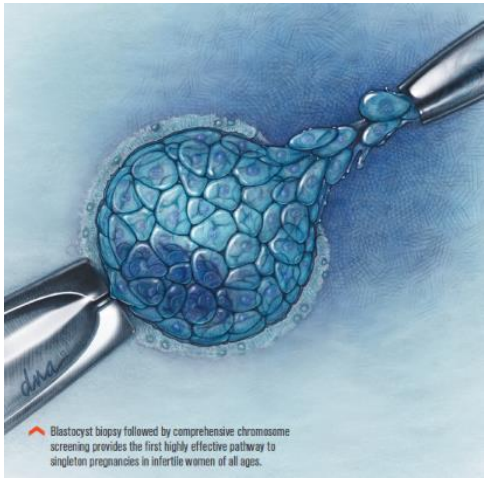
Using Preimplantation Genetic Screening (PGS) to Optimize Elective Single Embryo Transfer (eSET)



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Center for Women's Reproductive Care at Columbia University

ColumbiaDoctors

Objectives

- To discuss the advantages and safety benefits of elective single embryo transfer (eSET)
- To review the limitations of morphology-based selection for eSET
- To summarize the development of a validated technology for preimplantation genetic screening (PGS)
- To highlight future directions to further enhance safety and efficacy of IVF

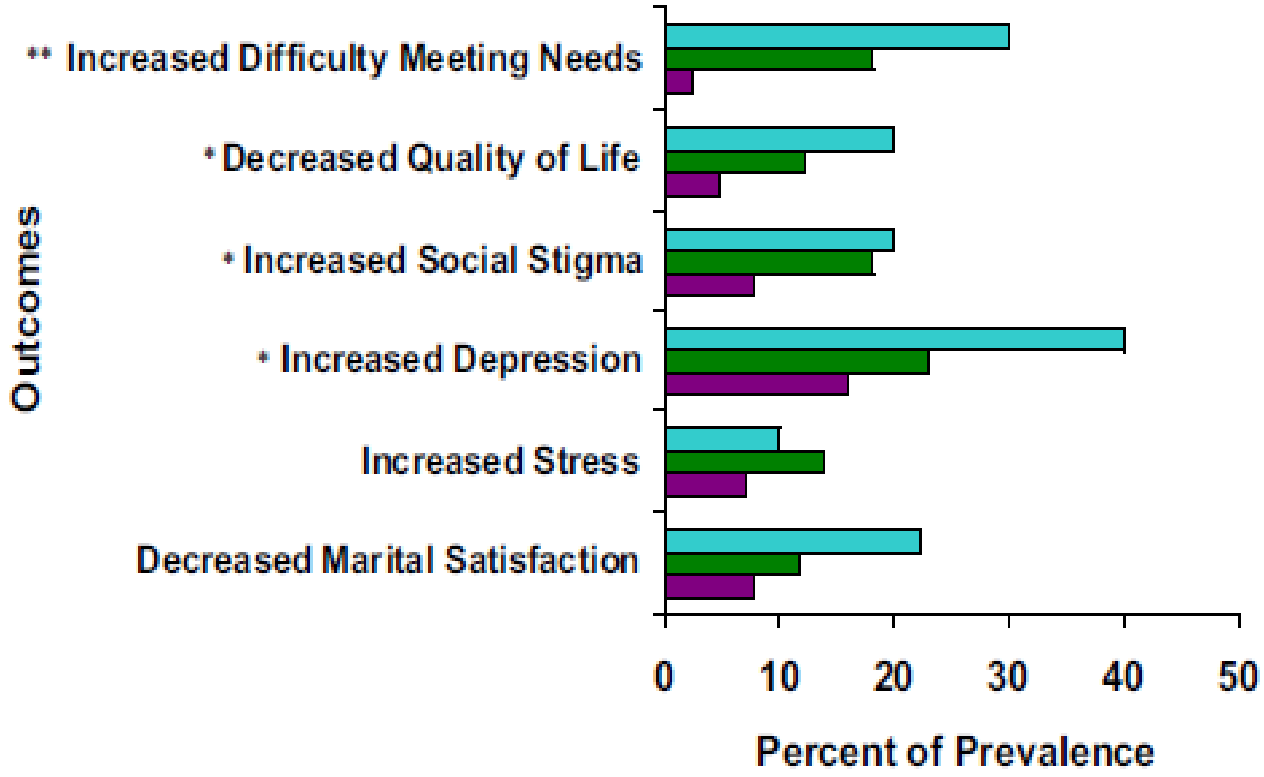
Singleton Term Delivery: The Ideal IVF Outcome

- IVF twin pregnancies are at an increased risk of:
  - Preeclampsia (2-fold risk increase)[1]
  - Extreme prematurity (7.4-fold increase delivery <32 wks) [2]
  - NICU admission (3.8-fold increased risk)[2]
  - Perinatal Death (2-fold increase)[2]
- Two IVF singleton deliveries have better obstetrical outcomes than one IVF twin delivery[3]

1. ASRM Practice Committee, *Fertil Steril*, 2012. PMID: 22192352
2. Pinborg A, et al., *Acta Obstet Gynecol Scand*, 2004. PMID: 15488125
3. Sazonova A ,et al., *Fertil Steril*, 2013. PMID: 23219009

Multiple Births Increase the Psychological Burden of Infertility Care

Prevalence (%) in outcomes by multiplicity in full-term births: *purple* = singletons; *green* = twins; *turquoise* = triplets. *P <.05, **P <.001.



Ellison et al., *Fertil Steril,* 2005. PMID: 15866579

ASRM Guidelines Do Not Eliminate Multiples

TABLE 1

Recommended limits on the numbers of embryos to transfer.

Prognosis	Age < 35 yrs	35–37 yrs	38–40 yrs	41–42 yrs
Cleavage-stage embryos[a]				
Favorable[b]	1–2	2	3	5
All others	2	3	4	5
Blastocysts[a]				
Favorable[b]	1	2	2	3
All others	2	2	3	3

[a] See text for more complete explanations. Justification for transfering one additional embryo more than the recommended limit should be clearly documented in the patient's medical record.

[b] Favorable = first cycle of IVF, good embryo quality, excess embryos available for cryopreservation, or previous successful IVF cycle.

Practice Committee Number of Embryos Transferred. Fertil Steril 2009.

- Nearly half of all babies born after IVF are part of a multiple birth[1]
- A mandatory SET policy for good-prognosis patients still resulted in a 19% multiple pregnancy rate[2]

1. MMWR Surveil Summ., 2009. PMID: 19521336
2. Ryan GL et al., *Fertil* Steril, 2007. PMID: 17490657

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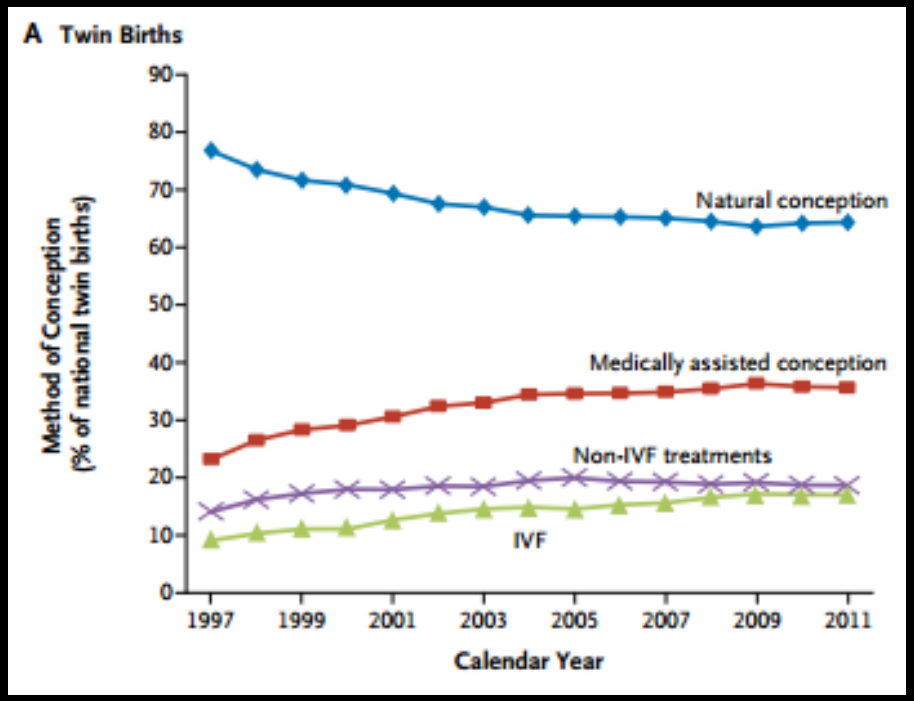
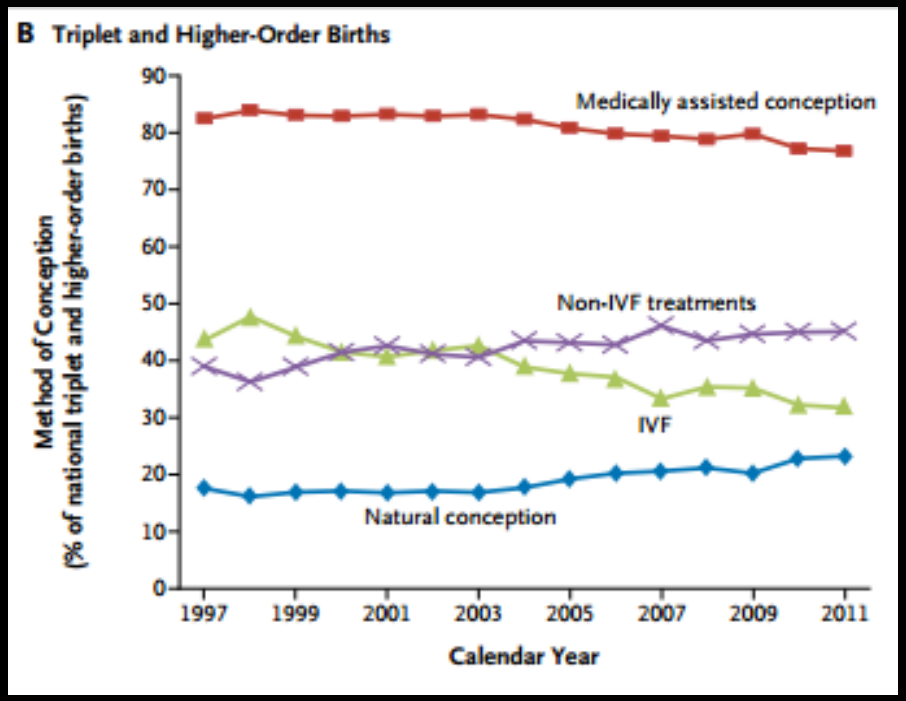
ORIGINAL ARTICLE

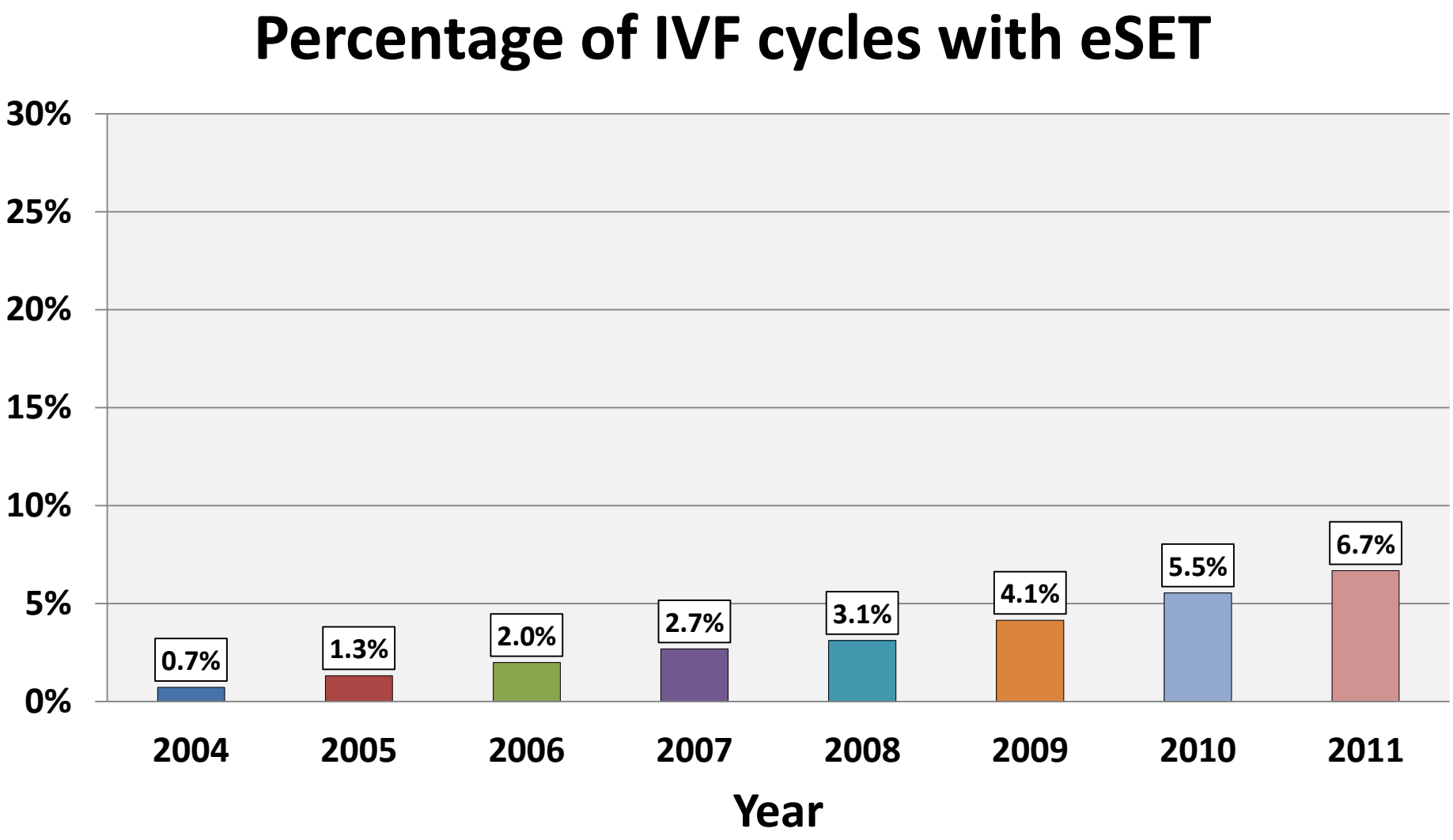
Fertility Treatments and Multiple Births in the United States





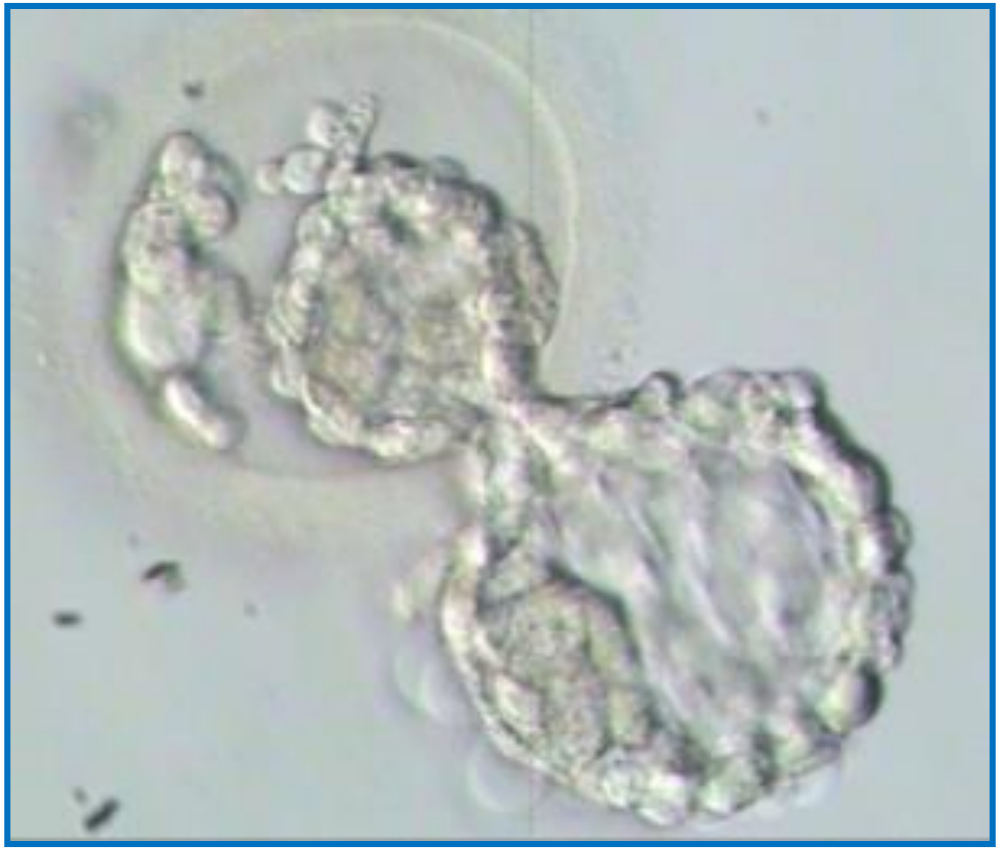
Kulkarni D et al, *New Engl J Med*, 2014. PMID: 24304051

Single embryo transfer is rare in the U.S.



Source: Sart.org

Why not transfer a single embryo every time?



How many failures before Louise Brown & Elizabeth Carr?

40th attempt was ectopic

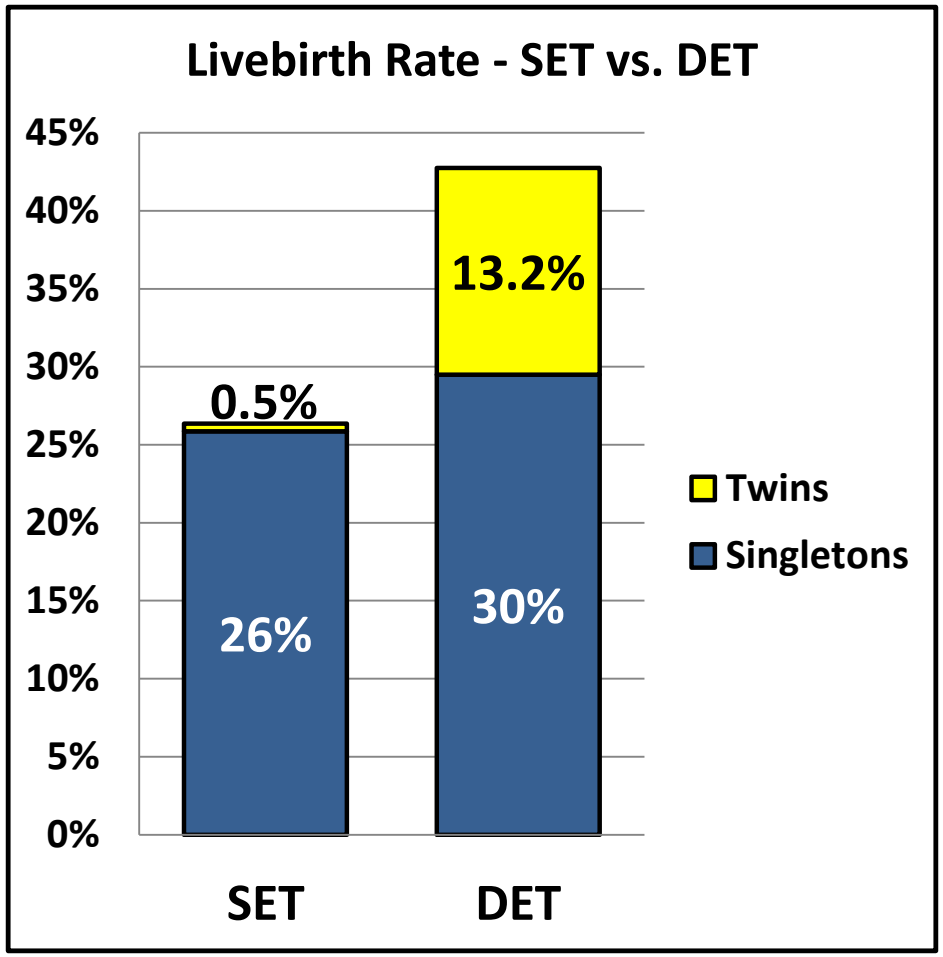
104th attempt Louise was born

41 natural cycles

13 hMG stimulations

54th attempt Elizabeth was born

FRESH SET RESULTS IN LOWER DELIVERY RATES THAN DOUBLE EMBRYO TRANSFER (DET)

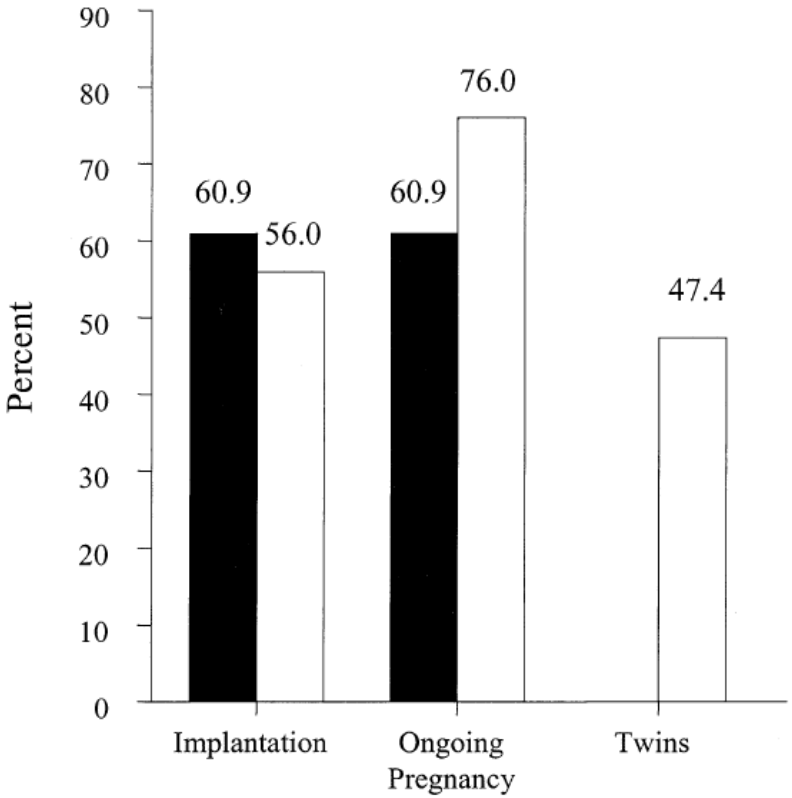


- Cochrane Review of 6 randomized trials from 1999-2006 (N = 1,257)
- Young, good prognosis patients with “top quality” embryos available
- Slightly more singletons after DET

Pandian Z et al., *Cochrane Database Syst Rev*, 2009. PMID: 19370588

Single blastocyst transfer: a prospective randomized trial

David K. Gardner, D.Phil., Eric Surrey, M.D., Debra Minjarez, M.D., Annette Leitz, L.P.N., John Stevens, B.S., and William B. Schoolcraft, M.D.



- 95% CI of Risk Difference[1]: -41% to +11%
- RCT of single blast vs. single cleavage (32% vs. 22% delivery rate)[2]
- Did not significantly impact clinical practice as eSET still underutilized

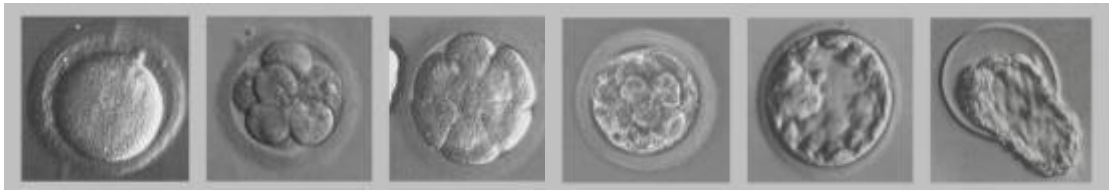
1. Gardner DK *et al*, *Fertil Steril*, 2004
2. Papanikolaou EG *et al*, *NEJM*, 2006

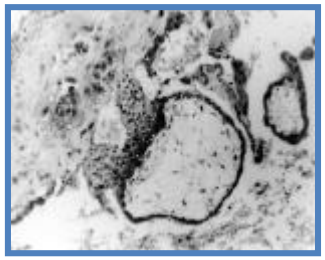
Success Rates and Acceptability of eSET

Table IV. Attitudes towards elective single embryo transfer (eSET)

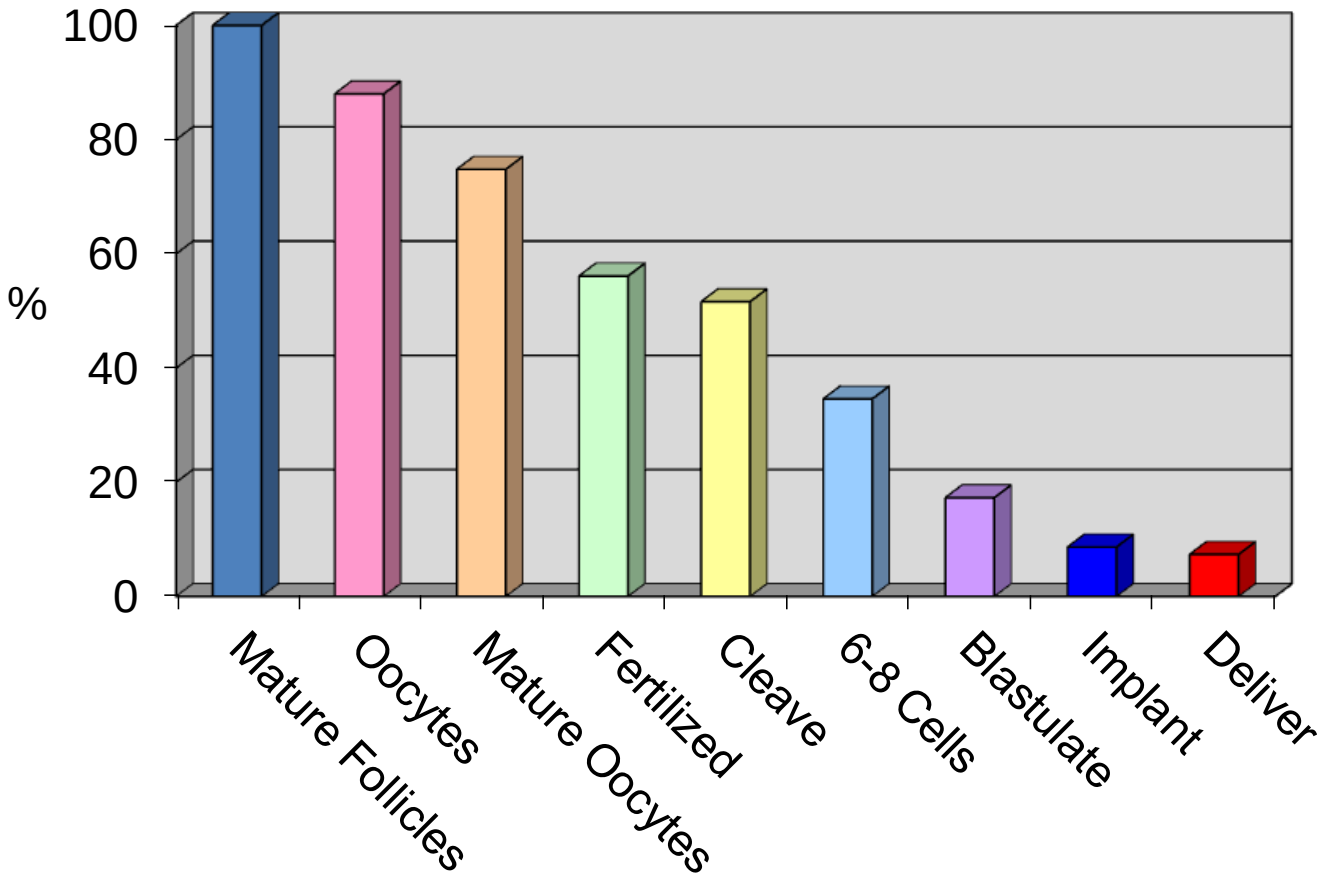
	Control max $n = 62$	Information leaflet max $n = 66$	Discussion max $n = 61$	P-value
Would elective single embryo transfer (eSET) be acceptable if this meant slightly reduced pregnancy rates?				
Yes	17/51 (27%)	20/66 (30%)	24/17 (32%)	0.39
Would eSET be more acceptable if this reduced the number of twins?				
Yes	25/61 (40%)	32/66 (49%)	26/61 (43%)	0.76
Would eSET be acceptable if the success rate was the same as DET?				
Yes	51/62 (82%)	55/66 (83%)	53/61 (87%)	0.76
Would finances affect your decision to have SET?				
Yes	16/62 (26%)	17/65 (26%)	19/61 (31%)	0.80
Would eSET be acceptable if the cost was fixed regardless of the number of cycles?				
Yes	35/61/(57%)	36/65 (55%)	38/58 (65%)	0.73
Would your opinion change if you were charged for the hospital care of premature twins?				
Yes	17/61 (27%)	20/64 (31%)	19/59 (31%)	0.69

Murray S *et al*, *Hum Reprod*, 2004





Efficiency of Human Reproduction through IVF



Retrospective Eval.

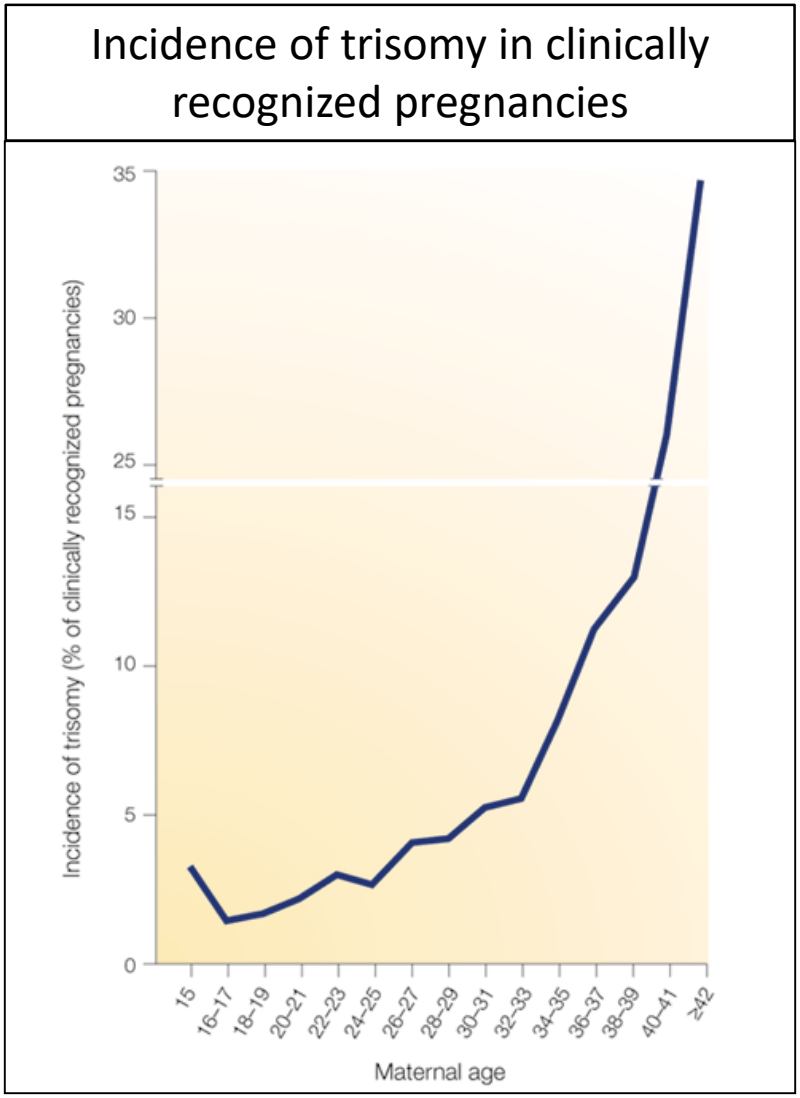
Proportion leading to delivery:

- 7% of Follicles
- 13% of Embryos
- 21% of 8 Cells
- 42% of Blastocysts

N=132,874 Mature Follicles

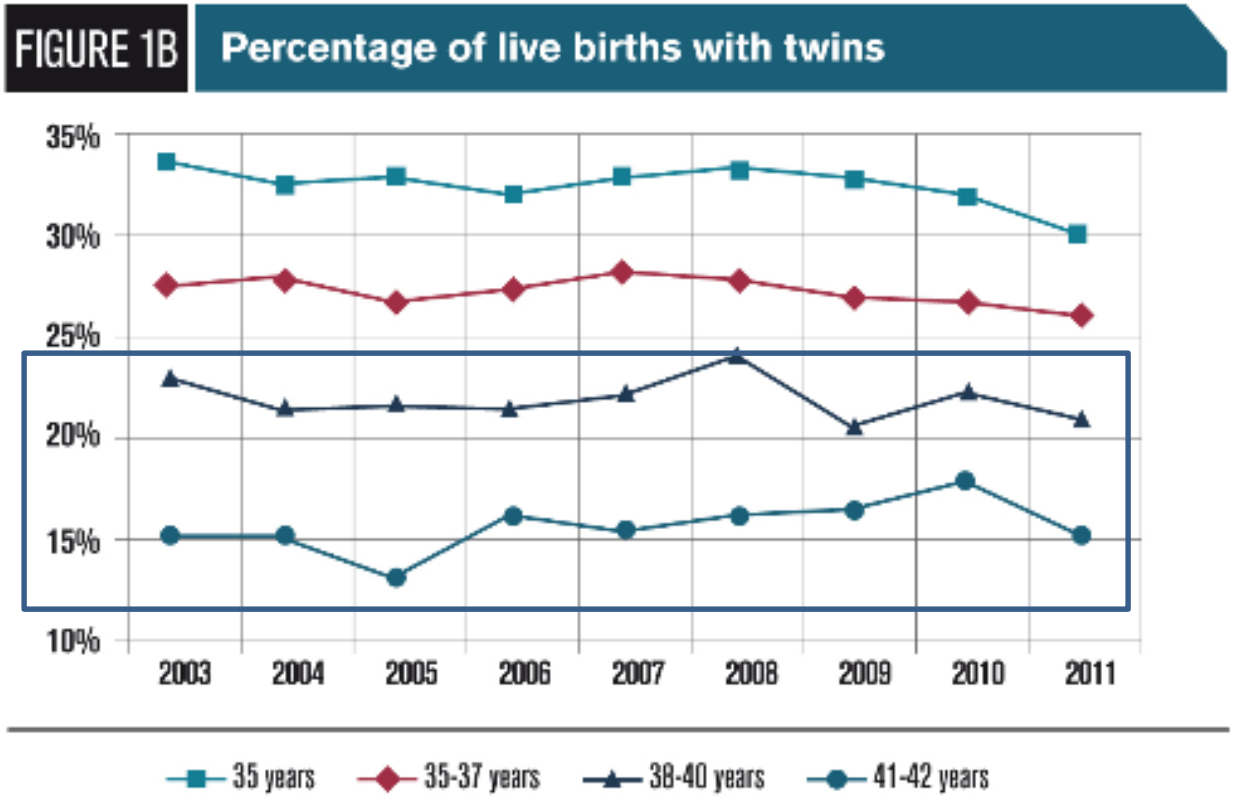
What might explain the high failure rate?

Embryos have a high risk of aneuploidy



Hassold T & Hunt P, *Nat Rev Genet*, 2001. PMID: 11283700

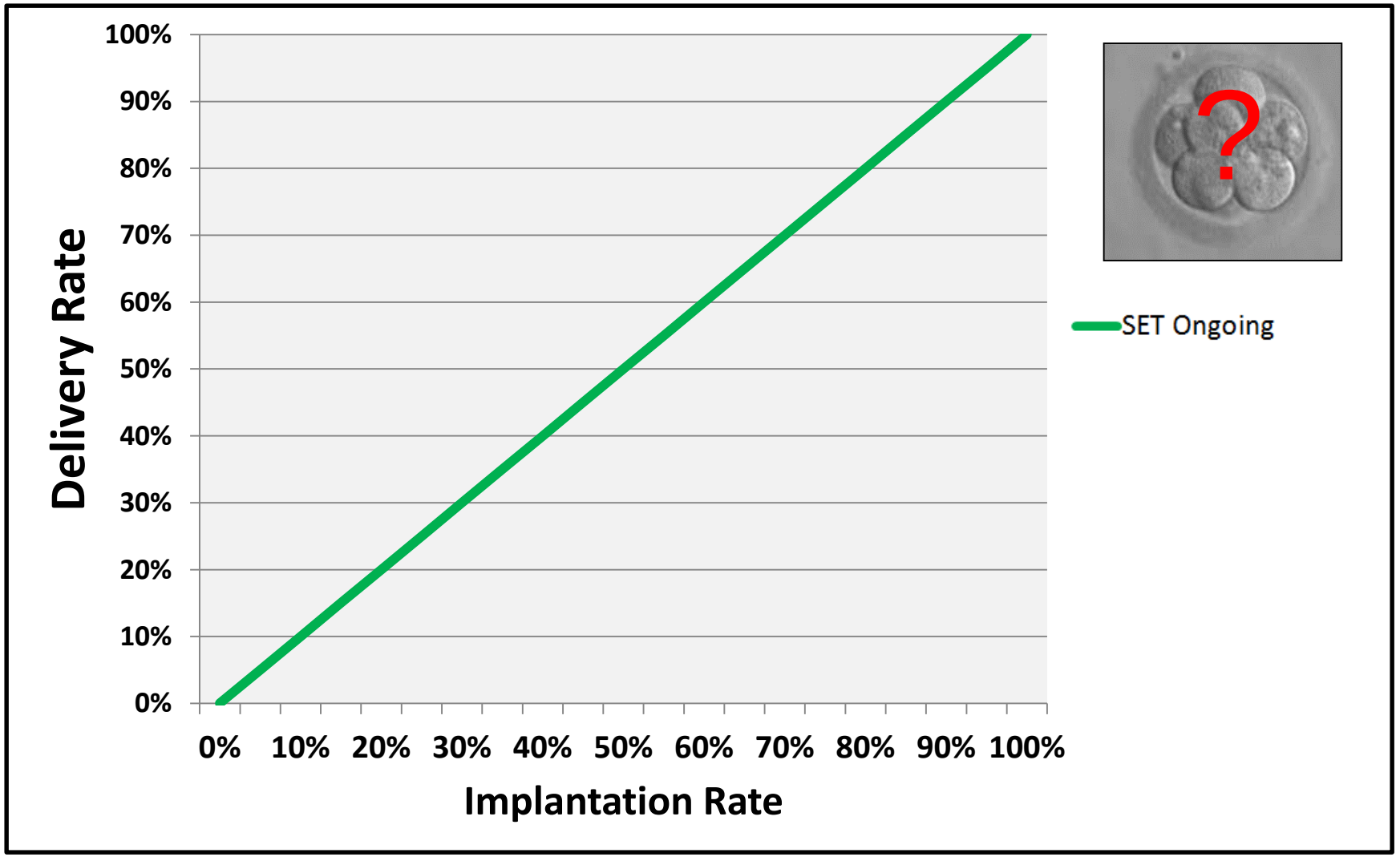
The risk of twins remains high even among older patients not traditionally offered eSET



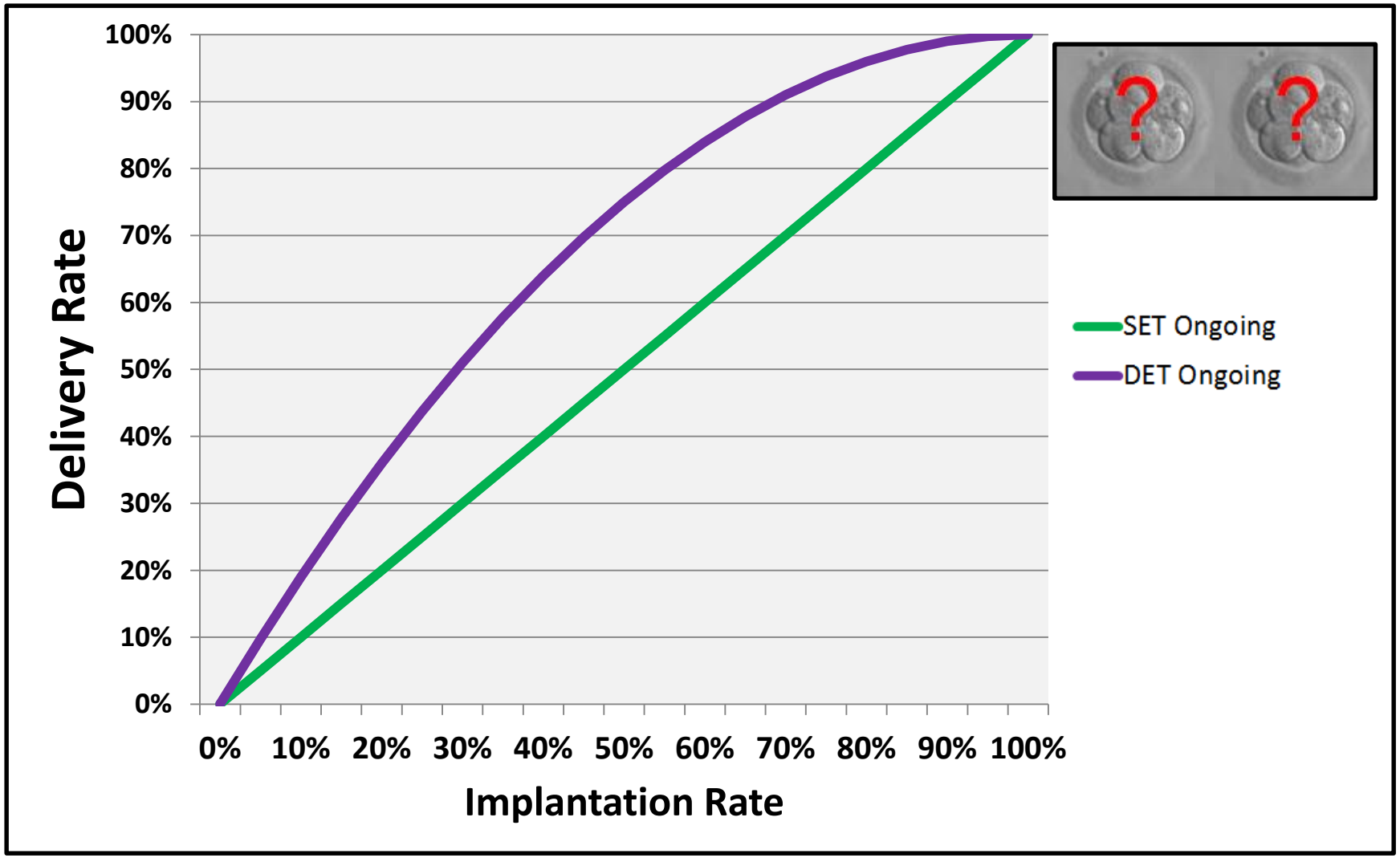
The proportion of live births after IVF in the United States that were twin births. The twin delivery rate has remained stable across age groups from 2004 to 2011.

Source: Society for Assisted Reproductive Technology

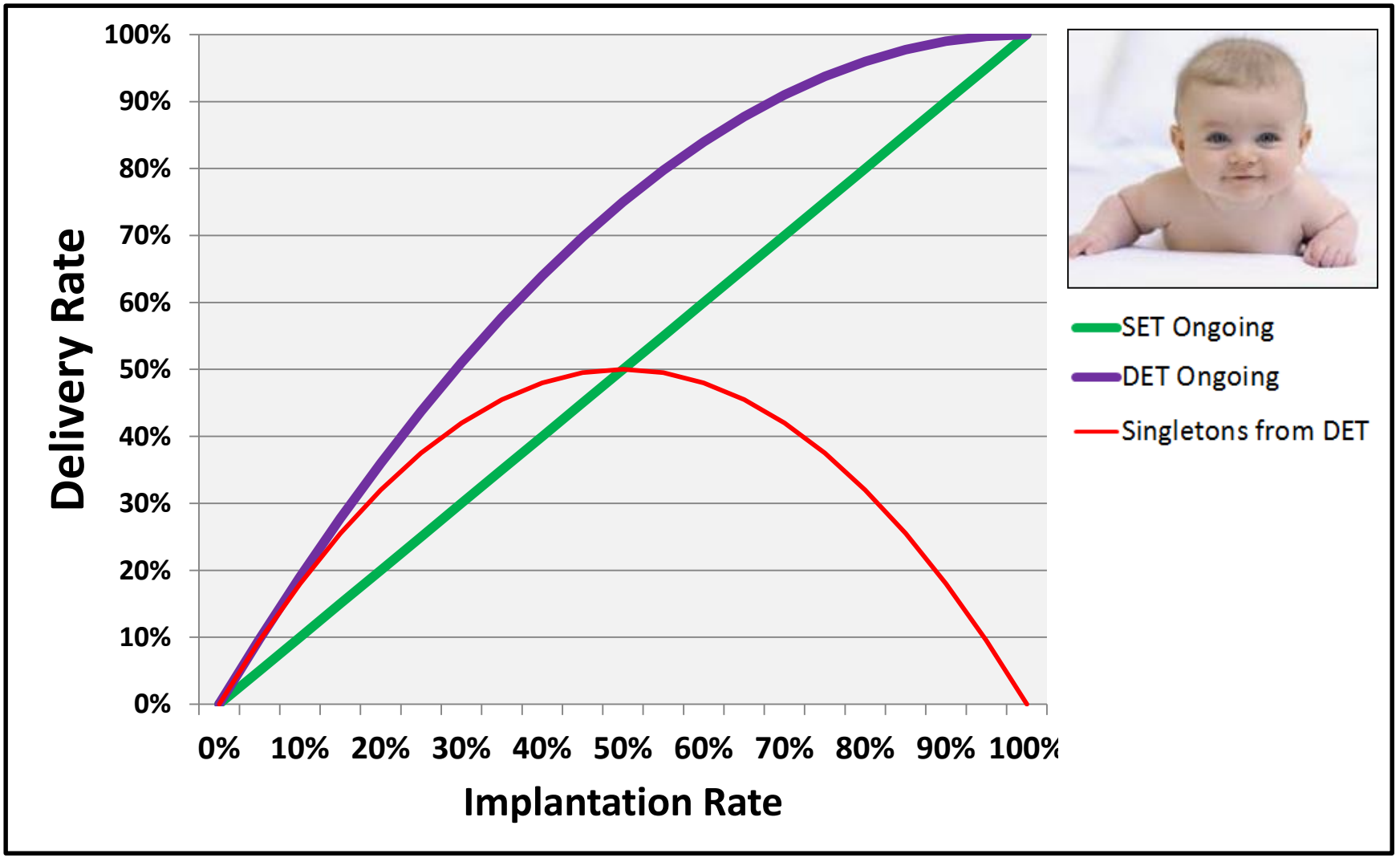
SET delivery rate ≈ Implantation Rate



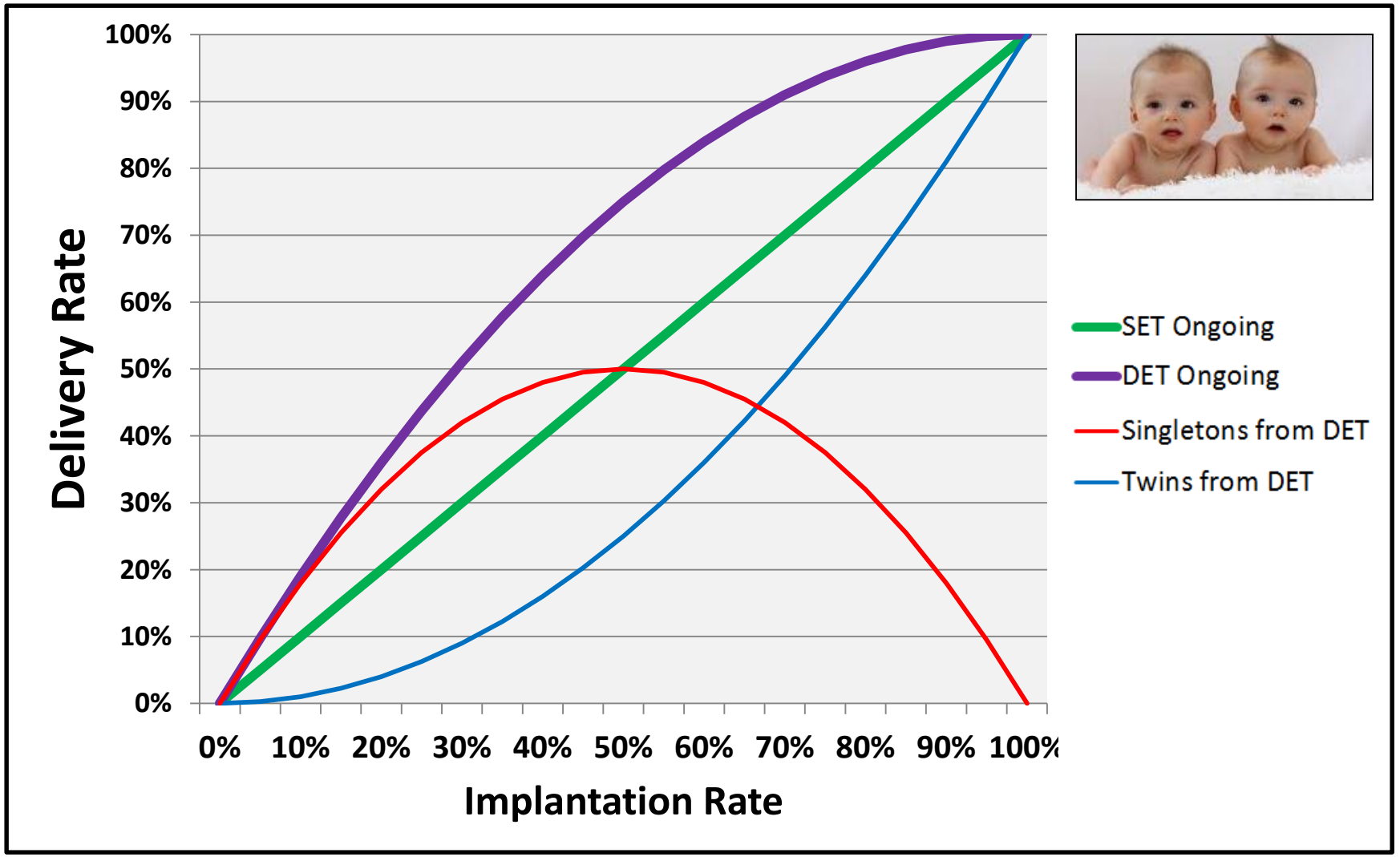
Double embryo transfer (DET) results in higher delivery rates



DET usually results in more singletons

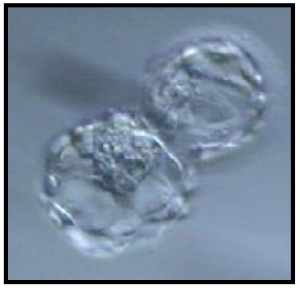
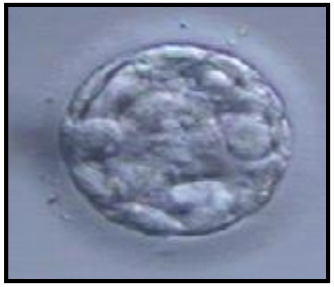


…At the cost of more twins



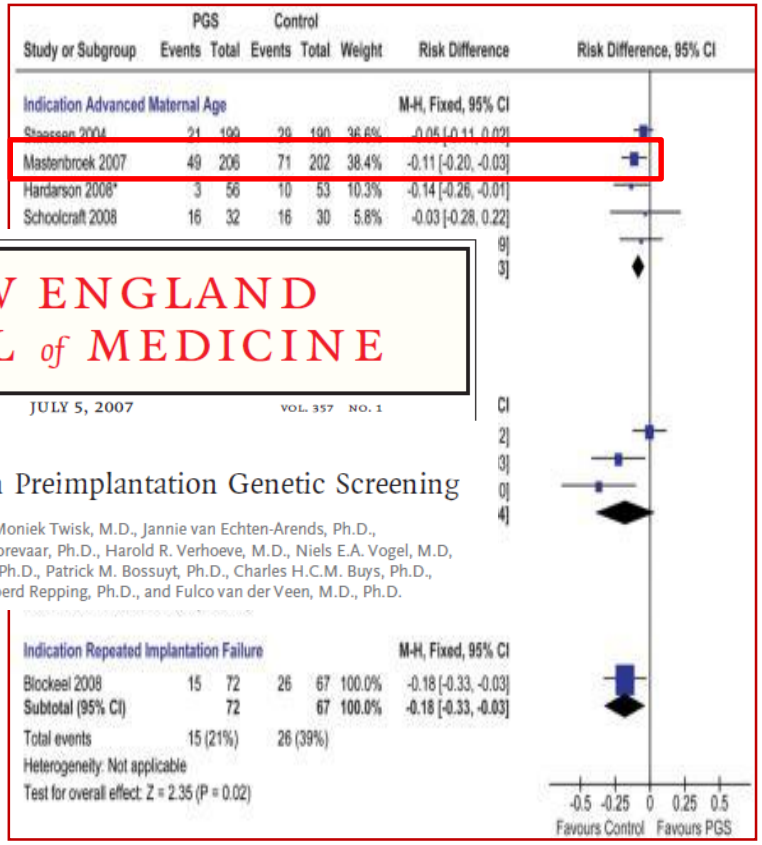
Aneuploidy screening can only improve outcomes if morphology is sometimes selected against…

SET Based on Embryo Morphology

Screening Result					
					
Aneuploid	Aneuploid	Euploid	Aneuploid	Euploid	Euploid

SET Based on Aneuploidy Screening and Embryo Morphology

FISH for Aneuploidy Screening



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ESTABLISHED IN 1812 JULY 5, 2007 VOL. 357 NO. 1

In Vitro Fertilization with Preimplantation Genetic Screening

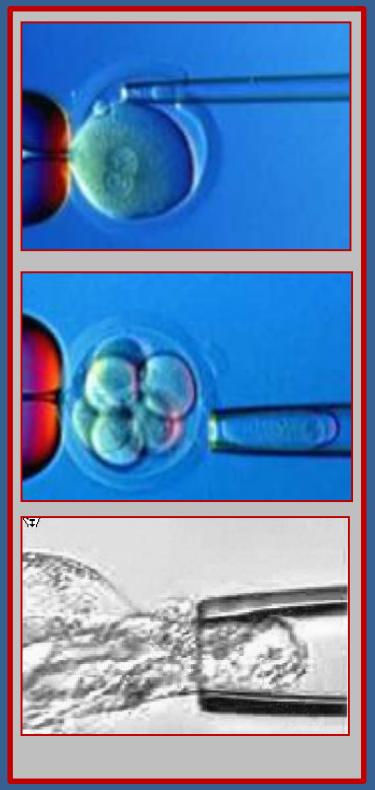
Sebastiaan Mastenbroek, M.Sc., Moniek Twisk, M.D., Jannie van Echten-Arends, Ph.D., Birgit Sikkema-Raddatz, Ph.D., Johanna C. Korevaar, Ph.D., Harold R. Verhoeve, M.D., Niels E.A. Vogel, M.D, Eus G.J.M. Arts, Ph.D., Jan W.A. de Vries, Ph.D., Patrick M. Bossuyt, Ph.D., Charles H.C.M. Buys, Ph.D., Maas Jan Heineman, M.D., Ph.D., Sjoerd Repping, Ph.D., and Fulco van der Veen, M.D., Ph.D.

Anything can be done "sub-optimally"

Mastenbroek S et al, *Hum Reprod Update*, 2013. PMID: 21531751

Limitations of FISH-based PGS

- Impact of the biopsy
  - Could potentially outweigh benefit of selection
  - Safety not previously evaluated rigorously



Polar Body Biopsy

Blastomere Biopsy

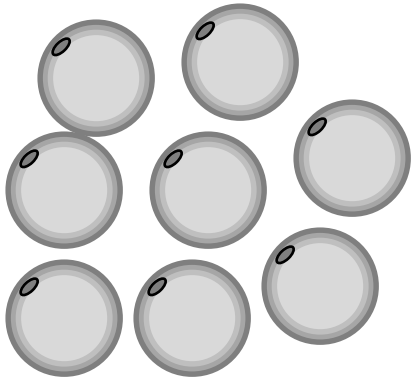
Trophectodem Biopsy

A trophectoderm biopsy

https://www.youtube.com/watch?v=FHBuwaE1CEM

Does Embryo Biopsy Impact the Developmental Potential of the Oocyte

Routine IVF
Care through Retrieval



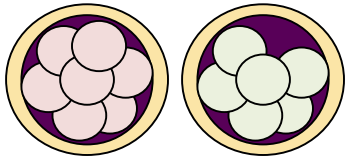
Identify mature oocytes

ICSI, culture, and select 2 best embryos for transfer

One embryo randomized to undergo biopsy

Cell submitted for eventual aneuploidy screening and fingerprinting

Transfer the embryos



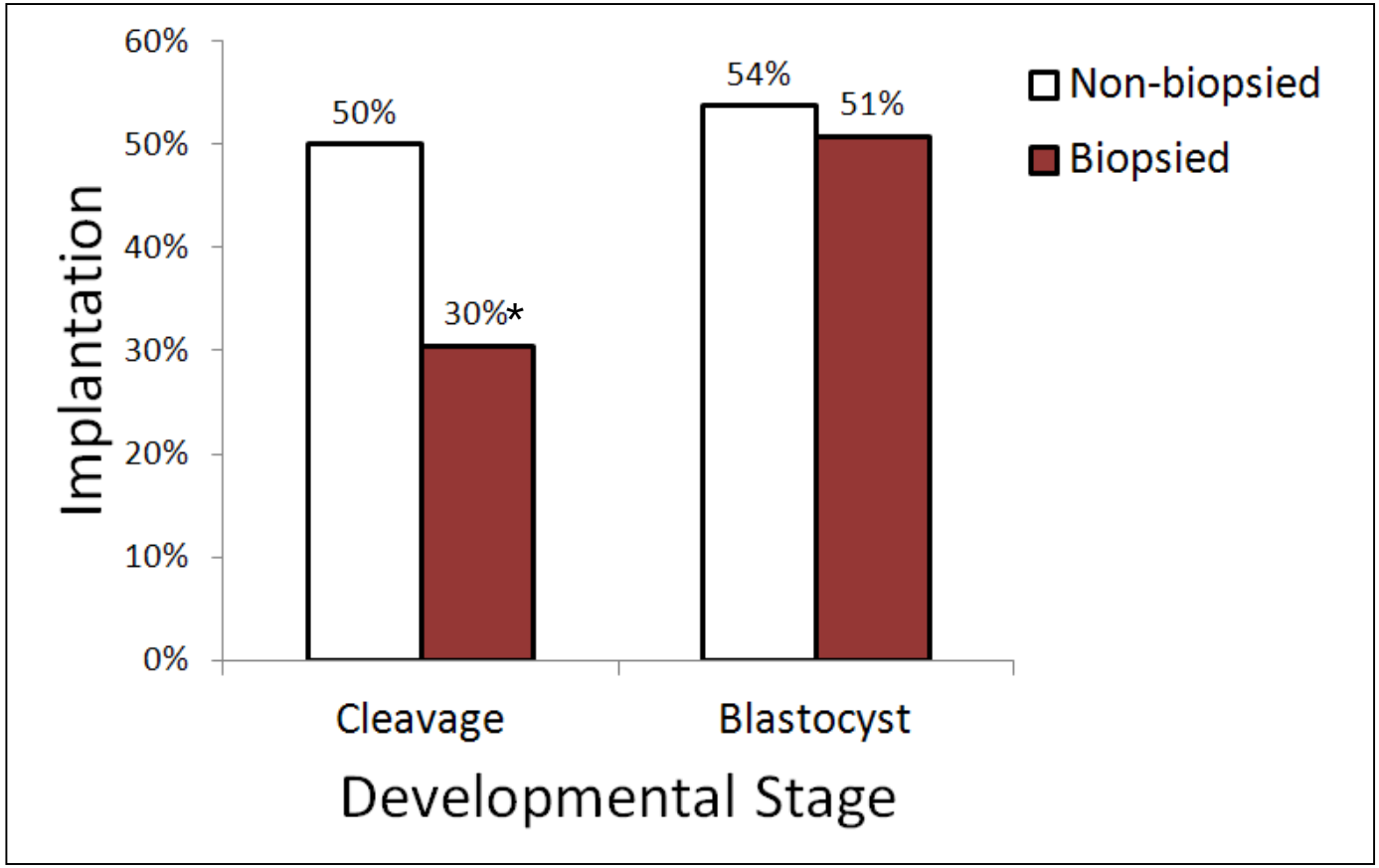
Implantation, Maternal serum sampling for free fetal DNA and Fingerprinting

N=113 pairs; 226 embryos

SEMINAL CONTRIBUTION

Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial

Richard T. Scott Jr., M.D.,[a,b] Kathleen M. Upham, B.S.,[a] Eric J. Forman, M.D.,[b] Tian Zhao, M.S.,[a] and Nathan R. Treff, Ph.D.[a,b,c]



*P<0.03, McNemar chi-square

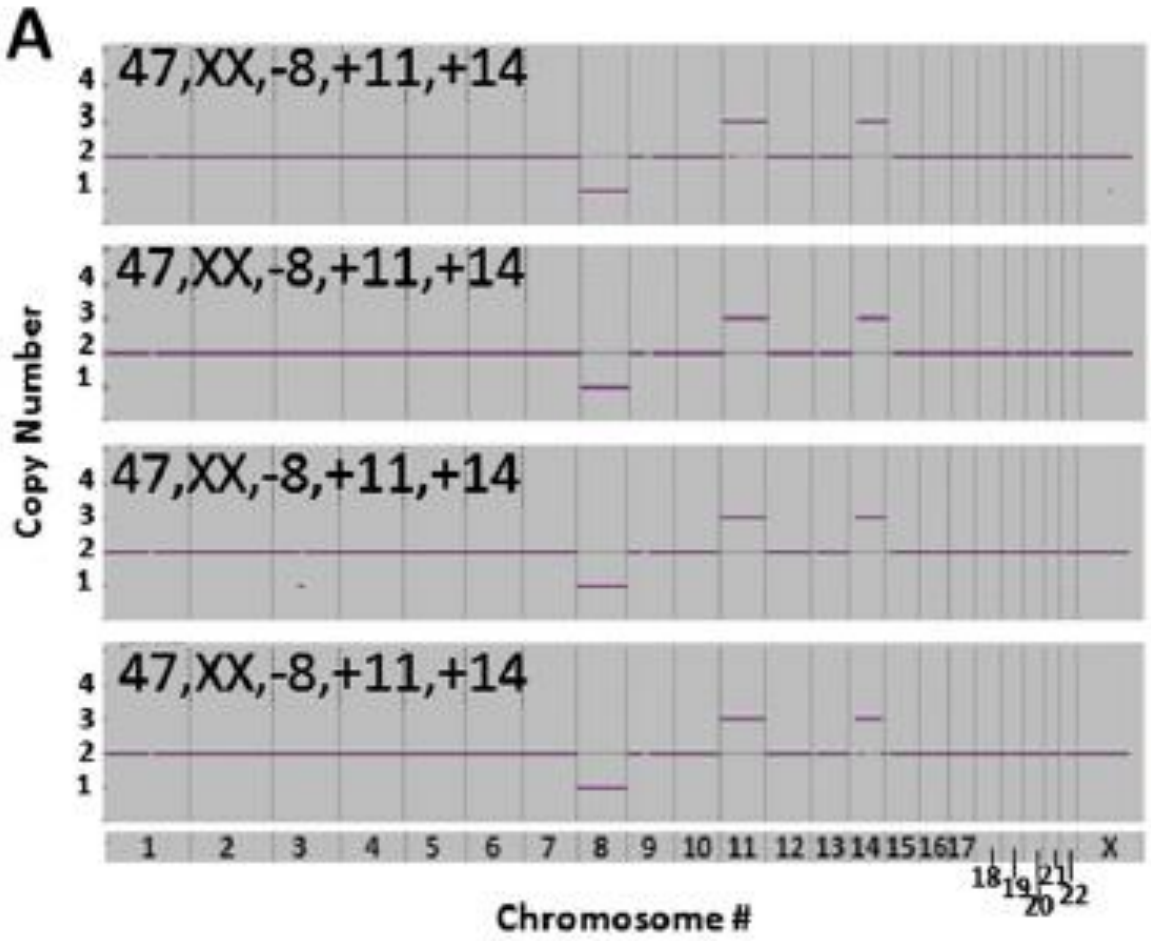
Scott RT et al, *Fertil Steril*, 2013. PMID: 23773313

Limitations of FISH-based PGS

- Impact of the biopsy
  - Could this outweigh benefit of selection
  - Not previously evaluated rigorously
- Accuracy of PGS result
  - Does FISH result reflect true state of embryo?
  - Limited number of chromosomes probed with FISH
  - High error rate of single cells FISH

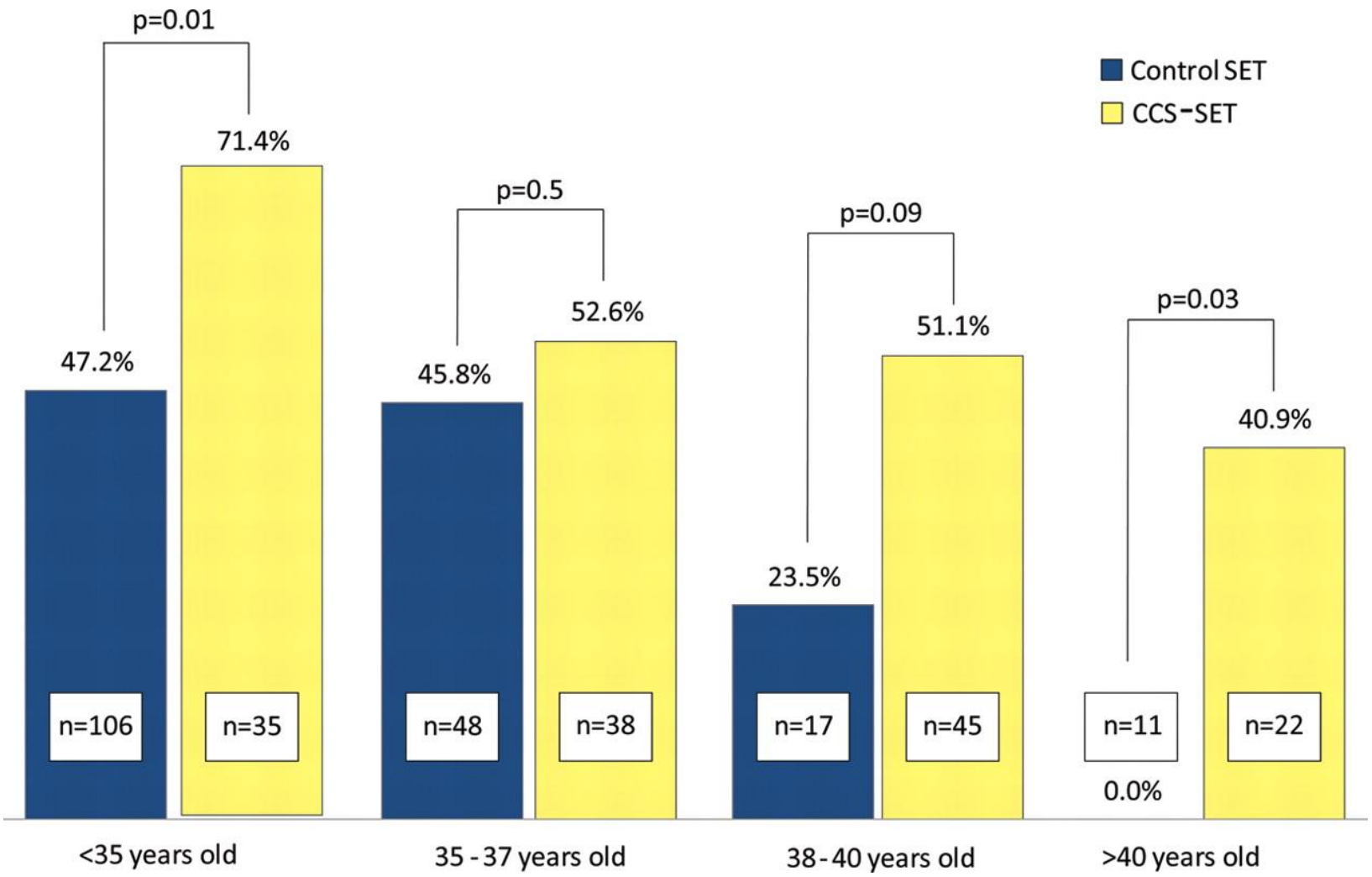
Accurate single cell 24 chromosome aneuploidy screening using whole genome amplification and single nucleotide polymorphism microarrays

Nathan R. Treff, Ph.D.,[a,b] *Jing Su, M.Sc.,*[a] *Xin Tao, M.Sc.,*[a] *Brynn Levy, Ph.D.,*[a,c] *and Richard T. Scott, Jr., M.D.*[a,b]



Treff NR et al, *Fertil Steril*, 2010. PMID: 20188357

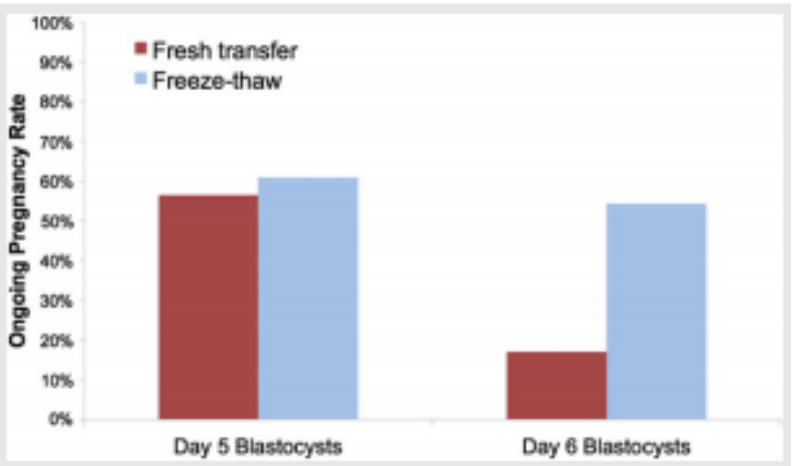
Delivery Rate by Age Group for Control SET versus CCS-SET



Forman E et al. Hum. Reprod. 2012;27:1217-1222

human reproduction

Timing Matters: Synchrony of the embryo and endometrium during IVF



Comparison of rates of ongoing pregnancy following fresh (*red*) and freeze-thaw (*blue*) transfers of day 5 or day 6 blastocysts.

Shapiro. Embryo-endometrium asynchrony. Fertil Steril 2013.

Critical Threshold

Progesterone

Secretory Transformation

Window of Receptivity

-2 -1 0 1 2 3 4 5 6 7

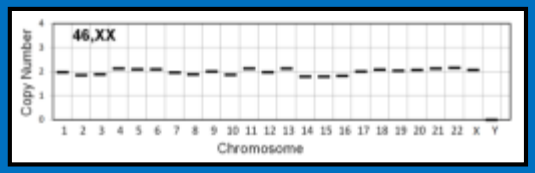
Days Relative to Retrieval

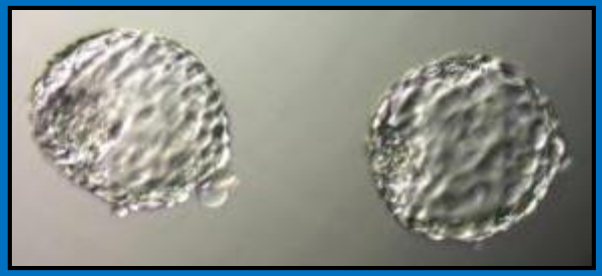
hCG Given

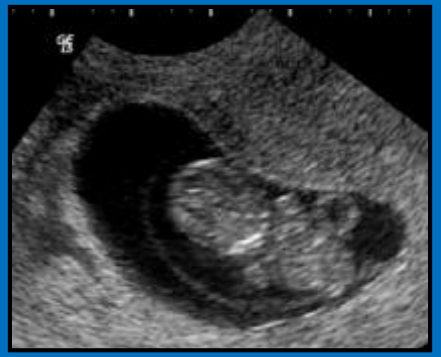
Oocyte Retrieval

Can

1 ≥ 2?


46,XX
Copy Number
0 1 2 3 4
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y
Chromosome





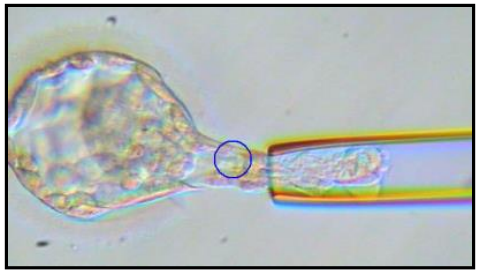
Blastocyst Euploid Selective Transfer (BEST) Trial

- Non-Inferiority Randomized Controlled Trial
  - Primary outcome: Ongoing pregnancy to viable gestation (≥24 weeks) after 1st synchronous blastocyst-stage embryo transfer (fresh day 5 blastocysts or frozen 6 blastocysts)
  - Study Group:

    Transfer single blastocyst after real-time qPCR based PGS
  - Control Group:

    Transfer best two blastocysts by traditional morphology

Trophectoderm Biopsy



Cell Lysis & Preamplification

384-Well Plate

Control Group

-Transfer best two blastocysts by traditional morphology criteria

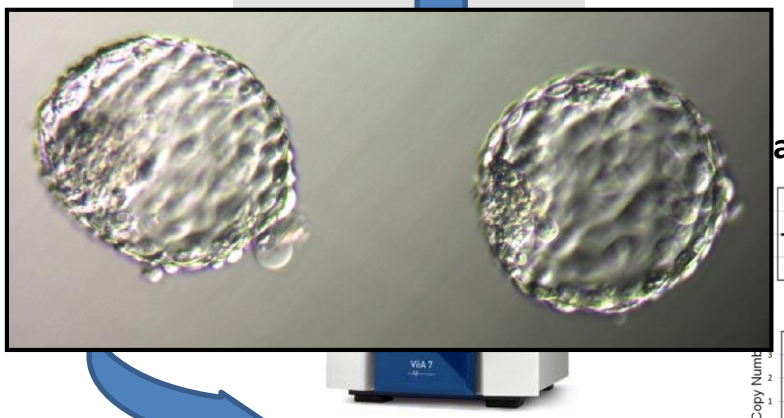
-All other good-quality blastocysts vitrified in pairs

Study Group

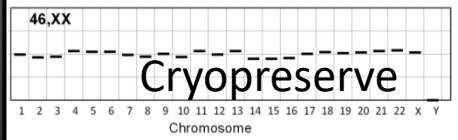
-All good-quality blastocysts undergo TE biopsy and PGS

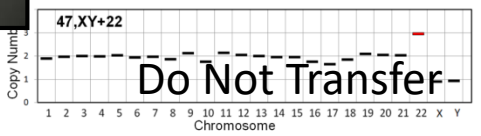
-Transfer single best euploid

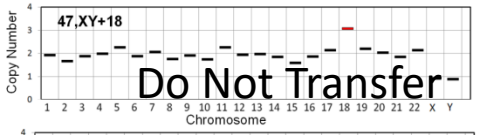
-All other euploid blastocysts vitrified individually

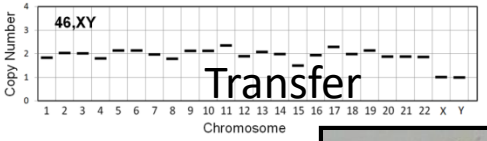


antation Genetic Screening Results

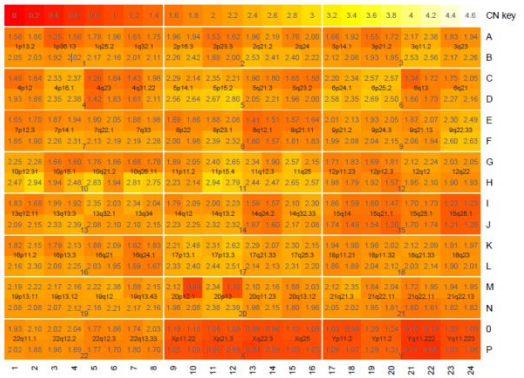








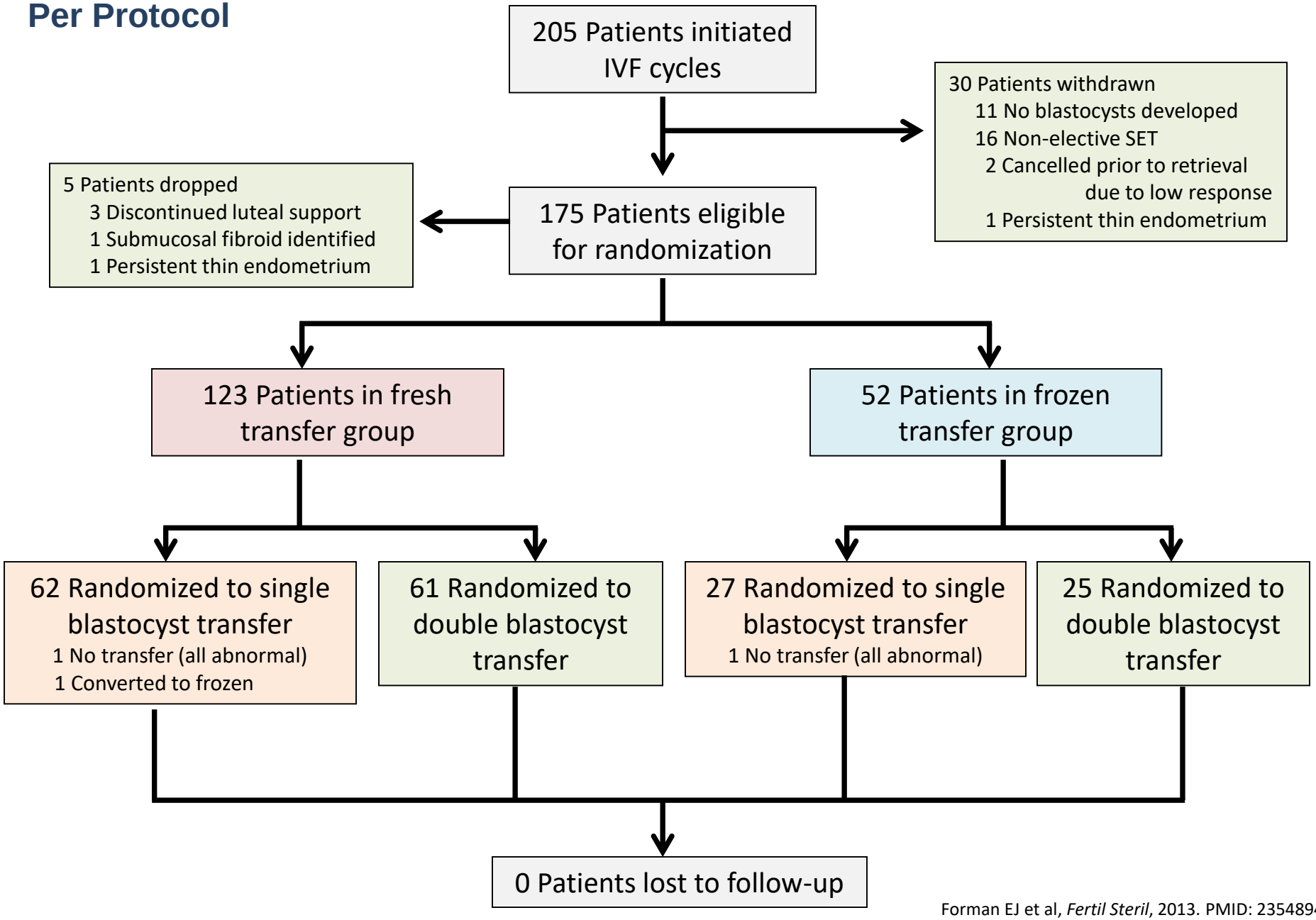
PCR Heatmap



Single Euploid Blastocyst Selected for transfer

Study flow of participants

Per Protocol



Forman EJ et al, *Fertil Steril*, 2013. PMID: 23548942

TABLE 1

Characteristics of the patients.

Characteristic	Single euploid blastocyst transfer (n = 89)	Double blastocyst transfer (n = 86)	*P* value
Age at oocyte retrieval, y			
Mean ± SD	35.1 ± 3.9	34.5 ± 4.7	.5
Range	25.1–41.4	22.9–42.6	
Body mass index, kg/m^2			
Mean ± SD	23.5 ± 3.5	23.8 ± 3.2	.4
Range	17.6–30.2	18.0–30.8	
Anti-Müllerian hormone level, ng/mL			
Mean ± SD	3.5 ± 2.4	4.0 ± 3.5	.2
Range	1.2–18.0	1.2–22.0	
Day 3 FSH, IU/L			
Mean ± SD	6.9 ± 1.8	6.6 ± 1.7	.2
Range	3.2–11.6	2.8–10.8	
Primary cause of infertility, n (%)			
Male factor	25 (28.1)	22 (25.6)	.9
Unexplained	24 (27.0)	24 (27.9)	
Ovulatory dysfunction	12 (13.5)	17 (19.8)	
Tubal factor	11 (12.4)	13 (15.1)	
Endometriosis	6 (6.7)	4 (4.7)	
Other	11 (12.4)	6 (7.0)	
History of previous pregnancies, n (%)	47 (52.8)	42 (48.8)	.6
Live birth	23 (25.8)	22 (25.6)	
Clinical miscarriage	25 (28.1)	14 (16.3)	
Termination of pregnancy	11 (12.4)	9 (10.5)	
History of prior treatment with IVF, n (%)	18 (20.2)	16 (18.6)	.8
Live birth	8 (9.0)	4 (4.7)	

Forman. Blastocyst Euploid Selective Transfer (BEST) Trial. Fertil Steril 2013.

Forman EJ et al, *Fertil Steril*, 2013. PMID: 23548942

TABLE 2

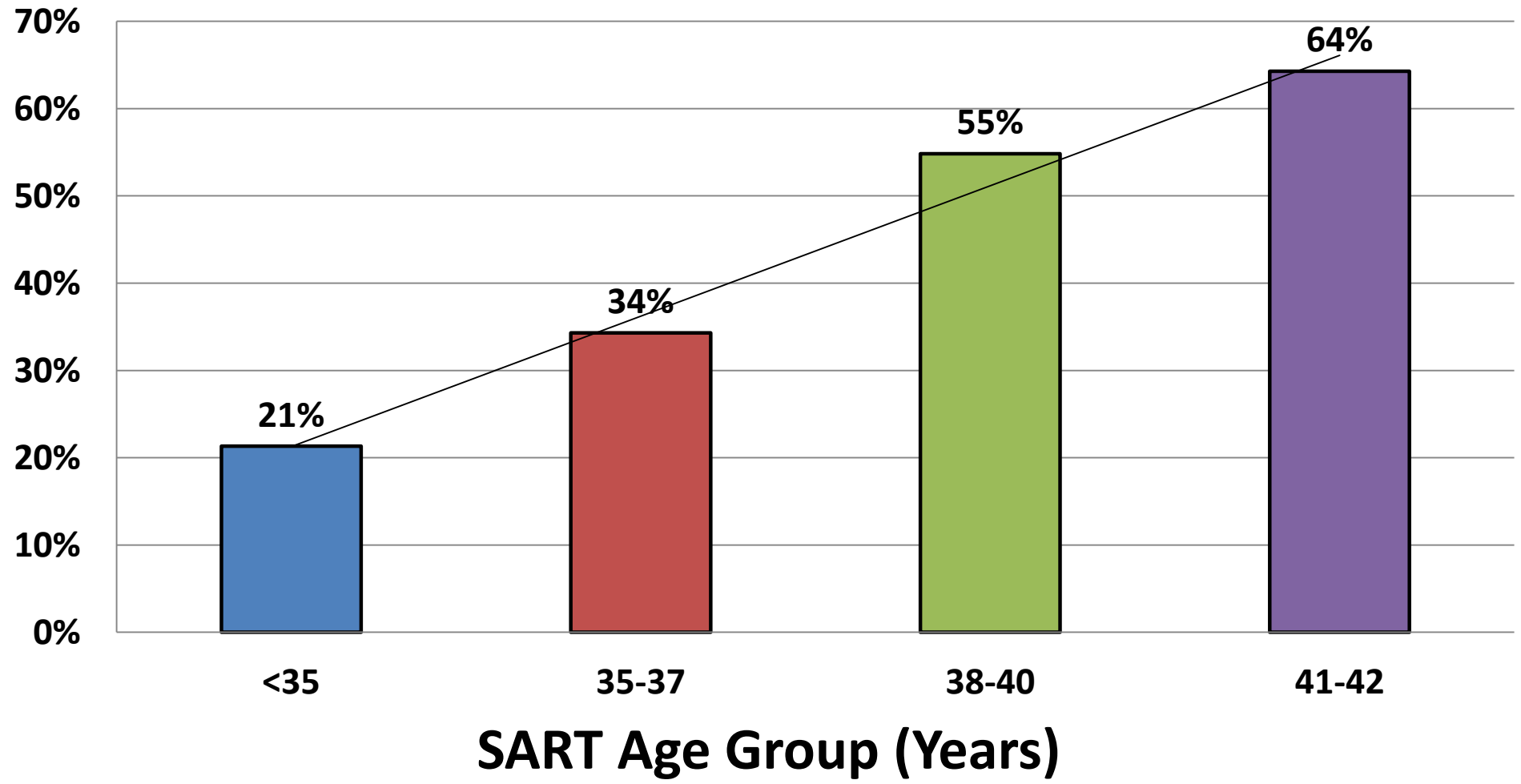
Outcomes according to treatment group (intention-to-treat analysis).

Outcome	Single euploid blastocyst transfer (n = 89)	Double blastocyst transfer (n = 86)	*P* value
Total dose of gonadotropins, ampules			
Mean ± SD	37.8 ± 12.9	37.0 ± 13.6	.7
Range	15.5–72	14.5–81	
E_2, pg/mL, at surge			
Mean ± SD	2,437 ± 1,212	2,540 ± 1,236	.6
Range	513–6,267	605–6,000	
Retrieved oocytes			
Mean ± SD	16.9 ± 8.4	15.7 ± 7.1	.6
Range	5–45	3–42	
Fertilized oocytes (two pronuclei)			
Mean ± SD	11.1 ± 5.9	10.8 ± 5.7	.9
Range	4–30	3–33	
High-quality blastocysts			
Mean ± SD	5.8 ± 3.6	5.3 ± 3.0	.5
Range	2–22	2–18	
Vitrified blastocysts			
Mean ± SD	3.7 ± 2.9	3.9 ± 2.8	.5
Range	0–17	0–16	
Vitrified euploid blastocysts			
Mean ± SD	3.2 ± 2.8	NA	
Range	0–17	NA	
Patients who received fresh ET (%)	60 (67)	61 (71)	.6
Patients who received frozen embryo transfer due to			
Embryo-endometrial dyssynchrony	22	20	
Ovarian hyperstimulation syndrome risk	5	3	
Hydrosalpinx	0	2	
Nondiagnostic result of embryo biopsy	1	0	

Forman. Blastocyst Euploid Selective Transfer (BEST) Trial. Fertil Steril 2013.

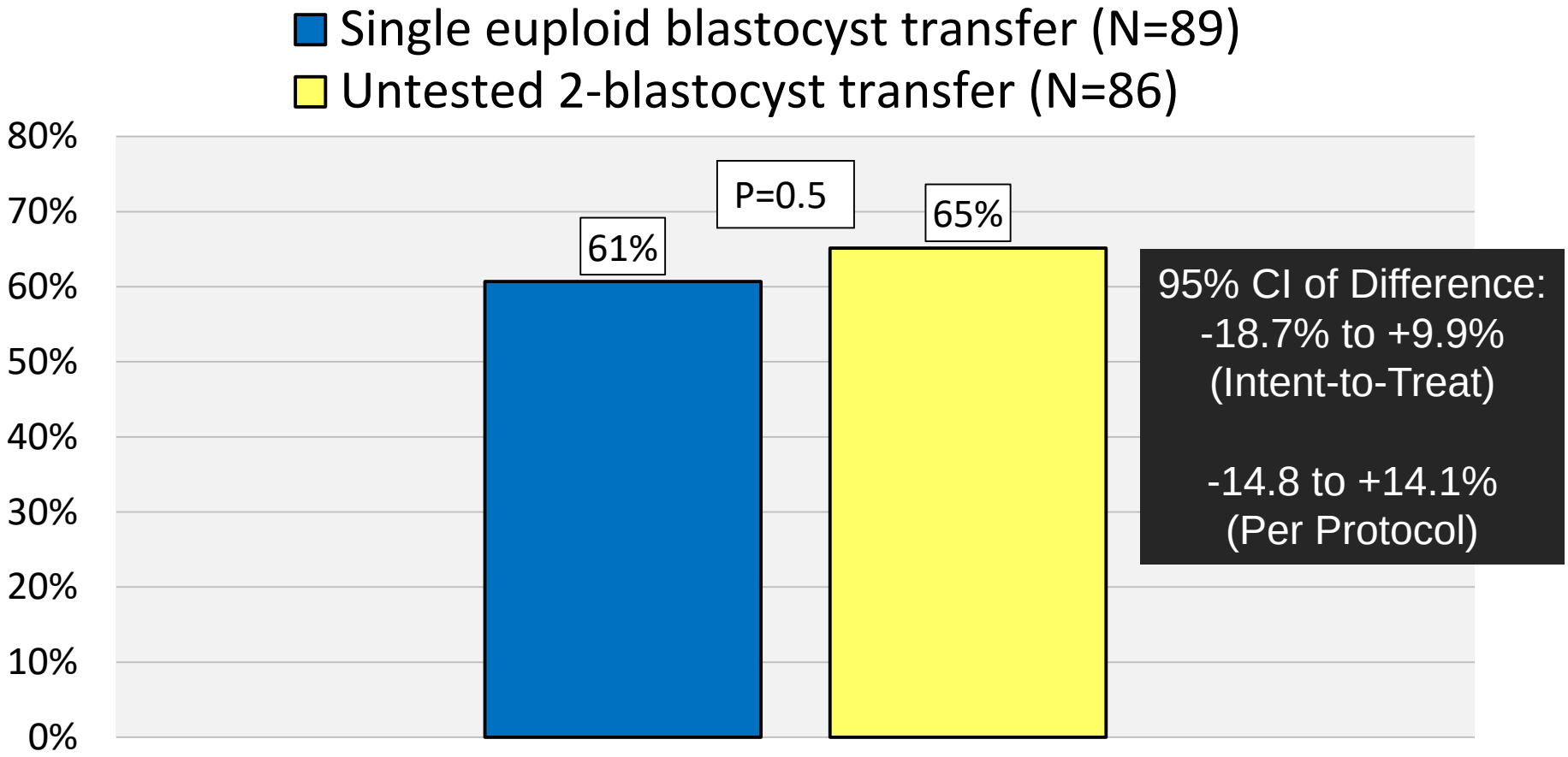
Forman EJ et al, *Fertil Steril*, 2013. PMID: 23548942

Aneuploidy Rate of Transferrable Blastocysts



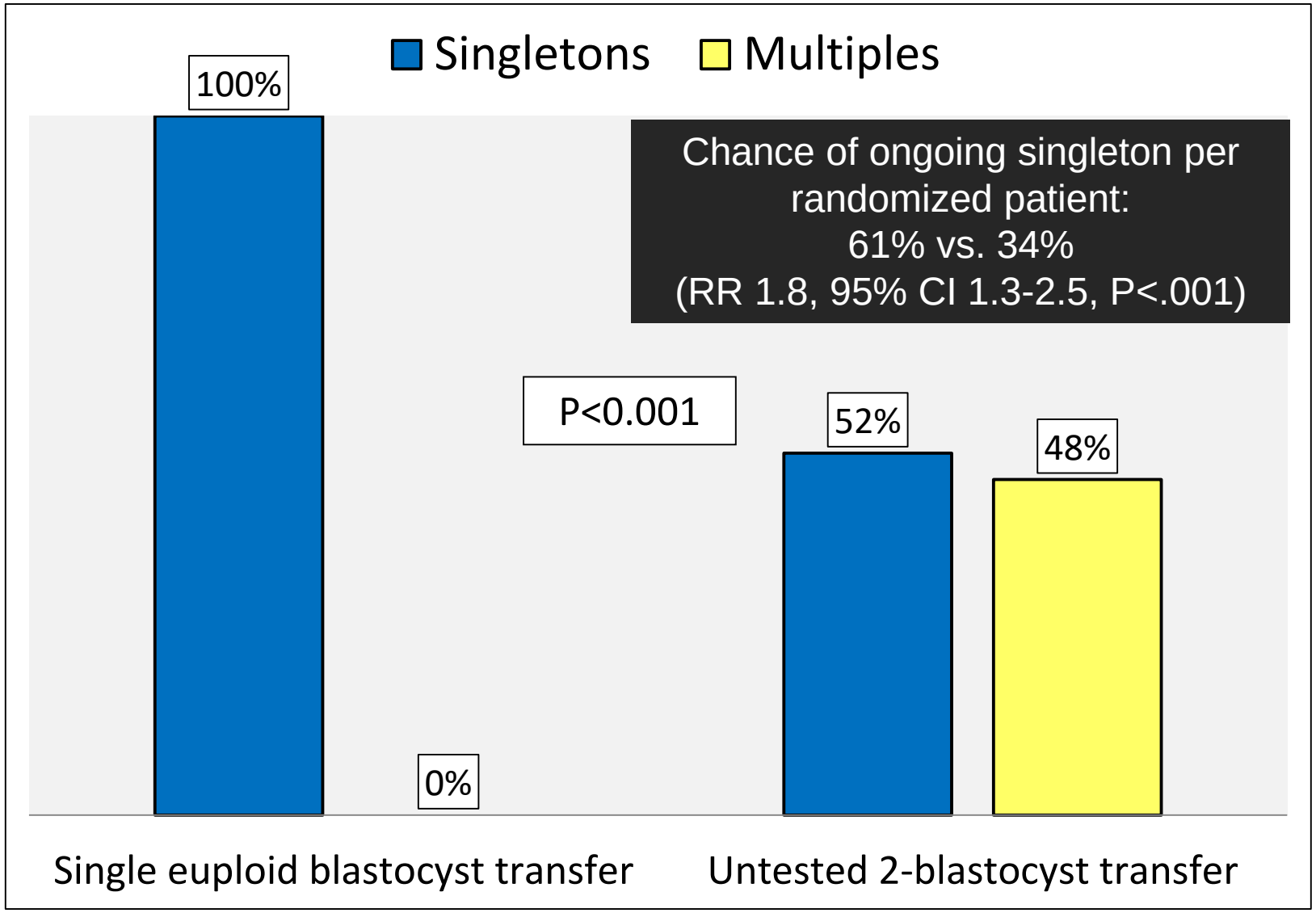
Aneuploidy increases with increasing age (P<0.01)
N=506 blastocysts, Overall aneuploidy rate = 31%

Delivery Rate Per Patient (n=175)



Forman EJ et al, *Fertil Steril*, 2013. PMID: 23548942

Proportion of Ongoing Pregnancies



Forman EJ et al, *Fertil Steril*, 2013. PMID: 23548942

Delivery Follow Up

REPRODUCTIVE ENDOCRINOLOGY AND INFERTILITY

Obstetrical and neonatal outcomes from the BEST Trial: single embryo transfer with aneuploidy screening improves outcomes after in vitro fertilization without compromising delivery rates

Eric J. Forman, MD; Kathleen H. Hong, MD; Jason M. Franasiak, MD; Richard T. Scott Jr, MD

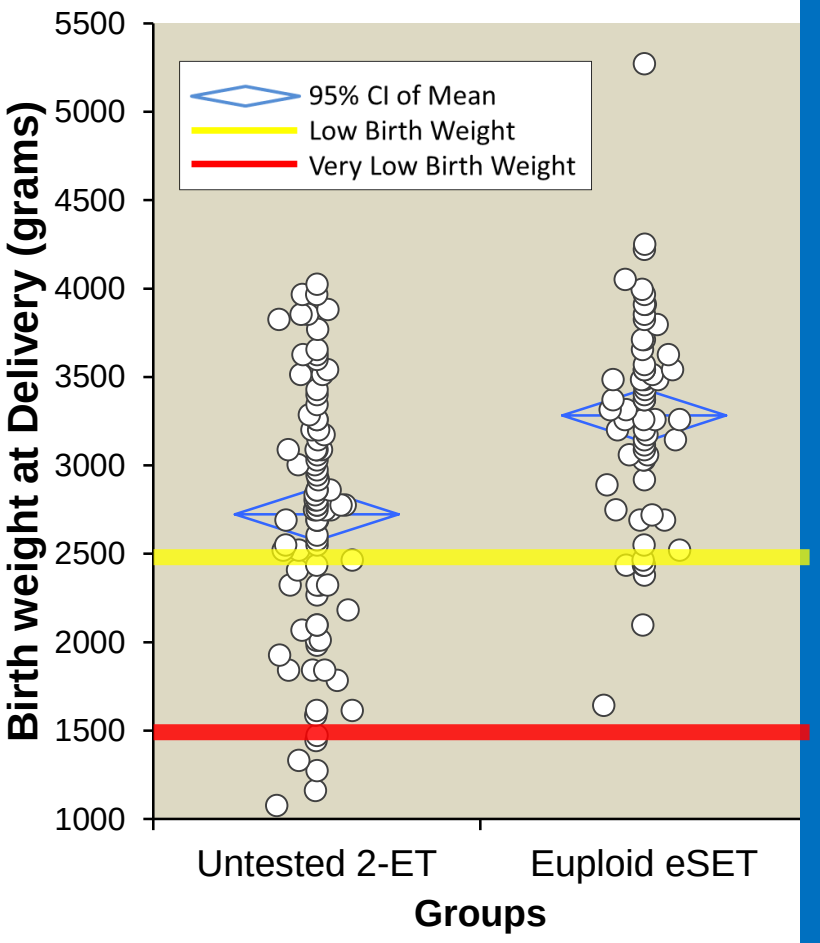
Forman EJ et al, *Am J Obstet Gynecol*, 2014. PMID: 24145186

Delivery Follow Up

- Followed outcomes from 1st embryo transfer to delivery
- Encouraged patients who did not deliver to have a frozen transfer
- Lower risk of preterm delivery after euploid eSET: 13% vs. 29% (P=0.03)
- Nearly twice as likely to have a term, singleton delivery: 60% vs. 31% (P<0.001)

Forman EJ et al, *Am J Obstet Gynecol*, 2014. PMID: 24145186

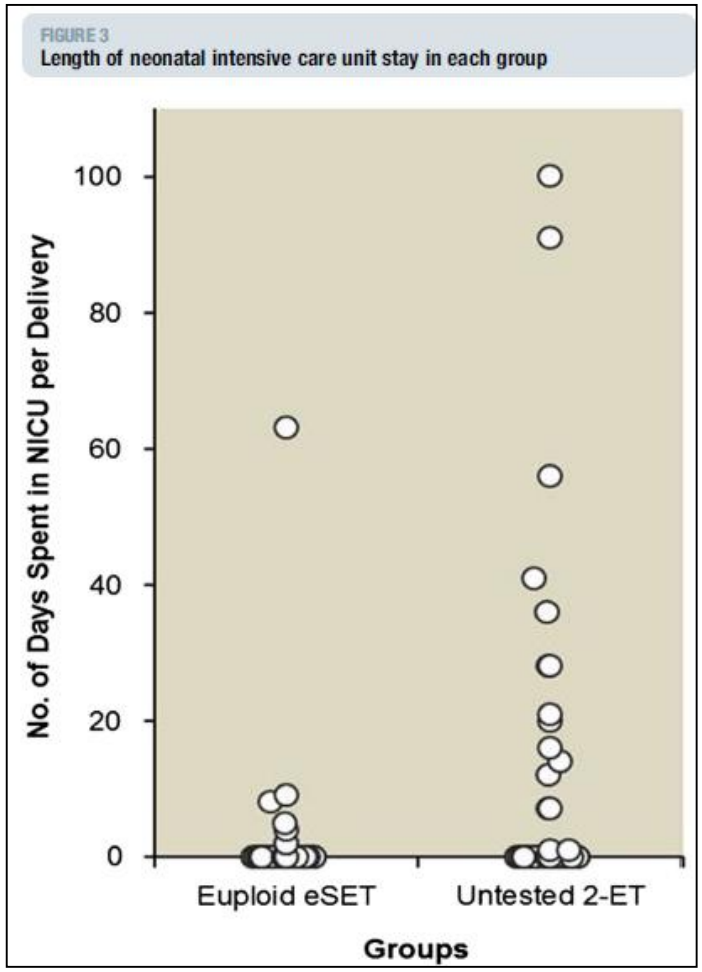
Better obstetrical outcomes



- Median Birthweight:
  - 3,317g – Single euploid
  - 2,778g – 2-Blastocyst (P<0.001)
- Low birthweight (<2,500g):
  - 11% (7/62) – Single Euploid
  - 33% (30/92) – 2-Blastocyst (P=0.002)
- Very low birthweight (<1,500g):
  - 0% (0/62) – Single Euploid
  - 7% (6/92) – 2-Blastocyst (P=0.08)

Forman EJ et al, *Am J Obstet Gynecol*, 2014. PMID: 24145186

Better Obstetrical Outcomes
Reduced Risk of NICU Admission

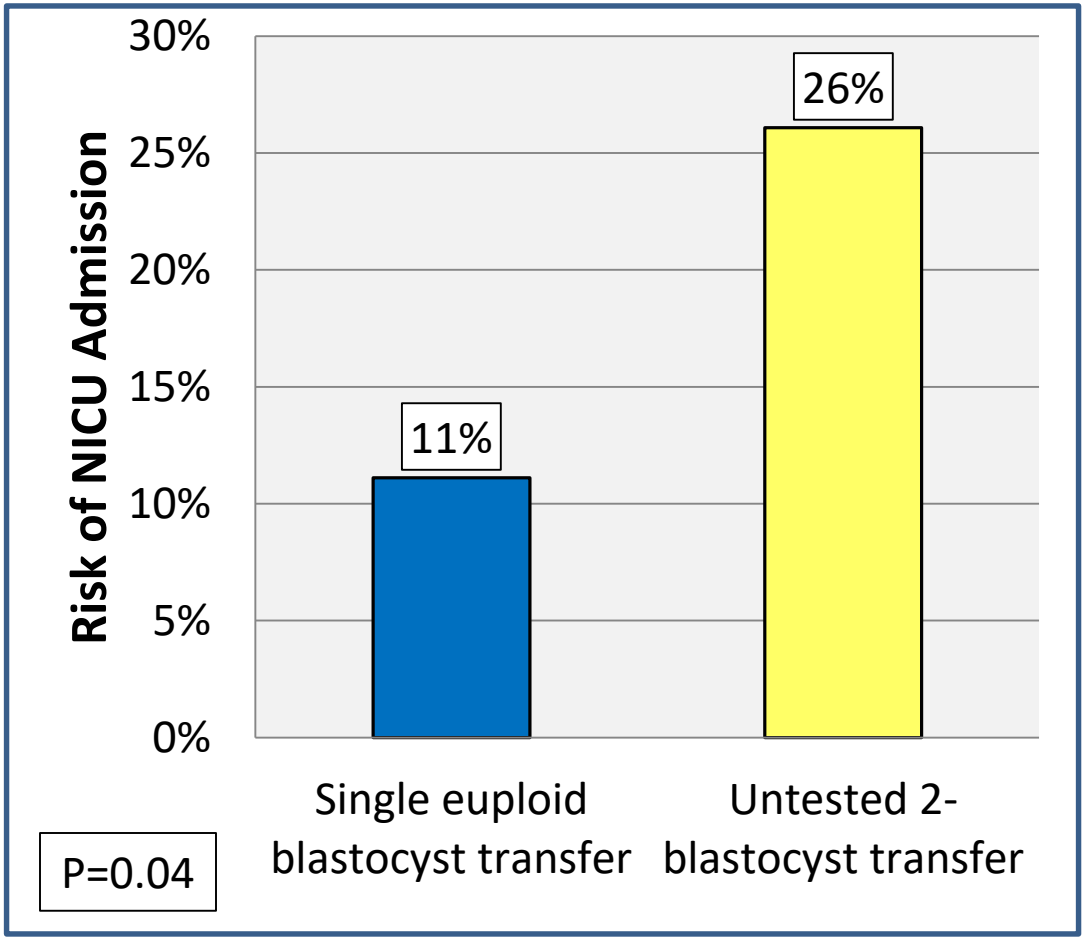


Euploid eSET:
93 total days spent in NICU

Untested 2-Embryo Transfer:
479 total days spent in NICU

Forman EJ et al, *Am J Obstet Gynecol*, 2014. PMID: 24145186

Better Obstetrical Outcomes
Reduced Risk of NICU Admission



Forman EJ et al, *Am J Obstet Gynecol*, 2014. PMID: 24145186

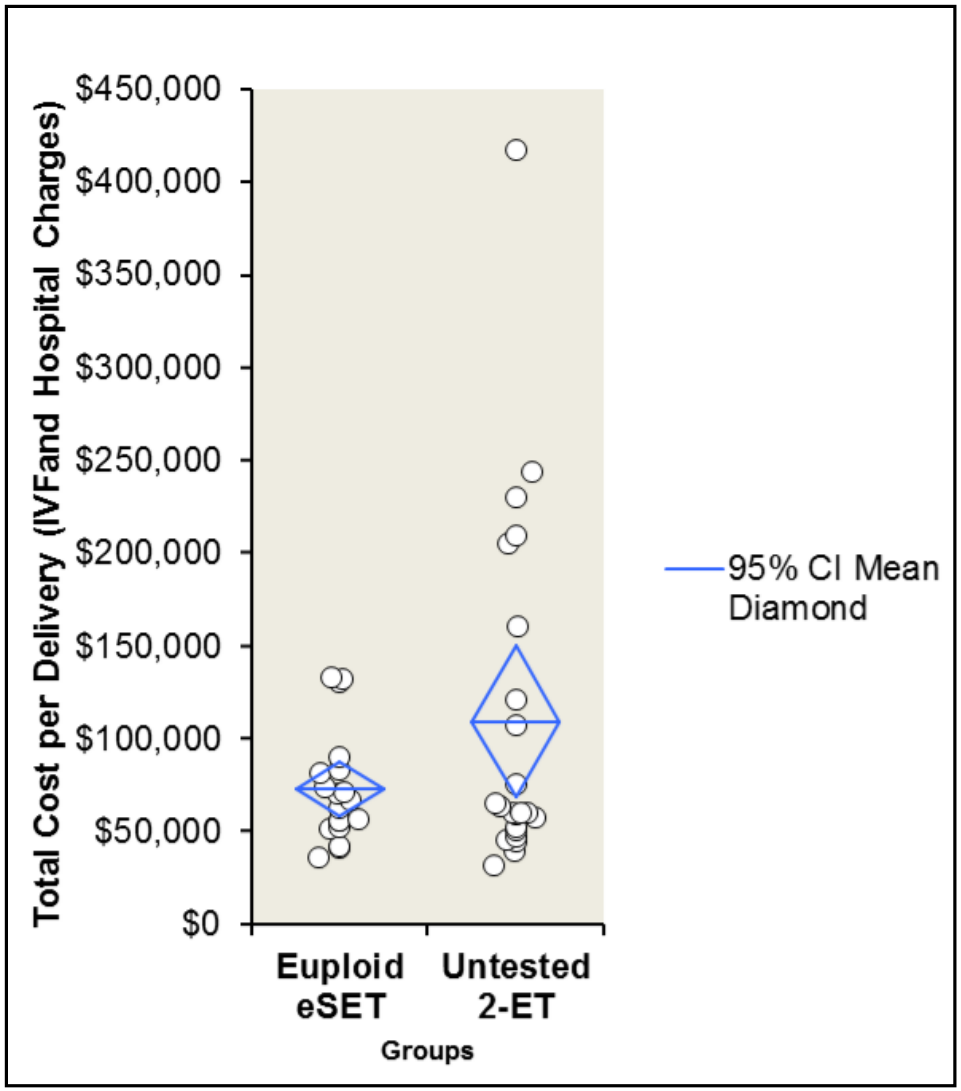
Obstetrical Costs Likely Outweigh Additional ART Costs

Costs per Delivery*	
Singleton	$21,458
Twins	$104,831
Triplets	$407,199

- Does not include:
  - Disability costs during bed rest
  - Loss of productivity in the work place

Lemos *et al Am J Obstet Gynecol* 2013; PMID: 24238479

Fewer “Big Ticket” Hospital Admissions

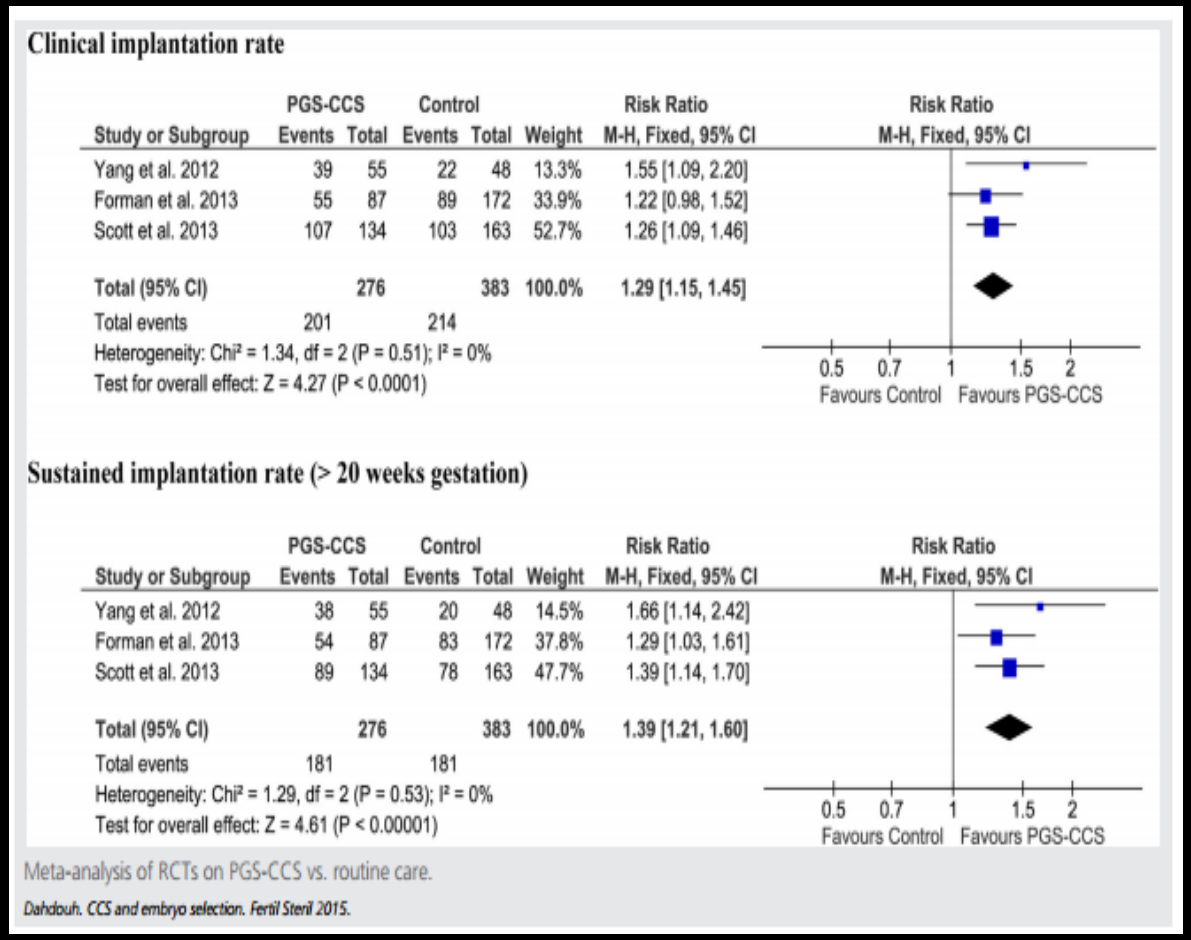


- Mean total hospital and IVF charges per delivery:
  - $73,407 (euploid eSET)
  - $111,488 (untested 2-ET)

  P=0.09
- ~33% reduction in costs per delivery
- A $38,000 difference per IVF delivery would represent $1.9 B savings on health care costs with ~50,000 IVF deliveries annually

Forman EJ et al, ASRM 2013

PGS for whole chromosome aneuploidy improves IVF outcomes:



Dahdouh EM et al, *Fertil Steril*, 2015

Guidance on the limits to the number of embryos to transfer: a committee opinion

Practice Committee of the American Society for Reproductive Medicine, and the Practice Committee of the Society for Assisted Reproductive Technology

American Society for Reproductive Medicine; and Society for Assisted Reproductive Technology, Birmingham, Alabama

TABLE 1

Recommendations for the limit to the number of embryos to transfer.

Prognosis	Age (y) < 35	35–37	38–40	41–42
Cleavage-stage embryos[a]				
Euploid	1	1	1	1
Other favorable[b]	1	1	≤3	≤4
All others	≤2	≤3	≤4	≤5
Blastocysts[a]				
Euploid	1	1	1	1
Other favorable[b]	1	1	≤2	≤3
All others	≤2	≤2	≤3	≤3

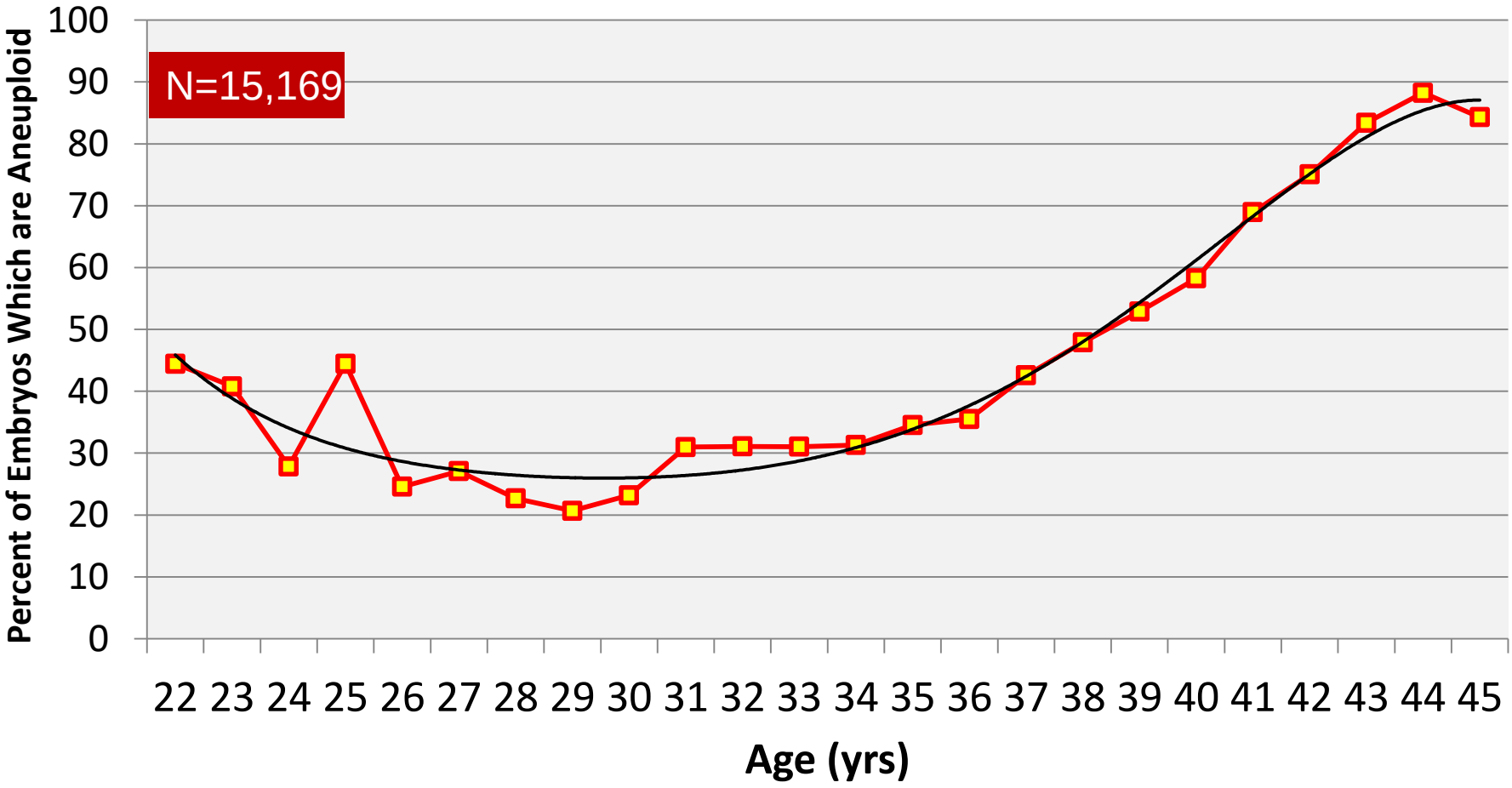
[a] See text for more complete explanations.

[b] Other favorable = Any ONE of these criteria: *Fresh cycle*: expectation of 1 or more high-quality embryos available for cryopreservation, or previous live birth after an IVF cycle; *FET cycle*: availability of vitrified day-5 or day-6 blastocysts, euploid embryos, 1st FET cycle, or previous live birth after an IVF cycle.

Please note: Justification for transferring additional embryos beyond recommended limits should be clearly documented in the patient's medical record.

ASRM. Limits on number of embryos to transfer. Fertil Steril 2017.

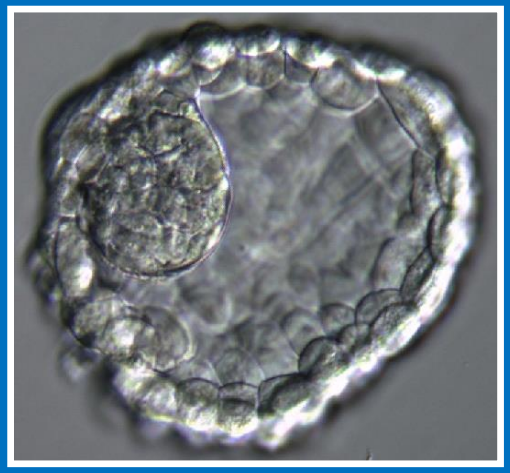
Clinical PGS: Contemporary Understanding of Maternal Age and Human Embryonic Aneuploidy

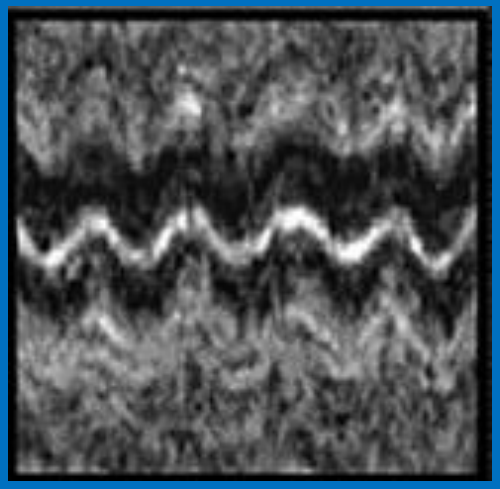


Franasiak JM, Forman EJ et al, *Fertil Steril*, 2014. PMID: 24355045

Why do 30-40% of high-quality embryos still fail to implant?

- Embryonic factors
  - Non-genomic – indels, methylation
  - Mosaicism
  - Mitochondrial function
- Uterine factors
  - Endometrial receptivity
    - Timing
    - Infection/Inflammation, Microbiome?
    - "Endometrial Disruption"
  - Endometrial contractility





Conclusions

- The future is now: the paradigm of clinical infertility care is shifting
- Healthy singleton delivery should be the goal of fertility treatment
- Blastocyst culture and synchronous transfer improves success of eSET
- Blastocyst-stage PGS and selective transfer of frozen-thawed euploid embryos enhances eSET and improves delivery outcomes after IVF

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