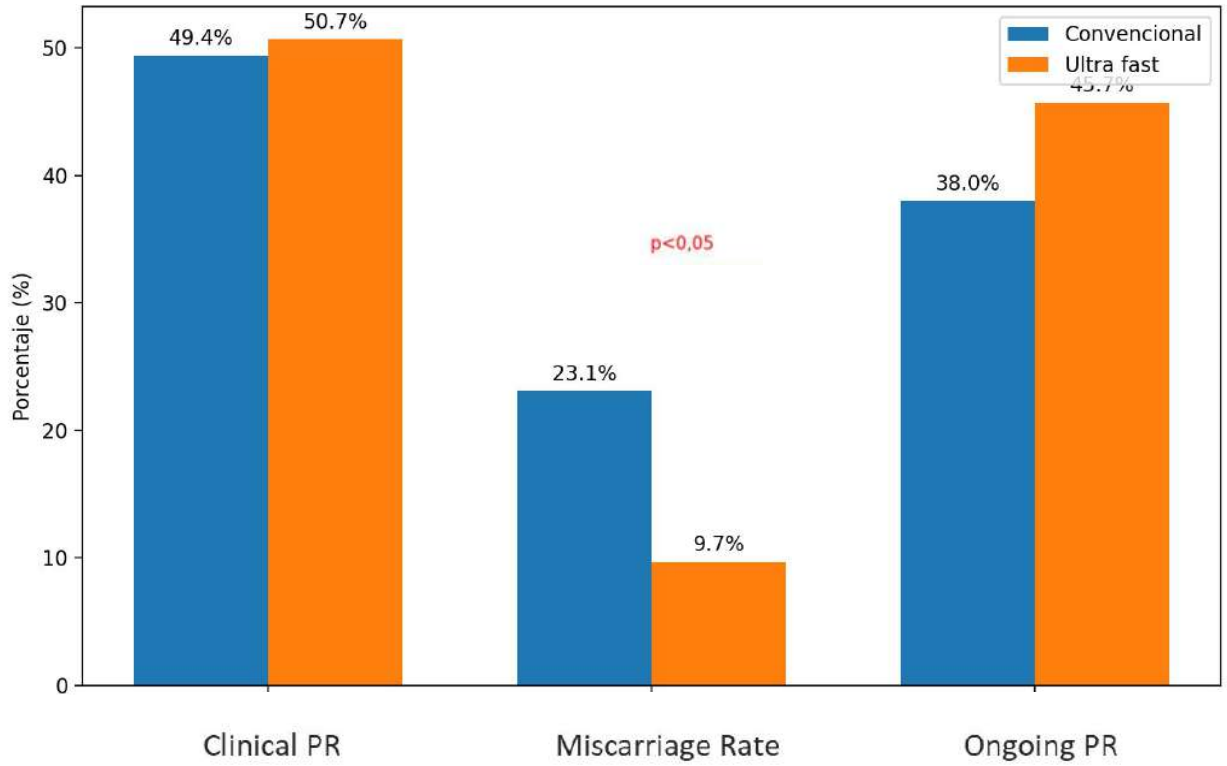
Protocol	FETs	Clinical PR	Miscarriage Rate	Ongoing PR
Conventional	184	91 (49,4%)	21 (23,1%)	70 (38%)
Ultra-fast Warming	142	72 (50,7%)	7 **(9,7%)***	65 (45,8%)

- **Clinical Pregnancy Rate:**
  - Conventional: 49,4%
  - Ultra-fast: 50,7%

- **Miscarriage Rate:**
  - Conventional: 23.1%
  - Ultra-fast: 9,7%

- **Ongoing Pregnancy Rate:**
  - Conventional: 38%
  - Ultra-fast: 45,7%



Ultra-fast Warming Protocol:

Lower miscarriage rate (-13.4%)

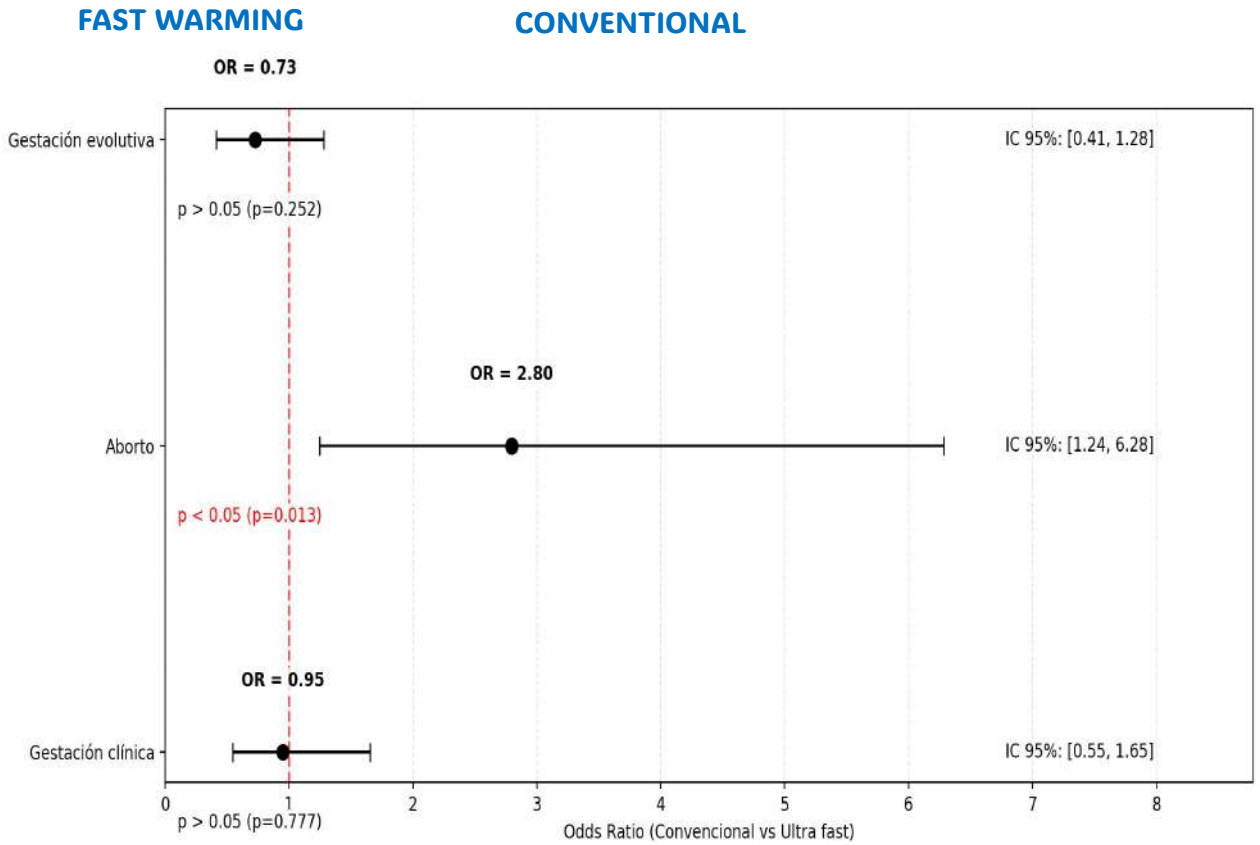
Higher ongoing pregnancy rate (+7.8%)

Ongoing PR:

OR: 0.73 (IC 95%: 0.41-1.28)
p: 0.25

Miscarriage R:
OR: 2.80 (IC 95%: 1.24-6,28)
p: 0.013

Clinical PR:
OR: 0,95 (IC 95%: 0.55-1.65)
p: 0.77



SUMMARY OF OUR EXPERIENCE

- Optimization of laboratory processes:

  ✓ less time
  ✓ fewer steps = lower risk

- Higher embryonic survival (in collapsed embryos)

- Faster expansion rate

- Higher hatching rate (assisted hatching)

Lower miscarriage rate

Universal post-warming dilution of vitrified embryos: impact of different vitrification/warming kits, warming volume and rapid dilution/rehydration steps on survival and clinical outcomes

Lodovico Parmegiani[1]*, Gabor Vajta[2], Colleen Lynch[3], Alessandra Arnone[1], Silvia Bernardi[1], Antonio Manuel Maccarini[1], Sara Lanzilotti[1], Azzurra Rastellini[1], Enzo Troilo[1], Elena Nardi[4] Walter Ciampaglia[1]

Research Question

Is the universal warming protocol (UW) efficient to warm vitrified human blastocysts when combining different brands of vitrification and warming kits, when reducing warming droplets volume and when using just a single rehydration step (universal single-step warming - USSW)?

Universal post-warming dilution of vitrified embryos: impact of different vitrification/warming kits, warming volume and rapid dilution/rehydration steps on survival and clinical outcomes

Lodovico Parmegiani[1]*, Gabor Vajta[2], Colleen Lynch[3], Alessandra Arnone[1], Silvia Bernardi[1], Antonio Manuel Maccarini[1], Sara Lanzilotti[1], Azzurra Rastellini[1], Enzo Troilo[1], Elena Nardi[4] Walter Ciampaglia[1]

Is posible to **combine various kits for vitrification/warming** (diferent brands: Kitazato, Sage, Irvine - Scientific, Vita-vitro)

The universal warming can be efficient and safe when **reducing the volumen of 1M sucrose/trehalose** solution

The universal warming can be efficient and safe when **rehydrating blastocyst with a single step**

(different brands / small drops / 1´) **Protocol for the future?**

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