Extended Culture of Blastocysts: Advantages and Disadvantages and In Which Patients?

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2017 AAB Educational Conference/21st Annual CRB Symposium
Westin Galleria Hotel, Houston, Texas
May 17th-19th, 2017

DISCLOSURES

- Catherine Racowsky, PhD, HCLD
- Extended culture of blastocyst: Advantages and disadvantages & which patients?

FINANCIAL DISCLOSURES (during last 12 months)

Consultant: World Health Organization
LifeGlobal, Inc

Speaker's Bureau: Ferring Pharmaceuticals, Inc
LifeGlobal, Inc

UNLABELED/UNAPPROVED USES DISCLOSURE

None

Discussion Outline

1. Consider the source of the oocytes we handle
2. Review requirements for optimizing the culture conditions
3. Discuss the rationale for extending culture to the blastocyst stage
4. Review the evidence for day 3 vs. day 5 transfer
5. Outline a protocol for selection of optimal day of transfer for each patient

ARS Slide 1

Please indicate the percentage of your patients who have blastocyst transfer:

A. Less than 10%

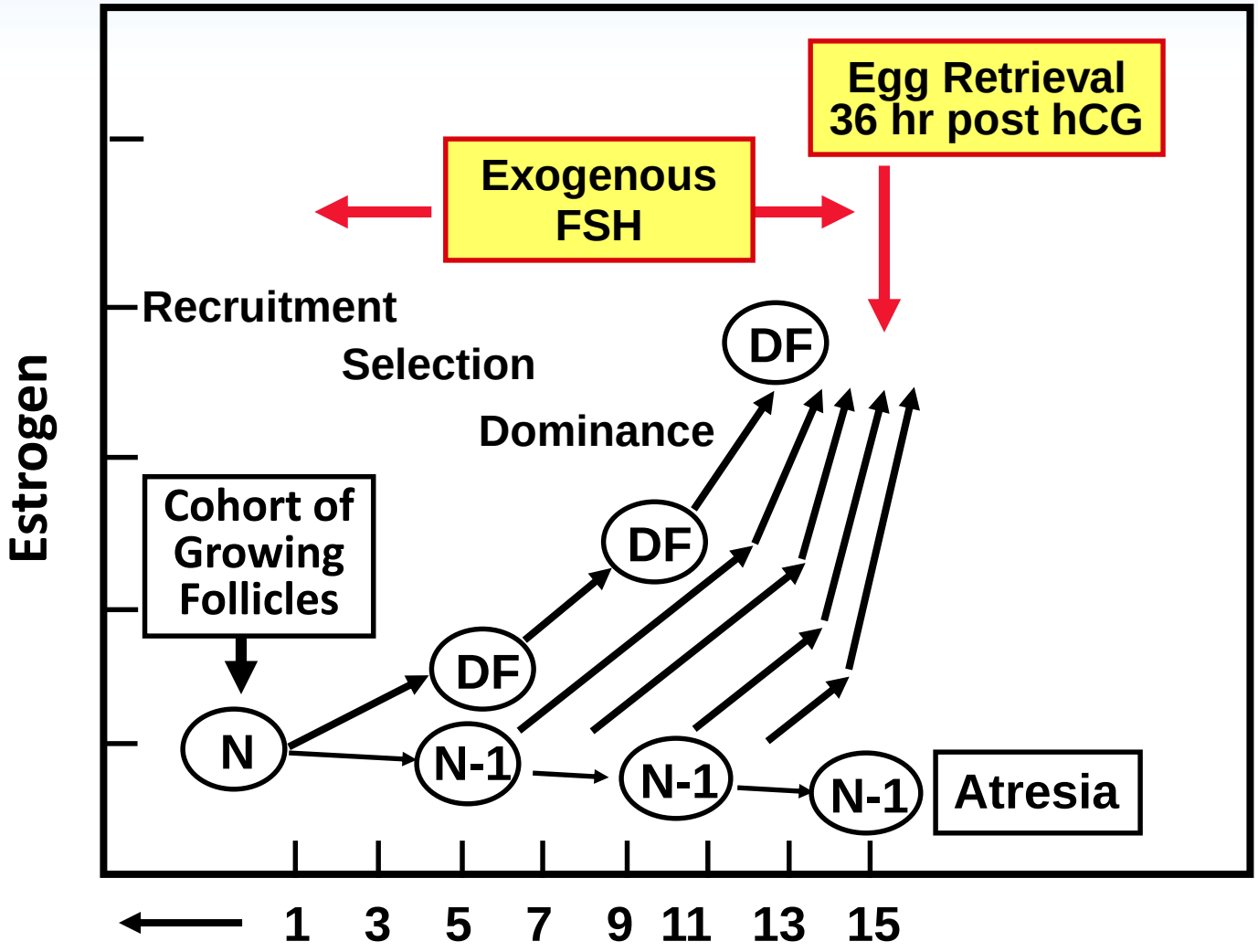
B. Approximately 25%

C. Approximately 50%

D. Approximately 75%

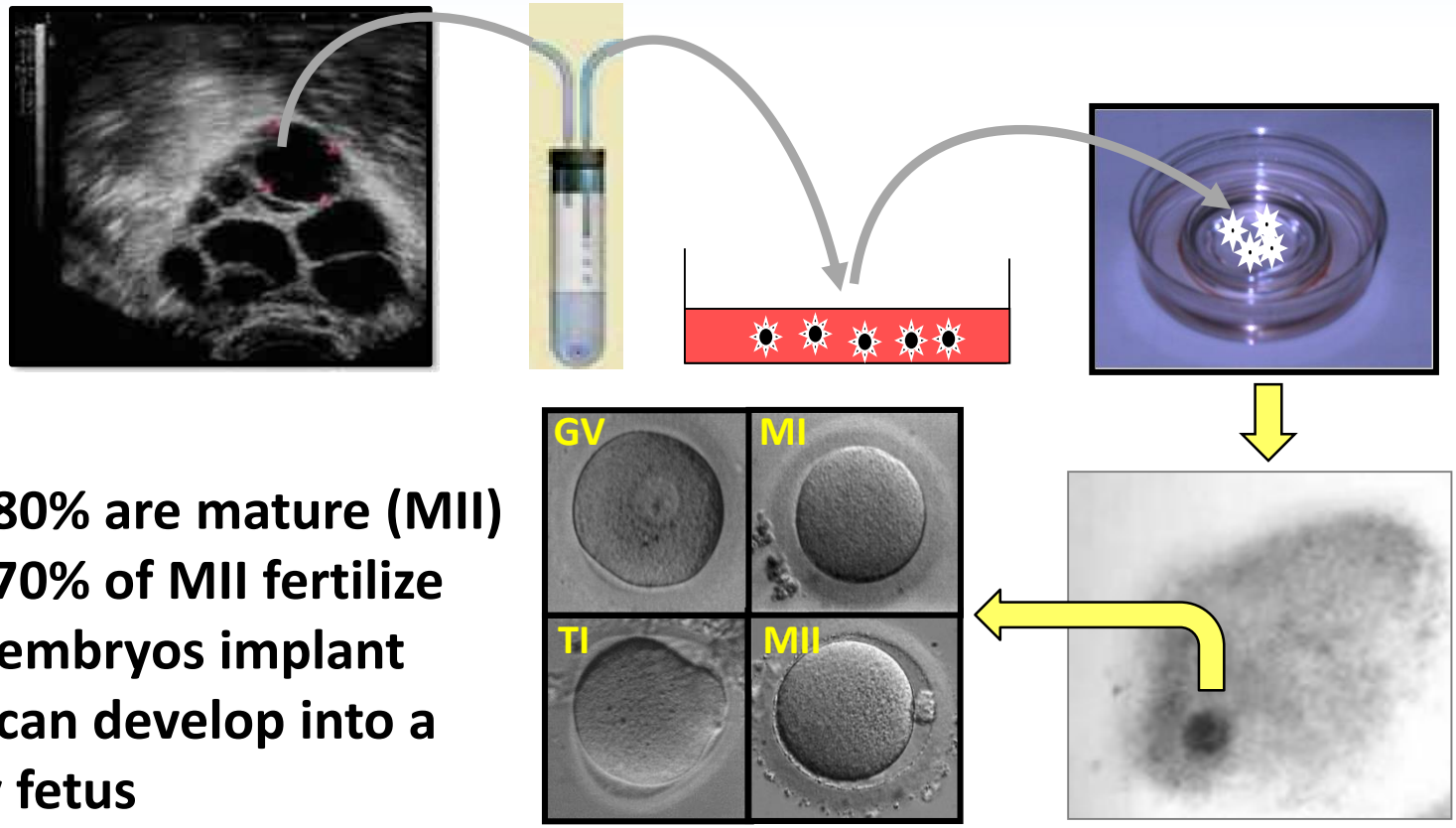
E. 100%

Source of the Oocytes: Follicle Growth & Selection



Adapted from Hodgen '82

Ovarian stimulation & oocyte wastage

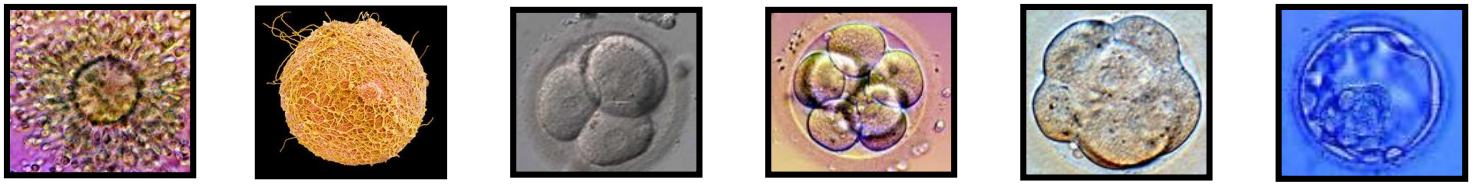


- Only ~ 80% are mature (MII)
- Only ~ 70% of MII fertilize
- Not all embryos implant
- Not all can develop into a healthy fetus

Ovarian stimulation typically results in a high number of abnormal, developmentally incompetent oocytes

The Goals of ART

- To maximize the likelihood of pregnancy for each patient
- To produce a healthy, genetically normal full-term delivery
- To minimize the risk of a multiple gestation



The Critical Questions are …

- **How can we achieve these goals for each patient by:**
  - Optimizing the culture conditions
  - Choosing the optimal day to transfer AND
  - Selecting the most developmentally competent embryo available

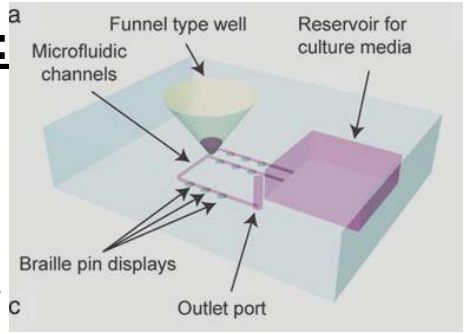
Our culture systems are very complex!

The Complexity of the Culture System

- Culture dish
- Embryo density
- Gas phase: O_2 tension
- Culture medium: type & protein
- Oil and “contact” materials
- Incubator type
- Culture platform
- Air quality

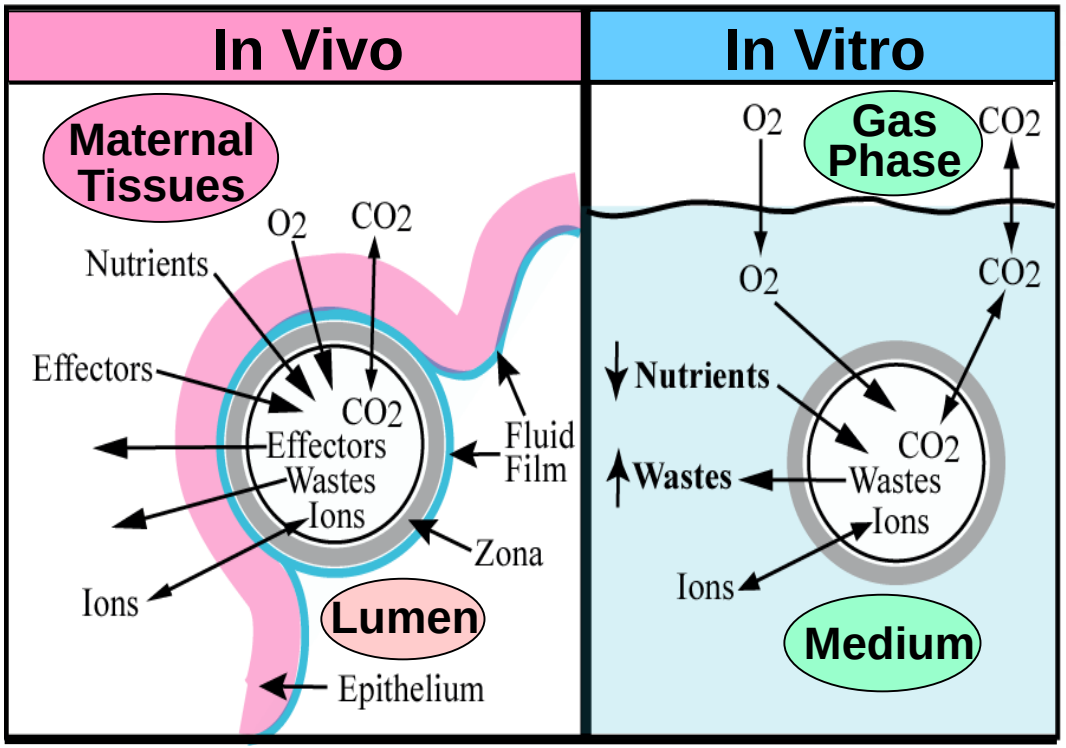
Dynamic platforms:

- Shaking/rotation
- Tilting
- Vibration
- Controlled fluid flow



The in vitro environment is quite different from that in vivo

The In Vivo vs. In Vitro Environments



Courtesy of Don Rieger

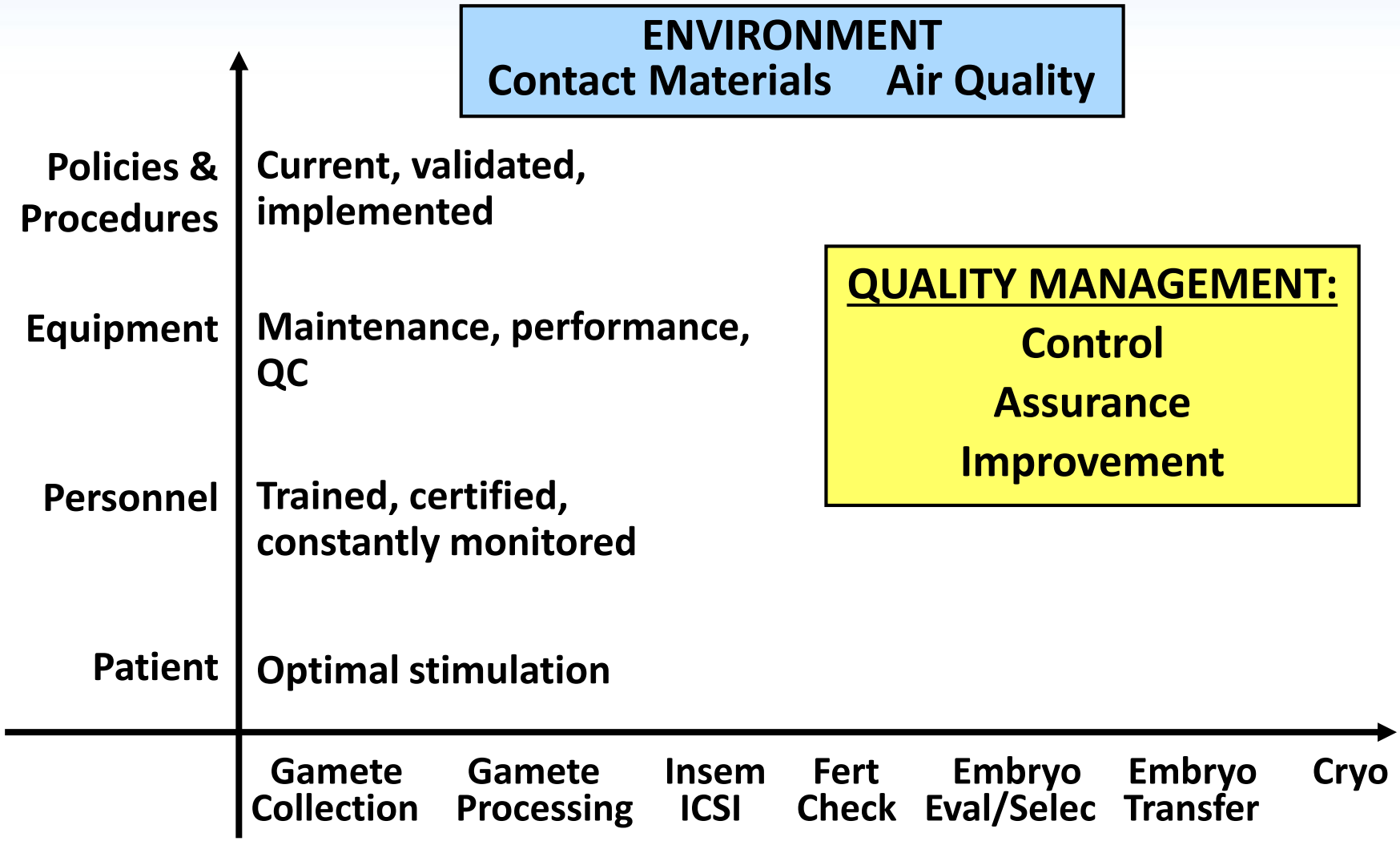
In vivo environment is:

- Moist, not fluid
- Micro, not macro
- Moving, not stagnant
- Chemically dynamic, not static
- Epithelial surfaces are glycoprotein rich, not inert

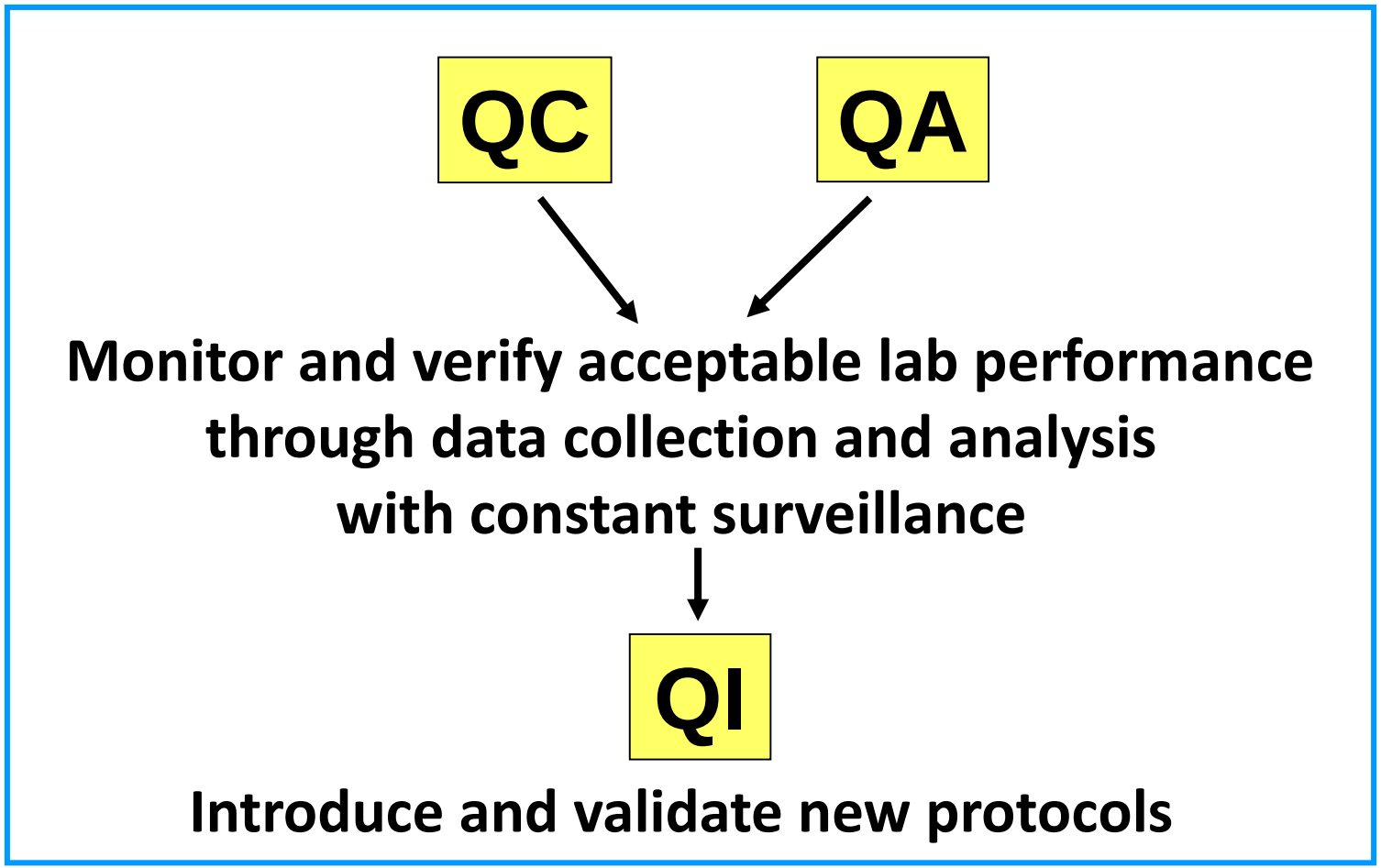
Current embryo culture systems are non-physiological and are likely to be sub-optimal

Requirements for Optimizing the Culture Conditions

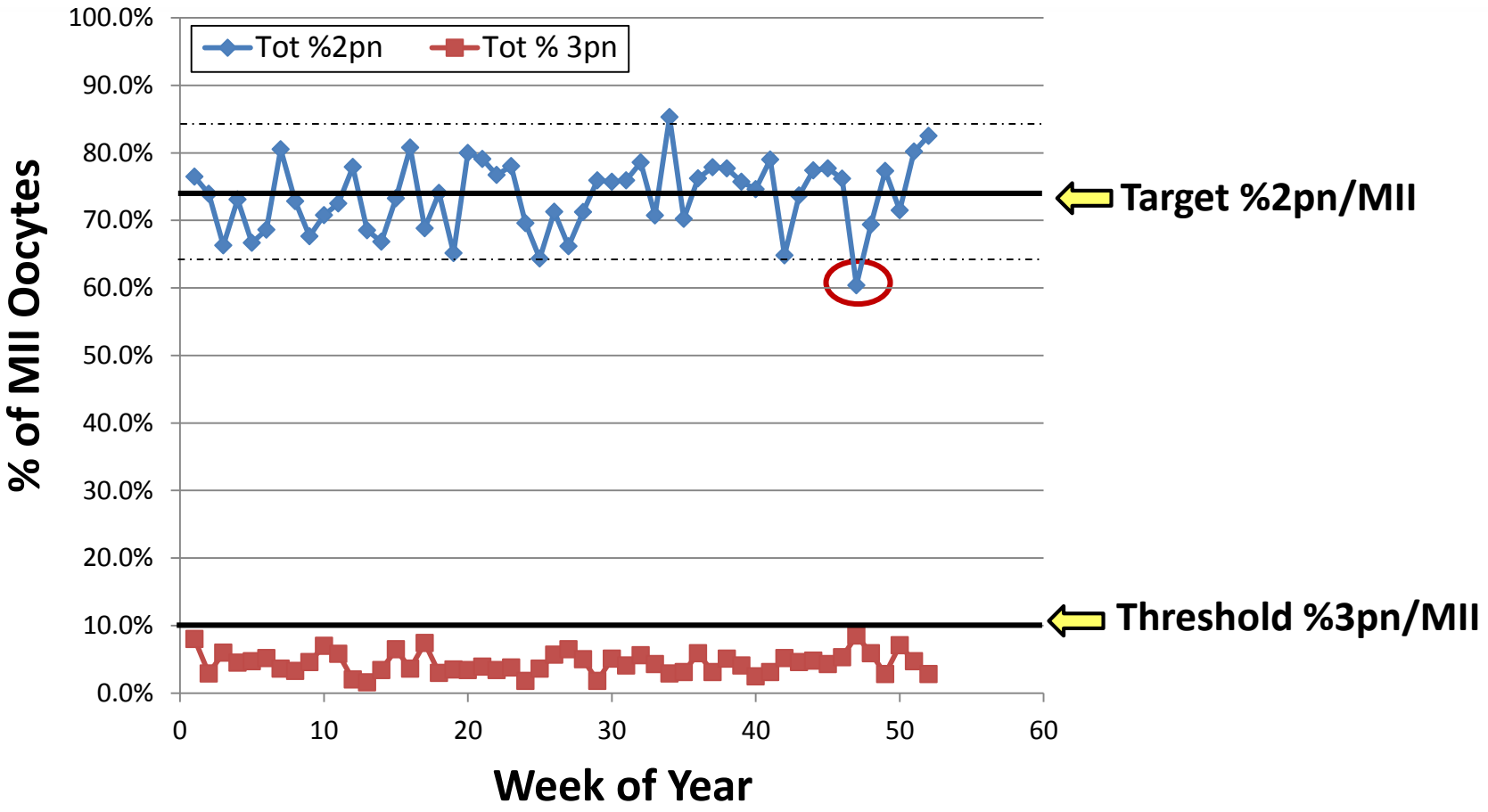
Quality Management in the IVF Laboratory



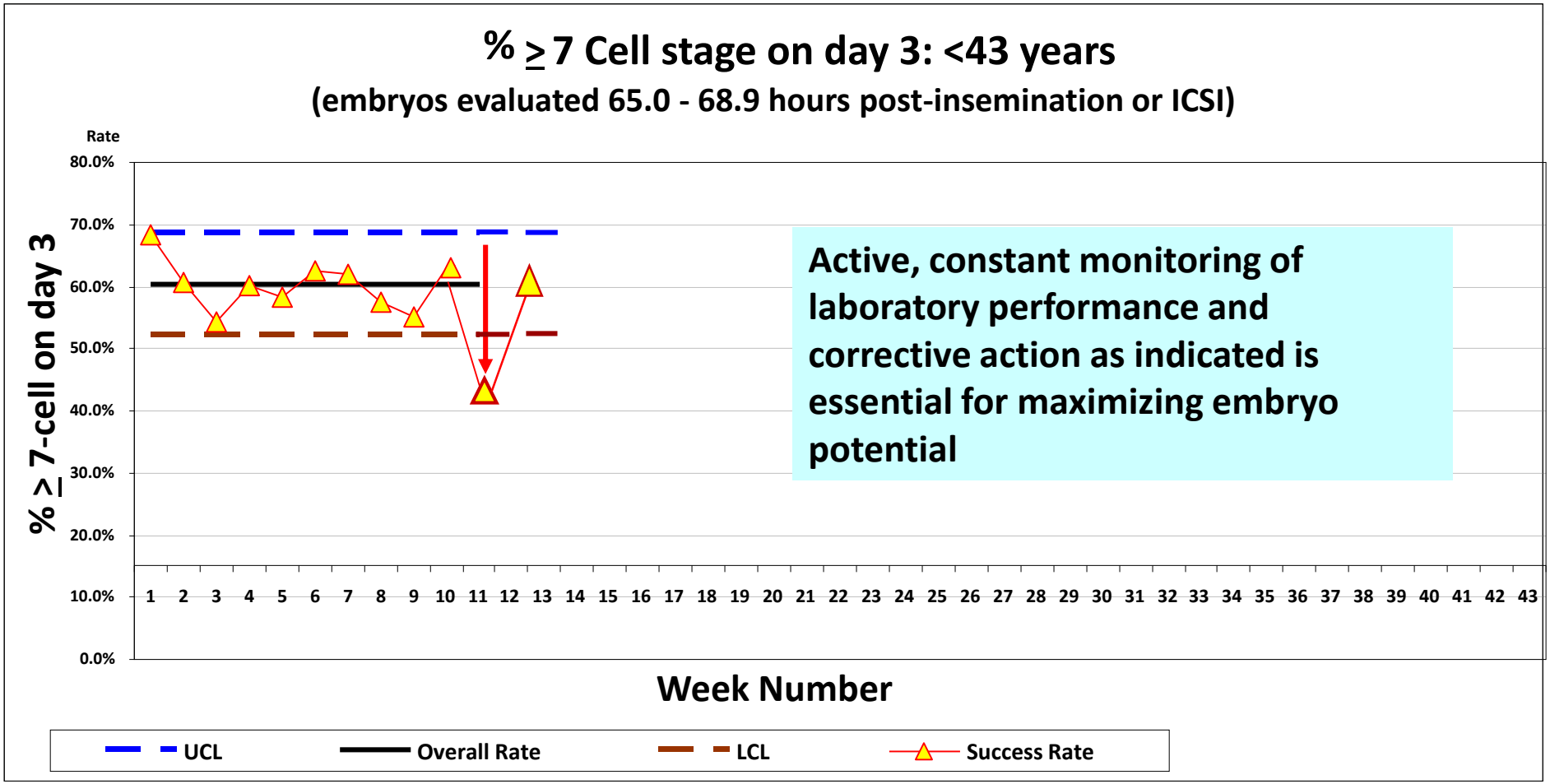
Quality Management (QM) in the IVF Laboratory



QM Program: Fertilization rate as an indicator



QM Program: Embryo development as an indicator



UCL=upper confidence limit, LCL =lower confidence limit

Oocyte Source and Optimizing the Culture System

Summary

- All the oocytes but 1 (or 2) in a retrieved cohort would have undergone atresia in a natural cycle
- A cohort of retrieved oocytes is typically heterogeneous in quality
- The embryology lab is challenged to identify the “best” oocyte/embryo and to optimize culture conditions
- An effective QM program, involving quantifiable indicators in the IVF lab, is mandatory

Rationale for Extending Culture

ARS Slide 2

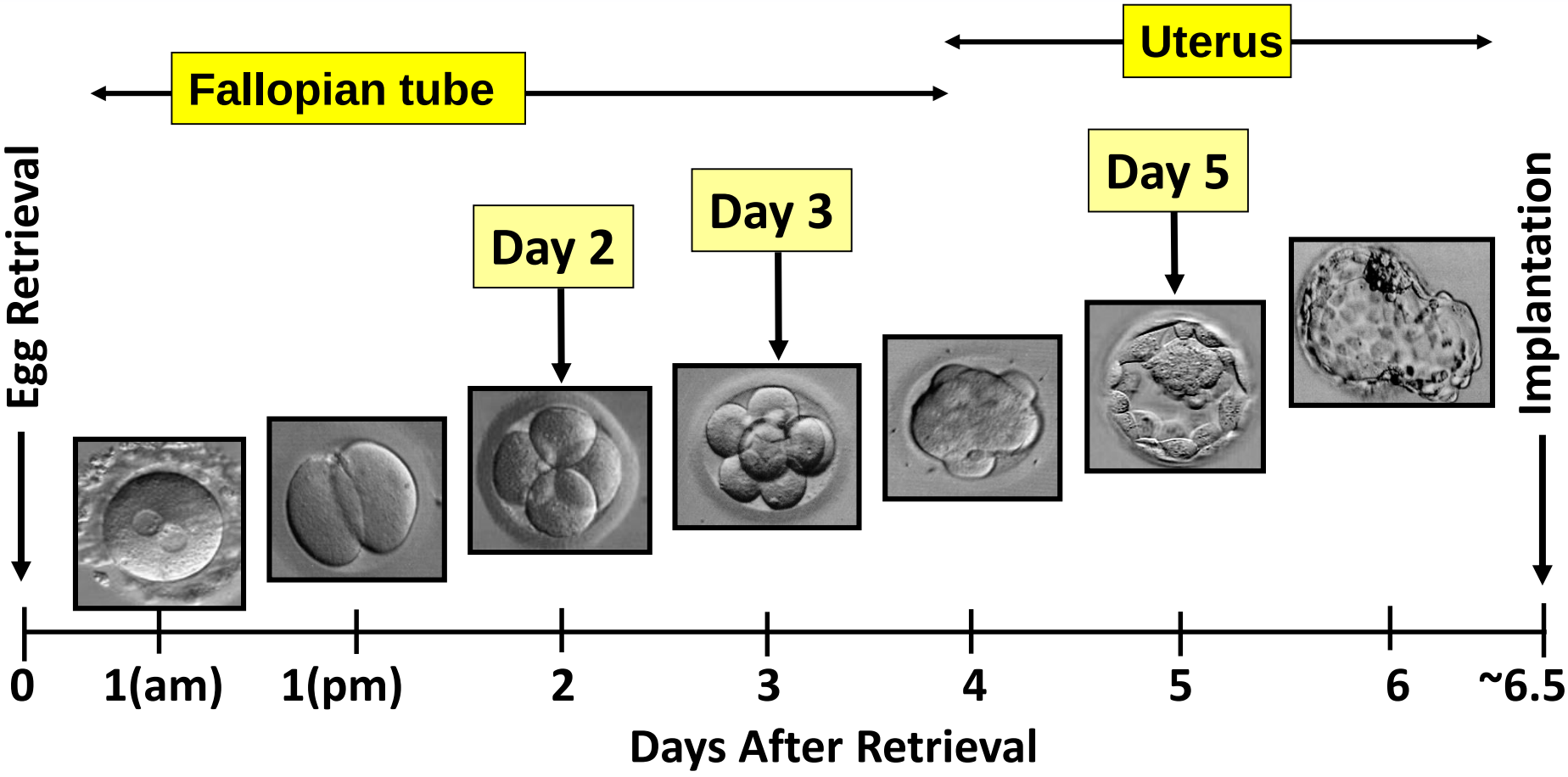
What are the key benefits of extended (i.e. blastocyst) culture?

A. This improves embryo development

B. This eliminates the non-viable embryos

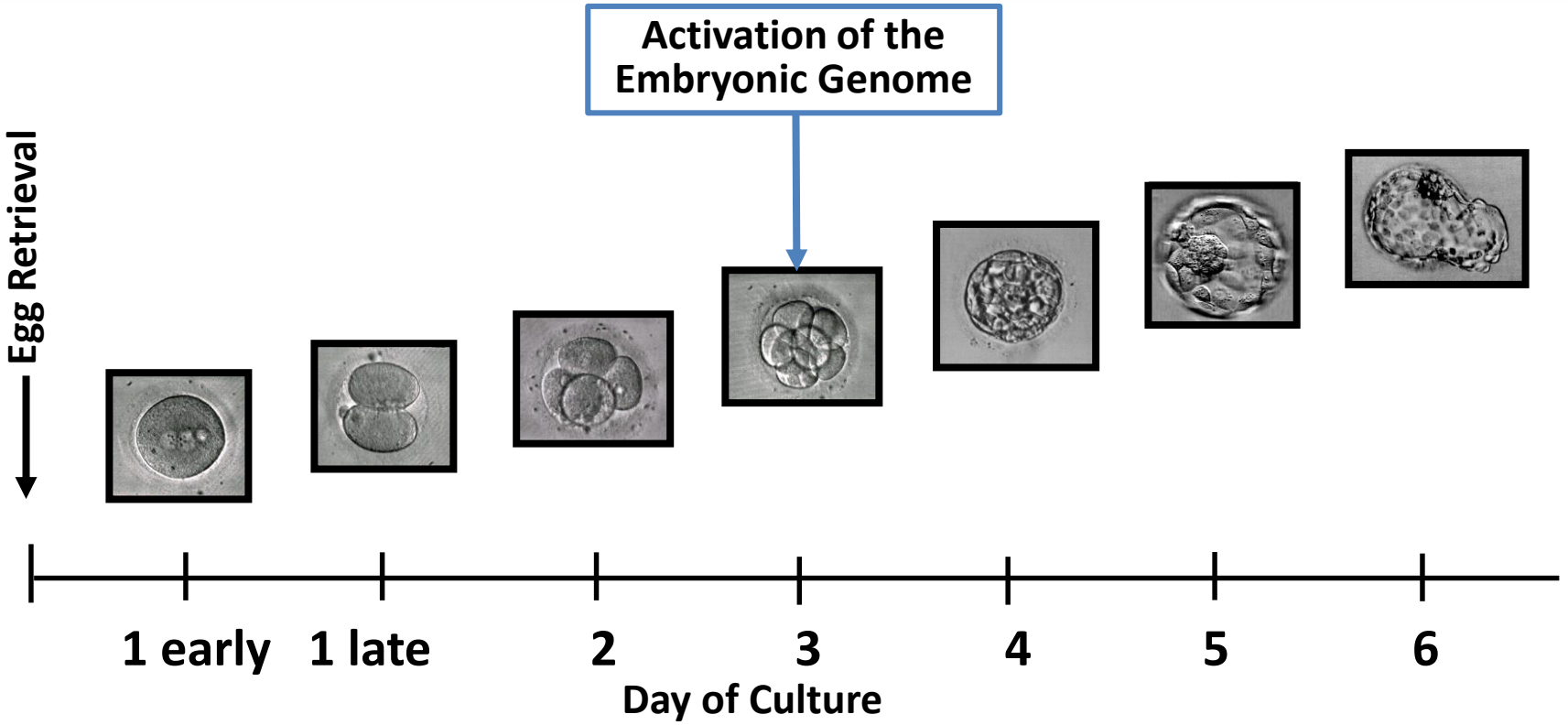
C. This helps embryologists choose the better embryo(s)

The Normal Human Preimplantation Timeline



Rationale for Extending Culture

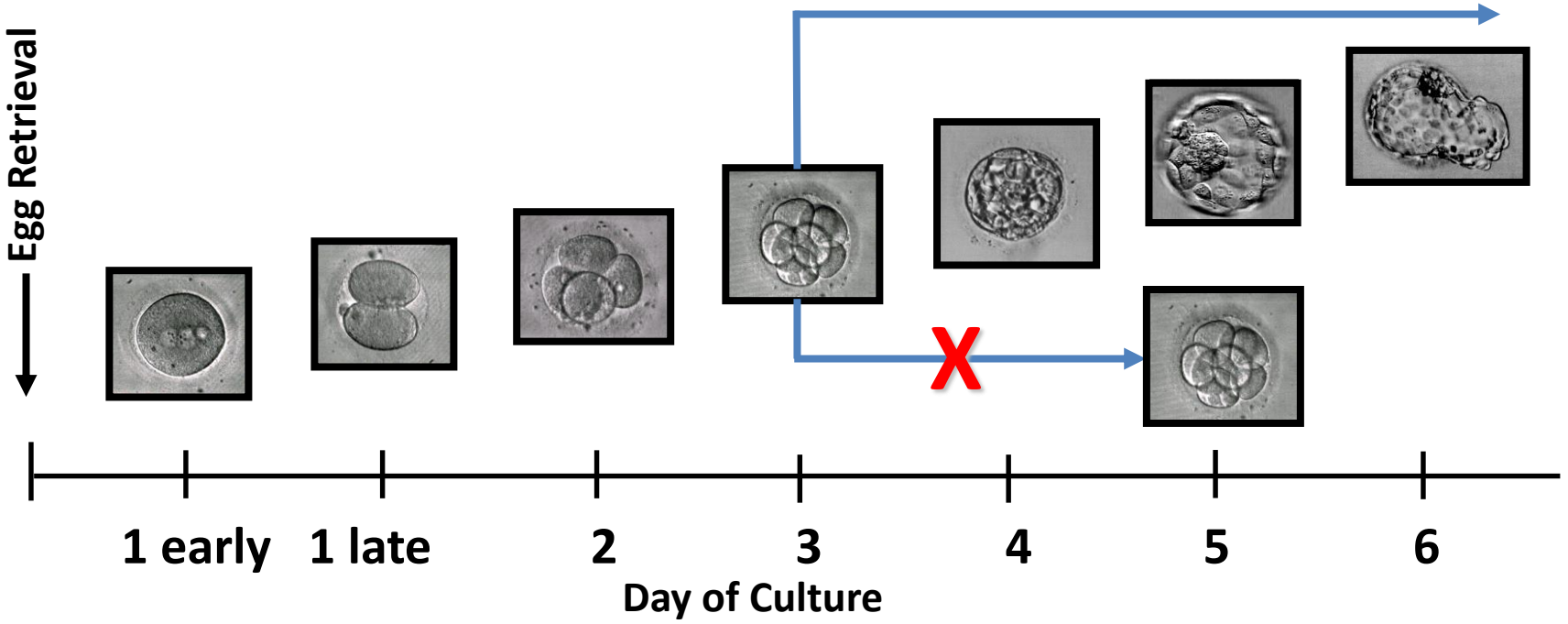
Embryo Developmental Issues



Day 5 transfer allows self-selection of the morphologically "best" embryos

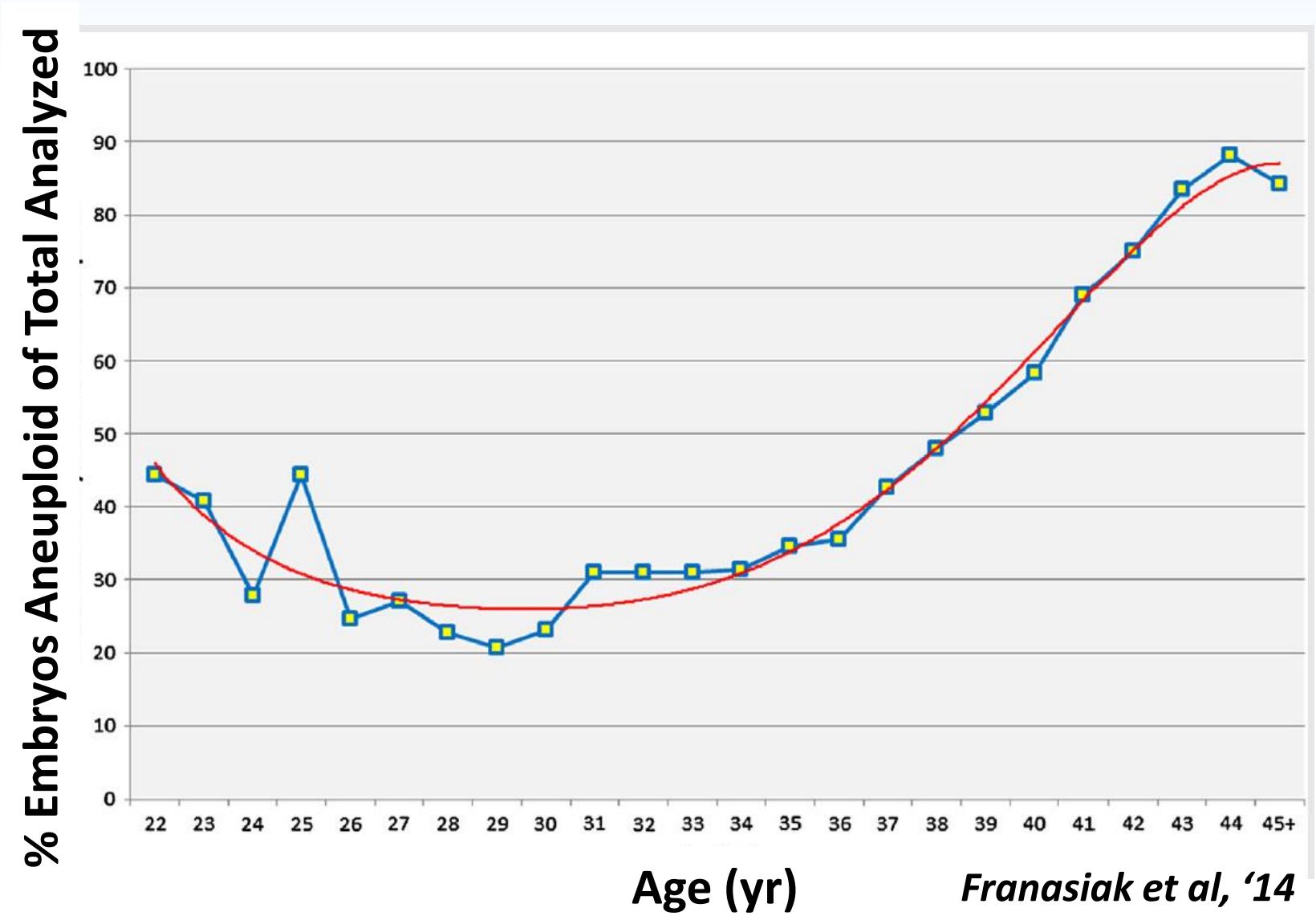
Rationale for Extending Culture

Embryo Developmental Issues



Day 5 transfer allows self-selection of the morphologically "best" embryos

Aneuploidy and Female Age in the Human

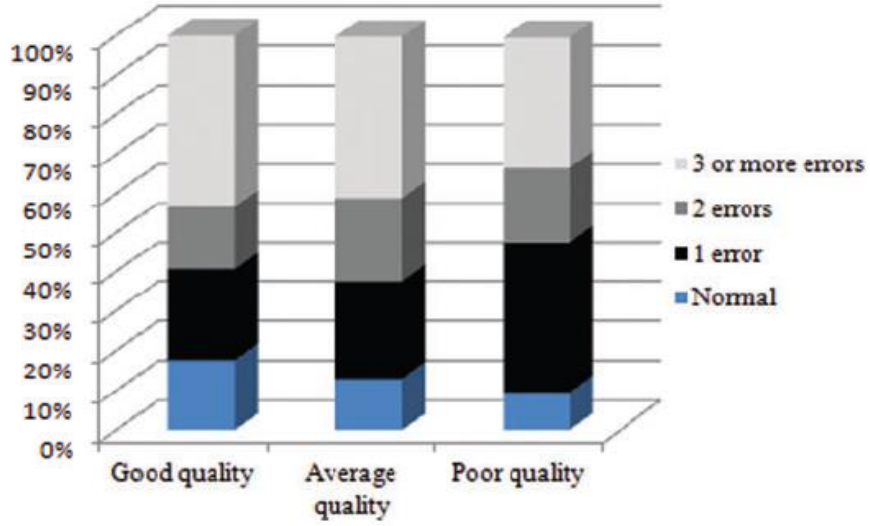


Franasiak et al, '14

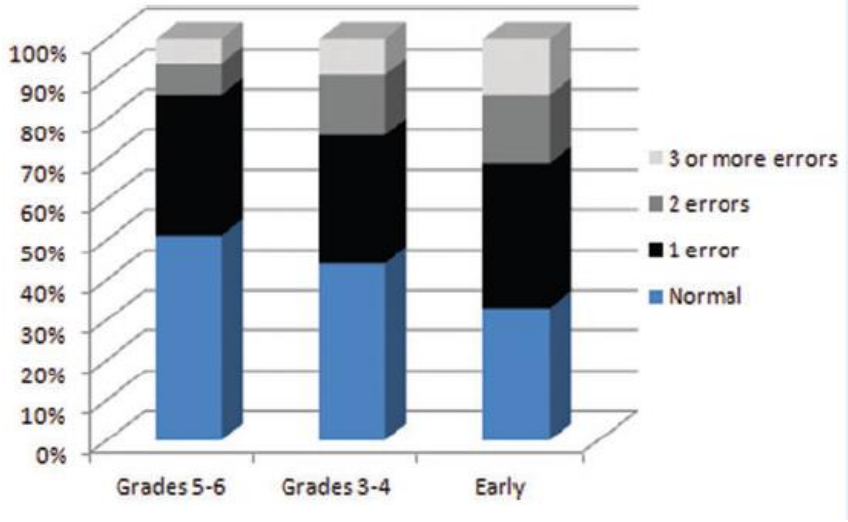
Rationale for Extending Culture

Embryo Developmental Issues

Day 3: 84% aneuploidy



Day 5: 56% aneuploidy

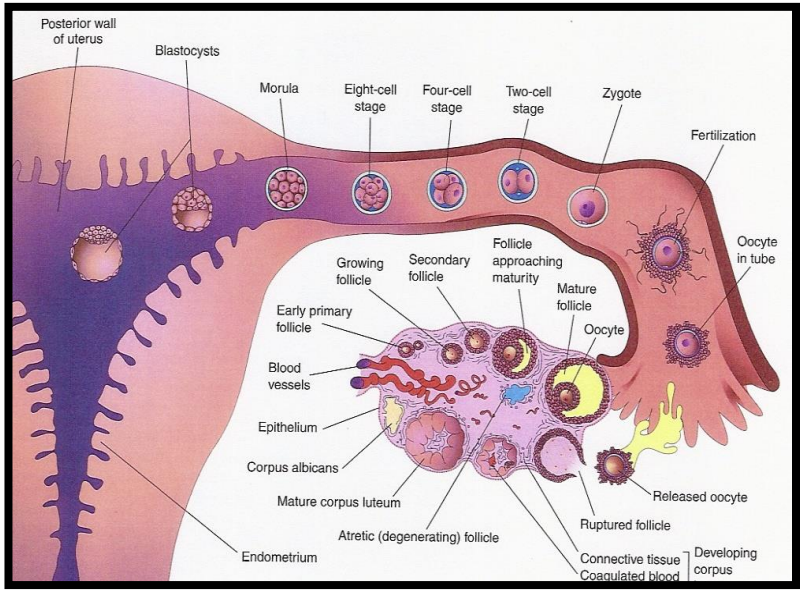


Culture to day 5 may allow for greater selection of euploid embryos

Fragouli et al., '00

Rationale for Extending Culture
Uterine Issues

- Improved synchrony between embryonic stage and uterine environment: Disturbance due to elevated estradiol[1] and progesterone[2]
- Reduced uterine contractility with blastocyst transfer[3]
- Reduced risk of embryo expulsion[4]



Moore & Persaud '98; The developing human embryo

Blastocyst transfer confers advantages on uterine

[1]Valbuena et al '01; [2]Healy et al '16; [3]Fanchin et al '01; [4]Fanchin et al '09

Rationale for Extending Culture

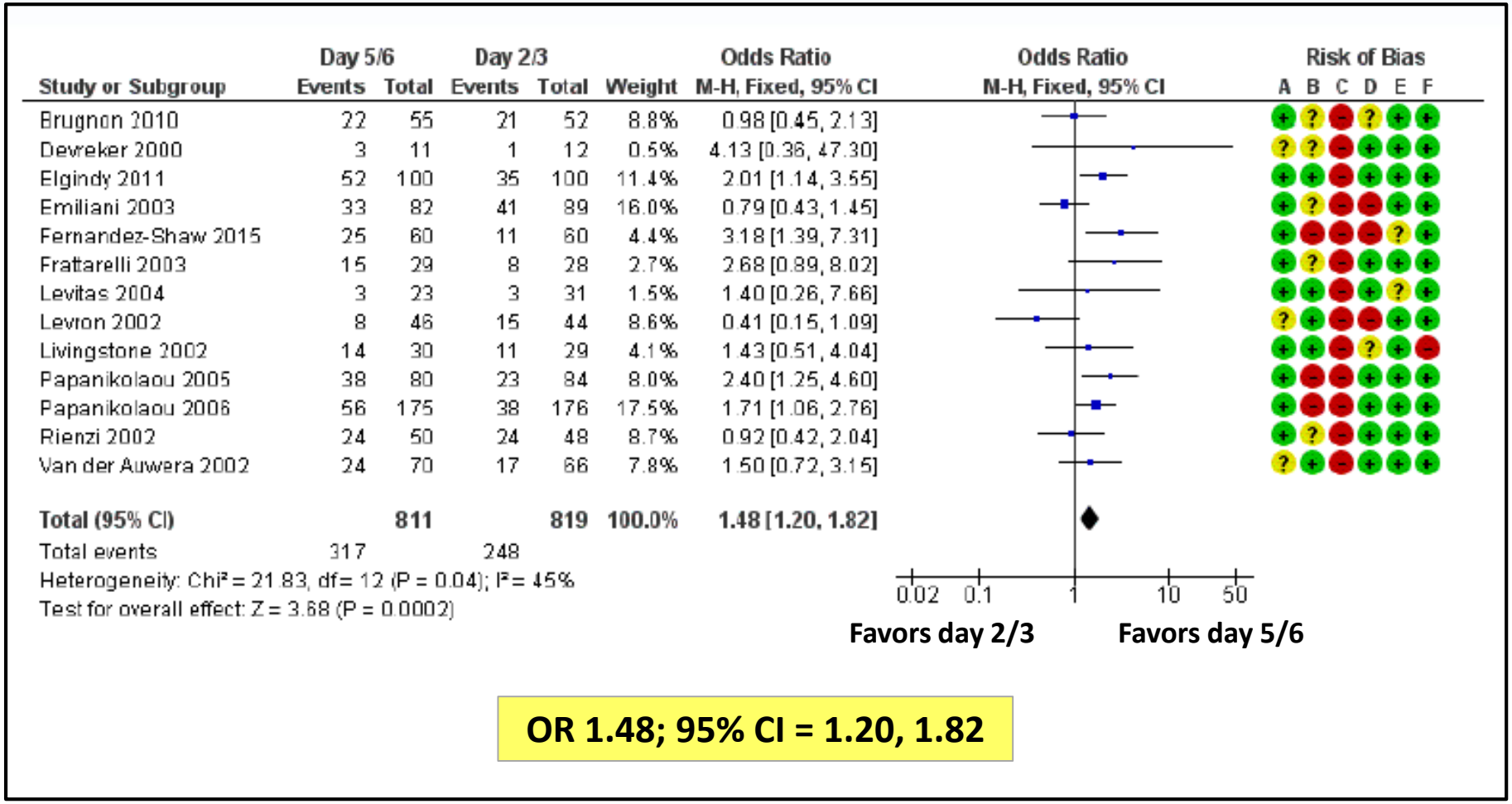
Summary

- Self-selection of embryos results in:
  - Higher quality embryos developing to the blastocyst stage
  - A lower incidence of aneuploidy in developing embryos
- The uterine environment may be more favorable for blastocyst transfer
- Therefore, extended culture should enable transfer of fewer embryos of higher quality in a more receptive uterus
- Together, higher implantation rates and lower multiple birth rates should result following blastocyst transfer

What does the evidence from RCTs tell us?

What Is the Evidence For and Against Blastocyst Culture?

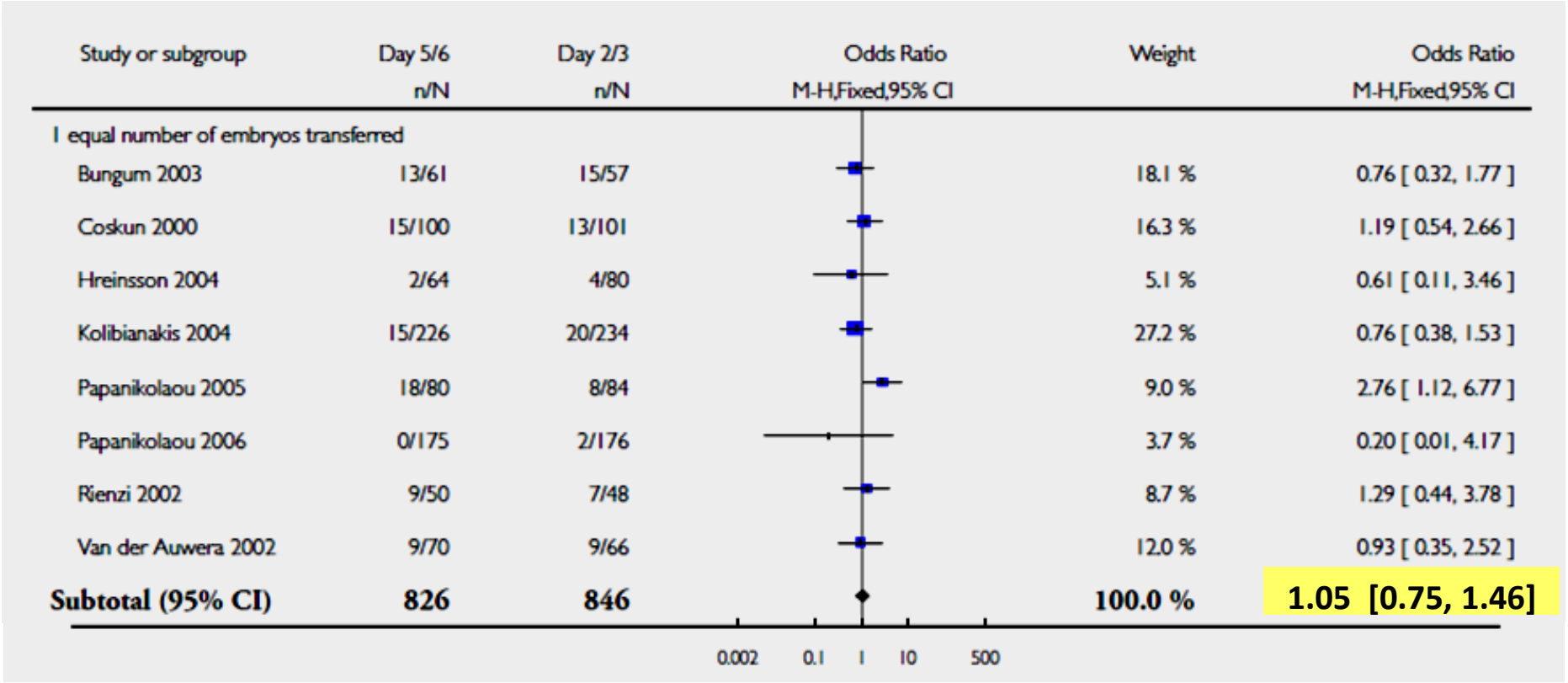
Live Birth Rate: Fresh Transfers (RCTs)



OR 1.48; 95% CI = 1.20, 1.82

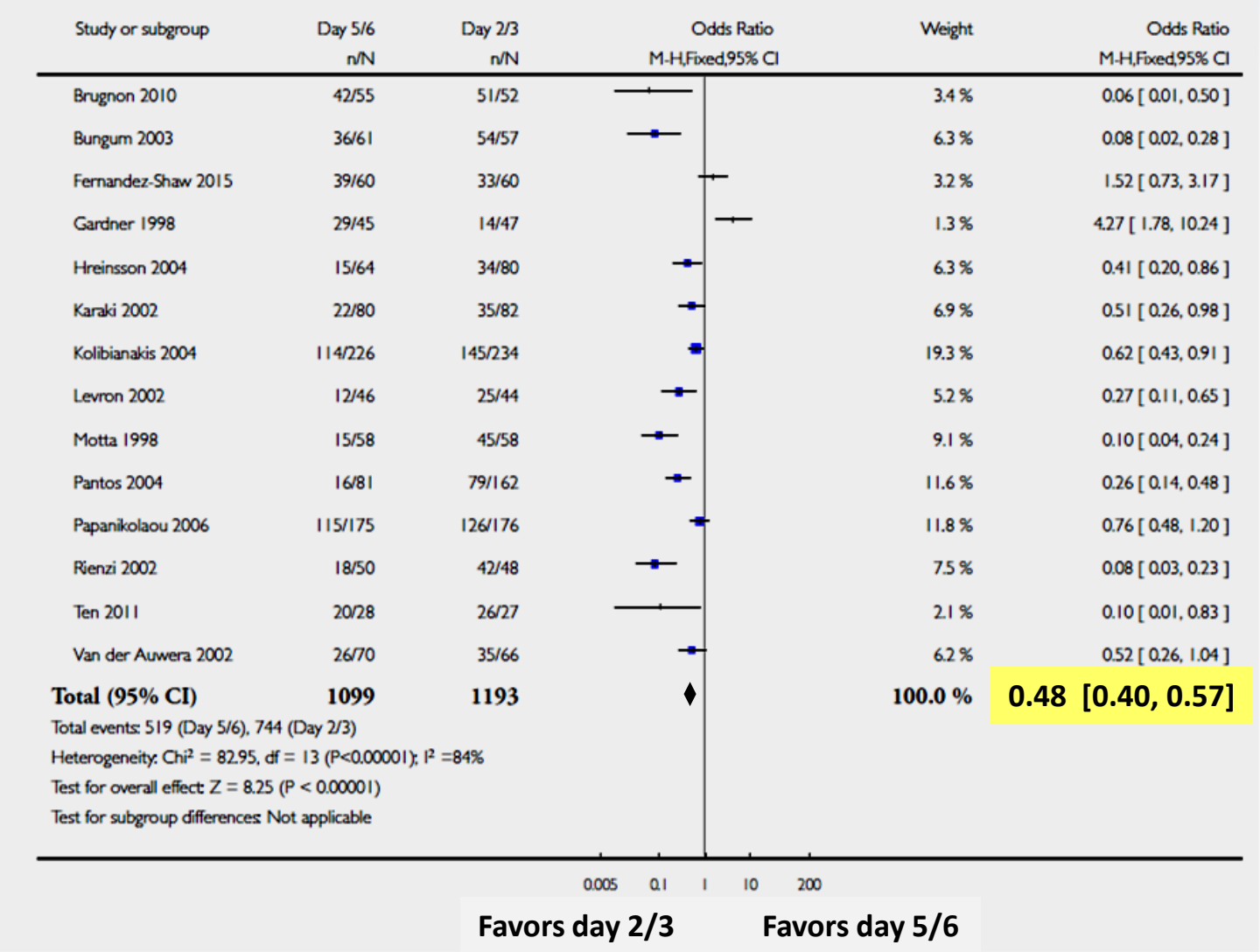
Glujovsky et al., '16

Multiple Birth Rate: Fresh Transfers



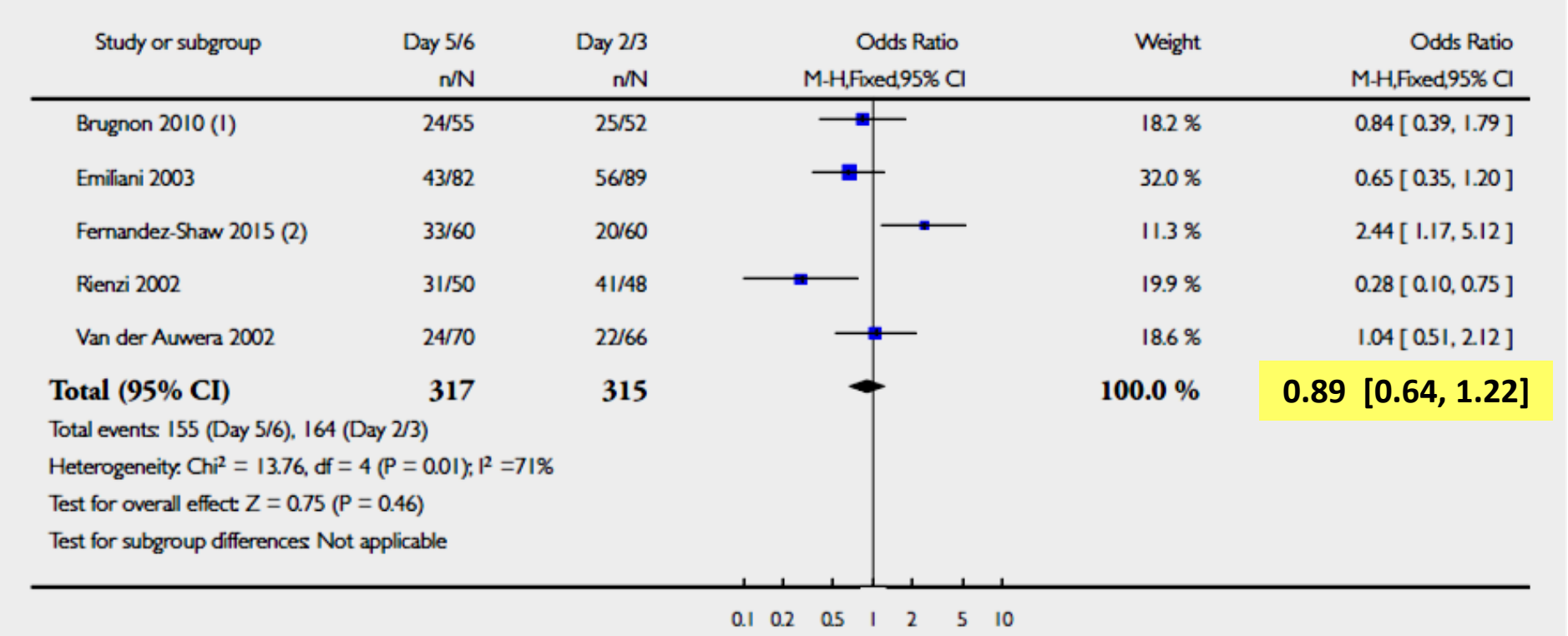
Glujovsky et al., '16

Embryo Freezing: Per Retrieval



Glujovsky et al., '16

Cumulative Live Birth Rate: Undefined # CETs



Glujovsky et al., '16

Fresh blastocyst versus cleavage transfers: Results

	# Trials	Day 5/6 Events/Total	Day 2/3 Events/Total	AOR (95% CI)
Live birth rate (LBR)	13	317/811 (39.1%)	248/819 (30.3%)	1.48 (1.20, 1.82)
Transfer cancellation rate (unselected patients	17	108/1274 (8.5%)	47/1303 (3.6%)	2.50 (1.76, 3.55)
Multiple birth (equal # embryos transferred)	8	81/826 (9.8%)	78/846 (9.2%)	1.05 (0.75, 1.46)
Embryo freezing per retrieval	14	519/1099 (47.2%)	744/1193 (62.4%)	0.48 (0.40, 0.57)
Cumulative LBR (undefined # CETs)	5	155/317 (48.9%)	164/315 (52.1%)	0.89 (0.64, 1.22)

Glujovsky et al., 2016

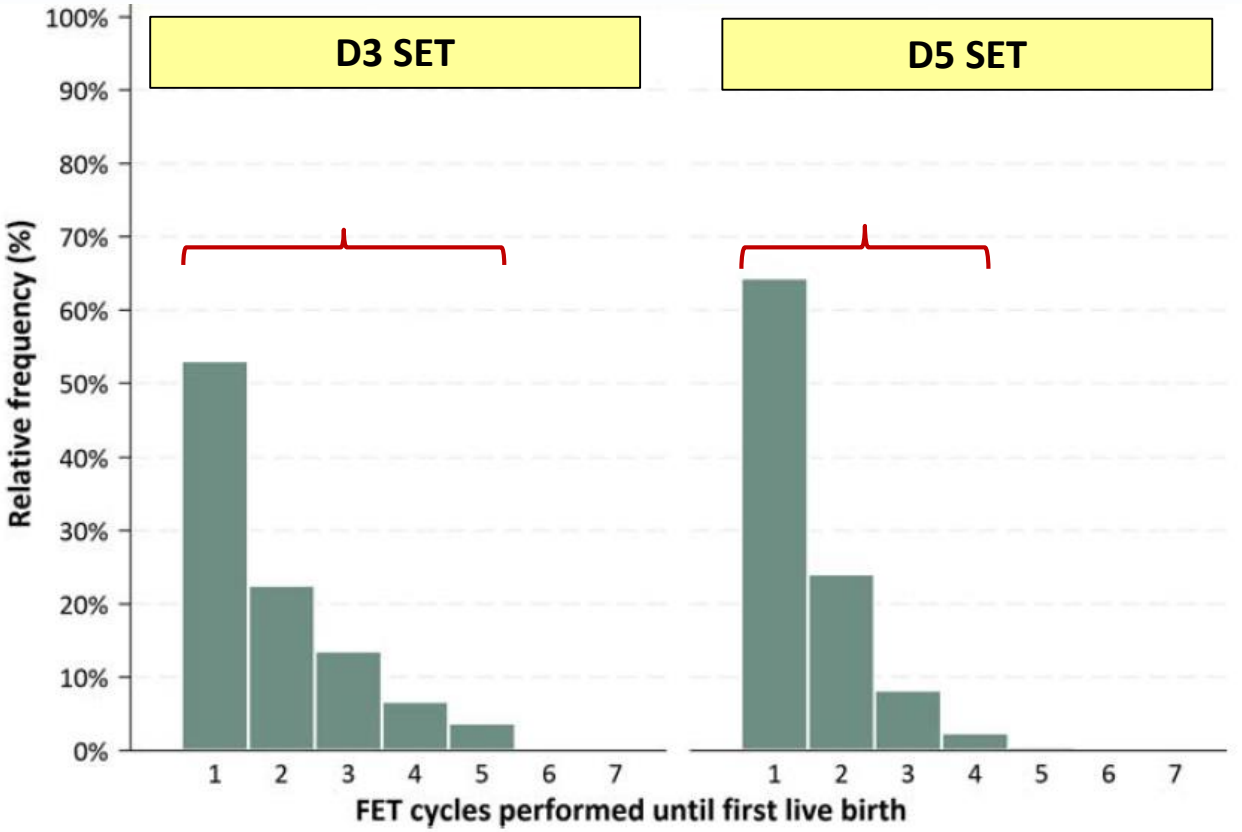
Cumulative Live Birth Rate: CETs within 1 yr of retrieval

Table II Treatment cycle live birth outcomes.

	SET Day 3 ($n = 377$)	SET Day 5 ($n = 623$)	*P* value
Fresh cycles	377	623	
Transfer rate (%)	370/377 (98.1%)	588/623 (94.4%)	0.004
Deliveries with live birth per cycle[a]	115 (30.5%)	229 (36.8%)	0.044
Singletons	115	225	
Twins	0	4[b]	
FET cycles[c]	329	325	
Transfer rate (%)	320/329 (97.3%)	296/325 (91.1%)	0.001
Double embryo transfer cycles (%)	156/320 (48.8%)	91/296 (30.7%)	<0.001
Deliveries with live birth per cycle[d]	68 (20.7%)	70 (21.5%)	0.785
Singletons	62	62	
Twins	5	7	
Triplets	1	1	
Cumulative live birth per patient			
Per innitiated fresh cycle[e]	183/377 **48.5%**	299/623 **48.0%**	0.867
Adjusted[f]	51.8%	45.9%	0.103

De Vos et al., '16

Cumulative Live Birth Rate: CETs within 1 yr of retrieval



Time to pregnancy is shorter with blastocyst transfer

De Vos et al., '16

Monozygotic Twinning from Fresh Transfers

	Incidence of MZ Twins		Fold Increased Risk
	Day 3	Day 5	
Rijinders et al ’98	0.7%	2.7%	4.0
Milki et al ’03	2.0%	5.6%	2.8
Da Costa et al ’01	0.7%	3.9%	5.6
Wright et al ’04	0.4%	1.5%	3.8
Behr et al ’00	--	5.0%	n/a

Blastocyst transfer is associated with an increased risk of monozygotic twinning

Day 2/3 vs. Day 5/6: Monozygotic Twinning

Table II The association between ART parameters and monozygosity.

	OR (95% CI)	aOR (95% CI)
Embryo stage		
Cleavage	Reference	Reference
Compaction	0.63 (0.24–1.65)	0.91 (0.34–2.38)
Early blastocyst	**2.20 (1.20–4.06)**	**2.70 (1.36–5.34)**
Advanced blastocyst	**1.73 (1.12–2.65)**	**2.05 (1.29–3.26)**

OR, univariable logistic regression odds ratio; aOR, adjusted multivariable logistic regression odds ratio.

N= 6,103 clinical pregnancies following SET

Mateizel et al., '16

Day 2/3 vs. Day 5/6: Monochorionic Twinning

Day ET	ICSI	N (cases)	OR	95% CI
3	No	1326 (12)	1.00	Referent
3	Yes	902 (18)	1.87	0.88 – 3.97
5	No	245 (7)	4.31	1.59 – 11.68
5	Yes	28 (4)	24.42	7.03 – 24.42

Blastocyst transfer is associated with an increased risk of monochorionic twinning

Skiadas et al '08

Summary of Obstetrical Outcomes

Outcome per Singleton Birth	# Studies/Subgroups	RR (95% CI)
Perinatal mortality	3	1.48 (1.09-2.02)
Pre-term birth	13	1.12 (1.02-1.23)
Very pre-term birth	10	1.14 (1.04-1.24)
Large for gestational age	7	1.12 (1.03-2.51)
Small for gestational age	8	0.84 (0.75-0.94)

However, the evidence for each of the above is of low/very low quality and most of the absolute incidences are very small

Martins et al., '16

Clinical and Obstetrical Outcomes from Day 3 vs. Day 5 ET

Summary

Day 5/6 transfers are associated with:

- An increase in live birth rate following fresh transfer
- No difference in the multiple birth rate
- An increase in monozygotic and monochorionic twinning rates
- A decrease in the number of embryos frozen
- No difference in cumulative live birth rate within 1 yr of retrieval
- A shorter time to pregnancy
- An increase in transfer cancellation rate in unselected patients
- Several adverse obstetrical outcomes, but absolute risks are low

However, the evidence supporting the above is of low/very low quality

ARS Slide 3

Which of the following is blastocyst *versus* cleavage stage transfer associated with:

A. An increased risk of monozygotic and monochorionic twinning
B. An increase in live birth rate following fresh transfer
C. A decrease in the number of embryos frozen
D. A shorter time to pregnancy
E. No difference in cumulative pregnancy rate within 1 year of the retrieval
F. All of the above

Which Patients Should Have Blastocyst Culture?

Which Patients Should Have Blastocyst Culture?

PGT patients: YES …… at least for now!

PGT

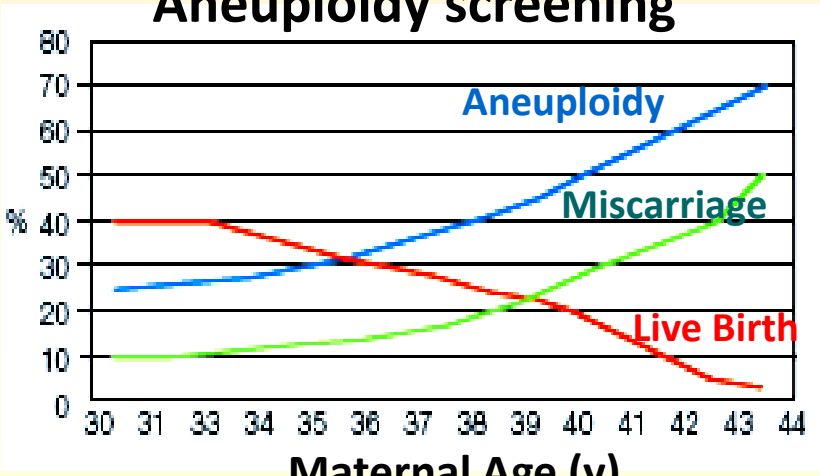
PGD

PGS

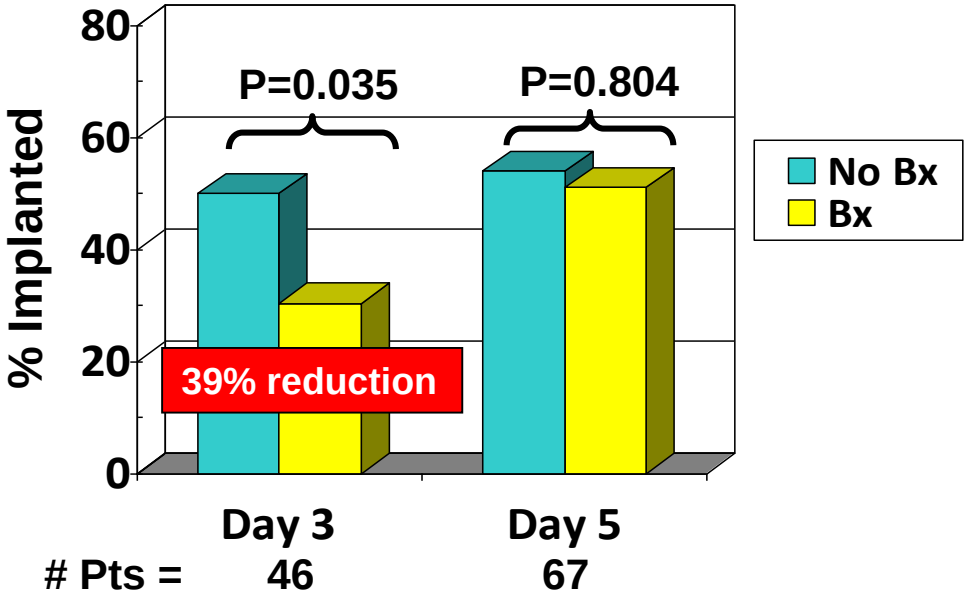
Known genetic disorders

- **Highly accurate**
- **Requires care in Bx/handling**
- **Potential allelic dropout**
- **Poor amplification**
- **Mosaicism**

Aneuploidy screening



Day 5 Biopsy Appears Not to Impact Implantation



- One of a sibling embryo pair was biopsied & the embryos transferred in pairs
- Conceptuses were DNA fingerprinted to determine whether implanted embryo was biopsied or not

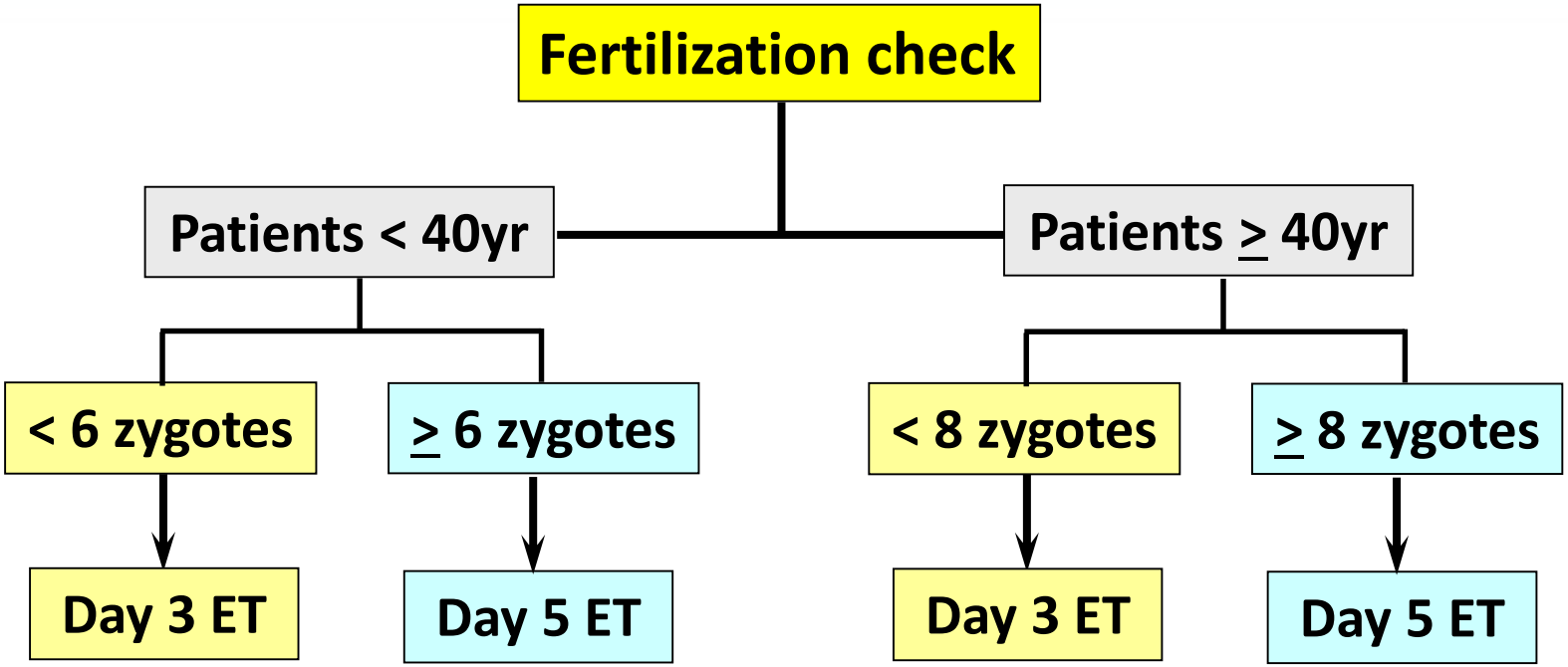
Which Patients Should Have Blastocyst Culture?

What About non-PGT patients?

- A definitive answer remains to be determined
- Appropriately powered RCTs with current technologies are required to resolve this issue
- Because of the risk of having no blastocysts to transfer, an algorithm for transfer day should be used
- Patients should be counseled regarding the pros and cons of each transfer day

Algorithm for Patient Selection to Day 3 *versus* Day 5 Transfer

Prospective Selection of ET Day for Non-PGT Patients



Day 3 is recommended for patients with poor previous blastocyst formation

ARS Slide 4

Please answer “yes” to only one of the following.

After listening to this lecture, do you think:

A. All patients should have blastocyst transfer

B. Selected patients should have blastocyst transfer

C. No patients should have blastocyst transfer

Key Points: Blastocyst versus Cleavage Transfer

- **Blastocyst culture requires that a lab is "in control" through implementation of a stable QM program**
- **The lab must have:**
  - An efficacious and reliable culture system
  - Adequate incubator space to keep all embryos safe
  - A proven vitrification protocol for blastocyst freezing
- **Acknowledgment that extended culture increases costs to the laboratory**

Key Points: Blastocyst versus Cleavage Transfer

- **The rationale for blastocyst culture rests on benefits from:**
  - Self-selection of those embryos capable of forming blastocysts (at least in vitro) and possibly some selection of euploid embryos
  - Potentially improved uterine receptivity

Key Points: Blastocyst versus Cleavage Transfer

- **Blastocyst transfer is associated with increased risks of monozygotic and monochorionic twinning, as well as some obstetrical and neonatal risks**
- **Blastocyst transfer is associated with a shortened time to pregnancy:**
  - Emotional value and reduced costs to patients
- **However, cumulative pregnancy rates between day 3 and day 5 transfer are very similar, if not identical**
- **If a lab offers blastocyst transfer, an ET algorithm is recommended**

Final Comments

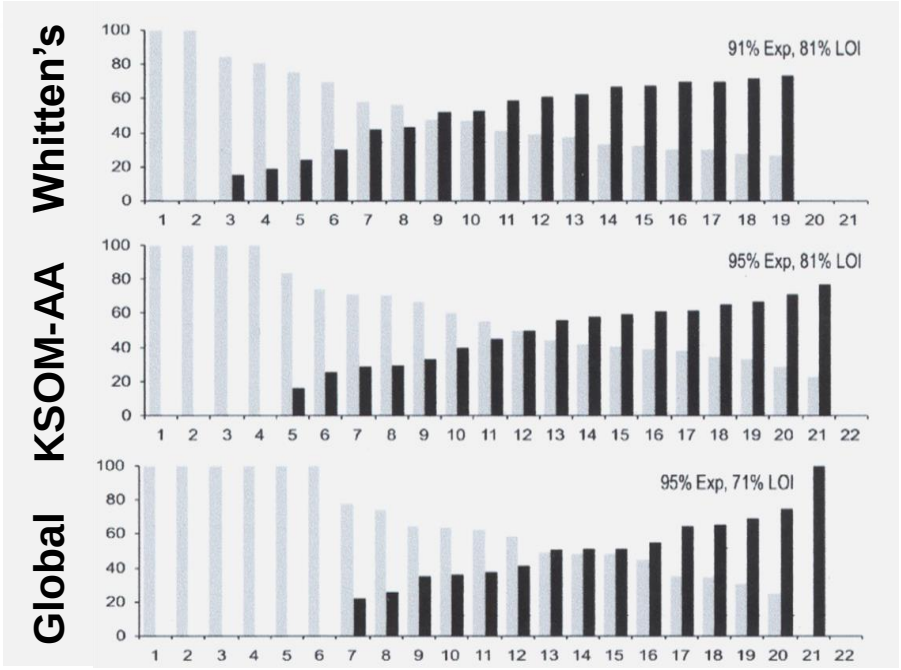
We have come a long way since the birth of Louise Brown nearly four decades ago

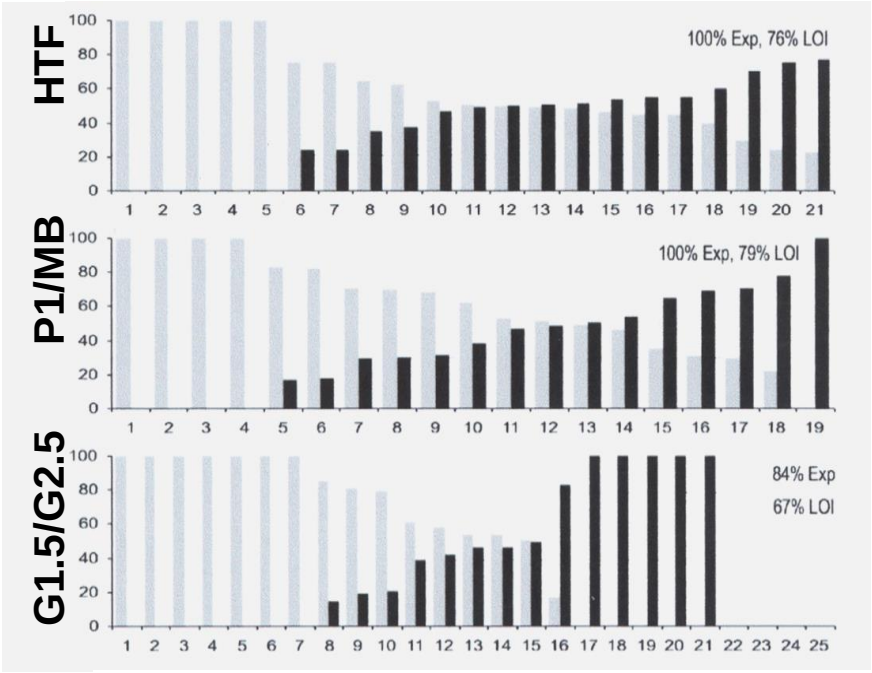
- **We have a greatly improved understanding regarding:**
  - The biology of human gametes and embryos, and the development of the pre-implantation embryo
  - The basic requirements in running an IVF laboratory, and in culturing embryos to the blastocyst stage
- **We have also made great advances in ovarian stimulation and transfer protocols**

More and more patients are leaving our clinics pregnant!!

HOWEVER ……..

H19 Expression in Mouse Embryos





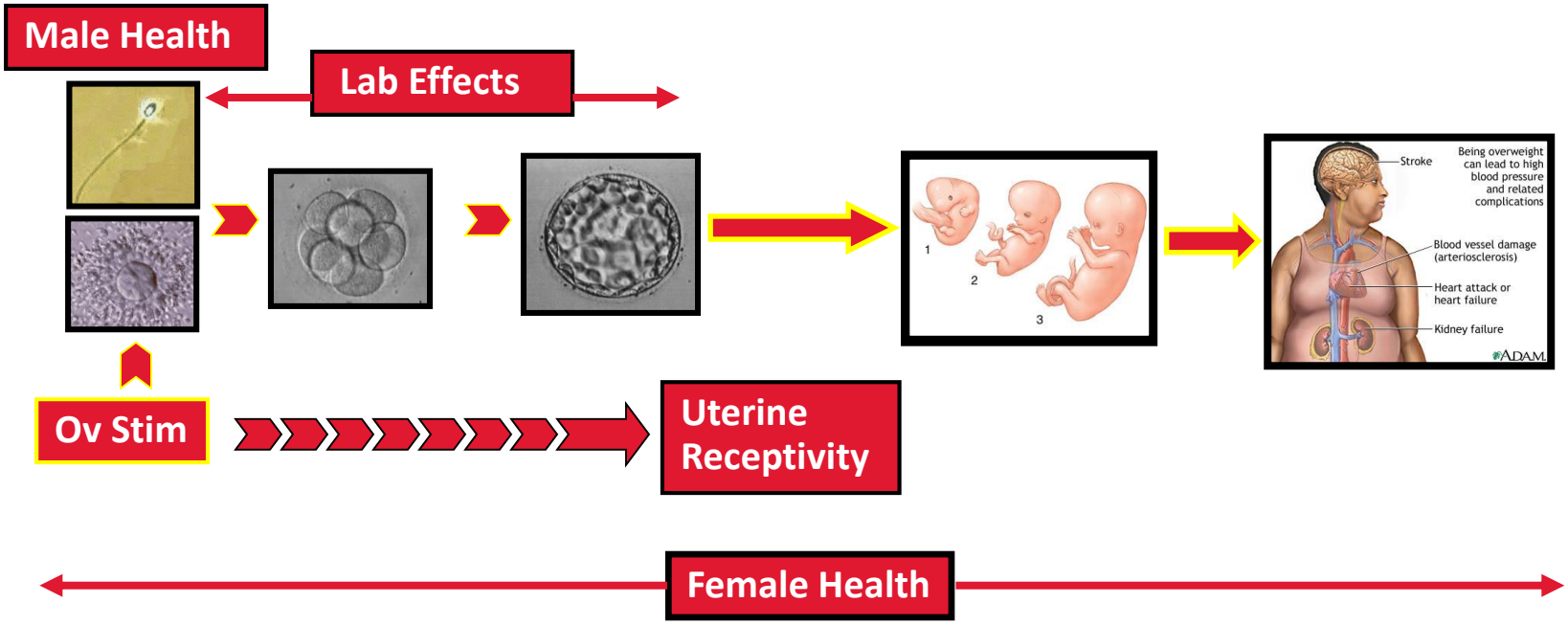
Maternal Paternal

It is currently unknown whether there are media-associated epigenome-wide alterations in human embryos during culture

Markert-Velker et al., '10

The Barker Hypothesis

A baby's nourishment before birth and during infancy, as manifest in patterns of fetal and infant growth, "programmes" the development of risk factors such as raised blood pressure and glucose intolerance that are key determinants of coronary heart disease



Barker DJ. Eur J Epidemiol. 2003;8:733-736

THANK YOU!!