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Embryo evaluation: Minimalist

BSRM
MARCH 20

UZ GENT
UNIVERSITEIT GENT

COI

Lecture honoraria from Ferring, Merck, Organon, Cooper Surgical

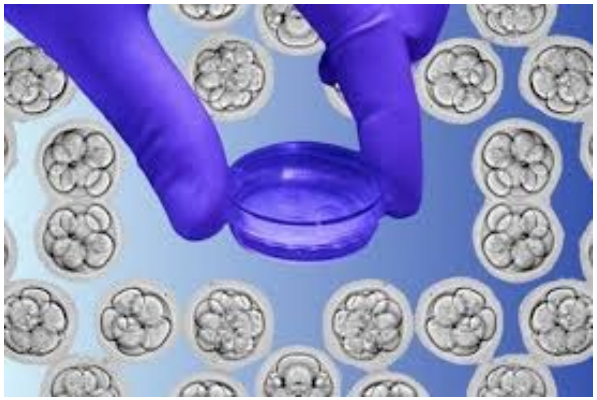
Travel/accommodation reimbursement
Ferring, Merck, Organon, Cooper Surgical, eFertility ~Vitrolife group

Key opinion leader FuijiFilm

Why do we evaluate embryos?

To decide on the disposition of embryos:

use them clinically



discard

How do we evaluate embryos?

	RECOMMENDATIONS
OOCYTE ASSESSMENT	• Giant oocytes should be excluded from clinical use. • The use of small/large oocytes and IVM-rescued oocytes should be documented for prognostic and traceability purposes due to their apparently lower developmental potential. • Finally, embryos derived from MII oocytes free of large or multiple vacuoles, SER-a, and very large first PB should be prioritized for clinical use. • Prenatal follow-up and the follow-up of babies born from oocytes with atypical phenotypes and rescue IVM demands attention.
ZYGOTE STATE ASSESSMENT	• Assessment of PN number should be carried out between 16 and 17 hpi in both conventional IVF and ICSI cases. • Zygotes with 2PN should be prioritized for clinical use. • 2.1PN and 1PN zygotes from IVF or ICSI may be considered for clinical use with appropriate counselling, especially if associated with PGT-A technology appropriate for biparental diploidy assessment. • The clinical use of 3PN zygotes is not recommended, while pre-clinical or pilot clinical studies should be encouraged. • Dynamic features such as PN size, PN position and juxtaposition, NPB pattern, and cytoplasmic halo cannot be accurately assessed during static observations. Thus, they cannot be consistently used as biomarkers of viability.

The Istanbul consensus update: a revised ESHRE/ALPHA consensus on oocyte and embryo static and dynamic morphological assessment[†,‡]

The Working Group on the update of the ESHRE/ALPHA Istanbul Consensus, Giovanni Coticchio[1,*], Aisling Ahlström[2], Gemma Arroyo[3], Basak Balaban[4], Alison Campbell[5,6], Maria José De Los Santos[7,8], Thomas Ebner[9], David K. Gardner[10,11], Borut Kovačič[12], Kersti Lundin[13], M. Cristina Magli[14], Saria Mcheik[15], Dean E. Morbeck[16,17], Laura Rienzi[18], Ioannis Sfontouris[19], Nathalie Vermeulen[15], and Mina Alikani[20,*]

How do we evaluate embryos?

DAY -1, -2 & -3 EMBRYO ASSESSMENT	• 2-cell embryos on Day-1, 4-cell embryos on Day- 2, 8-cell embryos on Day-3 showing <10% fragmentation, mononucleation, and stage-specific cell size should be prioritized in case of cleavage stage embryo transfer or cryopreservation. • Cleavage stage embryos with atypical features such as extensive fragmentation, multinucleation, vacuoles, cytoplasmic granularity, membrane, and zona irregularities, can be considered suitable for clinical use. However, extended culture of these embryos for further evaluation should be considered.
DAY-4 EMBRYO ASSESSMENT	• Day-4 embryos showing full compaction or early cavitation should be prioritized in case of Day-4 transfer or vitrification. • Embryos with partial compaction can form blastocysts and should be considered for clinical use. Extended culture of these embryos for further evaluation should be considered.
DAY-5, -6 & -7 EMBRYO ASSESSMENT	• The Gardner grading system for blastocyst scoring (Table 8) should be used. This system is distinguished from the prior Consensus grading by using letters for the ICM/TE grades and adding additional expansion stages (e.g. hatched blastocyst). • Non-viable blastocysts should be graded as "D" as opposed to "C" based on degenerative features or absence of a distinct ICM. • The common features that are clearly associated with implantation potential include day of blastocyst formation (Day 4-7), stage of expansion (3,4,5,6), and grade of ICM (A, B, C) and TE (A, B, C). • Blastocysts with grade C ICM and/or TE and Day 7 blastocysts can be viable and could be considered suitable for clinical use. • Blastocysts with 2 ICM indicating potential monozygotic twinning should not be transferred without thorough patient counselling. • Assigning relative importance of each variable requires systematic multivariate analysis with a large dataset and is further complicated when assessing fresh versus frozen untested and euploid blastocysts.

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How do we evaluate embryos?

DURATION OF EMBRYO CULTURE AND FREQUENCY OF ASSESSMENTS	• Extended embryo culture is an accepted and standard practice. • The length of embryo culture and frequency of static embryo observations must be adjusted to the equipment in the laboratory and staff skill, ensuring minimal changes in culture conditions that could affect embryo development.

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Undisturbed culture: a clinical examination of this culture strategy on embryo in vitro development and clinical outcomes

María Ángeles Valera, M.Sc.,[a,b] Akhil Garg, M.Sc.,[b] Lorena Bori, Ph.D.,[a,b] Fernando Meseguer, M.Sc.,[a,b] José María de los Santos, Ph.D.,[b] and Marcos Meseguer, Ph.D.[a,b]

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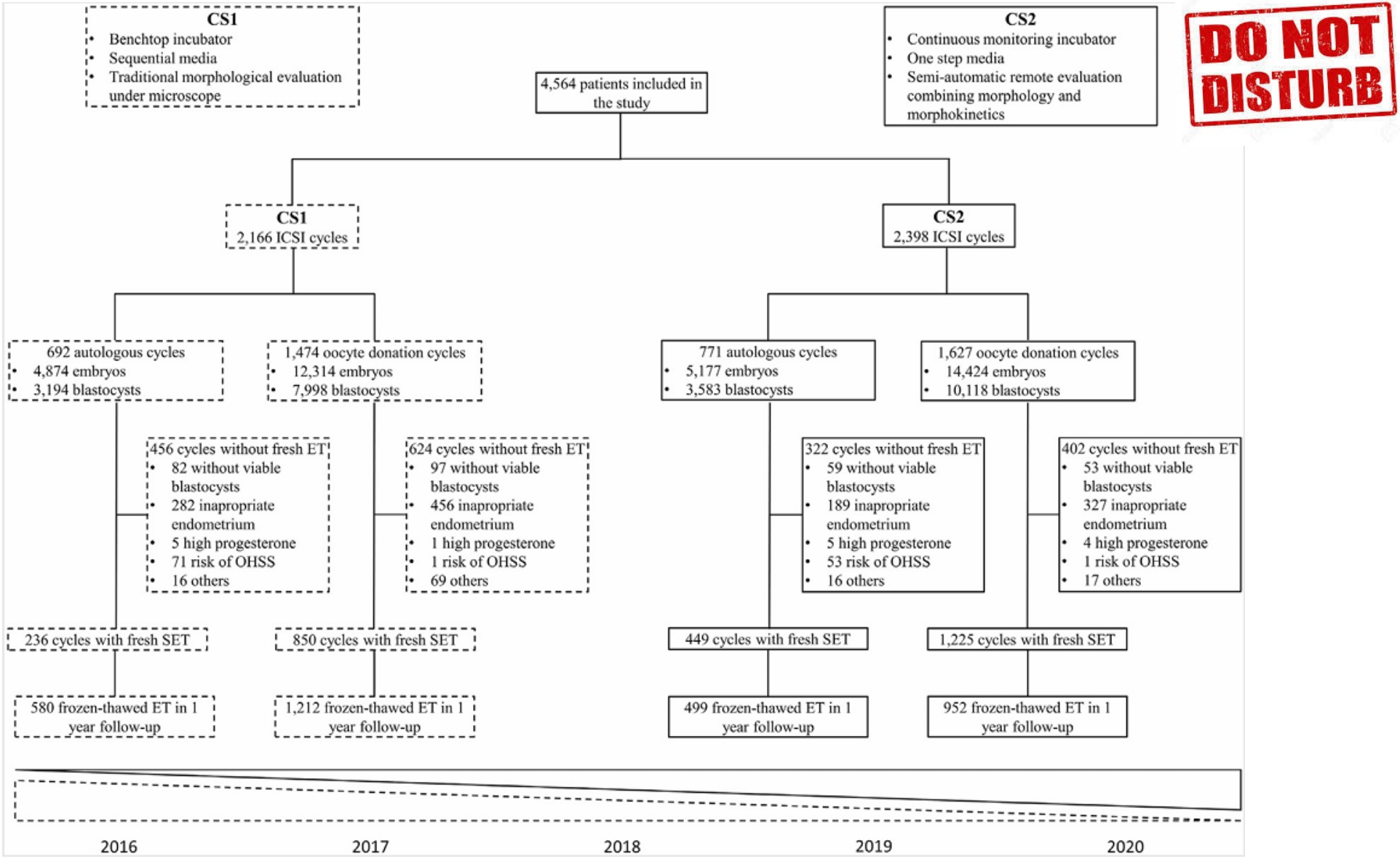

CS1
Benchtop incubator
Sequential media
Traditional morphological evaluation under microscope
CS2
Continuous monitoring incubator
One step media
Semi-automatic remote evaluation combining morphology and morphokinetics
DO NOT DISTURB
4,564 patients included in the study
CS1
2,166 ICSI cycles
CS2
2,398 ICSI cycles
692 autologous cycles
4,874 embryos
3,194 blastocysts
1,474 oocyte donation cycles
12,314 embryos
7,998 blastocysts
771 autologous cycles
5,177 embryos
3,583 blastocysts
1,627 oocyte donation cycles
14,424 embryos
10,118 blastocysts
456 cycles without fresh ET
82 without viable blastocysts
282 inapropriate endometrium
5 high progesterone
71 risk of OHSS
16 others
624 cycles without fresh ET
97 without viable blastocysts
456 inapropriate endometrium
1 high progesterone
1 risk of OHSS
69 others
322 cycles without fresh ET
59 without viable blastocysts
189 inapropriate endometrium
5 high progesterone
53 risk of OHSS
16 others
402 cycles without fresh ET
53 without viable blastocysts
327 inapropriate endometrium
4 high progesterone
1 risk of OHSS
17 others
236 cycles with fresh SET
850 cycles with fresh SET
449 cycles with fresh SET
1,225 cycles with fresh SET
580 frozen-thawed ET in 1 year follow-up
1,212 frozen-thawed ET in 1 year follow-up
499 frozen-thawed ET in 1 year follow-up
952 frozen-thawed ET in 1 year follow-up
2016
2017
2018
2019
2020

CS1

- Benchtop incubator
- Sequential media
- Traditional morphological evaluation under microscope

CS2

- Continuous monitoring incubator
- One step media
- Semi-automatic remote evaluation combining morphology and morphokinetics

Embryo development in culture strategy 1 and culture strategy 2 and weighted comparison.

	CS1		CS2		CS2 vs. CS1		CS2 vs. CS1	
Outcome	**Mean (95% CI)**	**SD**	**Mean (95% CI)**	**SD**	**Unweighted OR (95% CI)**	***P* value**	**Weighted OR (95% CI)**	***P* value**
Autologous ICSI								
Blastocyst by day 5/2PN	58.6 (56.2–61)	31.8	64.2 (62–66.3)	29.8	1.057 (1.024–1.091)	.001[a]	1.023 (0.98–1.067)	.306
Blastocyst by day 6/2PN	63.5 (61.3–65.8)	30.1	67.7 (65.7–69.7)	28.2	1.043 (1.012–1.074)	.006[a]	1.019 (0.977–1.063)	.378
A/B classified blastocysts/ 2PN	33.3 (31.1–35.4)	28.9	37.9 (35.9–39.9)	28.1	1.047 (1.017–1.078)	.002[a]	1.009 (0.964–1.055)	.712
Transferred or vitrified blastocysts/2PN	51.9 (49.7–54.2)	30	56.4 (54.4–58.5)	28.6	1.046 (1.015–1.078)	.003[a]	1.024 (0.98–1.071)	.291
Oocyte donation ICSI								
Blastocyst by day 5/2PN	62.7 (61.4–64)	25.4	68.5 (67.4–69.6)	22.8	1.06 (1.043–1.079)	<.001[a]	1.04 (1.016–1.064)	.001[a]
Blastocyst by day 6/2PN	65 (63.7–66.2)	24.3	70 (69–71.1)	21.7	1.052 (1.035–1.069)	<.001[a]	1.033 (1.011–1.056)	.003[a]
A/B classified blastocysts/ 2PN	35 (33.9–36.2)	23.5	40.2 (39.1–41.2)	22.4	1.052 (1.035–1.07)	<.001[a]	1.027 (1.004–1.05)	.02[a]
Transferred or vitrified blastocysts/2PN	50.9 (49.5–52.2)	25.9	55.6 (54.4–56.7)	23.5	1.048 (1.03–1.067)	<.001[a]	1.036 (1.011–1.062)	.004[a]

Note: A/B = top blastocyst morphological classifications by Asociación para el Estudio de la Biología de la Reproducción (ASEBIR) criteria; CI = confidence interval; CS1 = culture strategy 1; CS2 = culture strategy 2; ICSI = intracytoplasmic sperm injection; OR = odds ratio; PN = pronuclei.

[a] *P*< .05, statistical significance.

Valera. Undisturbed vs. sequential culture. Fertil Steril 2024.

CS1

- Benchtop incubator
- Sequential media
- Traditional morphological evaluation under microscope

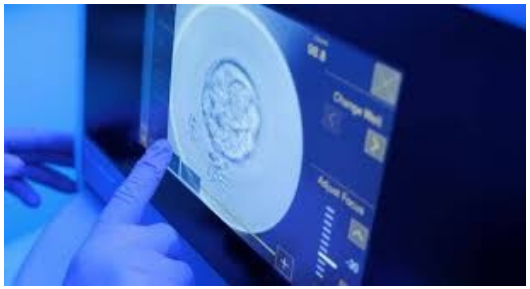
Clinical outcomes of intracytoplasmic sperm injection treatments cultured in culture strategy 1 and culture strategy 2, and weighted comparison.

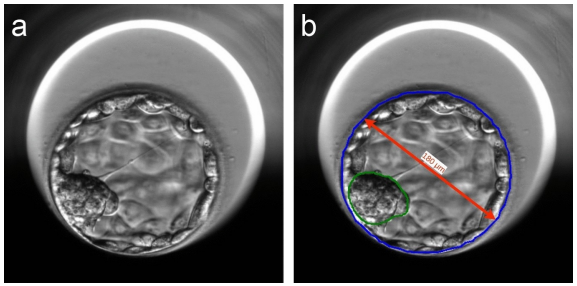
	CS1		CS2		CS2 vs. CS1		CS2 vs. CS1	
Outcome	**n**	**Proportion (95% CI)**	**n**	**Proportion (95% CI)**	**Unweighted OR (95% CI)**	***P* value**	**Weighted OR (95% CI)**	***P* value**
Autologous ICSI								
Cycles without viable embryos	82/692	11.8 (9.4–14.3)	59/771	7.7 (5.8–9.5)	0.616 (0.434–0.876)	.007[a]	0.614 (0.372–1.014)	.057
Fresh single embryo transfer outcomes (intention to treat)								
Clinical pregnancy rate	128/692	18.5 (15.6–21.4)	269/771	34.9 (31.5–38.3)	2.361 (1.853–3.009)	<.001[a]	1.605 (1.068–2.412)	.023[a]
Ongoing pregnancy rate	114/692	16.5 (13.7–19.2)	232/771	30.1 (26.9–33.3)	2.182 (1.694–2.811)	<.001[a]	1.877 (1.322–2.666)	<.001[a]
Live birth rate	82/692	11.8 (9.4–14.3)	136/771	17.6 (14.9–20.3)	1.593 (1.185–2.141)	.002[a]	1.617 (1.074–2.435)	.021[a]
Miscarriage rate/pregnancies	21/128	16.4 (10–22.8)	33/269	12.3 (8.3–16.2)	0.712 (0.394–1.289)	.262	0.873 (0.402–1.894)	.731
Cumulative outcomes (intention to treat)								
Cumulative clinical pregnancy rate	453/692	65.5 (3.5–61.9)	514/771	66.7 (3.3–63.3)	1.055 (0.85–1.311)	.627	0.961 (0.702–1.316)	.805
Cumulative ongoing pregnancy rate	351/692	50.7 (3.7–47)	403/771	52.3 (3.5–48.7)	1.064 (0.866–1.306)	.554	1.055 (0.78–1.428)	.727
Cumulative live birth rate	351/692	50.7 (3.7–47)	402/771	52.1 (3.5–48.6)	1.058 (0.862–1.3)	.588	1.051 (0.777–1.423)	.746
Cumulative miscarriage rate	93/692	13.4 (2.5–10.9)	87/771	11.3 (2.2–9.1)	0.819 (0.6–1.119)	.211	0.923 (0.611–1.394)	.702
Oocyte donation ICSI								
Cycles without viable embryos	97/1474	6.6 (5.3–7.8)	53/1627	3.3 (2.4–4.1)	0.478 (0.339–0.673)	<.001[a]	0.688 (0.438–1.079)	.103
Fresh single embryo transfer outcomes (intention to treat)								
Clinical pregnancy rate	548/1474	37.2 (34.7–39.6)	856/1627	52.6 (50.2–55)	1.876 (1.625–2.166)	<.001[a]	1.346 (1.077–1.683)	.009[a]
Ongoing pregnancy rate	482/1474	32.7 (30.3–35.1)	771/1627	47.4 (45–49.8)	1.854 (1.602–2.145)	<.001[a]	1.389 (1.113–1.734)	.004[a]
Live birth rate	320/1474	21.7 (19.6–23.8)	496/1627	30.5 (28.2–32.7)	1.582 (1.344–1.861)	<.001[a]	1.316 (1.036–1.672)	.024[a]
Miscarriage rate/pregnancies	90/548	16.4 (13.3–19.5)	103/856	12 (9.9–14.2)	0.696 (0.513–0.945)	.020[a]	0.872 (0.601–1.266)	.472
Cumulative outcomes (intention to treat)								
Cumulative clinical pregnancy rate	1124/1474	76.3 (2.2–74.1)	1322/1627	81.3 (1.9–79.4)	1.35 (1.135–1.604)	.001[a]	1.231 (0.925–1.639)	.154
Cumulative ongoing pregnancy rate	882/1474	59.8 (2.5–57.3)	1097/1627	67.4 (2.3–65.1)	1.389 (1.199–1.609)	<.001[a]	1.516 (1.19–1.93)	.001[a]
Cumulative live birth rate	877/1474	59.5 (2.5–57)	1090/1627	67 (2.3–64.7)	1.382 (1.193–1.6)	<.001[a]	1.5 (1.179–1.909)	.001[a]
Cumulative miscarriage rate	201/1474	13.6 (1.8–11.9)	178/1627	10.9 (1.5–9.4)	0.778 (0.627–0.965)	.022[a]	0.605 (0.407–0.899)	.013[a]

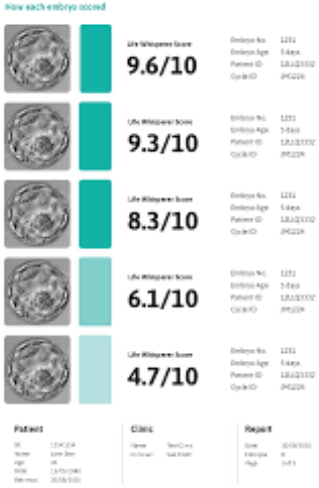
Note: CI = confidence interval; CS1 = culture strategy 1; CS2 = culture strategy 2; ICSI = intracytoplasmic sperm injection; OR = odds ratio.
[a] *P*< .05, statistical significance.

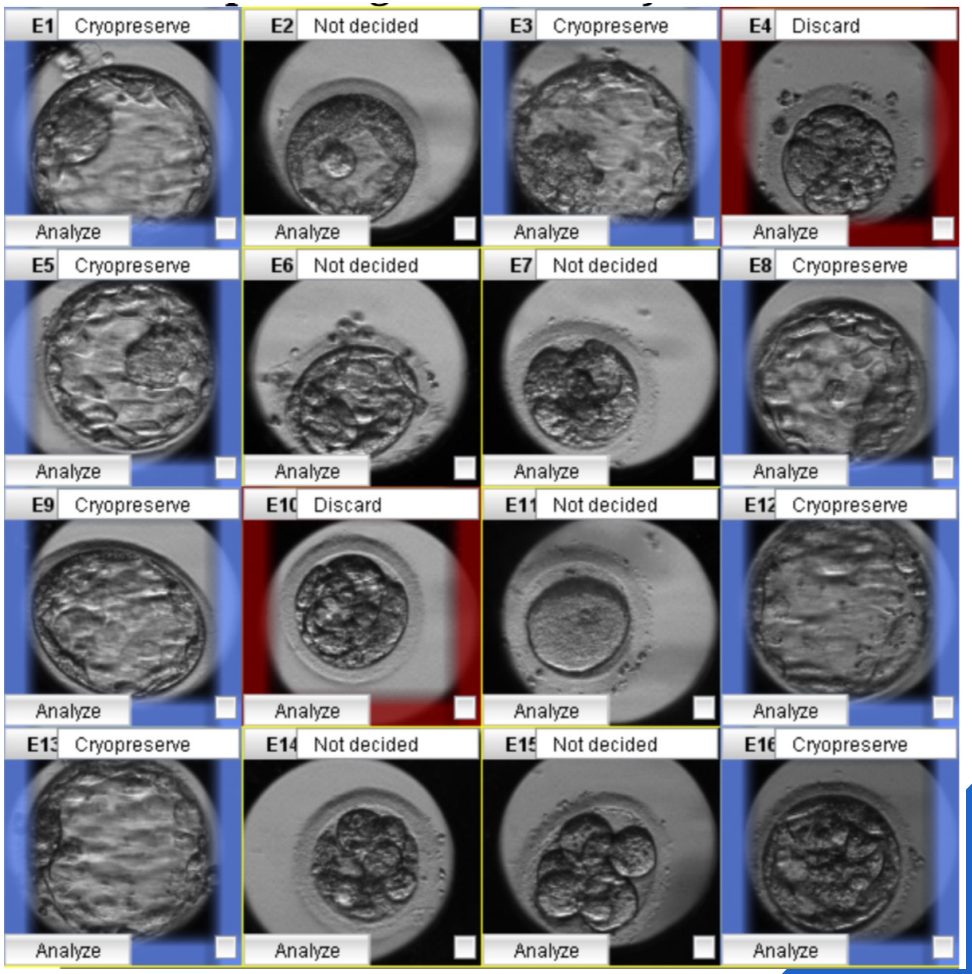
Valera. Undisturbed vs. sequential culture. Fertil Steril 2024.

“We like looking at embryos”

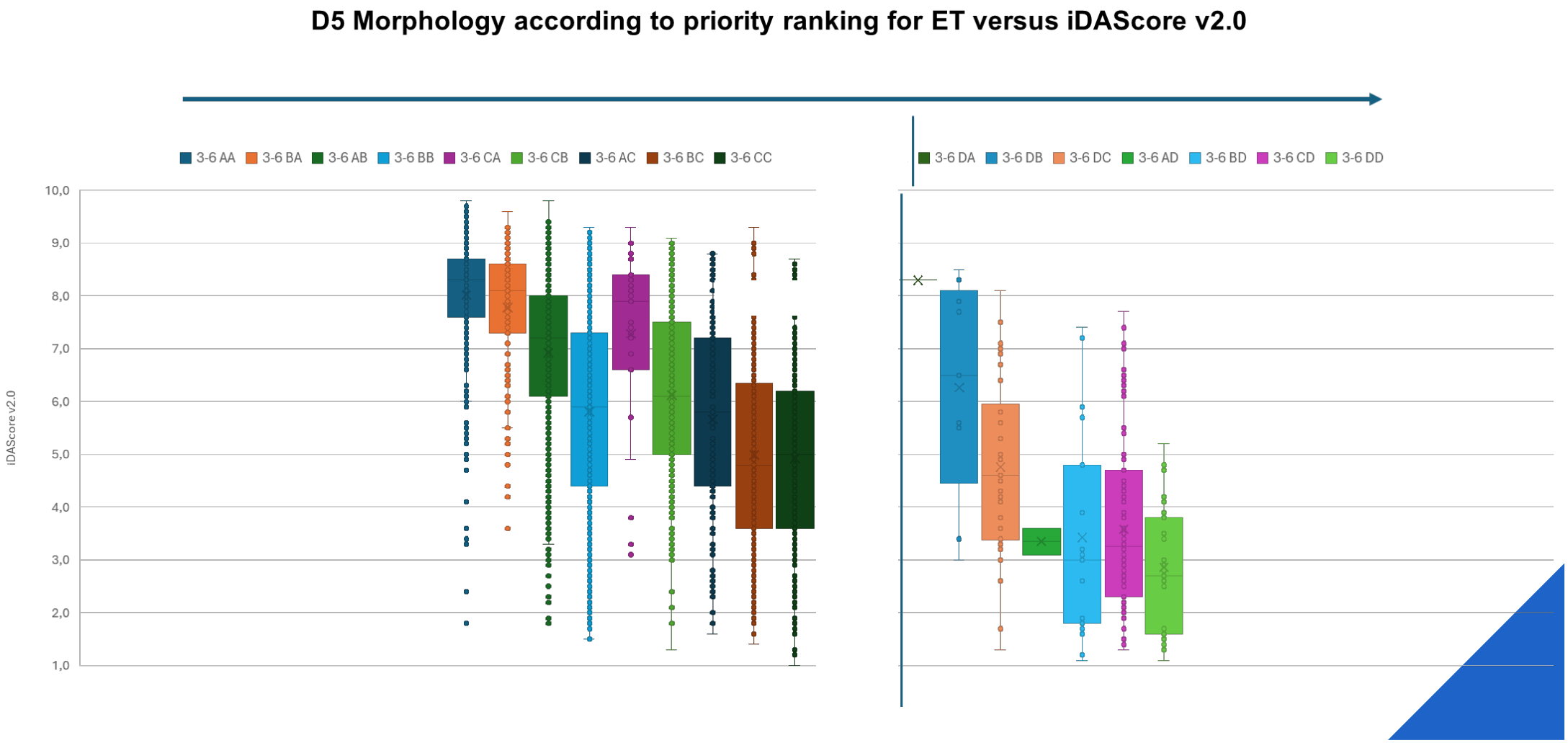








What about D-score = non-viable



Why do we evaluate embryos?

To decide on the disposition of embryos:

use them clinically 

discard 

How should we evaluate embryos?

D0: MII oocyte: giant or not?

D1: PN assessment

D2:

D3:

D4:

D5 – D7: decision making ⟶ use a tool

“We need to look at embryos to make decisions”

We are causing them more harm than good

Extended culture will determine the true developmental potential

We can use our time more wisely

Therefore …

“Embrace the minimalist way of embryo evaluation, we must”