Finding Blastocyst Vitrification and Warming Efficiencies

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MGH 1811
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VE RI TAS
HARVARD MEDICAL SCHOOL

IVF Demand Worldwide

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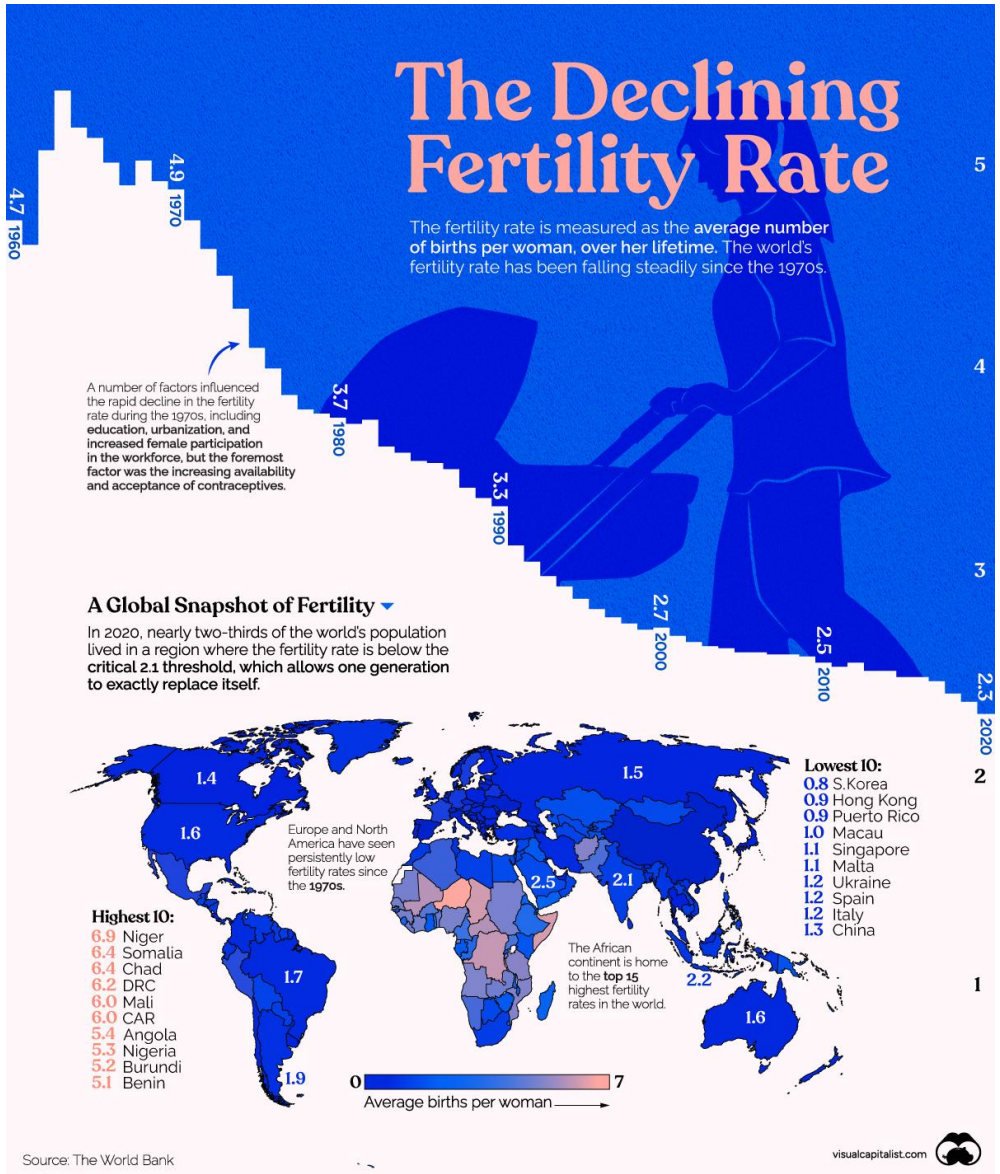
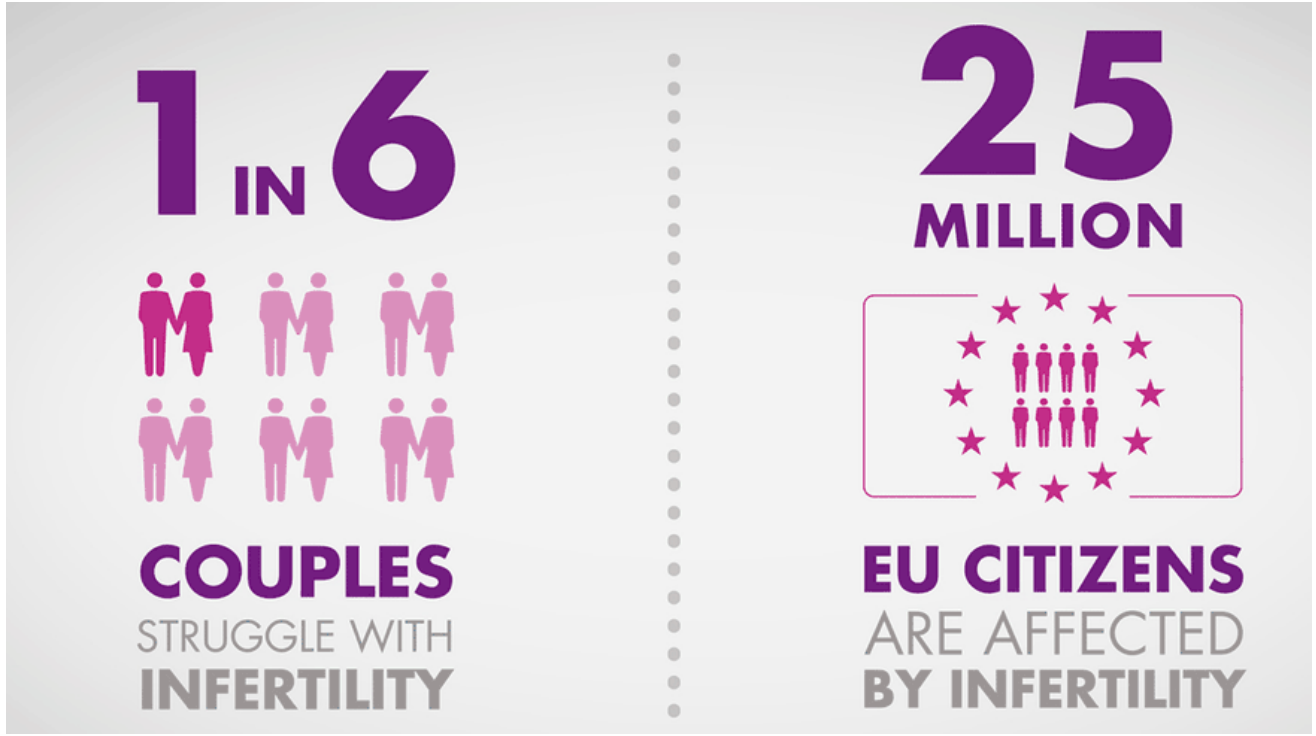


World Health Organization



SART Preliminary Data 2021-2022

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SART Cycles	2021	2022	% Increase
Total Cycles	301316	368502	22.3%
Embryo Banking for Fertility Preservation	5383	10345	92.2%
Oocyte Banking for Fertility Preservation	16786	24560	46.3%
Donor Oocyte Banking	1374	1944	41.5%
Thaw for Refreeze	4780	6809	42.4%

Estimated: USA needs to increase IVF cycles 10-fold to meet true demand for infertility services.

SART Preliminary Data 2022

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Age of woman	< 35	35 - 37	38 - 40	41 - 42	> 42
Number of cycle starts	51074	33093	33267	17035	12232
Cryopreservation Rate	87.1%	81.2%	72.8%	63.7%	47.3%

Requires Undivided Attention and Extensive Time Commitment

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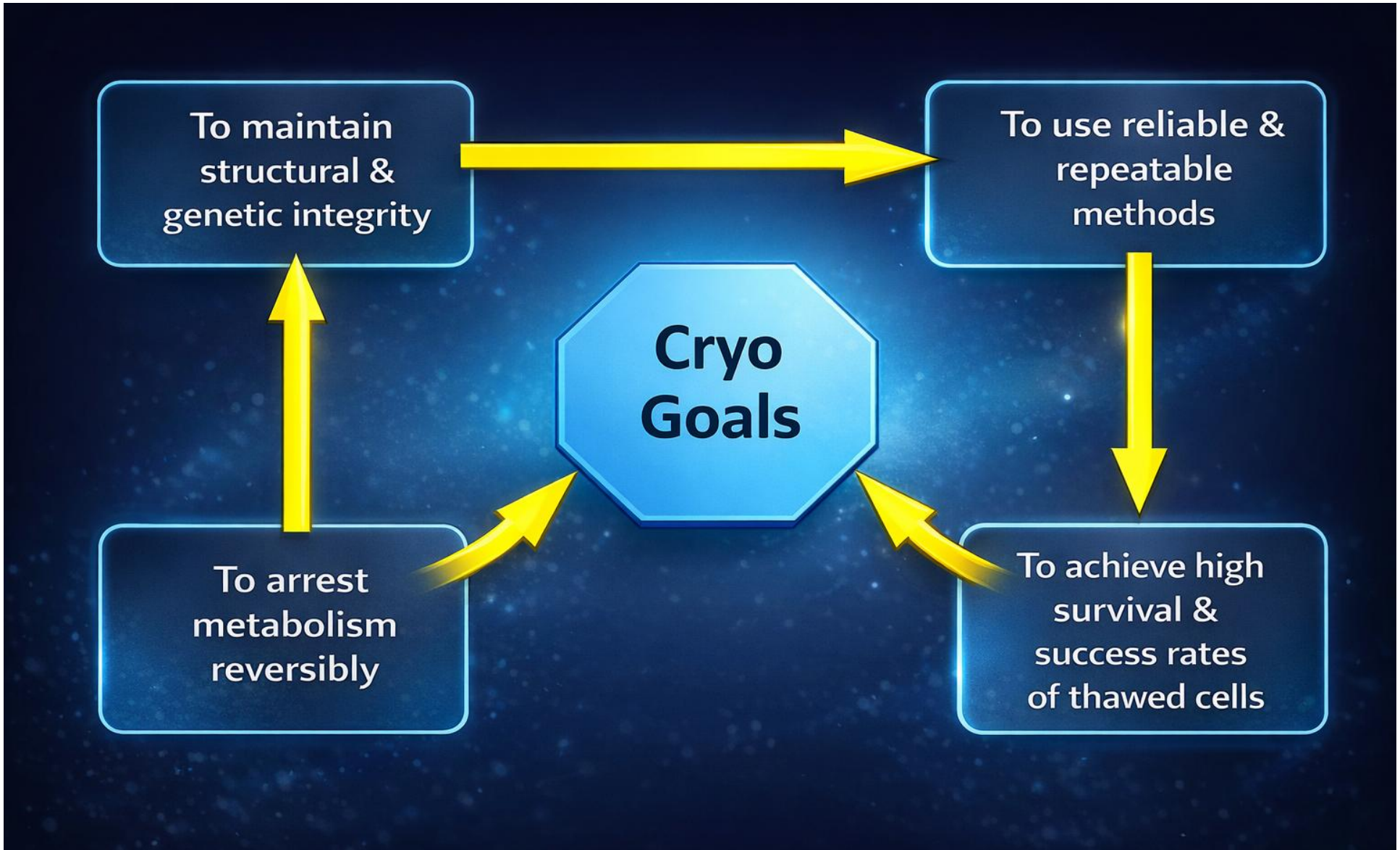
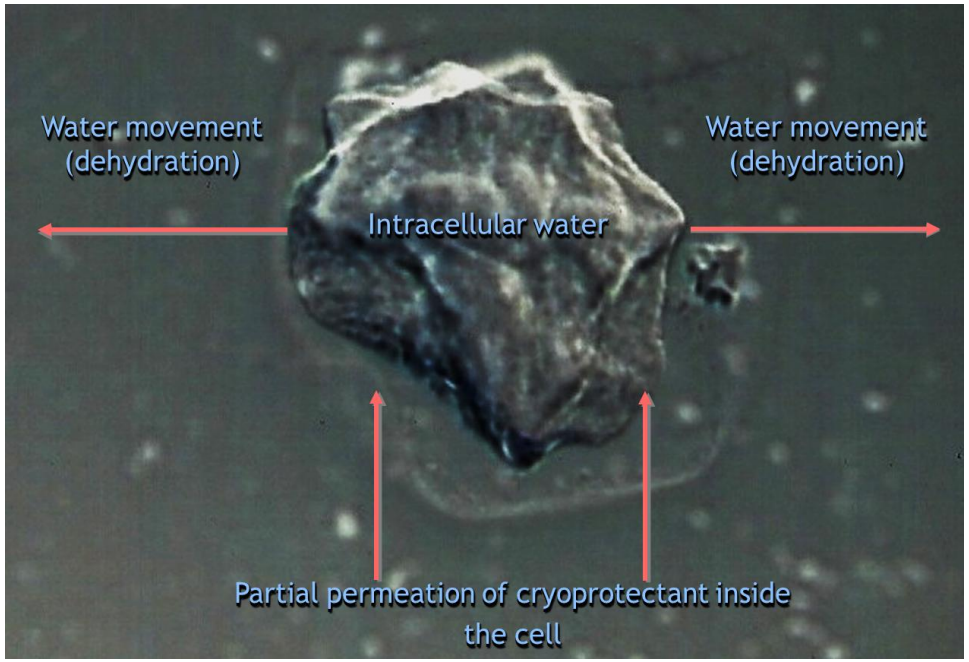
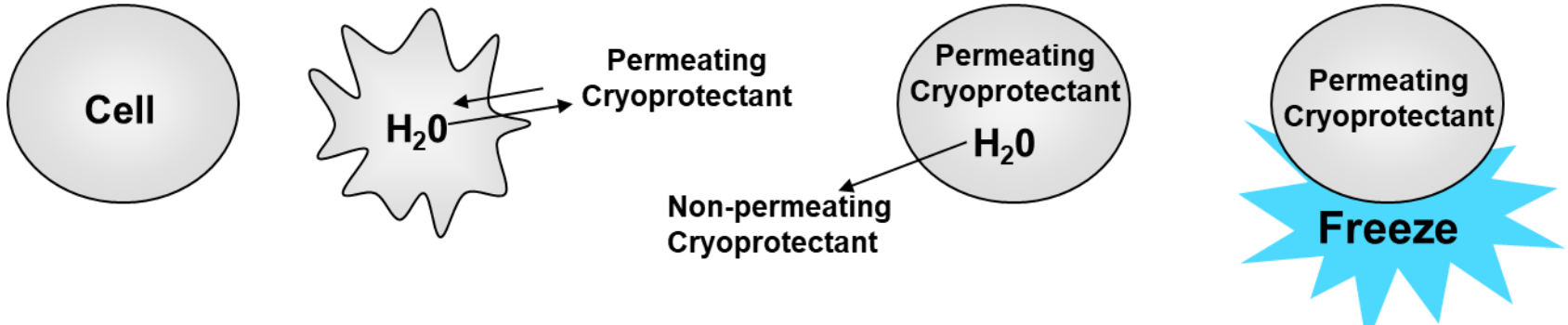
The Goals of Cryopreservation



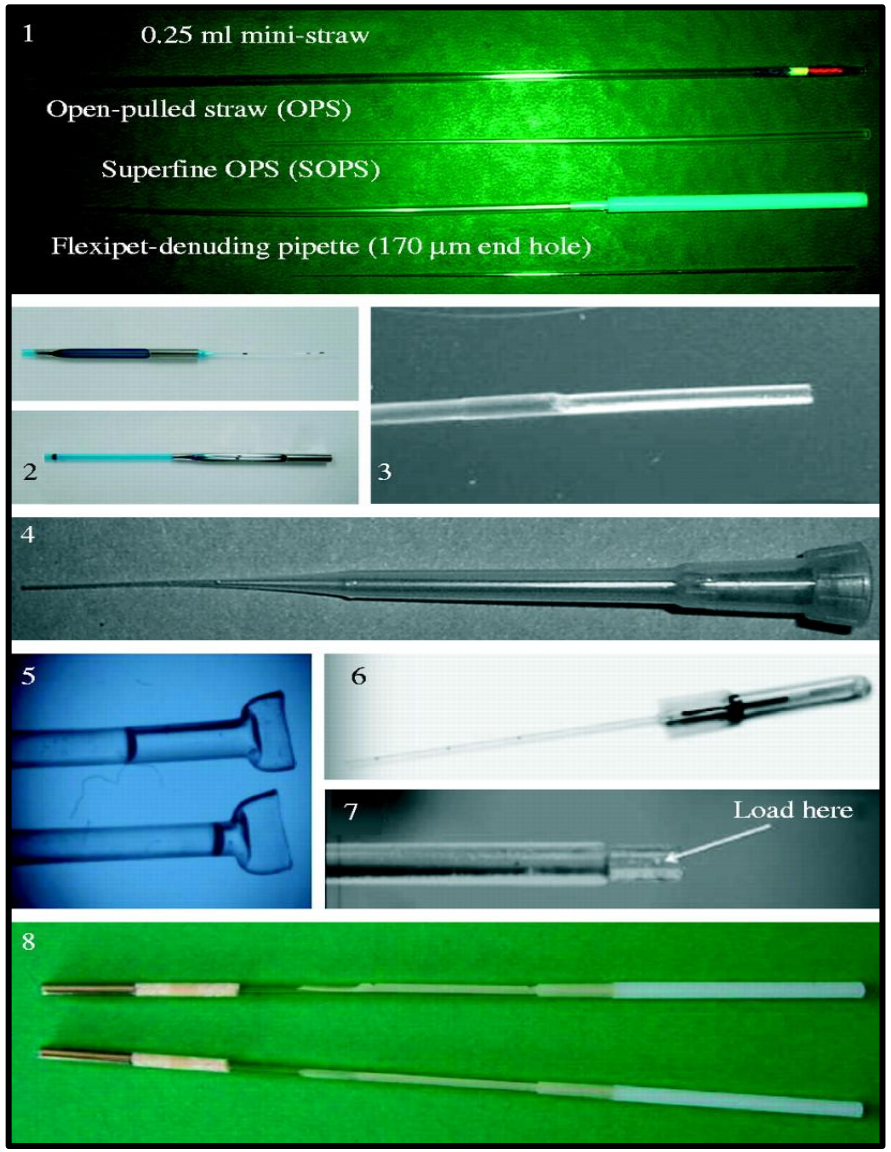
Underlying Principles for Cryo Success

- Freezing Procedure
  - Expose to cryoprotectants & dehydrate
  - Freeze/Cool from 37°C to -196°C without damage
  - Safe storage of the cryopreserved cells
- Thawing/Warming Procedure
  - Dilute/remove cryoprotectants
  - Thaw/Warm (rehydration)
- Return to Physiological Conditions





Vitrification Carrier Devices



"Open" Systems

Higher cooling rates (> 20,000°C) because no heat transfer

"Closed" Systems

No direct contact with LN2

Lower cooling rates due to reduced heat of transfer by the carrier wall

Gardner Embryo Grading

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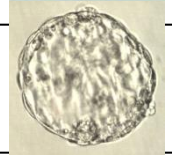
Grade		Stage	Description
1		Early Blastocyst	Blastocoele less than half the volume of the embryo, little or no expansion in overall size; ZP thick
2		Expanding Blastocyst	Blastocoele more than half the volume of the embryo, some expansion in overall size; ZP beginning to thin
3		Full Blastocyst	Blastocoele completely filling embryo; ZP not completely thinned
4		Expanded Blastocyst	Blastocoele completely filling embryo; fully expanded embryo and ZP very thin
5		Hatching Blastocyst	Hatching blastocyst, TE starting to herniate through the ZP
6		Hatched Blastocyst	Blastocyst completely hatched (i.e. completely out of the ZP)

Implement strict embryo grading criteria to help determine which embryos are best suited for vitrification. Your criteria may change based on success!

Gardner Embryo Grading

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ICM Grade	Description
A	ICM prominent & easily discernible with many cells, and cells compacted and tightly adhered together
B	ICM discernible but with fewer cells, and loosely adherent together
C	Very few cells visible, either compacted or loose, may be difficult to distinguish completely from TE
D	No ICM cells discernible in any focal plane or ICM cells appear degenerate or necrotic
TE Grade	**Description**
A	A continuous layer of small uniform eye-shaped cells bordering the blastocoele
B	Fewer, larger cells that may not form a continuous layer
C	Sparse TE cells, may be large
D	All TE cells degenerate

Freeze Criteria: ≥ 3BC or 3CB (no CC or D grades)

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Embryos Grades Stage 1-2 (too early for MGH vitrification)

Freeze Criteria: ≥ 3BC or 3CB (no CC or D grades)

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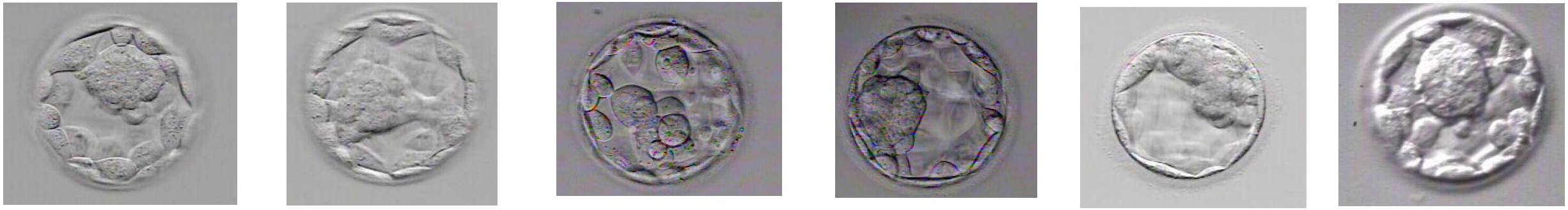
Embryos Grades Stage 1-2 (too early for MGH vitrification)

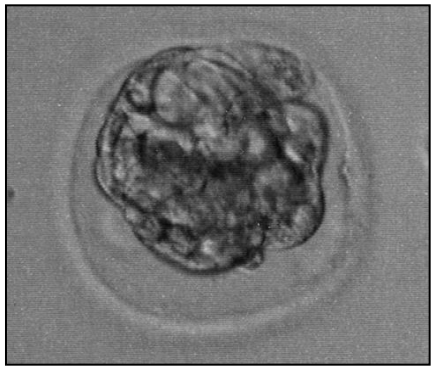


Embryos Grades Stage 3-4 (meets MGH vitrification criteria)

Pre-Vitrification Artificial Shrinkage of Blastocysts

Parameter	No Shrinkage	Shrinkage
Number of patients	76	245
Blastocyst survival rate	85.0%	97.2%*
Clinical pregnancy rate	34.1%	60.2%**

** P<0.05; ** P<0.01*



Mukaida et al, 2006

Collapsing with a Laser

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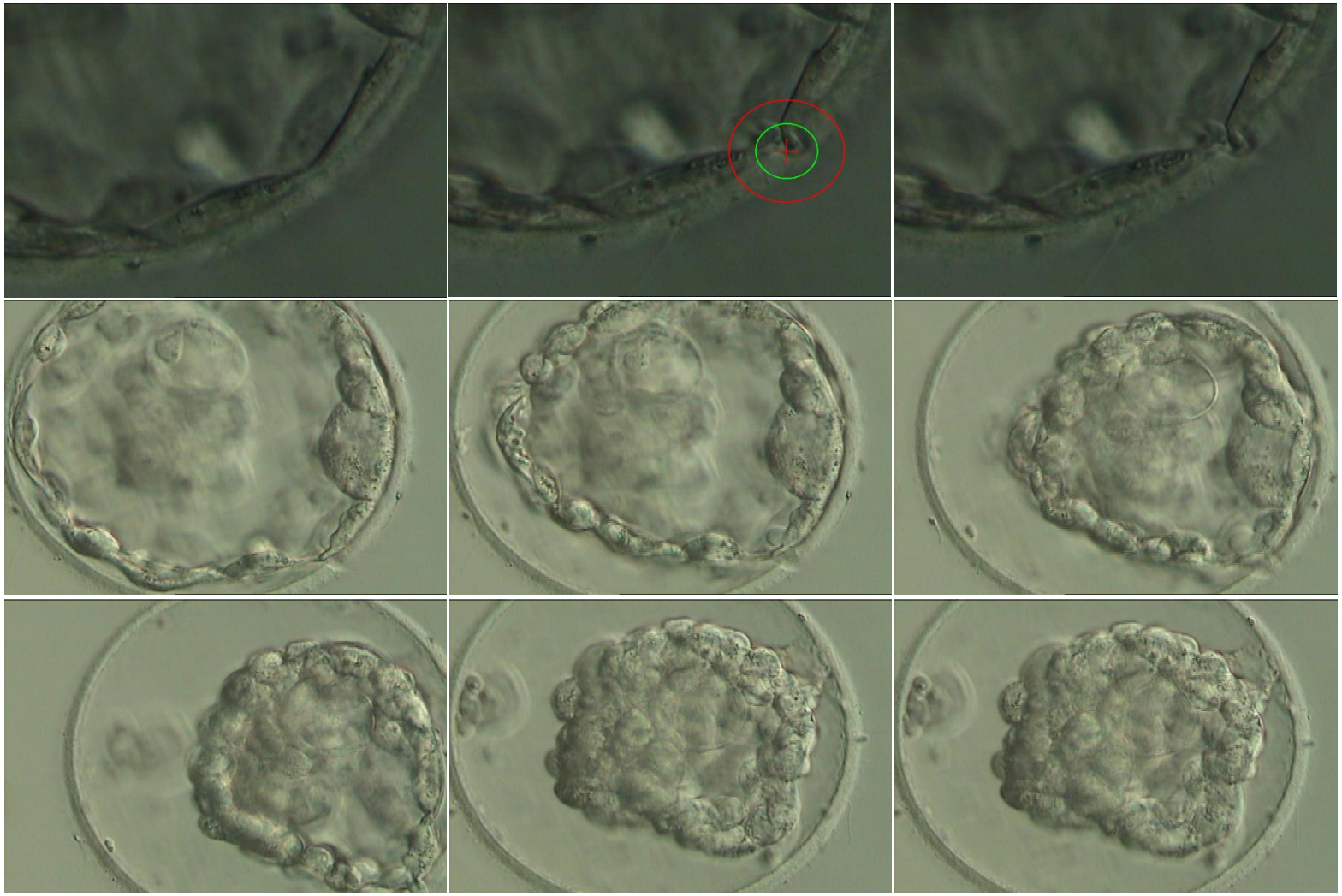

Joe Conaghan, 2013

Blastocyst Vitrification Timing

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biology

MDPI

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Article

A Shorter Equilibration Period Improves Post-Warming Outcomes after Vitrification and in Straw Dilution of In Vitro-Produced Bovine Embryos

Iris Martínez-Rodero [1], Tania García-Martínez [1], Erika Alina Ordóñez-León [1,2], Meritxell Vendrell-Flotats [1,3], Carlos Olegario Hidalgo [4], Joseba Esmoris [5], Xabier Mendibil [5], Sabino Azcarate [5], Manel López-Béjar [3], Marc Yeste [6] and Teresa Mogas [1,*]

Equilibration Solution: 12 min → 3 min

Table 2. Post-warming survival and hatching rates of day seven (D7) and day eight (D8) expanded blastocysts vitrified after shorter or longer exposure to the equilibration solution. Data are shown as mean ± standard error of the mean (SEM).

	Day 7 Blastocysts				Day 8 Blastocysts			
	n	Survival (%) (3 h)	Survival (%) (24 h)	Hatching Rate (%) (24 h)	n	Survival (%) (3 h)	Survival (%) (24 h)	Hatching Rate (%) (24 h)
Control	86	100 ^{a,A}	100 ^{a,A}	35.9 ± 4.0 ^{a,A}	40	100 ^{a,A}	100 ^{a,A}	50.0 ± 7.0 ^{a,B}
SE	86	60.6 ± 1.5 ^{b,A}	78.4 ± 2.0 ^{b,A}	31.4 ± 3.7 ^{a,A}	33	48.6 ± 5.3 ^{b,B}	63.0 ± 5.5 ^{b,B}	19.9 ± 2.7 ^{b,B}
LE	83	57.5 ±4.0 ^{b,A}	63.1 ± 2.6 ^{c,A}	10.1 ± 2.4 ^{b,A}	36	39.4 ± 4.7 ^{b,B}	55.3 ± 5.0 ^{c,B}	8.1 ± 2.7 ^{c,A}

^{a,b,c} Values within columns with different superscripts differ significantly ($p < 0.05$); ^{A,B} Same values within rows with different superscripts differ significantly ($p < 0.05$). Control: fresh non-vitrified expanded blastocysts; SE: expanded blastocysts vitrified after a short equilibration time (3 min); LE: expanded blastocysts vitrified after a long equilibration time (12 min).

Vitrification Kit: NX

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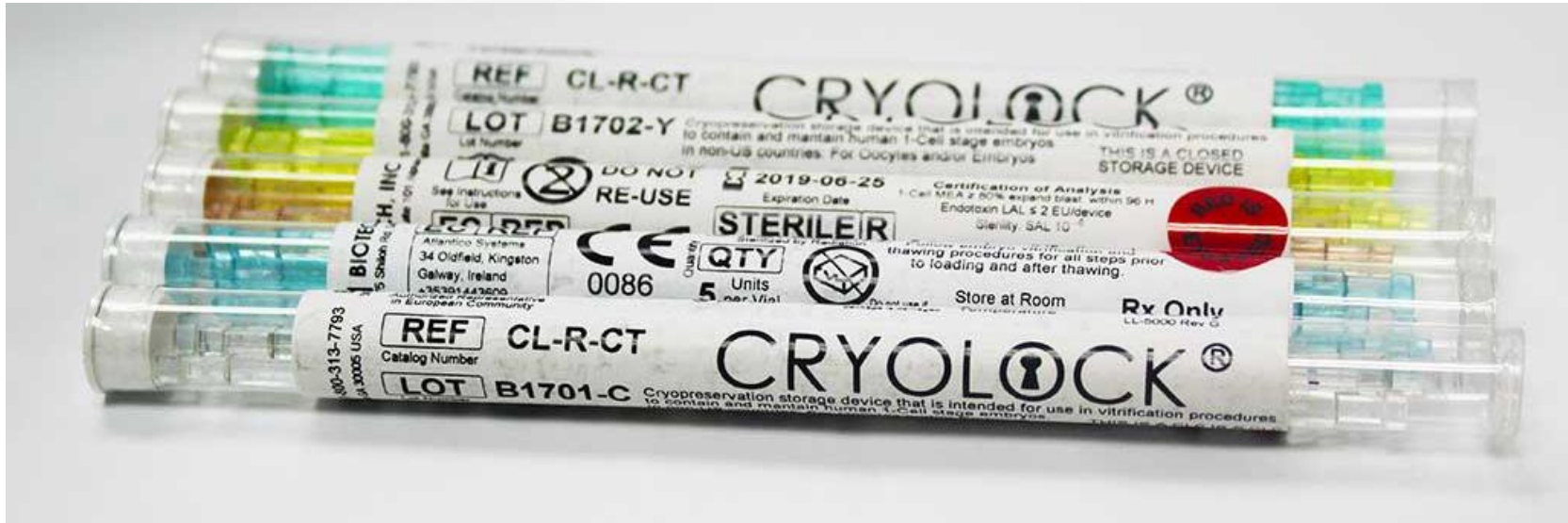

Standard Vitrification Protocol

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1. Aseptically dispense one (1) 50 µL drop of ES.

2. Transfer embryo(s) (2 maximum), to the ES drop and expose undisturbed for 6-10 minutes.

***NOTE**: The specimen(s) will shrink and then gradually return to original size, indicating that equilibration is complete.*

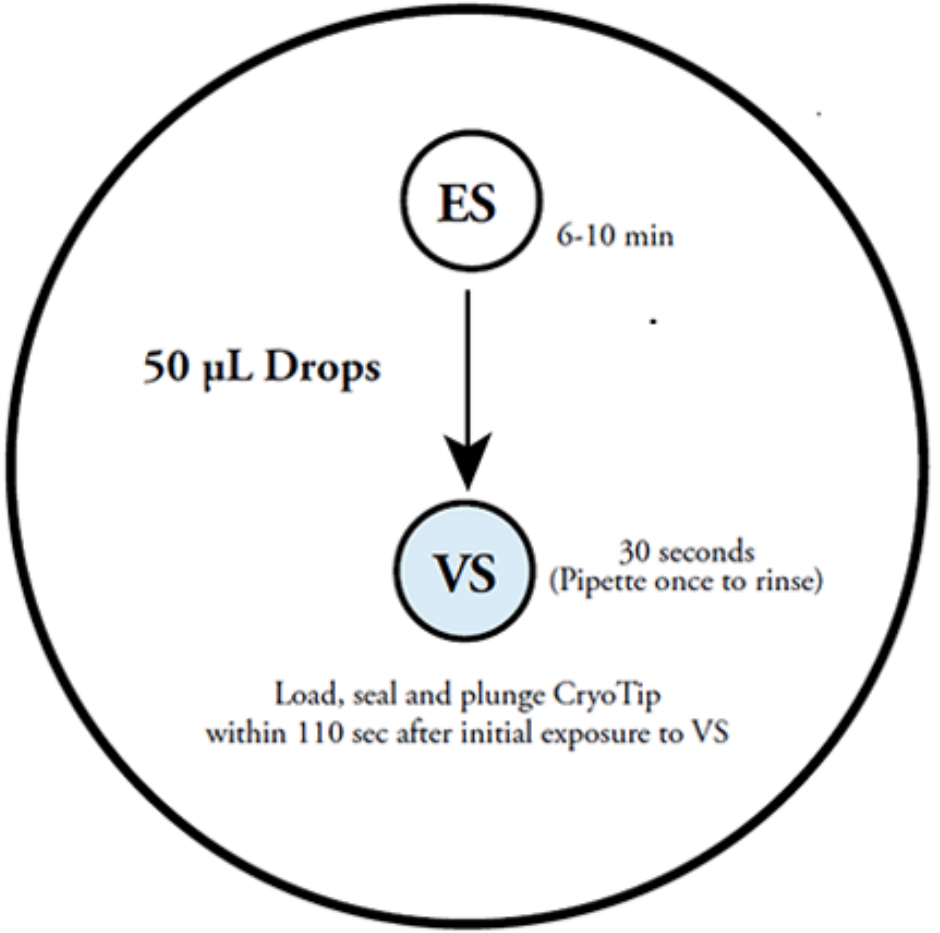
3. During above equilibration in ES, aseptically dispense one (1) 50 µL drop of VS 2 minutes prior to complete equilibration.

4. Transfer embryo(s) with minimal volume of medium from ES to the VS drop for 30 seconds before loading.

5. Gently pipette embryo(s) once within VS drop to ensure complete rinse with VS.

***NOTE**: To minimize floating, after 10 seconds pipette the specimen(s) to the bottom center of the VS drop.*

6. Load, seal and plunge device into LN_2 within 80 seconds, not to exceed 110 seconds after initial exposure to VS.



https://www.irvinesci.com/media/IrvineScientific/Resources/0/0/002777_embryo_vitrification_protocol.pdf

Fast Vitrification Protocol

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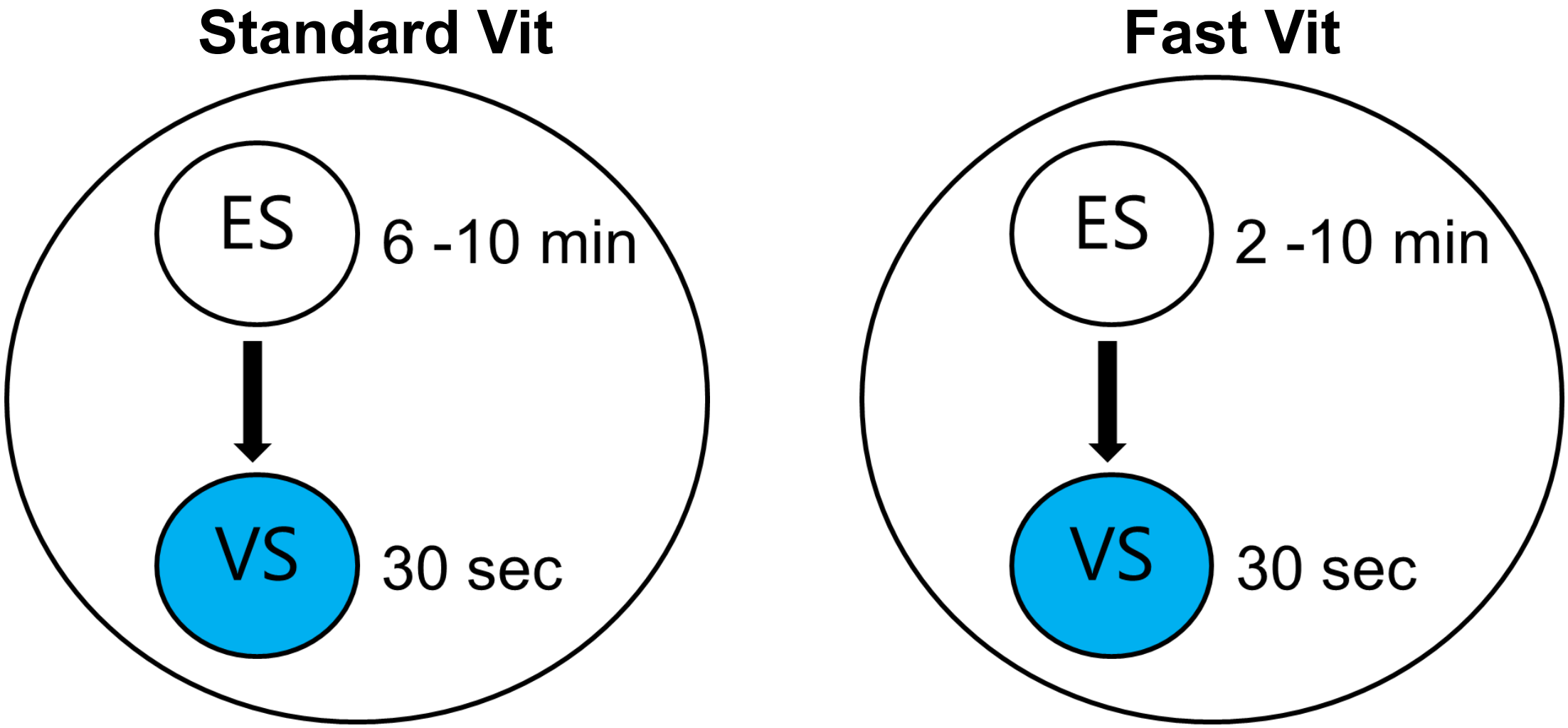

Load, seal and plunge cryo device within 110 sec after initial exposure to VS

Overall Pregnancy Outcomes with Fast Vitrification

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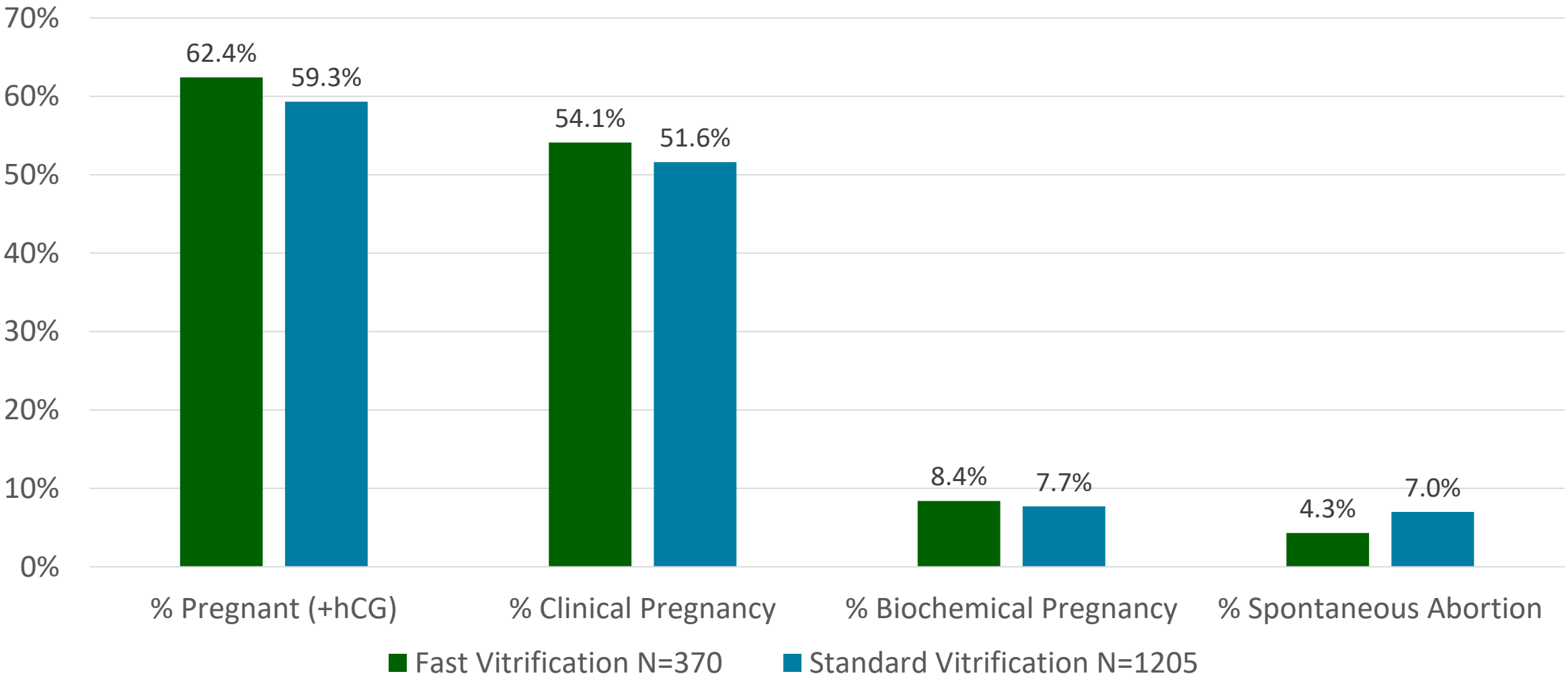

Impact of Day 5 vs Day 6 Vitrification

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Day of Vit	Outcome	Fast (%)	Standard (%)	p-Value	Significance
Day 5	% Pregnant (+hCG)	65.7%	61.6%	0.25	Not Significant
	% Clinical Pregnancy	58.2%	54.5%	0.31	Not Significant
	% Biochemical Pregnancy	7.5%	7.1%	0.96	Not Significant
	% Spontaneous Abortion	4.5%	6.5%	0.28	Not Significant

Impact of Day 5 vs Day 6 Vitrification

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Day of Vit	Outcome	Fast (%)	Standard (%)	p-Value	Significance
Day 5	% Pregnant (+hCG)	65.7%	61.6%	0.25	Not Significant
	% Clinical Pregnancy	58.2%	54.5%	0.31	Not Significant
	% Biochemical Pregnancy	7.5%	7.1%	0.96	Not Significant
	% Spontaneous Abortion	4.5%	6.5%	0.28	Not Significant
Day 6	% Pregnant (+hCG)	53.9%	51.3%	0.74	Not Significant
	% Clinical Pregnancy	43.1%	41.6%	0.87	Not Significant
	% Biochemical Pregnancy	10.8%	9.7%	0.91	Not Significant
	% Spontaneous Abortion	3.9%	8.6%	0.18	Not Significant

Blastocyst Stage at time of Vitrification

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Blastocyst Stage	Outcome	Fast (%)	Standard (%)	p-Value	Significance
Stages 3-4	% Pregnant (+hCG)	62.6%	59.6%	0.35	Not Significant
	% Clinical Pregnancy	54.3%	51.9%	0.47	Not Significant
	% Biochemical Pregnancy	8.3%	7.7%	0.80	Not Significant
	% Spontaneous Abortion	4.0%	7.7%	0.02	Significant

Blastocyst Stage at time of Vitrification

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Blastocyst Stage	Outcome	Fast (%)	Standard (%)	p-Value	Significance
Stages 3-4	% Pregnant (+hCG)	62.6%	59.6%	0.35	Not Significant
	% Clinical Pregnancy	54.3%	51.9%	0.47	Not Significant
	% Biochemical Pregnancy	8.3%	7.7%	0.80	Not Significant
	% Spontaneous Abortion	4.0%	7.7%	0.02	Significant
Stages 5-6	% Pregnant (+hCG)	59.1%	58.5%	1	Not Significant
	% Clinical Pregnancy	50.0%	50.7%	1	Not Significant
	% Biochemical Pregnancy	9.1%	7.7%	1	Not Significant
	% Spontaneous Abortion	9.1%	4.4%	0.63	Not Significant

Impact of Inner Cell Mass Grade

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ICM Grade	Outcome	Fast (%)	Standard (%)	p-Value	Significance
A	% Pregnant (+hCG)	66.9%	65.1%	0.75	Not Significant
	% Clinical Pregnancy	60.5%	57.9%	0.62	Not Significant
	% Biochemical Pregnancy	6.4%	7.2%	0.85	Not Significant
	% Spontaneous Abortion	2.9%	6.7%	0.10	Not Significant

Impact of Inner Cell Mass Grade

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ICM Grade	Outcome	Fast (%)	Standard (%)	p-Value	Significance
A	% Pregnant (+hCG)	66.9%	65.1%	0.75	Not Significant
	% Clinical Pregnancy	60.5%	57.9%	0.62	Not Significant
	% Biochemical Pregnancy	6.4%	7.2%	0.85	Not Significant
	% Spontaneous Abortion	2.9%	6.7%	0.10	Not Significant
B	% Pregnant (+hCG)	57.9%	57.1%	0.89	Not Significant
	% Clinical Pregnancy	48.2%	49.2%	0.87	Not Significant
	% Biochemical Pregnancy	9.7%	7.9%	0.50	Not Significant
	% Spontaneous Abortion	5.6%	7.2%	0.55	Not Significant

Impact of Inner Cell Mass Grade

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ICM Grade	Outcome	Fast (%)	Standard (%)	p-Value	Significance
A	% Pregnant (+hCG)	66.9%	65.1%	0.75	Not Significant
	% Clinical Pregnancy	60.5%	57.9%	0.62	Not Significant
	% Biochemical Pregnancy	6.4%	7.2%	0.85	Not Significant
	% Spontaneous Abortion	2.9%	6.7%	0.10	Not Significant
B	% Pregnant (+hCG)	57.9%	57.1%	0.89	Not Significant
	% Clinical Pregnancy	48.2%	49.2%	0.87	Not Significant
	% Biochemical Pregnancy	9.7%	7.9%	0.50	Not Significant
	% Spontaneous Abortion	5.6%	7.2%	0.55	Not Significant
C	% Pregnant (+hCG)	100%	34.8%	0.12	Not Significant
	% Clinical Pregnancy	66.7%	26.1%	0.44	Not Significant
	% Biochemical Pregnancy	33.3%	8.7%	0.76	Not Significant
	% Spontaneous Abortion	0%	4.3%	1	Not Significant

Impact of Trophectoderm Grade

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Trophectoderm Grade	Outcome	Fast (%)	Standard (%)	p-Value	Significance
A	% Pregnant (+hCG)	74.3%	73.3%	1	Not Significant
	% Clinical Pregnancy	64.3%	68.1%	0.68	Not Significant
	% Biochemical Pregnancy	10.0%	5.2%	0.31	Not Significant
	% Spontaneous Abortion	4.3%	8.1%	0.45	Not Significant

Impact of Trophectoderm Grade

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Trophectoderm Grade	Outcome	Fast (%)	Standard (%)	p-Value	Significance
A	% Pregnant (+hCG)	74.3%	73.3%	1	Not Significant
	% Clinical Pregnancy	64.3%	68.1%	0.68	Not Significant
	% Biochemical Pregnancy	10.0%	5.2%	0.31	Not Significant
	% Spontaneous Abortion	4.3%	8.1%	0.45	Not Significant
B	% Pregnant (+hCG)	60.5%	59.2%	0.77	Not Significant
	% Clinical Pregnancy	52.9%	51.2%	0.68	Not Significant
	% Biochemical Pregnancy	7.6%	8.0%	0.92	Not Significant
	% Spontaneous Abortion	3.8%	6.0%	0.21	Not Significant

Impact of Trophectoderm Grade

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Trophectoderm Grade	Outcome	Fast (%)	Standard (%)	p-Value	Significance
A	% Pregnant (+hCG)	74.3%	73.3%	1	Not Significant
	% Clinical Pregnancy	64.3%	68.1%	0.68	Not Significant
	% Biochemical Pregnancy	10.0%	5.2%	0.31	Not Significant
	% Spontaneous Abortion	4.3%	8.1%	0.45	Not Significant
B	% Pregnant (+hCG)	60.5%	59.2%	0.77	Not Significant
	% Clinical Pregnancy	52.9%	51.2%	0.68	Not Significant
	% Biochemical Pregnancy	7.6%	8.0%	0.92	Not Significant
	% Spontaneous Abortion	3.8%	6.0%	0.21	Not Significant
C	% Pregnant (+hCG)	54.1%	51.3%	0.89	Not Significant
	% Clinical Pregnancy	43.2%	43.3%	1	Not Significant
	% Biochemical Pregnancy	10.8%	8.0%	0.80	Not Significant
	% Spontaneous Abortion	8.1%	9.8%	0.97	Not Significant

Impact of PGT on Pregnancy Outcome

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PGT?	Outcome	Fast (%)	Standard (%)	p-Value	Significance
No PGT	% Pregnant (+hCG)	56.9%	57.4%	0.96	Not Significant
	% Clinical Pregnancy	48.9%	49.1%	1	Not Significant
	% Biochemical Pregnancy	8.0%	8.3%	0.99	Not Significant
	% Spontaneous Abortion	5.3%	7.8%	0.32	Not Significant

Impact of PGT on Pregnancy Outcome

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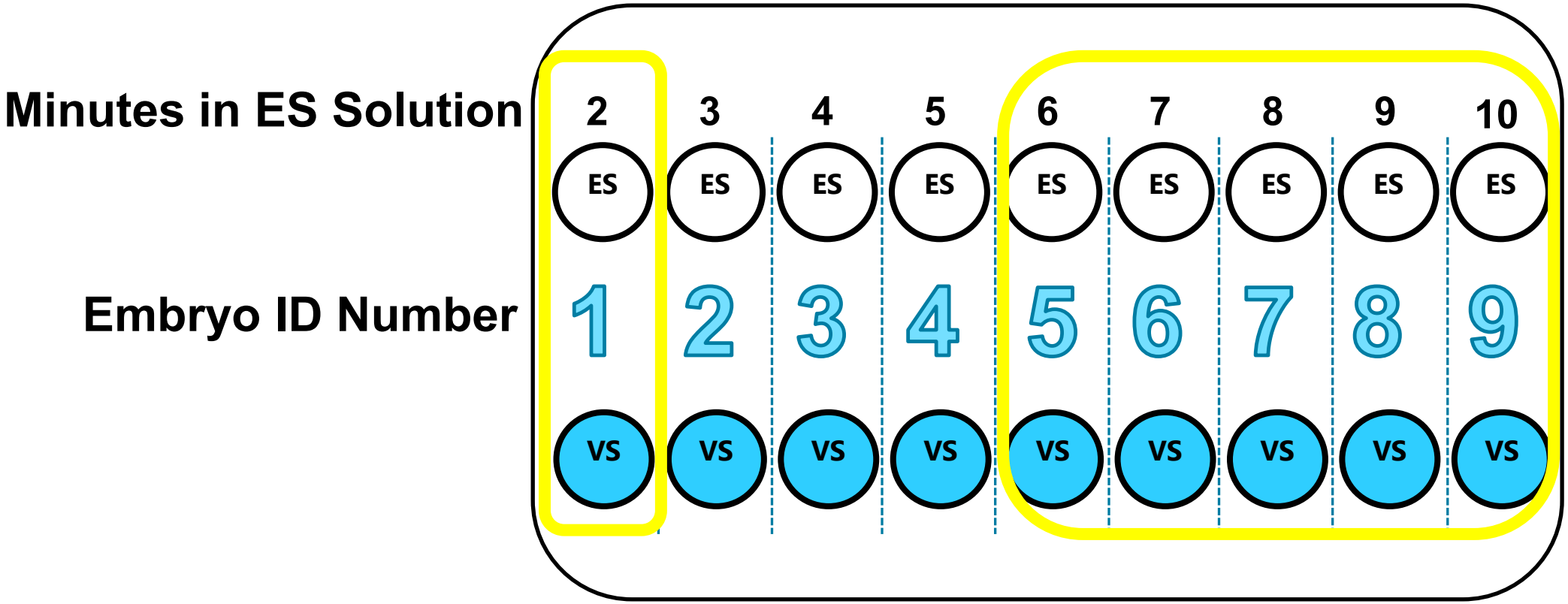
PGT?	Outcome	Fast (%)	Standard (%)	p-Value	Significance
No PGT	% Pregnant (+hCG)	56.9%	57.4%	0.96	Not Significant
	% Clinical Pregnancy	48.9%	49.1%	1	Not Significant
	% Biochemical Pregnancy	8.0%	8.3%	0.99	Not Significant
	% Spontaneous Abortion	5.3%	7.8%	0.32	Not Significant
With PGT	% Pregnant (+hCG)	68.1%	62.2%	0.18	Not Significant
	% Clinical Pregnancy	59.3%	55.4%	0.40	Not Significant
	% Biochemical Pregnancy	8.8%	6.8%	0.48	Not Significant
	% Spontaneous Abortion	3.3%	5.8%	0.27	Not Significant

Dish Setup for Multi Embryo Vitrification



2 Minute Vitrification vs Standard Vitrification

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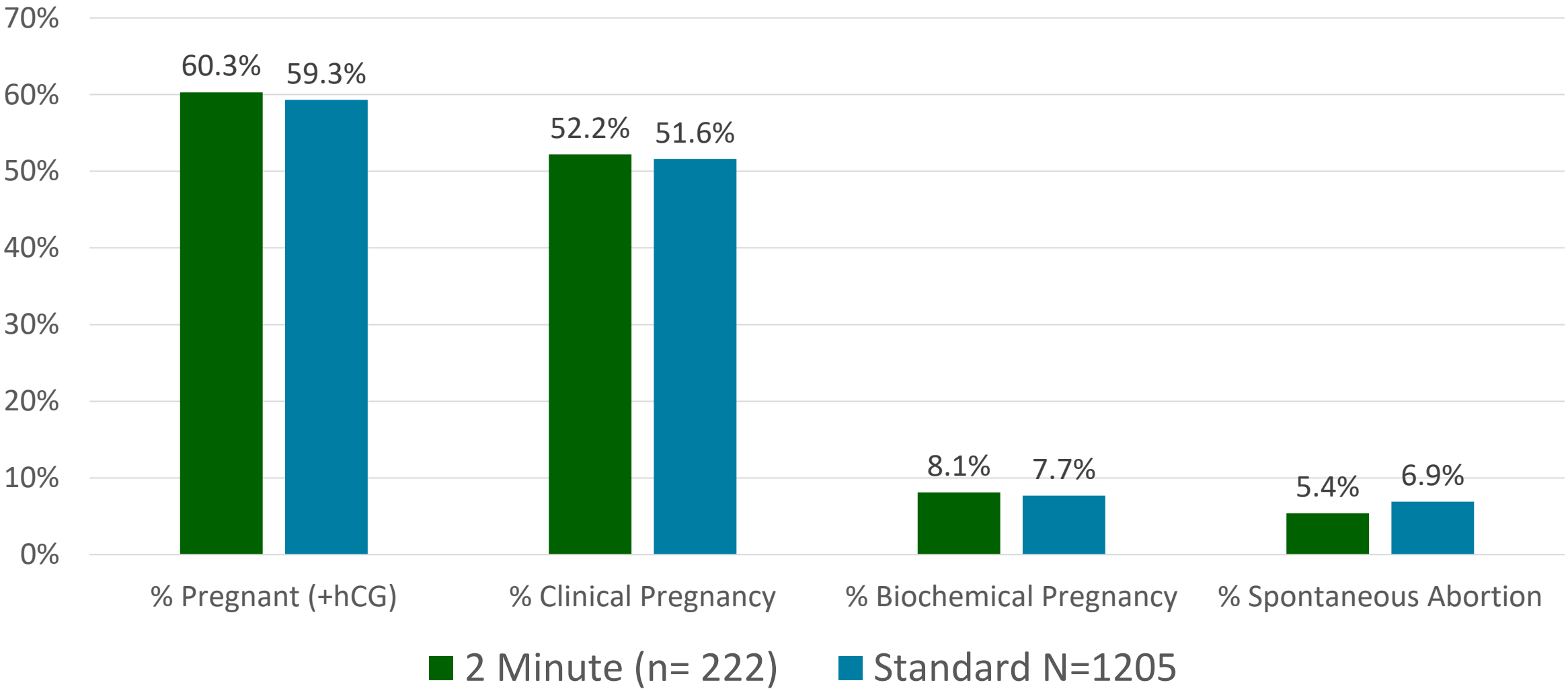
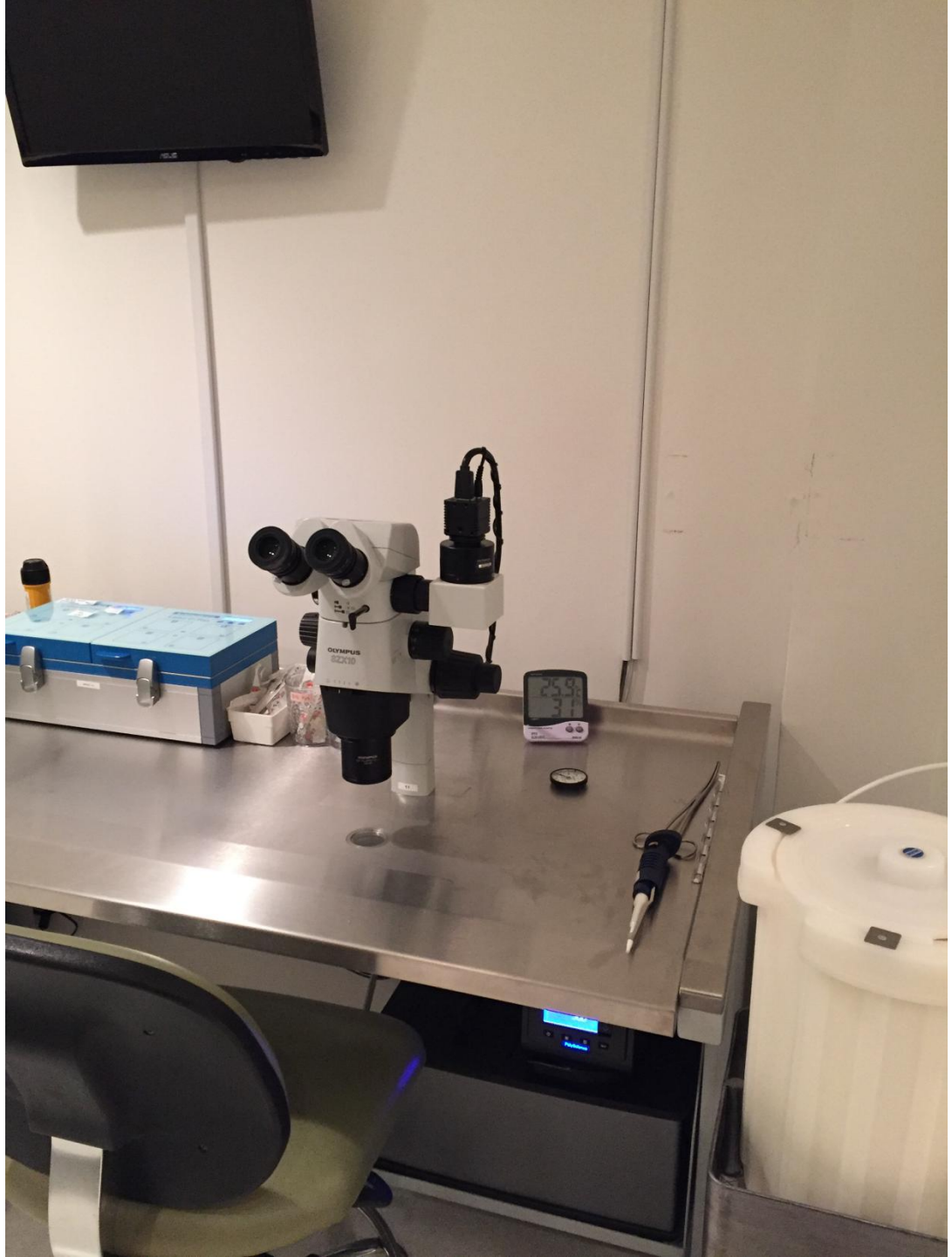
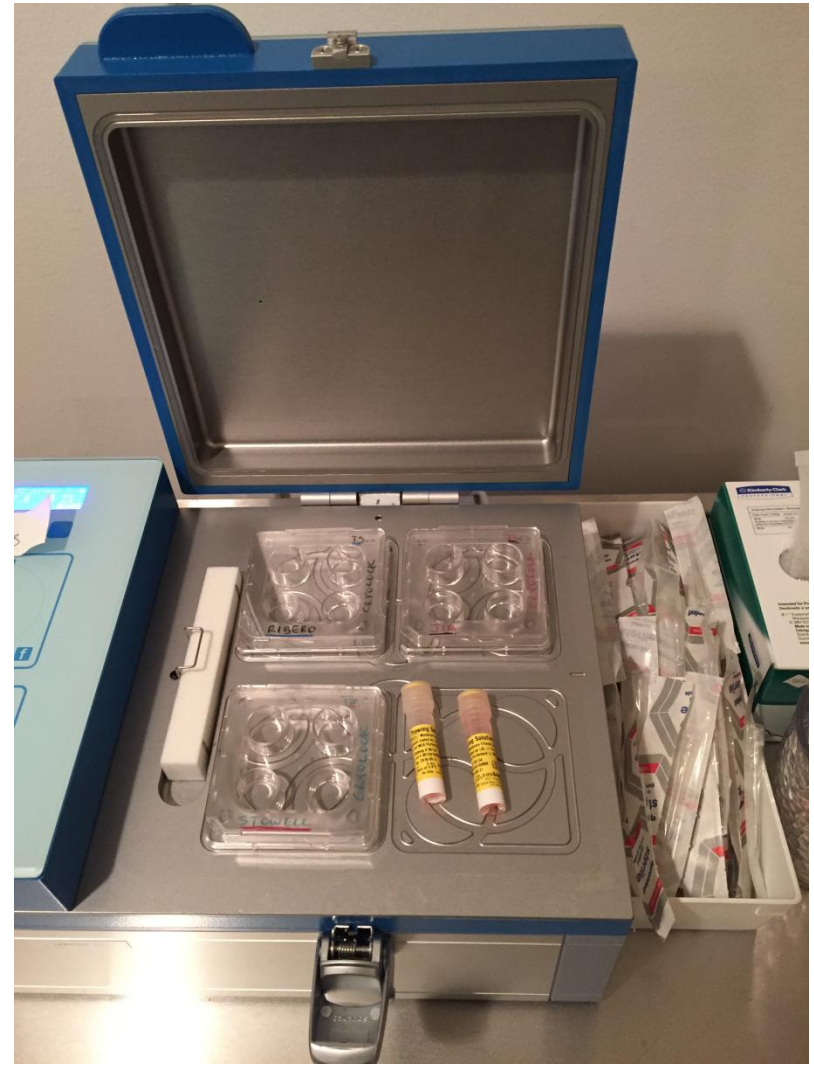
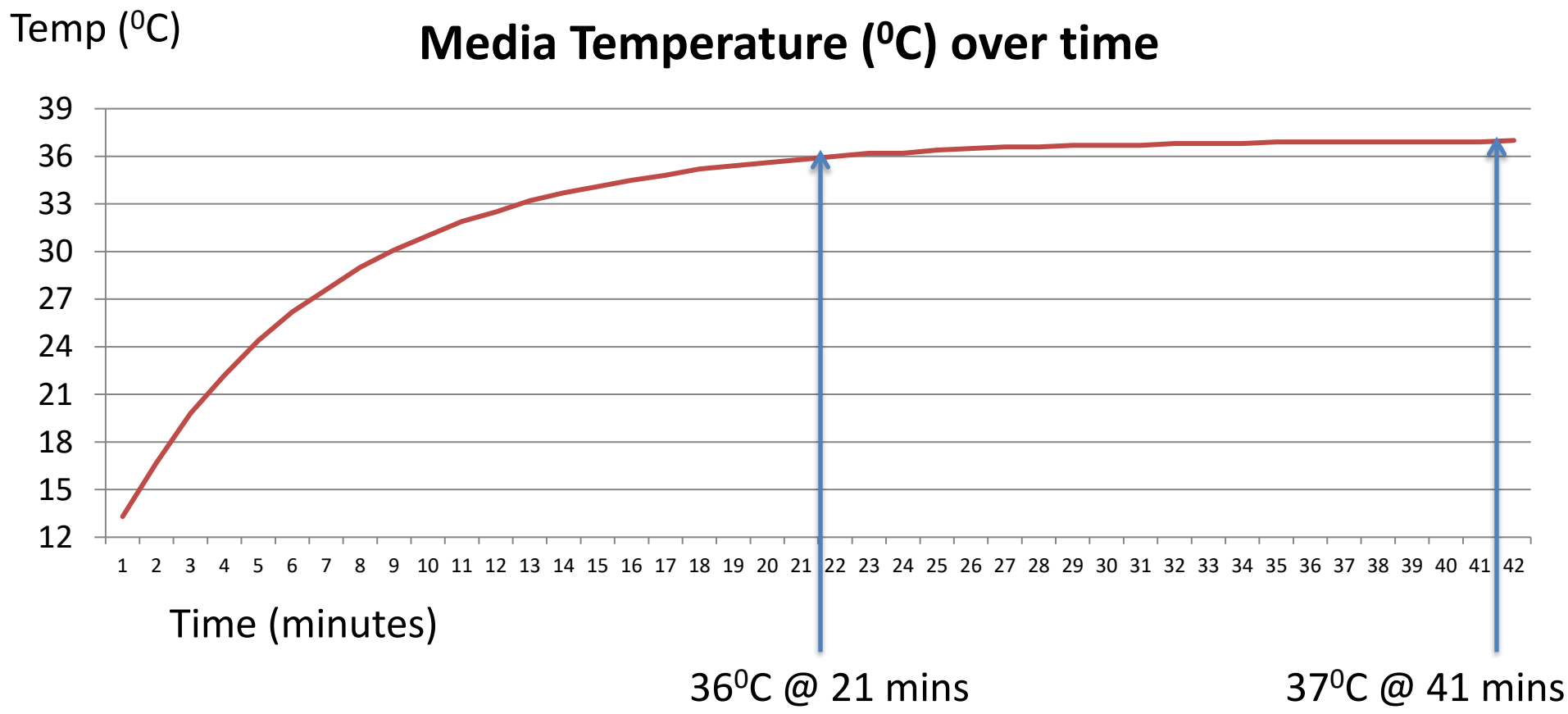


Warming Rate: Critically Important

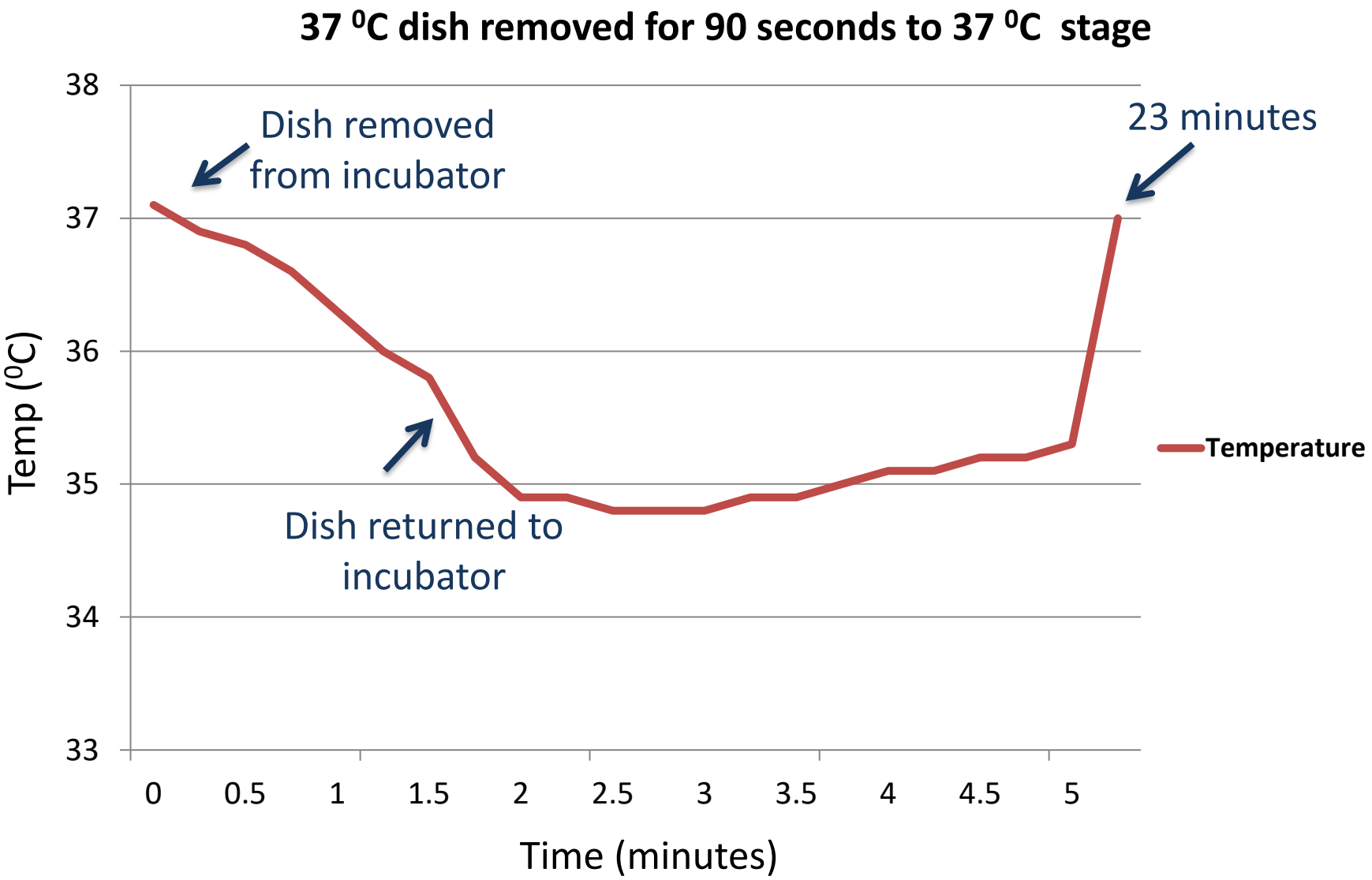

OLYMPUS
SZX10


RIBERO
CASTELLER
STEWELL

Nunc 4-well dish prepared with cold TS (1ml)



Nunc 4-well dish with 1ml TS



Blastocyst Warming Timing

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CLINICAL VALIDATION OF A NEW, ULTRAFAST WARMING PROTOCOL, RESULTING IN EQUIVALENT IMPLANTATION RATES AND SIGNIFICANT TIME SAVINGS VERSUS ROUTINE WARMING PROTOCOL, A PROSPECTIVE RANDOMIZED CONTROL.

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BACKGROUND: Over time, the procedures within the IVF lab have become more labor intensive resulting in longer procedure times and embryologist burnout. One of these procedures is embryo warming. Although not technically difficult, it takes approximately 15 minutes for an embryo to be moved between TS (thawing solution), DS (dilute solution), and WS (washing solution). We have modified the standard warming technique to merely moving the embryo to TS solution for 1 min and then immediately into culture media. This method eliminates the need for DS and WS while also decreasing time needed to complete the warming process.

OBJECTIVE: It is the objective of this study to compare outcomes of embryos warmed with the standard protocol (15 minutes) versus embryos warmed with the ultrafast protocol (1 min).

MATERIALS AND METHODS: All embryos were warmed with Fujifilm® warming media. Only patients undergoing a frozen embryo transfer (FET) of a single euploid blastocyst were included in this study. Patients were randomized between two groups: embryos warmed with the standard protocol (Group 1; 1 minute TS, 4 minutes DS, 8 minutes WS, and transfer to culture media) and embryos warmed using the ultrafast protocol (Group 2; 1 minute TS and transfer to culture media). All blastocysts were transferred utilizing standard of care protocols. Embryology time savings, pregnancy rates, and implantation rates were compared between groups.

RESULT(S): A total of 100 patients were randomized between the two groups; 51 in group 1 and 49 in group 2. The average age between group 1 and group 2 was not significant, 35.6±4.4 and 35.9±4.4 years, respectively (NS). Pregnancy rates between group 1 and group 2 was not significant, 34/51 (66.7%) and 40/49 (83.3%), respectively (0.1395). Implantation rate between group 1 and group 2 was not significant, 25/51 (49.0%) and 30/49 (62.5%), respectively (0.3052).

CONCLUSION(S): This study demonstrates that a significantly quicker embryo warming technique provides comparable pregnancy and implantation rates, while saving over 14 minutes of tech time per warming, a total of 1,400 tech minutes per 100 thaws. Additionally, there is a trend towards higher pregnancy and implantation rates with the ultrafast warming. Further studies are currently being conducted to determine if this trend is significant.

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Warming Oocytes and Embryos Protocol with Vit Kit - Warm NX

The following protocol is for use of Vit Kit - Warm NX (PN 90183) with vitrification/cryostorage devices that require direct plunge/contact into Thawing Solution. Vit Kit - Warm NX contains Thawing NX - TS (TS), Dilution NX - DS (DS), and Washing NX - WS (WS).

Have all necessary materials, tools, and equipment ready and easily accessible before starting procedure. The warming steps include plunging the device into the 37°C TS and subsequent diluting and washing in DS and WS at room temperature.

INITIAL PREPARATION

1. Set-up thawing dish (as shown in Figure 1):
   - **At 37°C:** Aseptically dispense a minimum volume of 1 mL of TS and warm to 37°C in a humidified incubator without CO_2 or on a heating stage at least 30 minutes prior to starting warming procedure.
2. Identify the device sample(s) to be warmed and quickly transfer from LN_2 storage to an LN_2 filled holding reservoir in preparation for warming procedure.
3. Place LN_2 filled holding reservoir in close proximity to the working area and stage of the microscope in order to achieve subsequent rapid manipulation from reservoir to TS.
4. Prepare the device for warming by referring to corresponding device IFU and internal laboratory procedure(s).
   - *Laboratory should consult their own procedures and protocols.*
5. Remove TS dish from 37°C incubator without CO_2 or heating stage and place it under focus on top of the microscope stage.
6. After specimen(s) are in TS following the device-specific protocol, leave the specimen(s) for a total of 1 minute.
   - Thirty (30) seconds following exposure into TS, gently pipette the specimen(s) if floating, and place at the bottom of the TS.
   - **At room temperature:** Aseptically dispense one (1) 50 µL drop of DS on a sterile Petri dish (see Figure 2).

Steps 7–10 must be performed at room temperature (20–27°C).

7. Transfer specimen(s) to DS for 4 minutes. Gently pipette specimen(s) once to ensure complete rinse in DS.
   - *The specimen(s) will remain shrunken during exposure to DS.*
8. During the 4-minute exposure in DS, aseptically dispense two (2) 50 µL drops of WS (WS1, WS2) as shown in Figure 2.
9. Transfer specimen(s) to WS1 then WS2 for 4 minutes each, undisturbed.
   - *The specimen(s) should rehydrate and reconstitute perivitelline space to the original size within 2–3 minutes in WS.*
10. Process the specimen(s) as indicated below:

    a) OOCYTE(S) should be transferred to pre-equilibrated culture medium in accordance with laboratory protocol for recovery (2–3 hours to allow time for spindle re-formation) prior to subsequent manipulations.

    b) There are two options for warmed EMBRYO(S):

    i) For immediate transfer to patient: Transfer EMBRYO(S) to pre-equilibrated transfer medium.

    ii) For further culture: Transfer EMBRYO(S) to pre-equilibrated culture medium for a 4-hour recovery period. After recovery period, transfer EMBRYO(S) to culture medium with 10% (v/v) protein (10% v/v when using Serum Substitute Supplement or Dextran Serum Supplement; 5% v/v when using Human Serum Albumin) and incubate accordingly until desired developmental stage has been reached for transfer to patient.

For additional details on the use of these products, each laboratory should consult its own laboratory procedures and protocols which have been specifically developed and optimized for your individual medical program.

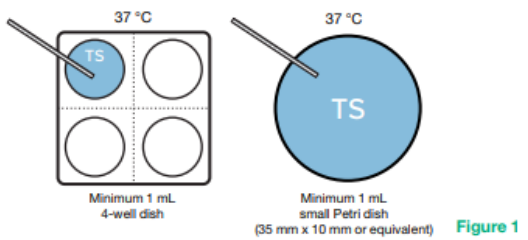

Figure 1

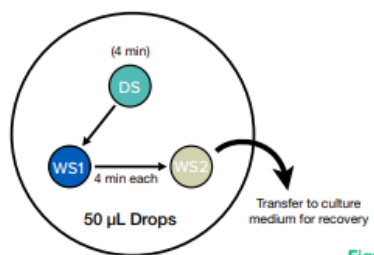

Figure 2

Standard Blastocyst Warming Protocol (FujiFilm)

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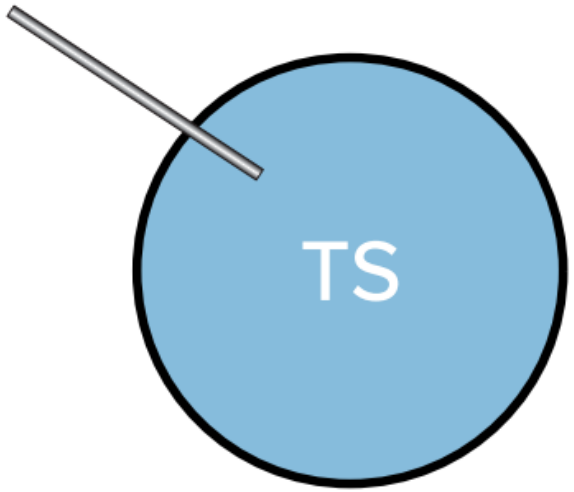
Thaw

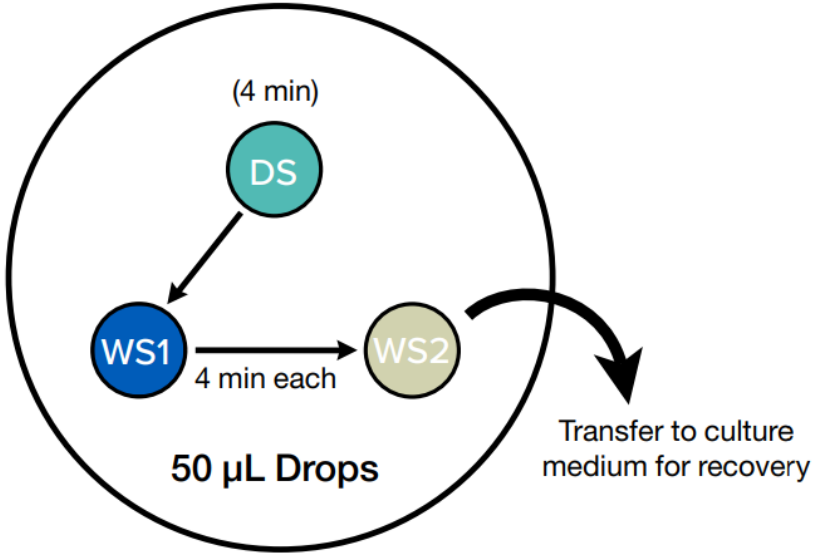
1 Minute
37ºC



Minimum 1 mL
small Petri dish
(35 mm x 10 mm or equivalent)

Dilution/Wash

12 Minutes
Room Temperature



Culture

~1 Minute Rinse
37ºC

Total Time: ~ 14 minutes

Standard Blastocyst Warming Protocol (FujiFilm)

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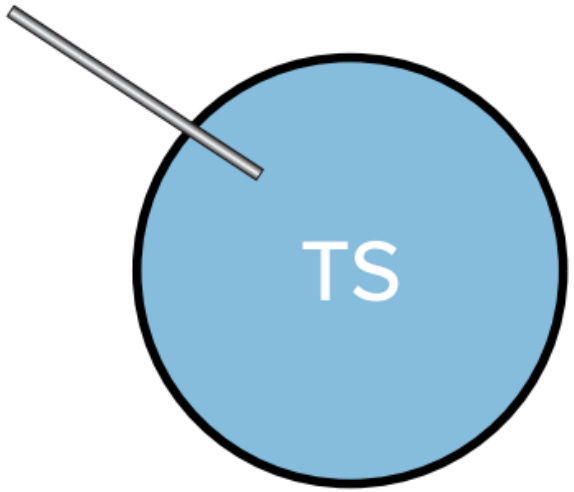
Thaw

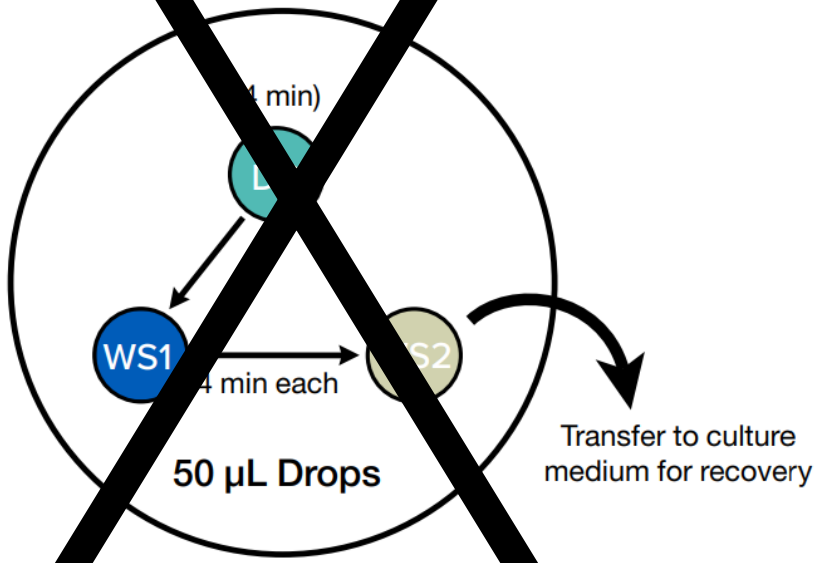
1 Minute
37ºC



Minimum 1 mL
small Petri dish
(35 mm x 10 mm or equivalent)

Dilution/Wash

12 Minutes
Room Temperature



Culture

~1 Minute Rinse
37ºC

Single Step Blastocyst Warming Protocol

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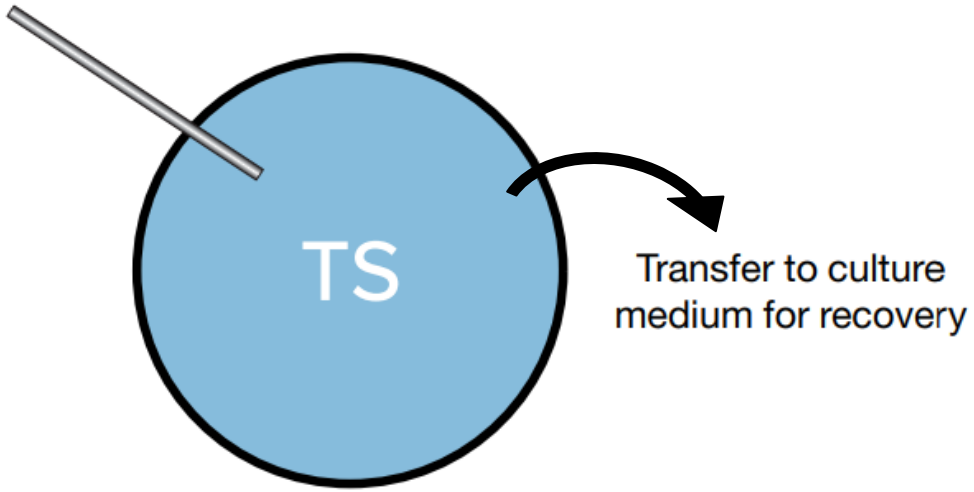
Thaw

1 Minute
37ºC



Minimum 1 mL
small Petri dish
(35 mm x 10 mm or equivalent)

Culture

~1 Minute Rinse
37ºC

Total Time: ~ 2 minutes

Single Step Blastocyst Warming Protocol

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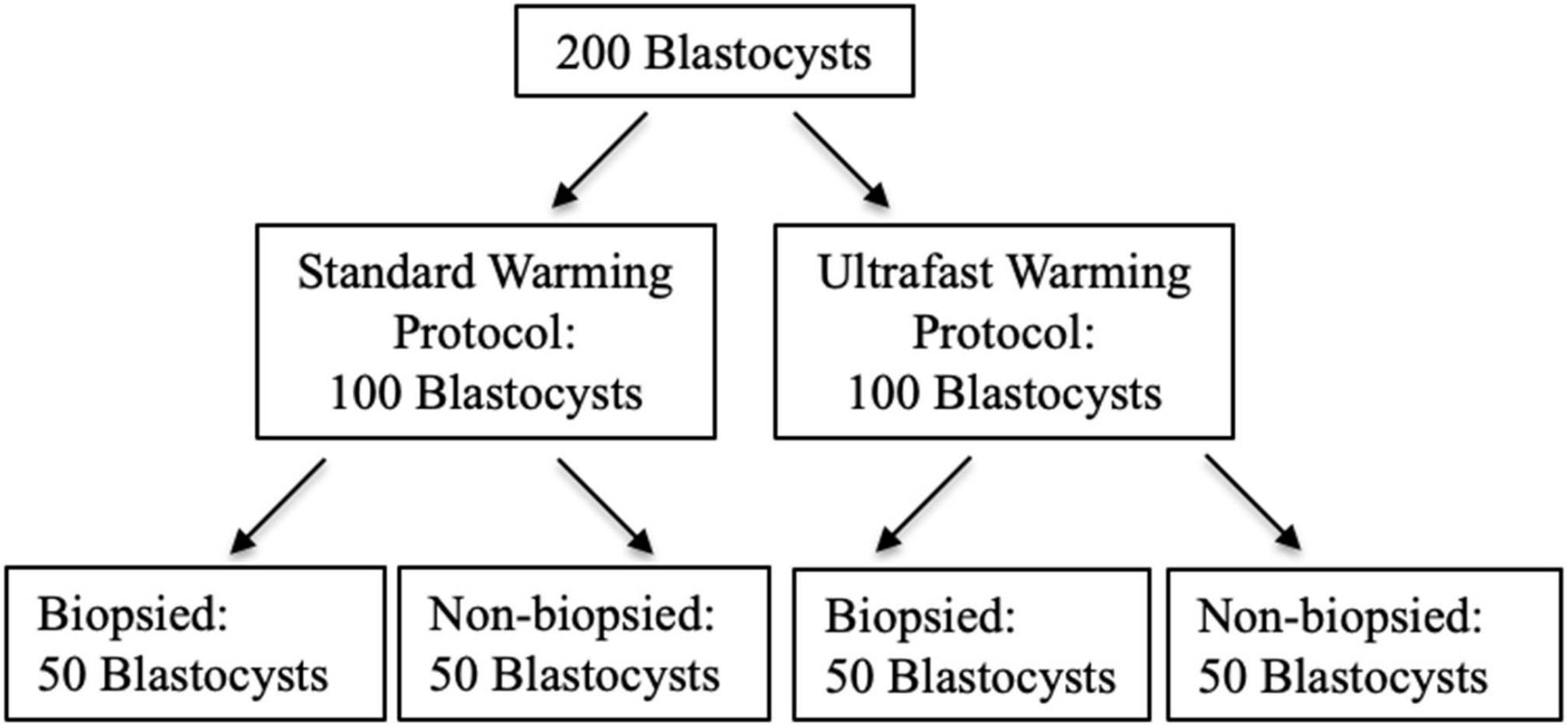

Early blastocyst: Stage 3; Expanded blastocyst: Stage 4; Hatched blastocyst: Stages 5 and 6

Blastocoel Expansion: Non-Biopsied Embryos

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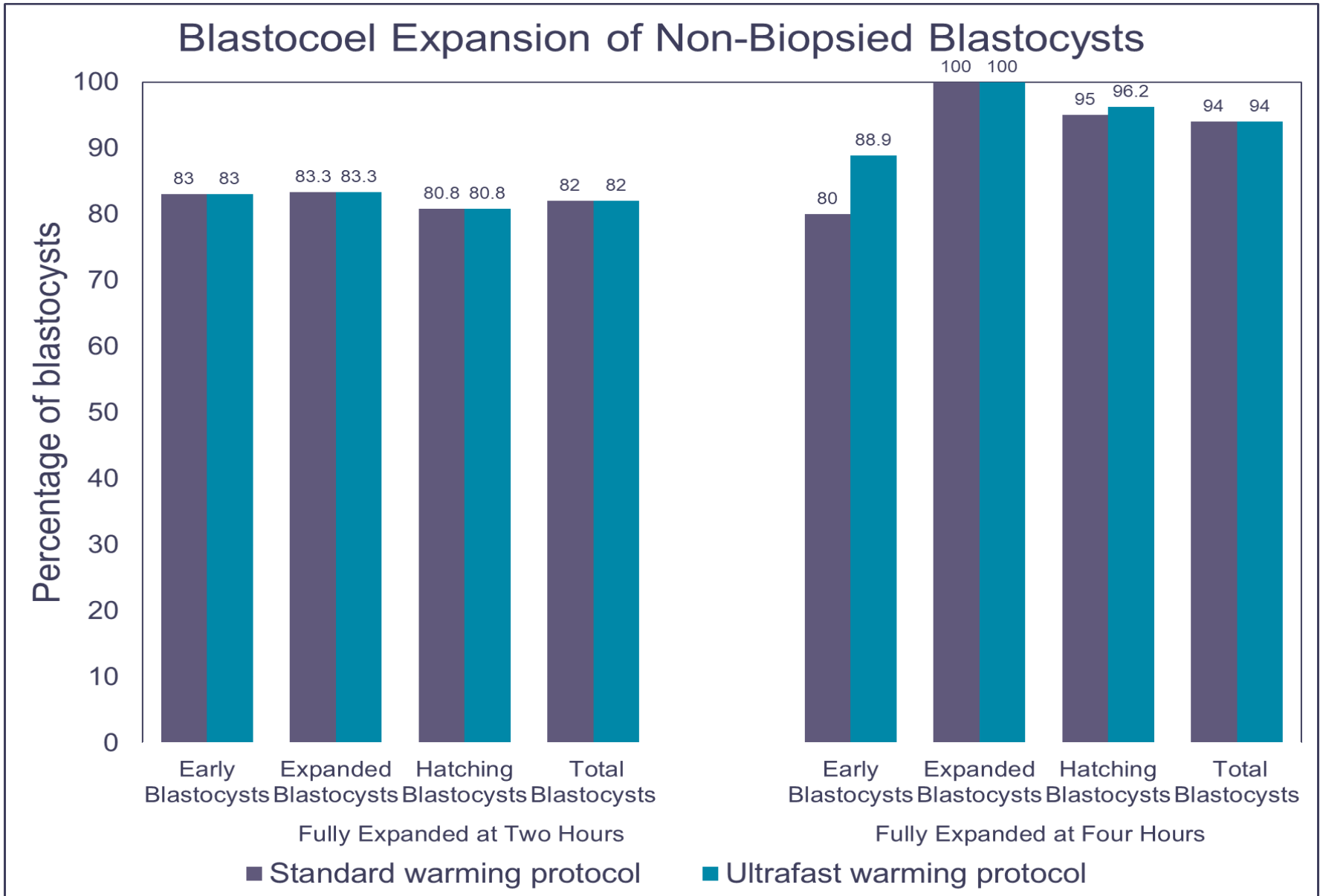

Blastocoel Expansion: PGT Embryos

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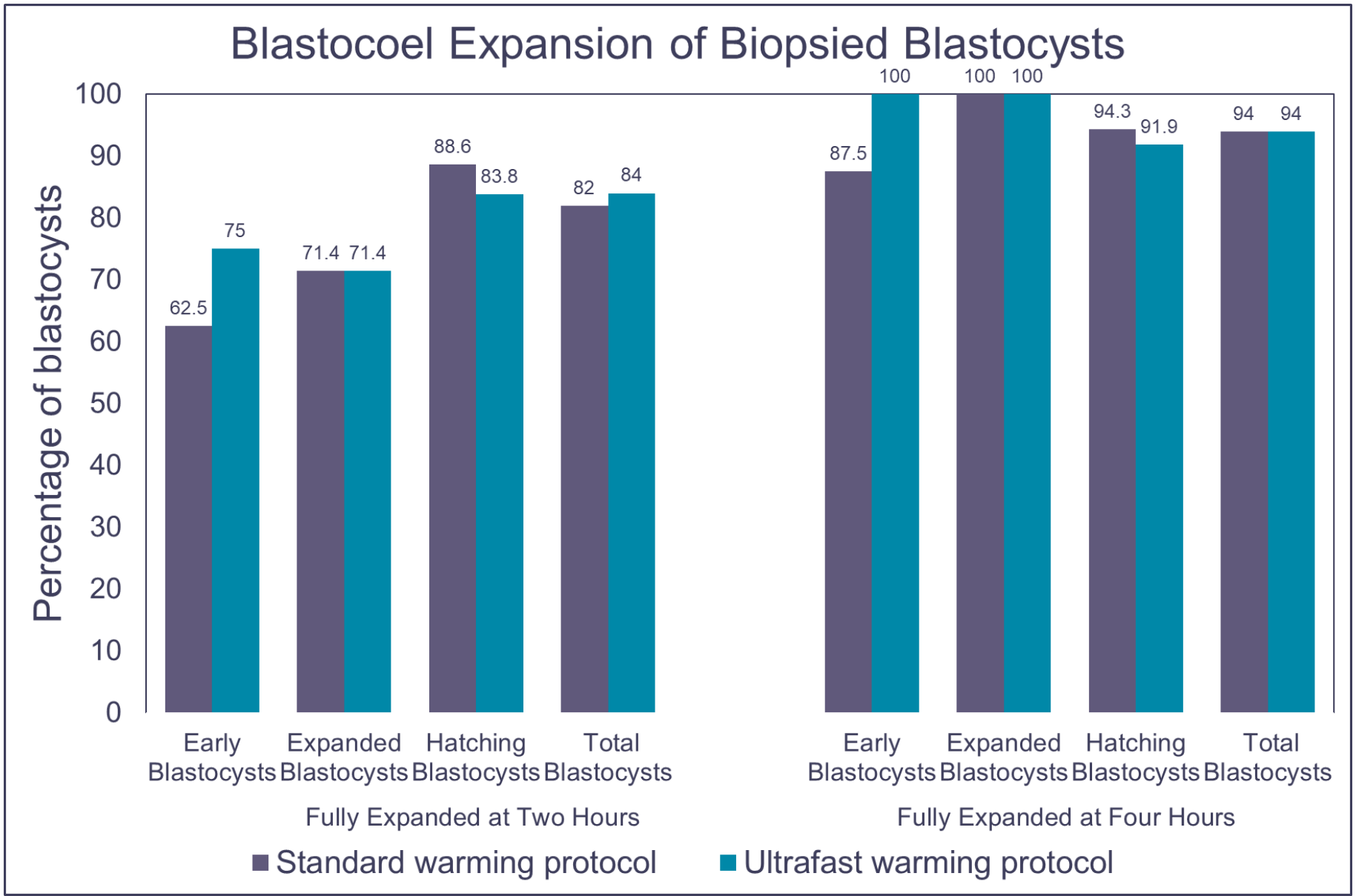

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ASSISTED REPRODUCTION TECHNOLOGIES

Live birth outcomes following single-step blastocyst warming technique – optimizing efficiency without impacting live birth rates

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Single Step Blastocyst Warming: Transfers

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Table 1. Baseline patient and cycle characteristics.

	Standard Warming (N=376 transfers)	Single-step Warming (N=376 transfers)	p-value
Maternal age (years)	34.3 (4.3)	34.7 (4.2)	0.148
AMH (ng/ml)	3.5 (2.6)	4.1 (3.5)	0.348
Day 3 FSH (mIU/mL)	6.9 (2.3)	6.8 (2.2)	0.587
BMI (kg/m^2)	25.8 (5.2)	26.1 (5.5)	0.742
Sperm collection method			0.763
Ejaculated	364 (96.8%)	361 (96.0%)	
Epididymal	5 (1.3%)	5 (1.3%)	
TESE	7 (1.9%)	10 (2.7%)	
IVF stimulation			0.939
Antagonist	205 (54.5%)	202 (53.7%)	
GnRH Agonist Flare	42 (11.2%)	45 (12.0%)	
LDLL	129 (34.3%)	129 (34.3%)	
# Oocytes retrieved	15.2 (7.2)	16.3 (8.4)	0.152
# M2 oocytes	12.4 (6.0)	13.0 (6.6)	0.330
# 2PN embryos	9.9 (4.9)	10.4 (5.5)	0.282
FET uterine prep			0.458
LEP	185 (49.2%)	176 (46.8%)	
Modified Natural	15 (4.0%)	22 (5.9%)	
Natural	176 (46.8%)	178 (47.3%)	

Single Step Blastocyst Warming: Clinical Outcomes

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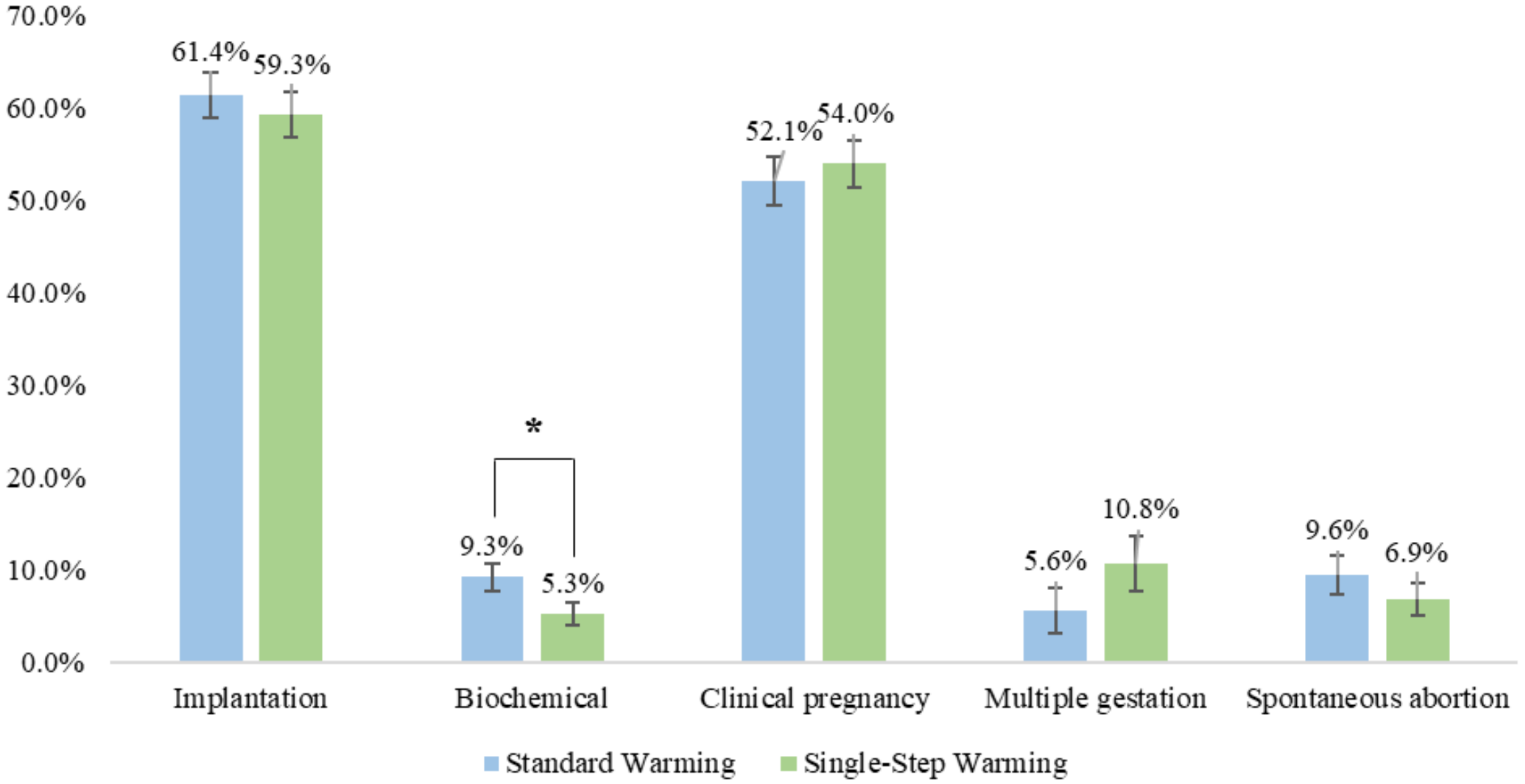

Single Step Warming: Live Birth Outcomes

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Warming Protocol	# Transfers	# Live Births	% Live Births	P-Value (Live Births)
Standard Warming	376	162	43.1%	0.713
Single-Step Warming	376	167	44.4%	

Single Step Warming: Live Birth Outcomes

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Warming Protocol	# Transfers	# Live Births	% Live Births	P-Value (Live Births)	Mean Birth Weight (g)	p-Value (Birth Weight)
Standard Warming	376	162	43.1%	0.713	3385.6 ± 555.1	0.374
Single-Step Warming	376	167	44.4%		3325.2 ± 536.2	

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INFERTILITY: ORIGINAL ARTICLE

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Ultra-Fast Warming Procedure of Vitrified Blastocysts Results in Maintained Embryology and Clinical Outcomes

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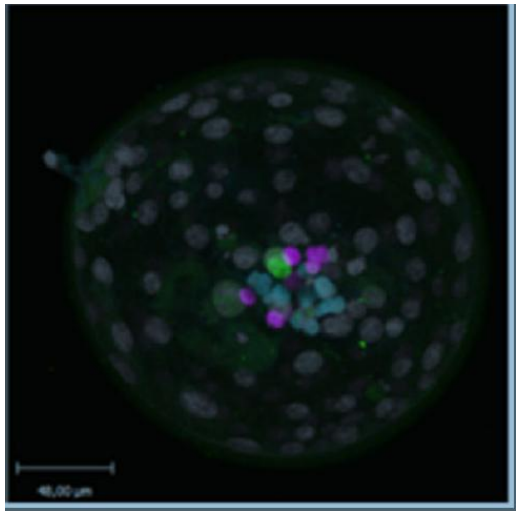

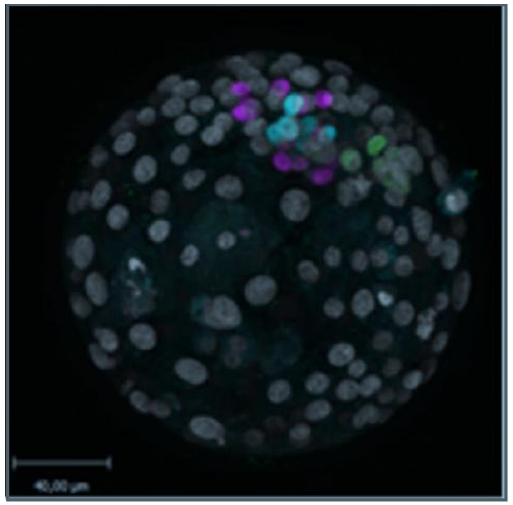

Expression of main cell fate markers at the blastocyst stage remain unchanged with this simplified warming protocol.

	Group 1 Conventional warming protocol (Jan-Jun 2022, n=547 FBT cycles, 578 embryos)	Group 2 Ultrafast warming protocol (Aug-Oct 2022, n=321 FBT cycles, 336 embryos)	*p*
Female age (years)	34.2±4.9	34.3±4.8	>.05
BMI (kg/m²)	24.9±5.4	25.0±5.5	>.05
Active smoker (%)	16.8	15.6	>.05
Primary infertility (%)	59	59.6	>.05
ICSI (%)	61.5	58.9	>.05
Day 5 blastocysts (n, %)	90.1	87.8	>.05
Single embryo transfer (%)	95.2	95	>.05
Survival rate (%)	97.6	97.8	>.05
Expansion rate (%)	80.5	80.3	>.05
Biochemical pregnancy rate (%)	41	42	>.05
Pregnancy loss rate (%)	29.9	29.6	>.05
Clinical pregnancy rate (%)	38.2	39.2	>.05
Live birth rate (%)	28.7	29.6	>.05

Results are presented as mean±standard deviation or proportion where appropriate. Biochemical pregnancy was defined by beta hCG serum level >100 IU/L. Pregnancy loss rate was defined by the proportion of biochemical pregnancies not leading to live birth

Vitrification + Warming Timing Efficiency

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Putting it all Together

Fast Vitrification: (2-10 minutes in ES)

Fast Single-Step Warming: (2 minutes)

Study Design

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Retrospective cohort study
- **Single academic fertility center** (Massachusetts General Hospital)
- **IRB approved**

Study Population
- **Single frozen embryo transfer (FET) cycles**
  - Blastocysts from oocytes retrieved **Nov 2015 – Nov 2024**
  - FETs performed **Jan 2022 – Dec 2024**
- **Exclusions:** donor oocytes, embryos from cryopreserved oocytes, imported embryos

Exposure Groups
- Standard Vitrification + Standard Warming (n=1025)
- Standard Vitrification + Fast Warming (n=926)
- Fast Vitrification + Fast Warming (n=471)

Outcomes
- **Primary:** Live birth rate
- **Secondary:** Pregnancy, biochemical pregnancy, clinical pregnancy, miscarriage, ectopic pregnancy

Outcomes based on Vitrification - Warming Protocol

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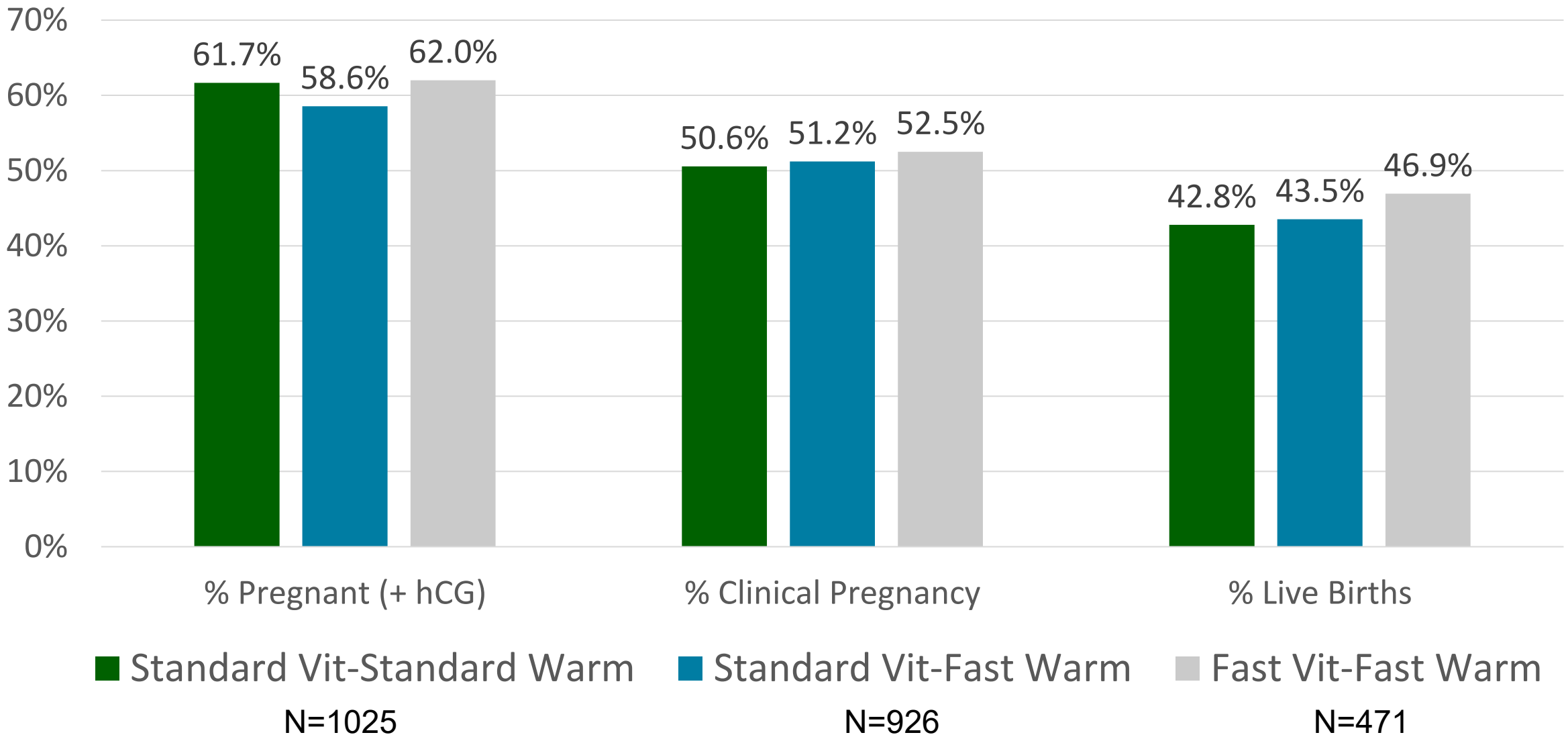

Outcomes based on Vitrification - Warming Protocol

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Adjusted pregnancy outcomes using Standard Vitrification-Standard Warming as reference (overall outcomes)

	Standard Vitrification-Fast Warming		Fast Vitrification-Fast Warming	
	Unadjusted OR (95% CI) p-value	Adjusted* OR (95% CI) p-value	Unadjusted OR (95% CI) p-value	Adjusted* OR (95% CI) p-value
HCG	0.90 (0.75-1.08) 0.252	0.91 (0.76-1.10) 0.350	1.16 (0.93-1.46) 0.188	1.18 (0.94-1.50) 0.159
Biochemical	0.91 (0.66-1.25) 0.538	0.97 (0.69-1.36) 0.867	0.95 (0.65-1.41) 0.806	1.06 (0.71-1.59) 0.778
Ectopic	0.27 (0.03-2.47) 0.250	0.26 (0.03-2.33) 0.227	1.64 (0.37-7.34) 0.520	1.50 (0.33-6.87) 0.599
Clinical pregnancy	0.94 (0.79-1.13) 0.507	0.94 (0.78-1.13) 0.493	1.16 (0.93-1.45) 0.180	1.15 (0.91-1.44) 0.244
Miscarriage	0.87 (0.60-1.24) 0.432	0.95 (0.65-1.40) 0.809	0.88 (0.56-1.38) 0.580	0.98 (0.62-1.56) 0.946
Livebirth	0.97 (0.81-1.16) 0.740	0.94 (0.78-1.14) 0.527	1.19 (0.96-1.49) 0.113	1.15 (0.91-1.44) 0.248

*Adjusted for oocyte age, BMI, date of cryopreservation, FET preparation

No Difference in Singleton Birthweight

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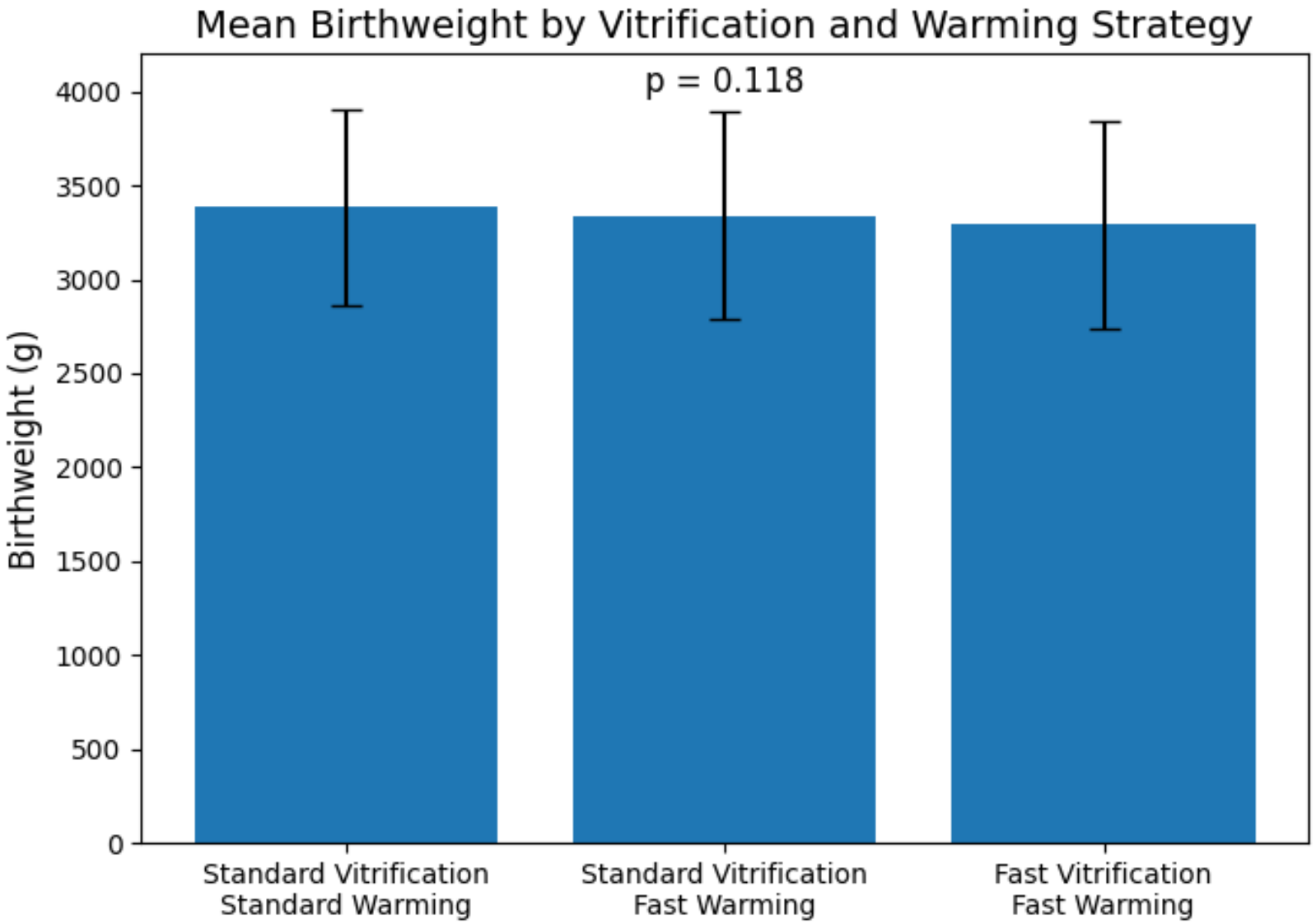

Clinical Reports of Fast Vitrification + Fast Warming

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EFFECT OF REDUCING THE VITRIFICATION AND WARMING TIMES ON BLASTOCYST SURVIVAL AND PREGNANCY RATES.

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OBJECTIVE: Even though embryo vitrification has been performed for nearly two decades, there is no consensus on exposure timing of embryos to vitrification and warming solutions. With embryo exposure timing in equilibration solution (ES) varying from 6-15 minutes, vitrification is time consuming in a busy IVF lab. Warming is a multistep process that can vary from 10-13 min. The aim of this study was to compare reducing the vitrification and warming time and evaluate the blastocyst survival rate and subsequent pregnancy rate.

MATERIALS AND METHODS: This was a retrospective analysis of 754 frozen embryo transfer cycles between January 2022 and March 2023. Embryos were divided into 3 groups: (1) traditional vitrification and warming, (2) traditional vitrification with rapid warming, and (3) reduced ES exposure with rapid warming. Traditional vitrification comprised of 6-10 min exposure to ES followed by 30-60 sec in vitrification solution (VS) (Vit Kit–Freeze NX, Irvine Scientific). Reduced ES comprised of 2min ES followed by 30-60sec VS exposure. Traditional warming comprised of 1min in thawing solution (TS) at 37°C, followed by 4min in dilution solution (DS) and 4min each in 2 drops of washing solution (WS) (Vit Kit-Warm NX, Irvine Scientific). Rapid warming only included 1min in TS before placing the embryo in culture. Main outcome measures were blastocyst survival rate and positive β-HCG pregnancy rate.

RESULTS: The data obtained are depicted in Table 1. No statistically significant differences were noted in the blastocyst survival (99.4%, 98.1%, and 100.0%) and pregnancy rates (65.6%, 71.7%, and 74.6%) between the three protocols. However, the reduced ES with rapid warming showed the highest survival and pregnancy rates.

Treatment Group	FET cycles	Embryos thawed	Embryos survived (%)	Positive β-HCG (%)
6min ES + 13min warm	492	505	502 (99.4%)[a,b]	322/491 (65.6%)[d,e]
6min ES + 1min warm	148	160	157 (98.1%)[a,c]	104/145 (71.7%)[e,f]
2min ES + 1min warm	114	119	119 (100.0%)[b,c]	85/114 (74.6%)[d,f]

Fertility and Sterility, Volume 120, Issue 4, e143

Acknowledgements

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