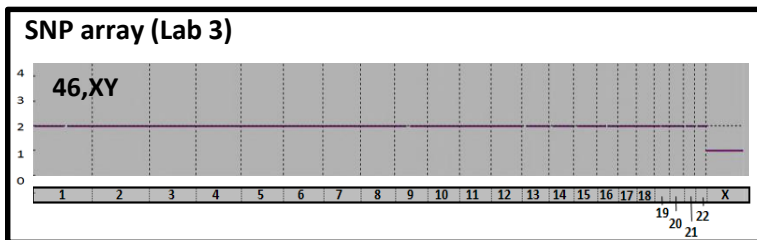
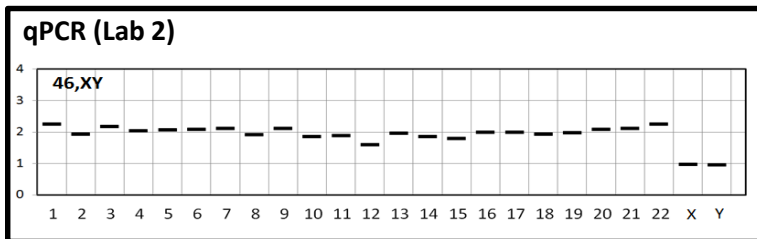
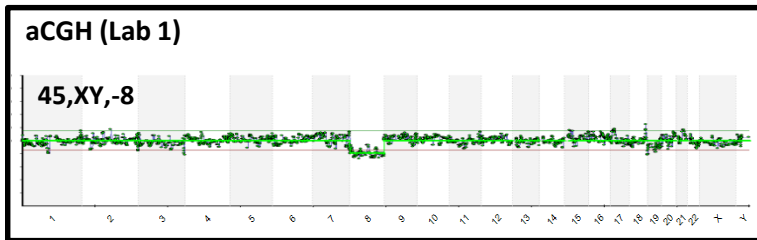


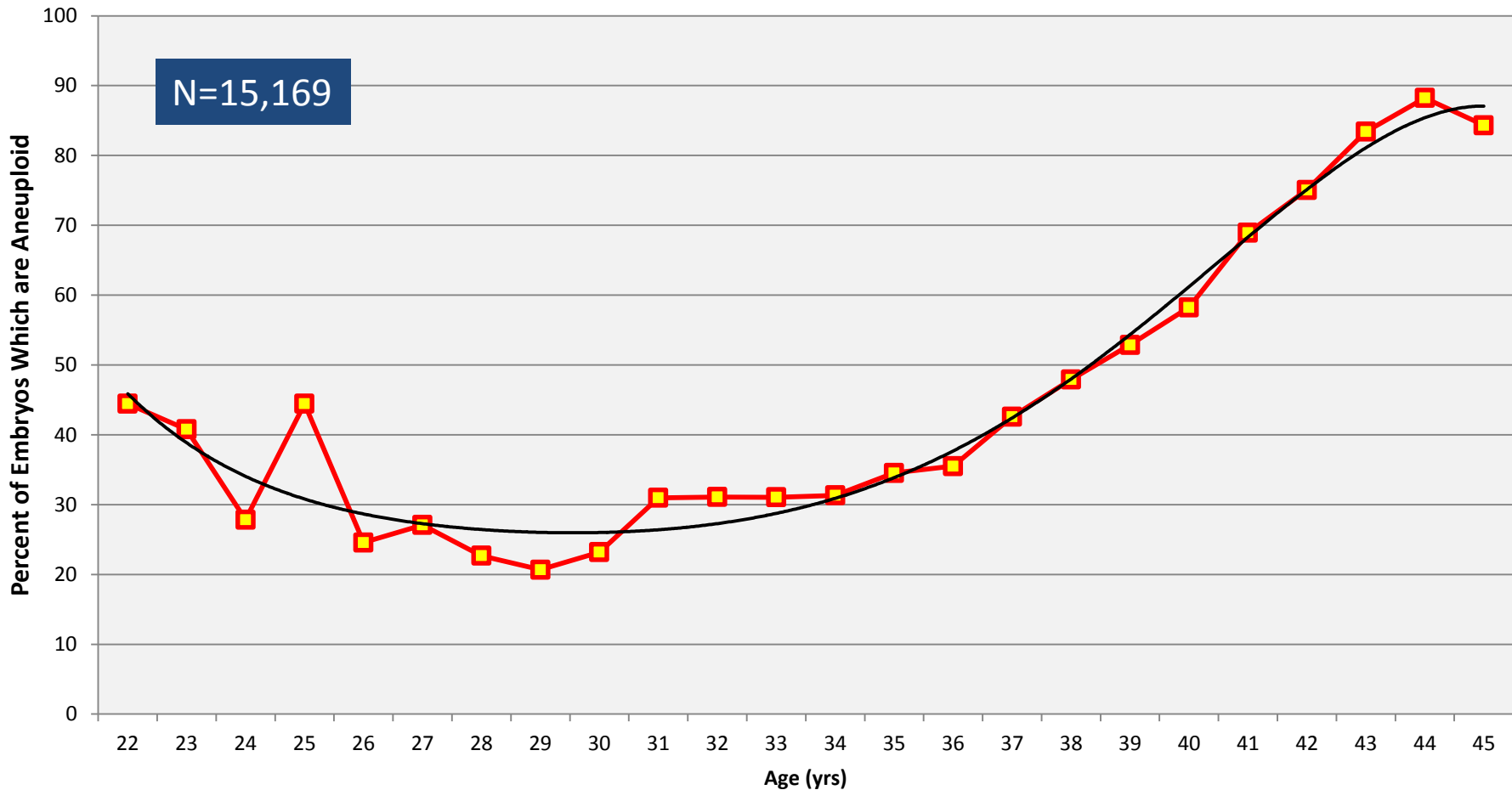
# Should Every Embryo be Screened or Frozen?

## What does the evidence say?

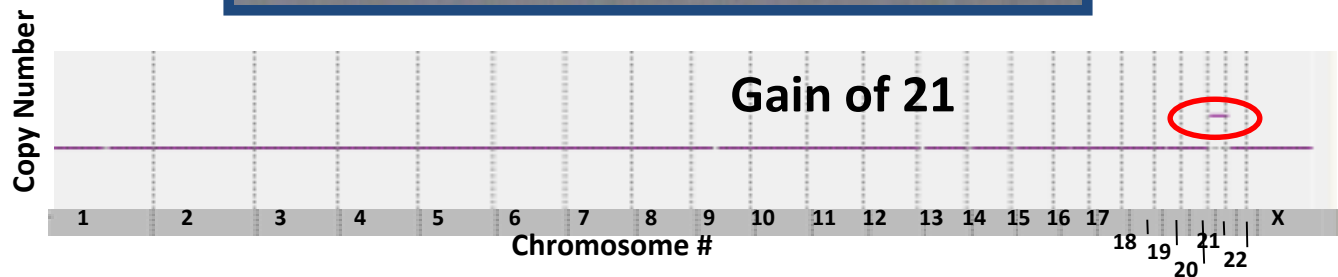
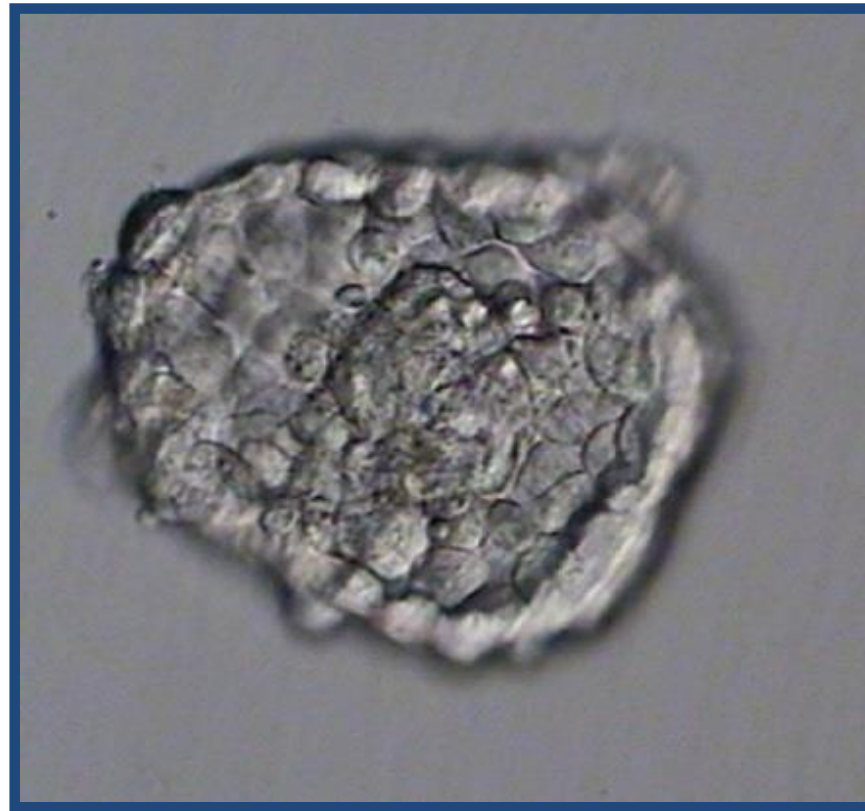


Richard T. Scott, Jr, MD, HCLD  
*Clinical and Scientific Director,  
Reproductive Medicine Associates of New Jersey  
Professor and Director, Reproductive Endocrinology  
Robert Wood Johnson Medical School, Rutgers University*

# Contemporary Understanding of Maternal Age and Human Embryonic Aneuploidy



# Is transferring an aneuploid embryo clinically useful?



# What are the “Burdens” of CCS



Thus the real questions are:

1. Safely attaining embryonic DNA
2. Predictive values of the techniques
3. Proportion of euploid embryos that will fail
4. Cost effectiveness

# Some Disagree with PGS

**Human Reproduction, Vol.29, No.9 pp. 1846–1850, 2014**

Advanced Access publication on July 8, 2014 doi:10.1093/humrep/deu163

human  
reproduction

OPINION

## Preimplantation genetic screening: back to the future

**Sebastiaan Mastenbroek\* and Sjoerd Repping**

Center for Reproductive Medicine, Academic Medical Center, University of Amsterdam, Q3-119, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands

\*Correspondence address. E-mail: s.mastenbroek@amc.uva.nl

*Submitted on April 1, 2014; resubmitted on May 9, 2014; accepted on June 3, 2014*

## Preimplantation genetic screening: back to the future

Sebastiaan Mastenbroek\* and Sjoerd Repping

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\*Correspondence address. E-mail: s.mastenbroek@amc.uva.nl

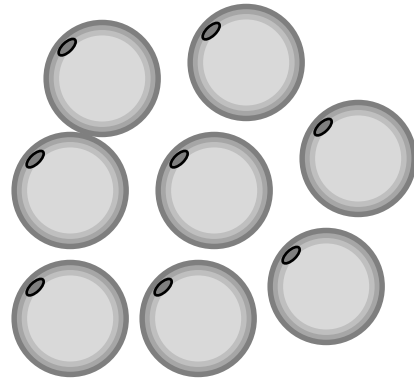
Submitted on April 1, 2014; resubmitted on May 9, 2014; accepted on June 3, 2014

# Some Disagree with PGS

- All embryo selection techniques are detrimental
- Inappropriate to use “Implantation Rates” as an endpoint
- “it can be questioned whether all patients will ever be able to understand all of the complexities concerning PGS”
- “cost-effectiveness is being forgotten”
- “evidence is now accumulating that all embryos in an IVF cycle can be cryopreserved and transferred in subsequent cycles without impairing, and maybe even improving, the cumulative pregnancy rate of that IVF cycle”
- Embryo selection should therefore not be used to select out embryos, but only to determine the order in which the embryos will be transferred, as the time to pregnancy can be improved by embryo selection, if embryos with the highest implantation potential are transferred first.
- Culturing to the blastocyst may be harmful

# Does Embryo Biopsy Impact the Developmental Potential of the Oocyte

Routine IVF Care through Retrieval



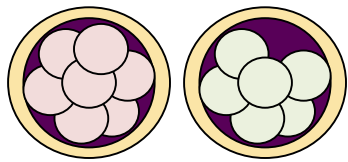
Identify mature oocytes

ICSI, culture, and select 2 best embryos for transfer



One embryo randomized to undergo biopsy

Transfer the embryos

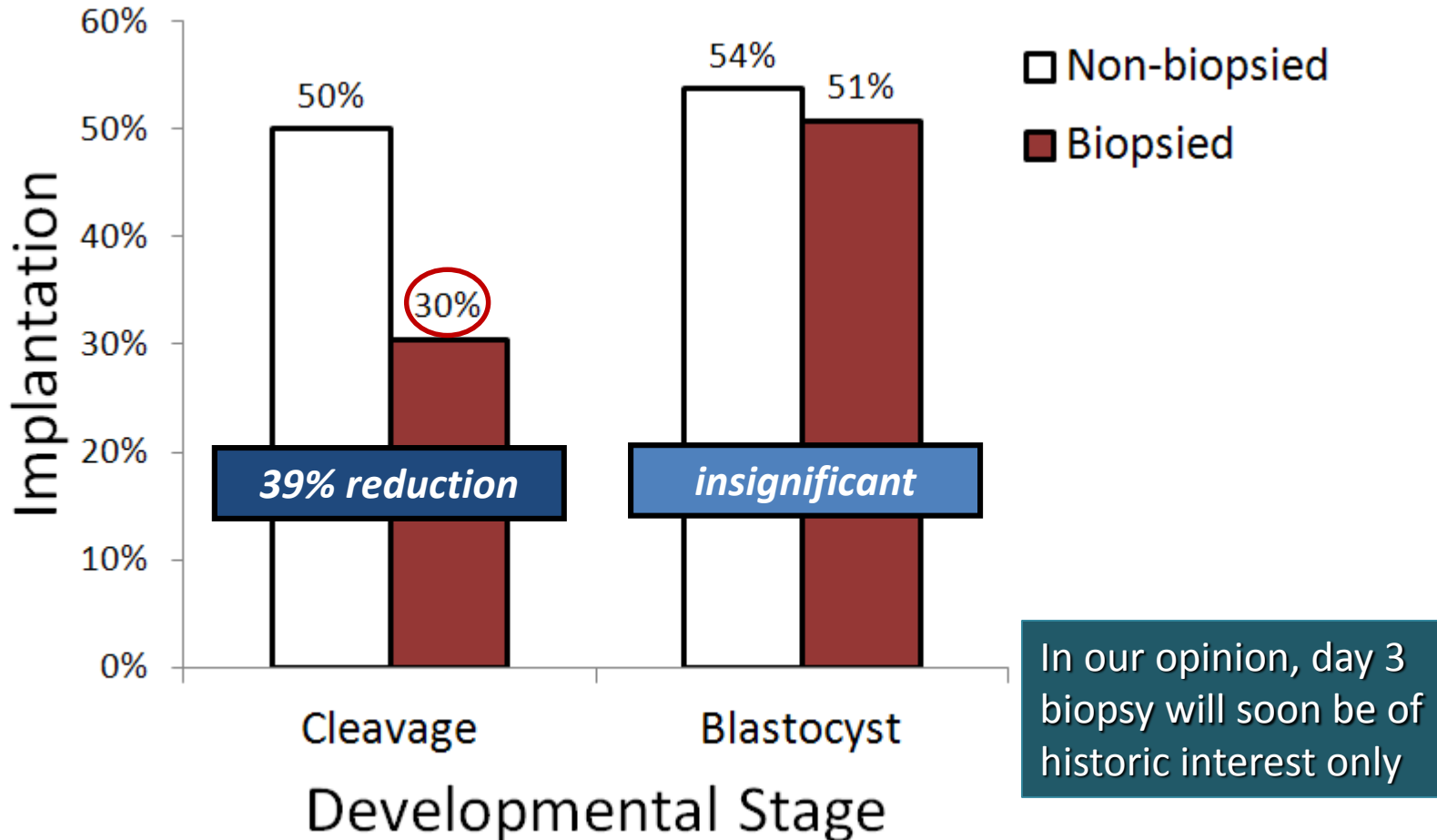


Cell submitted for eventual aneuploidy screening and fingerprinting

Implantation, Maternal serum sampling for free fetal DNA and Fingerprinting

N=113 pairs; 226 embryos

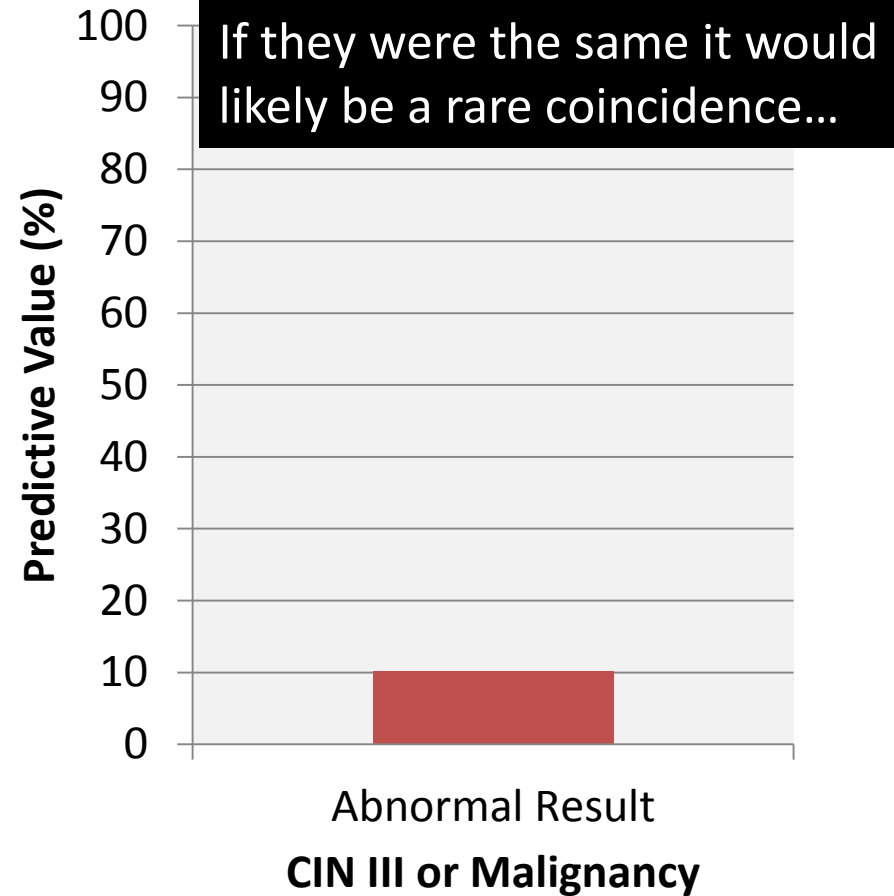
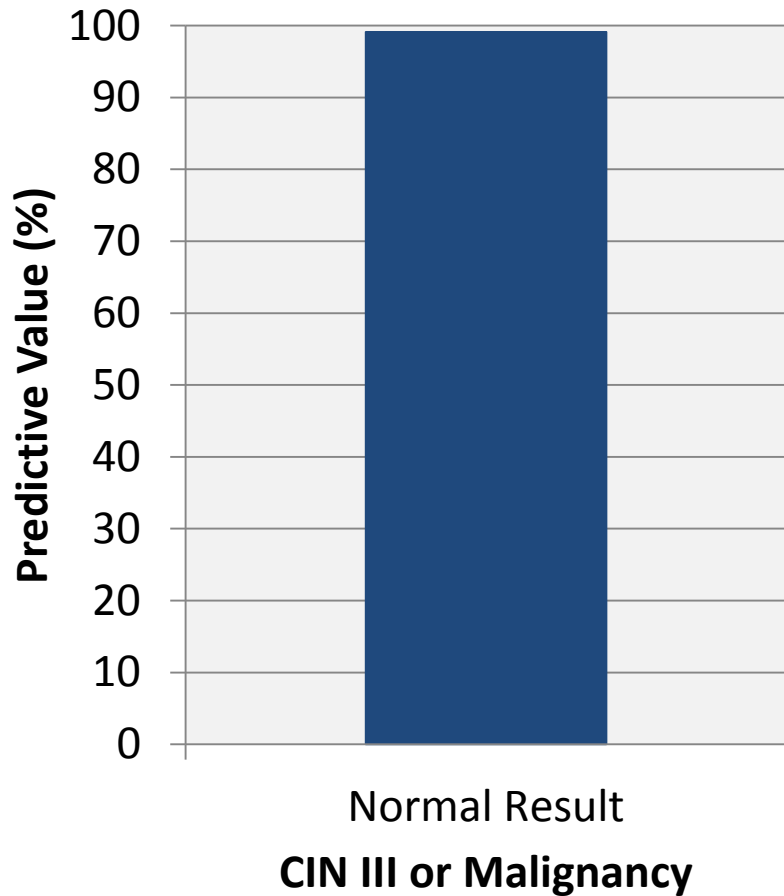
# Overall implantation rates



27% (mean maternal age 32) reported by Gutierrez-Mateo, C., et al. *Fertility and sterility* 92, 1544-1556 (2009)



# Is knowing the predictive value of a normal result sufficient?

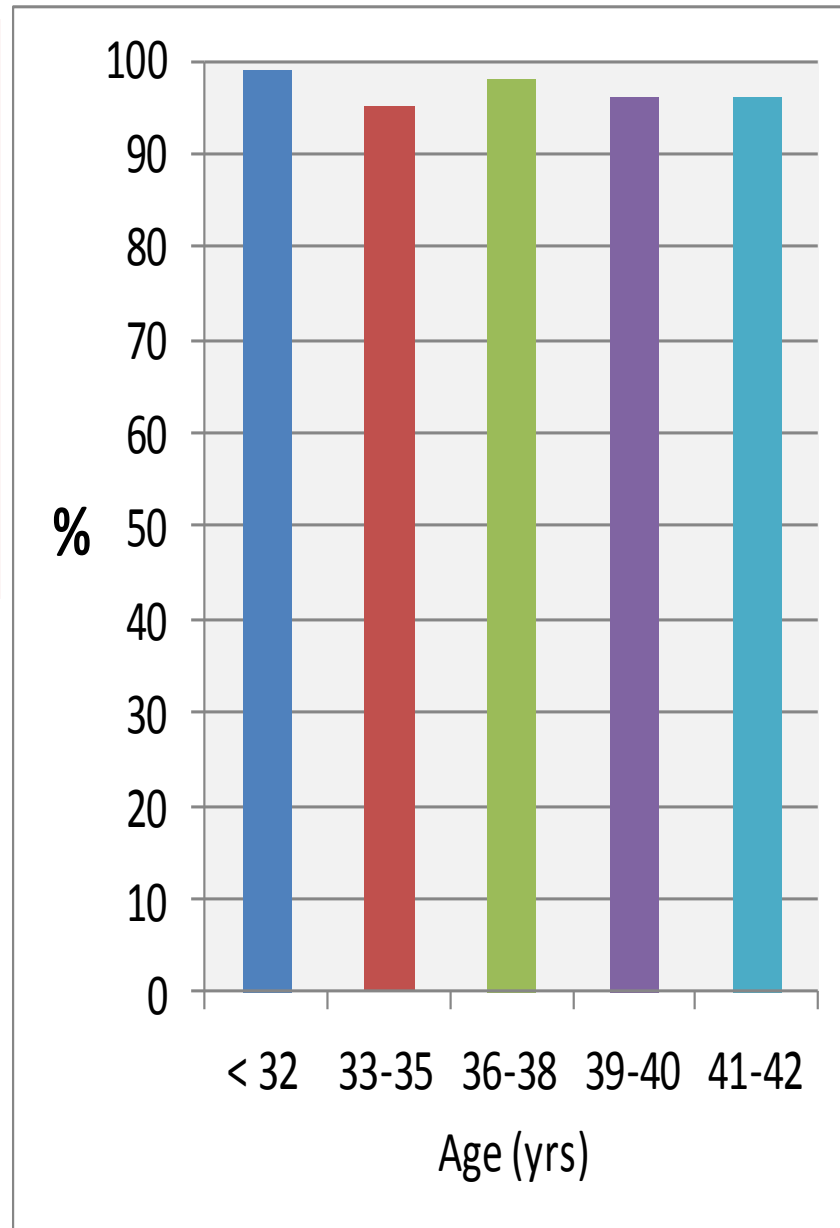


# Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study

Richard T. Scott Jr., M.D.,<sup>a,b</sup> Kathleen Ferry, B.S.,<sup>a</sup> Jing Su, M.S.,<sup>a</sup> Xin Tao, M.S.,<sup>a</sup> Katherine Scott, M.S.,<sup>a</sup> and Nathan R. Treff, Ph.D.<sup>a,b</sup>

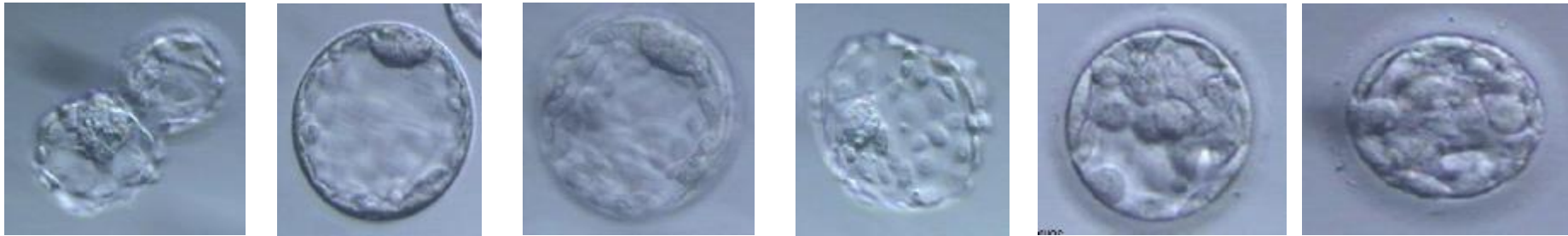
<sup>a</sup> Reproductive Medicine Associates of New Jersey, Morristown, New Jersey; and <sup>b</sup> Division of Reproductive Endocrinology, Department of Obstetrics Gynecology and Reproductive Science, University of Medicine and Dentistry of New Jersey–Robert Wood Johnson Medical School, New Brunswick, New Jersey

With greater experience,  
actual negative  
predictive value is  
~98.8%



*To have the opportunity for meaningful improvement, when you select for one criteria you most commonly deselect for another....*

Transfer Based on Embryo Morphology



Abnl

Abnl

NI

Abnl

NI

NI

Transfer Based on Aneuploidy Screening and Embryo Morphology

CCS changes the embryo selected  
40% of the time  
Forman et al ASRM 2012

METHODOLOGY

Open Access

## Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study

Zhihong Yang<sup>1</sup>, Jiaen Liu<sup>2</sup>, Gary S Collins<sup>3</sup>, Shala A Salem<sup>1</sup>, Xiaohong Liu<sup>2</sup>, Sarah S Lyle<sup>1</sup>, Alison C Peck<sup>1</sup>, E Scott Sills<sup>1\*</sup> and Rifaat D Salem<sup>1</sup>

**Table 3 Comparison of laboratory findings and clinical outcome among IVF patients undergoing SET with embryo assessment by aCGH + morphology (Group A) and blastocyst morphology alone (Group B)**

	A	B	p
Fresh blastocyst transfer <i>according to morphology assessment</i> :			
Grade 5/6	31 (56.4)	28 (58.3)	
Grade 4	21 (38.2)	19 (39.6)	0.677 <sup>a</sup>
Grade 3	3 (5.4)	1 (2.1)	
Clinical pregnancy	39 (70.9)	22 (45.8)	0.017 <sup>a</sup>
Ongoing pregnancy (≥20wks GA)	38 (69.1)	20 (41.7)	0.009 <sup>a</sup>
Missed abortion	1 (2.6)	2 (9.1)	0.597 <sup>b</sup>

Notes: All data reported as n (%). SET = single embryo transfer; aCGH = array comparative genomic hybridization; GA = gestational age <sup>a</sup> by Chi-squared test <sup>b</sup> by Fisher's exact test.

## aCGH enhances delivery rates – an RCT

- RCT
- Age
  - All < 35
  - Mean age of 31
- Sample Size
  - 55 aCGH
  - 48 control
- Significant improvement in outcomes
- Answers one of the four critical validation questions

Monosomy:Trisomy Ratio of 2

## 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.,<sup>a,b</sup> Kathleen M. Upham, B.S.,<sup>a</sup> Eric J. Forman, M.D.,<sup>b</sup> Tian Zhao, M.S.,<sup>a</sup> and Nathan R. Treff, Ph.D.,<sup>a,b,c</sup>

<sup>a</sup> Reproductive Medicine Associates of New Jersey, Morristown; <sup>b</sup> Division of Reproductive Endocrinology, Department of Obstetrics, Gynecology, and Reproductive Sciences, Robert Wood Johnson Medical School, Rutgers University, New Brunswick; and <sup>c</sup> Department of Genetics, Rutgers-State University of New Jersey, Piscataway, New Jersey

## Comprehensive chromosome screening alters traditional morphology-based embryo selection: a prospective study of 100 consecutive cycles of planned fresh euploid blastocyst transfer

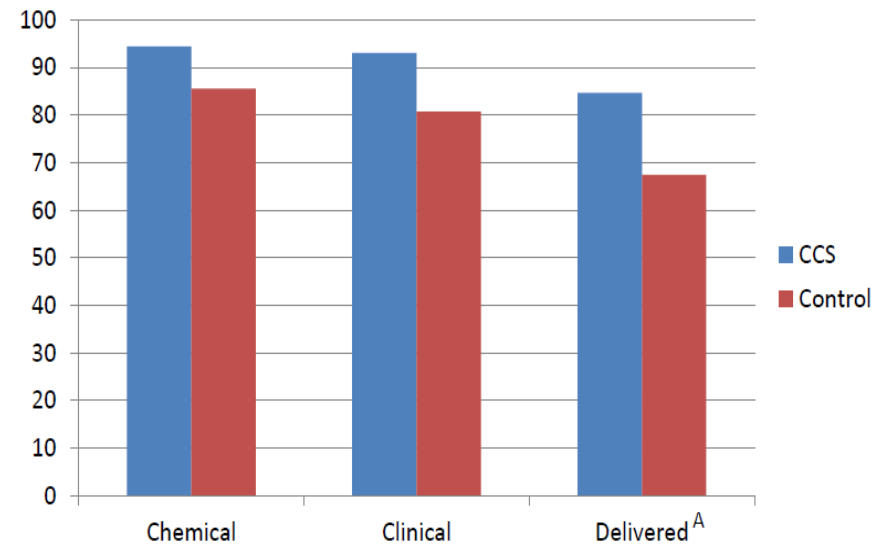
Eric J. Forman, M.D.,<sup>a,b</sup> Kathleen M. Upham, B.S.,<sup>a</sup> Michael Cheng, B.S.,<sup>a</sup> Tian Zhao, B.S.,<sup>a</sup> Kathleen H. Hong, M.D.,<sup>a,b</sup> Nathan R. Treff, Ph.D.,<sup>a,b</sup> and Richard T. Scott Jr., M.D.,<sup>a,b</sup>

<sup>a</sup> Reproductive Medicine Associates of New Jersey, Department of Reproductive Endocrinology, Basking Ridge; and <sup>b</sup> University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, Department of Obstetrics, Gynecology & Reproductive Sciences, New Brunswick, New Jersey

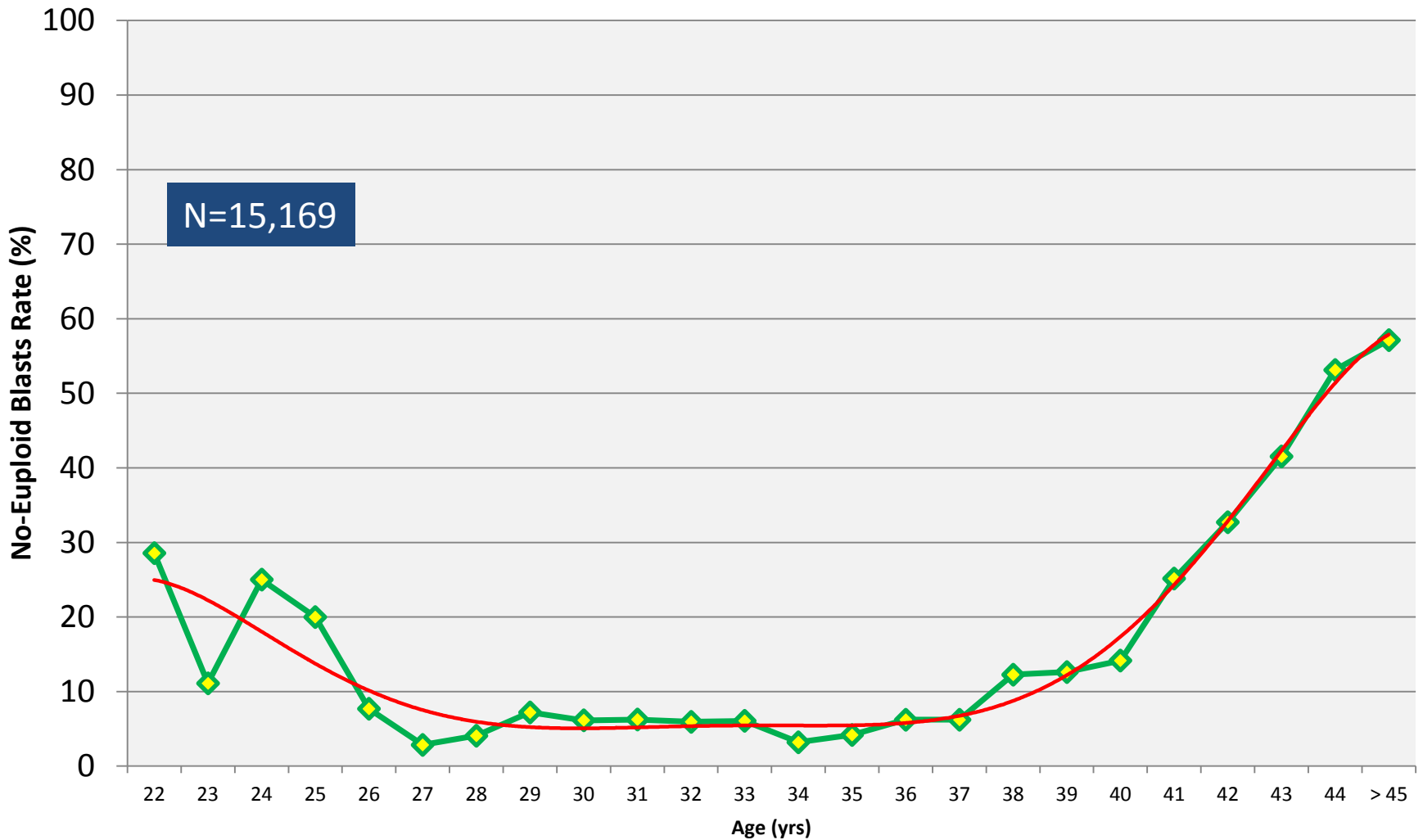
## Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial

Richard T. Scott Jr., M.D.,<sup>a,b</sup> Kathleen M. Upham, B.S.,<sup>a</sup> Eric J. Forman, M.D.,<sup>b</sup> Kathleen H. Hong, M.D.,<sup>b</sup> Katherine L. Scott, M.S.,<sup>a,c</sup> Deanne Taylor, Ph.D.,<sup>a,b</sup> Xin Tao, M.S.,<sup>a</sup> and Nathan R. Treff, Ph.D.,<sup>a,b</sup>

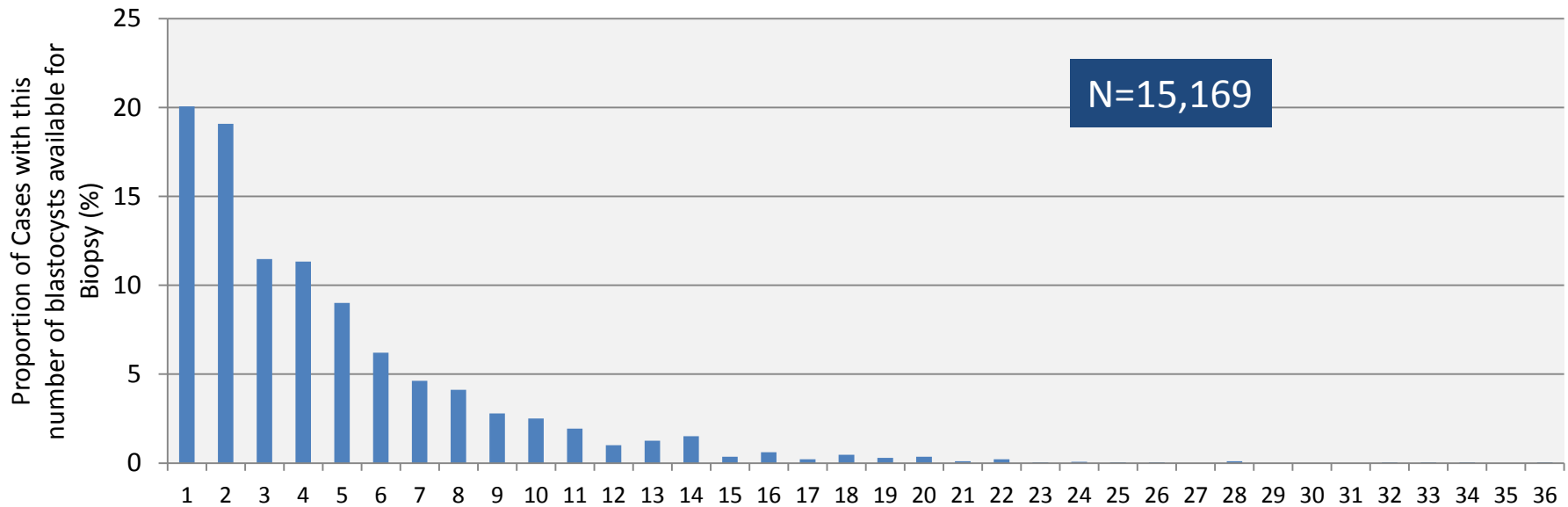
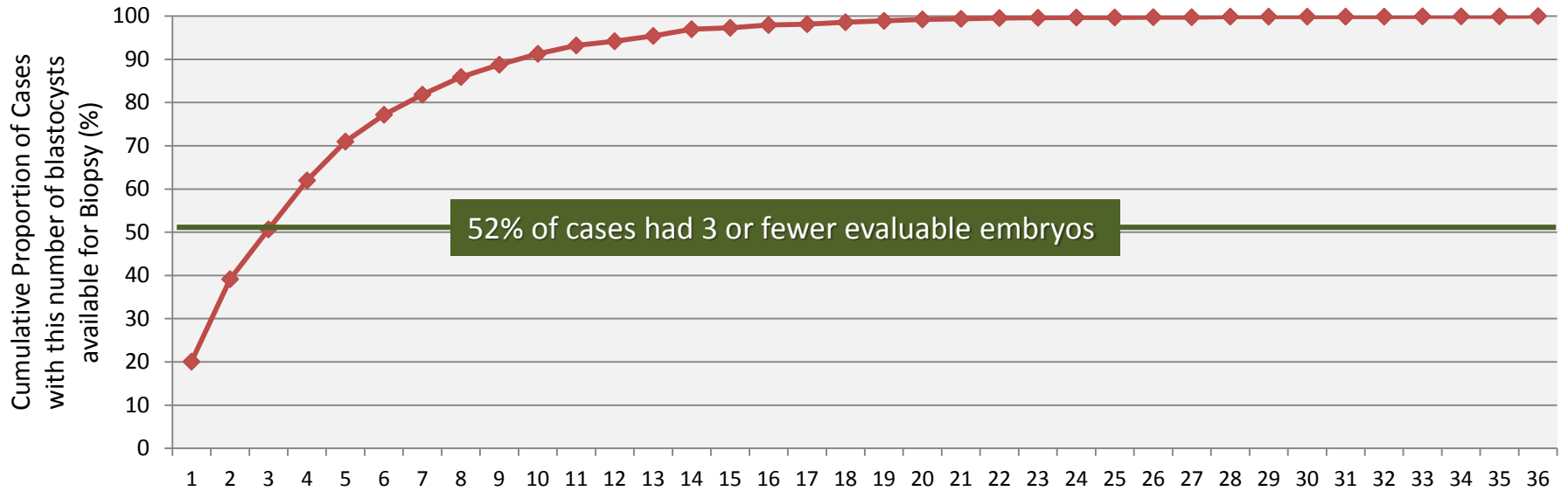
<sup>a</sup> Reproductive Medicine Associates of New Jersey, Morristown, New Jersey; <sup>b</sup> Division of Reproductive Endocrinology, Department of Obstetrics, Gynecology, and Reproductive Science, Robert Wood Johnson Medical School, Rutgers University, New Brunswick, New Jersey; and <sup>c</sup> Atlantic Reproductive Medicine Specialists, Raleigh, North Carolina



# The No Transfer Rate with CCS



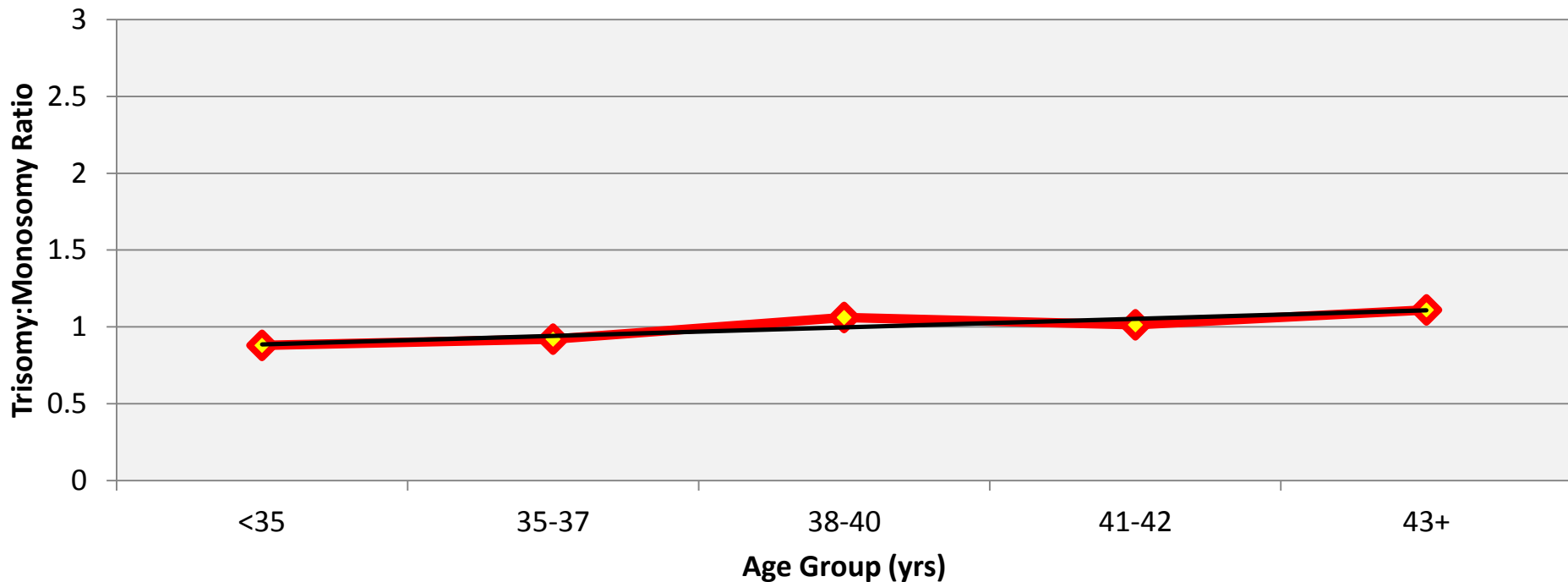
# How Many Embryos Do Patient Undergoing CCS Have?



# Trisomy:Monosomy Ratio by Age

N=15,169

Ratios consistent across nine programs



Key Indicator for QA of your assay



# Clinical Experience

## *Misdiagnoses*

### Clinical Error Rate

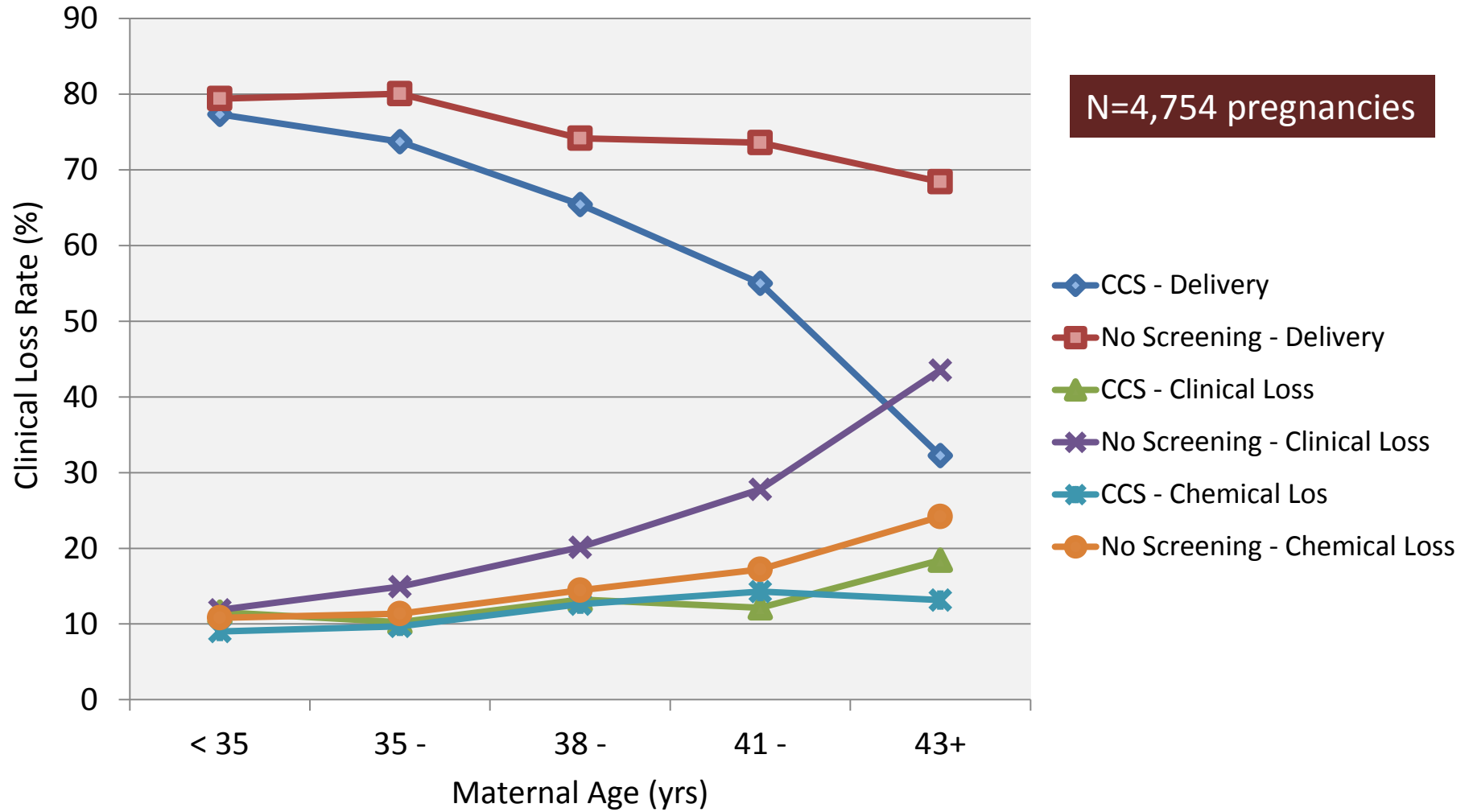
Per embryo	0.2%
Per transfer	0.3%
Per ongoing pregnancy	0.1%

- 4974 embryos
- 2976 gestations (62.1%)
- 10 errors
  - 1 tetraploid
  - 2 monosomies
  - 7 trisomies
- 3168 transfers
- 2354 ongoing / delivered (72.1%)
- Mean age 38.4 years
- 10 errors
  - 7 found in losses
  - 3 found in ongoing preg.

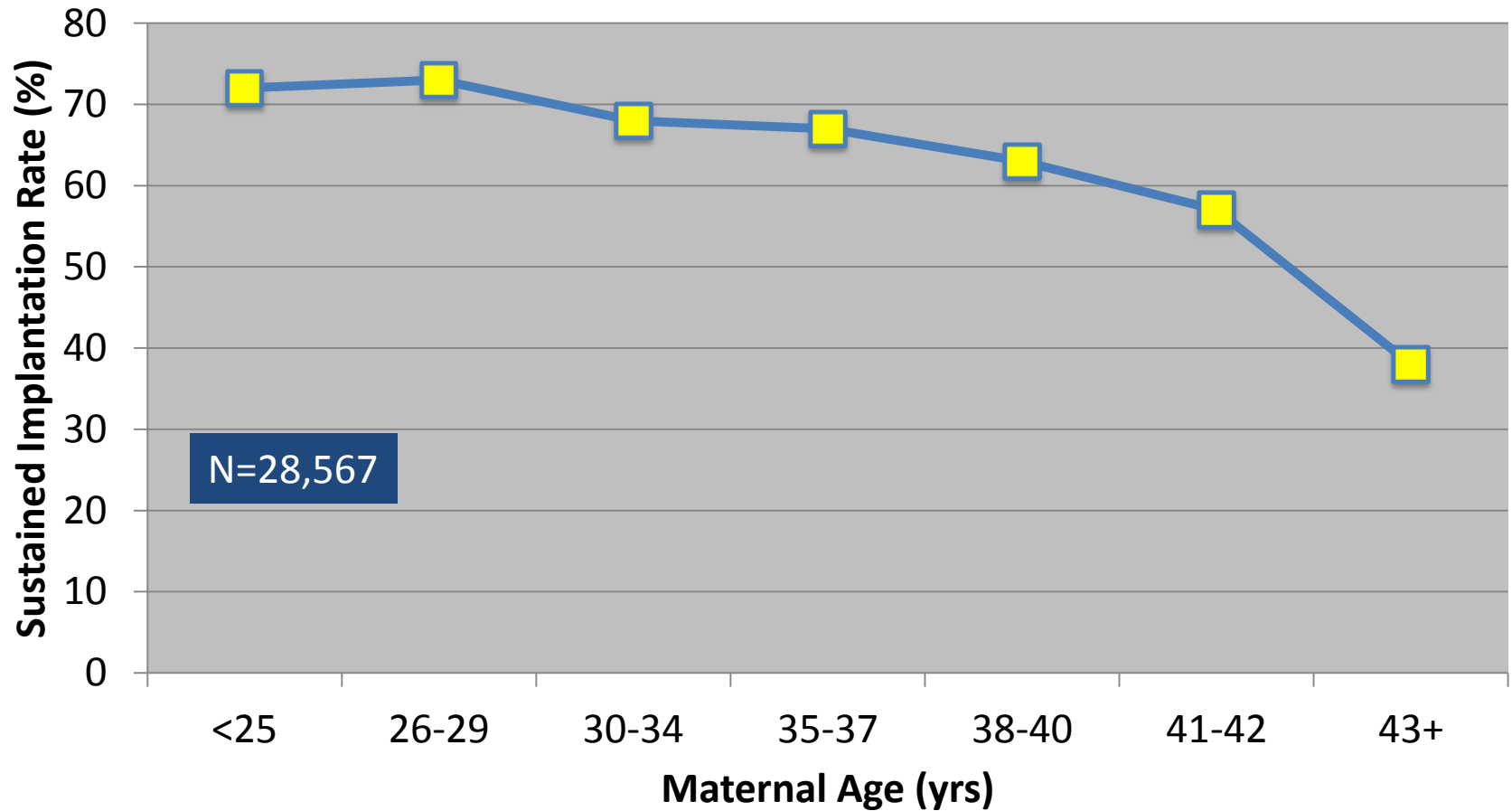
Mosaicism evaluated in 4 samples – 100% mosaic

# Consolidated Pregnancy Outcomes

## *Proportion of All Pregnancies*



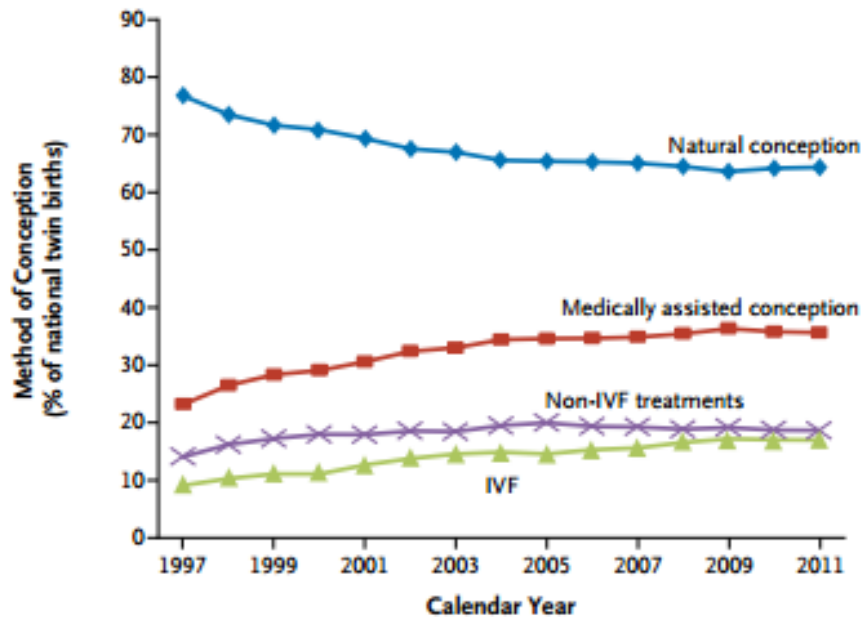
# PGS Improves but Does Not Normalize Implantation and Delivery Rates in Older Women



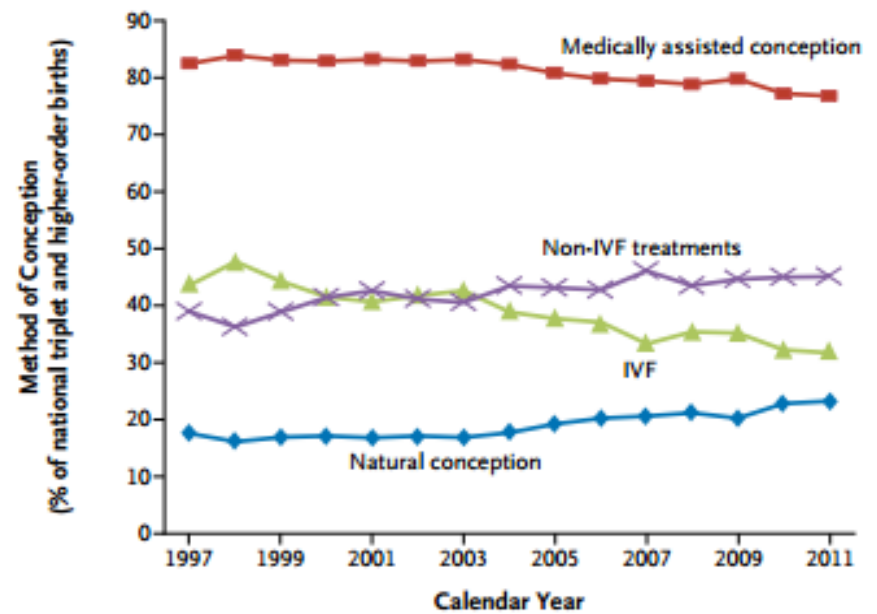
ORIGINAL ARTICLE

## Fertility Treatments and Multiple Births in the United States

**A** Twin Births

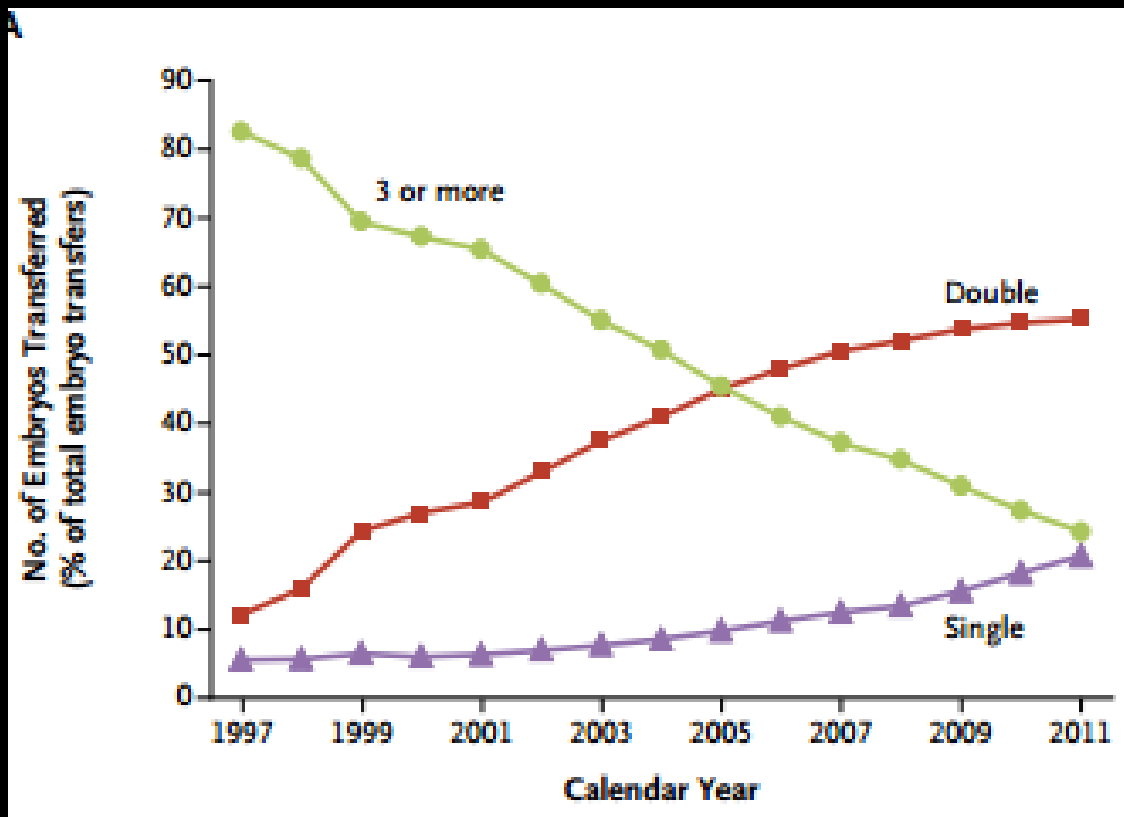


**B** Triplet and Higher-Order Births



ORIGINAL ARTICLE

## Fertility Treatments and Multiple Births in the United States



# Singleton Term Delivery: The Ideal IVF Outcome

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

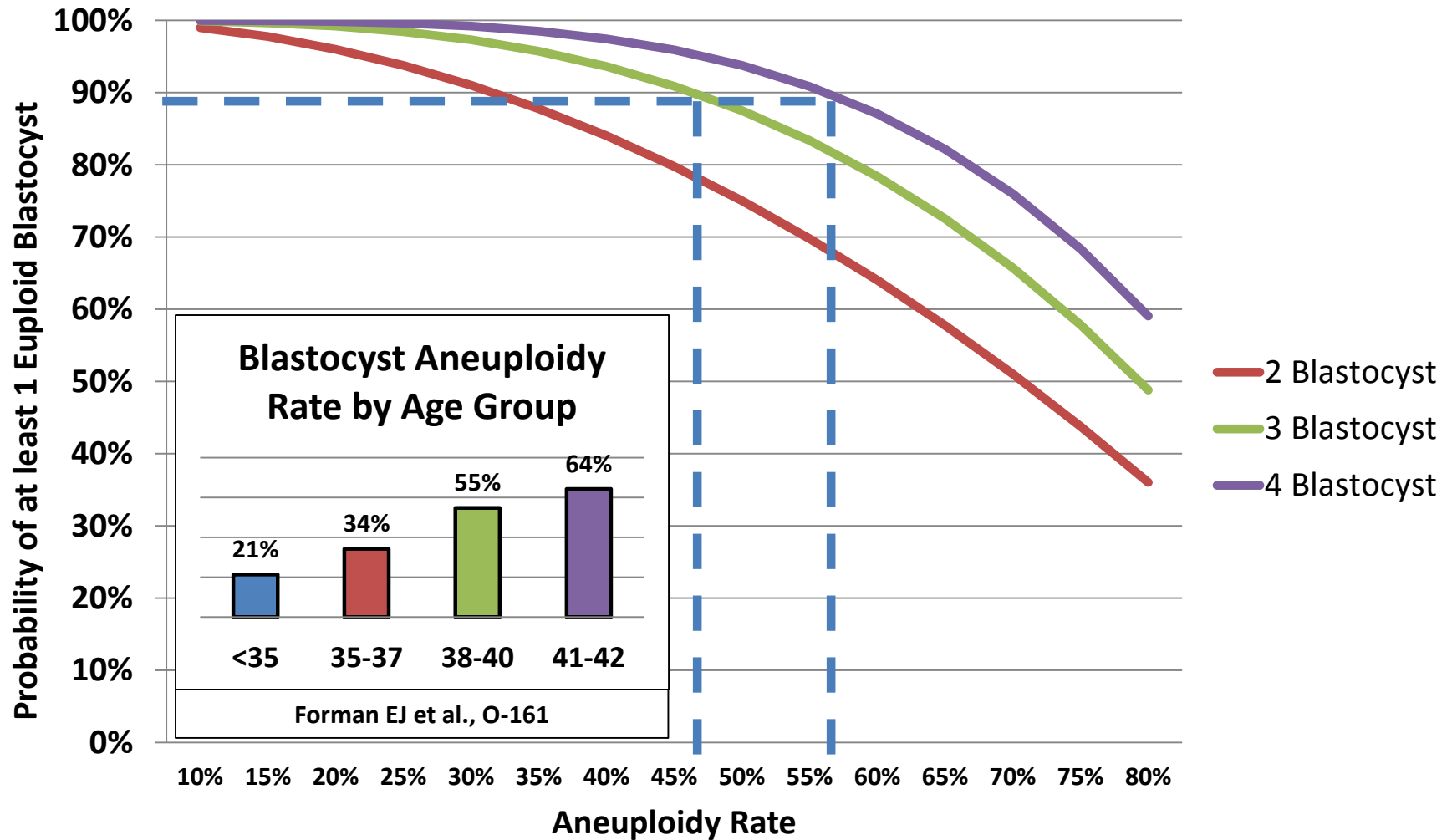
1. ASRM Practice Committee, *Fertil Steril*, 2012. PMID: 22192352
2. Pinborg A, et al., *Acta Obstet Gynecol Scand*, 2004. PMID: 15488125
3. Sazonova A ,et al., *Fertil Steril*, 2013. PMID: 23219009

# The Perception that Patients Love Multiples if They are Born Healthy is not Well Validated



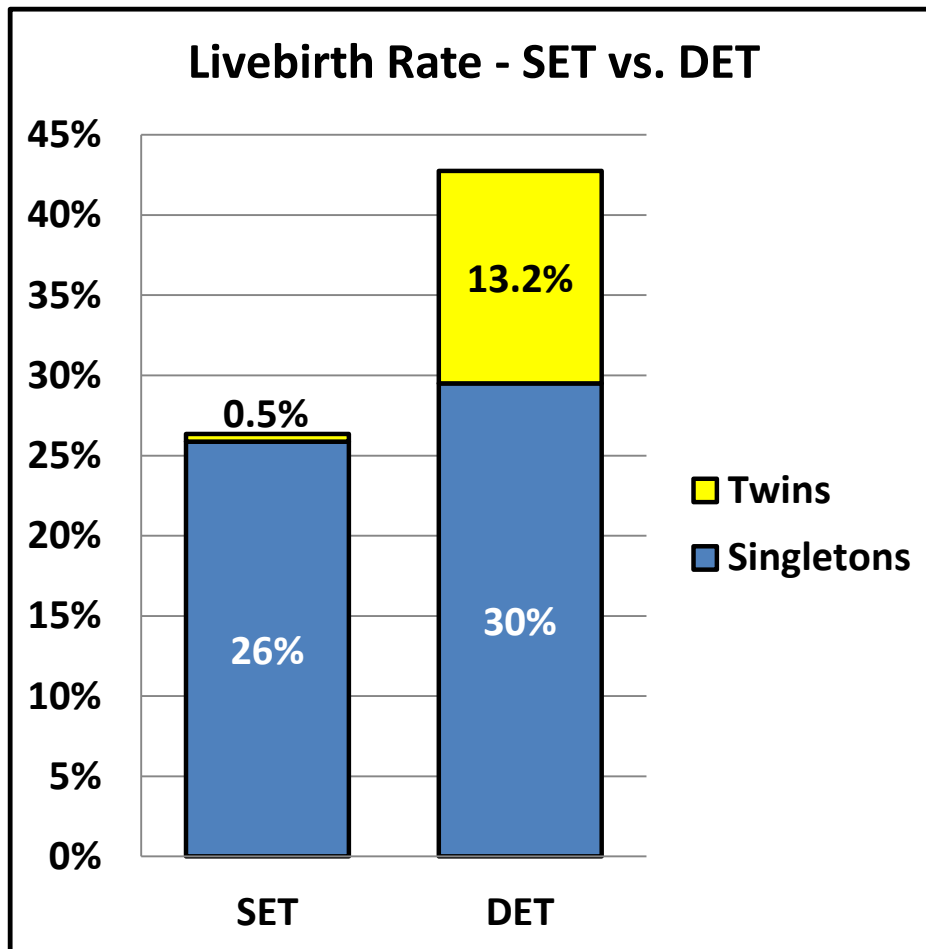
*Provided by a patient...*

# With >2 blastocysts, even patients at high aneuploidy risk are very likely to have a euploid blastocyst



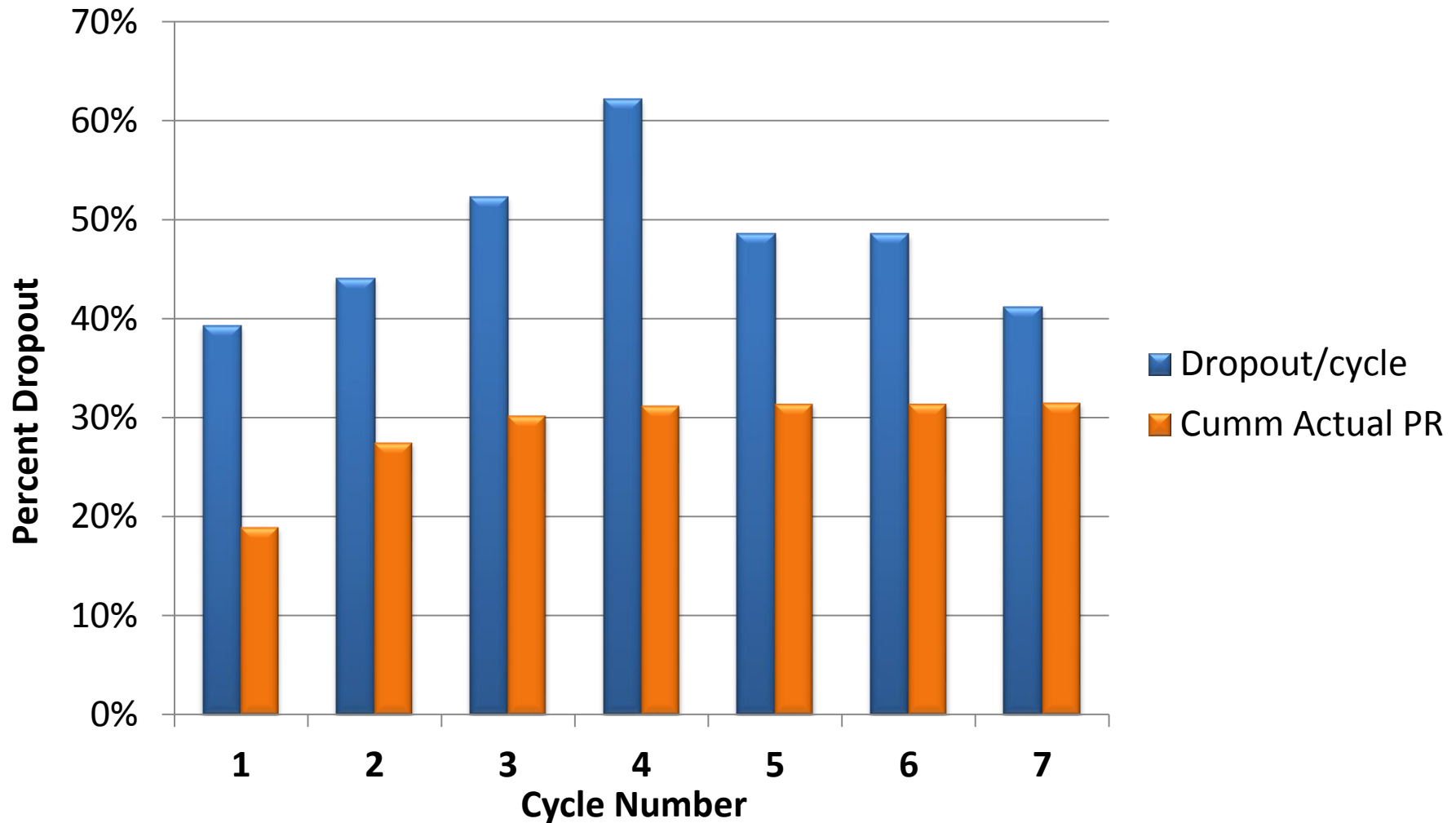


# FRESH SET RESULTS IN LOWER DELIVERY RATES THAN DOUBLE EMBRYO TRANSFER (DET)



- 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

# The Dropout Rate from IVF is Significant



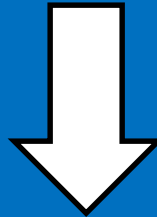
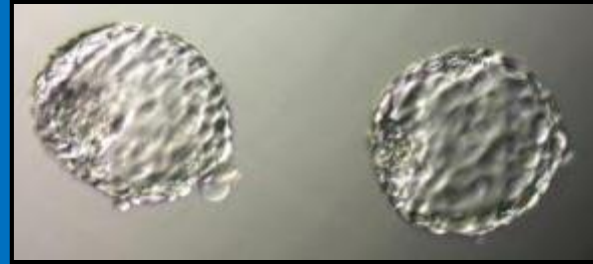
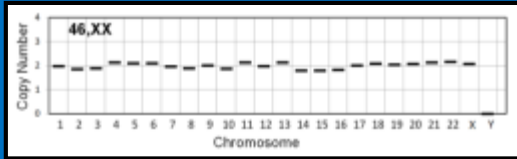
Source: Schroder AK: Cumulative pregnancy rates and drop out rates of a German IVF programme: 4, 102 cycles in 2,130 patients. *RBM Online* (2004) 8:600-606

# Can

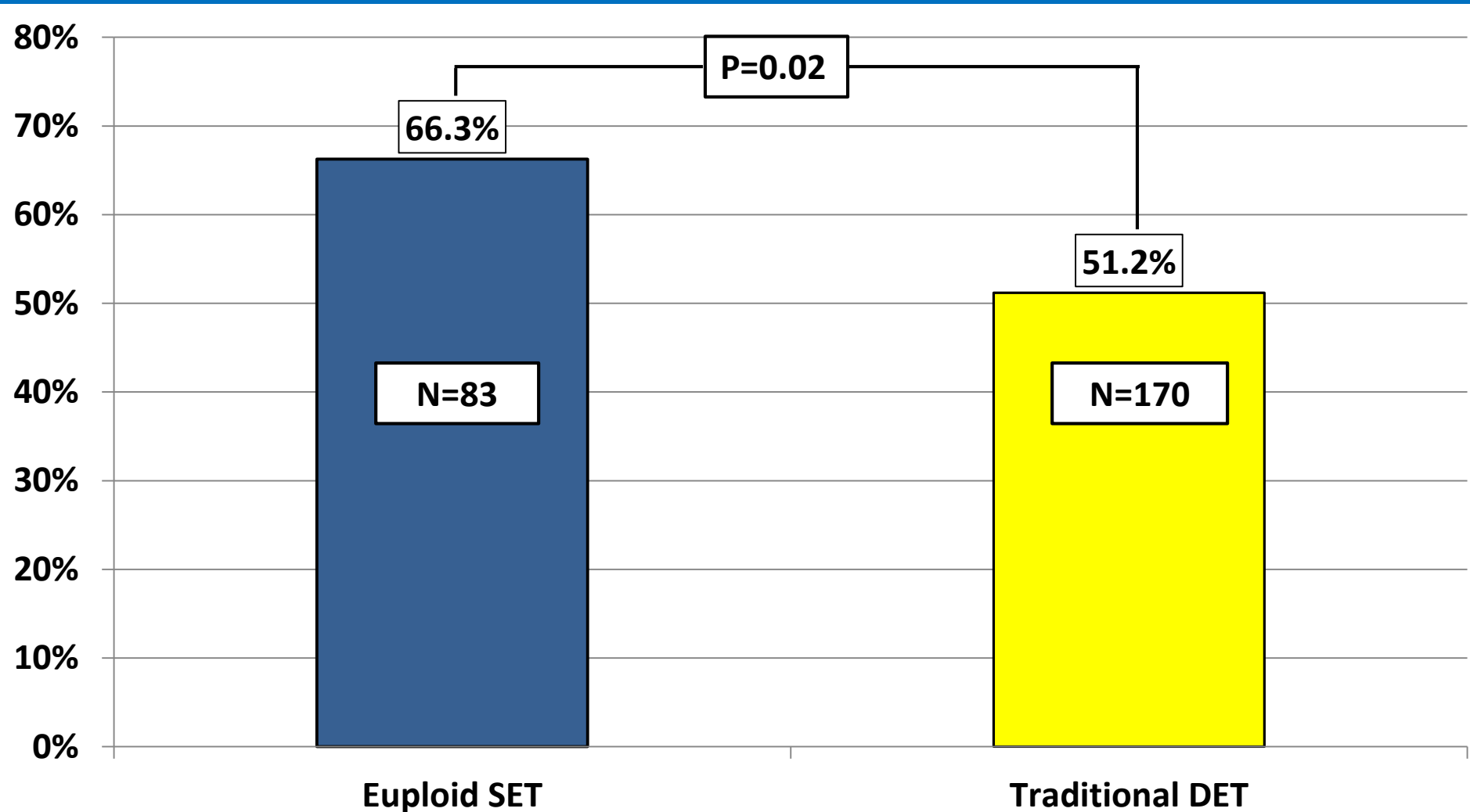
1

$\geq$

2?



# CCS Results in Higher Implantation Rates

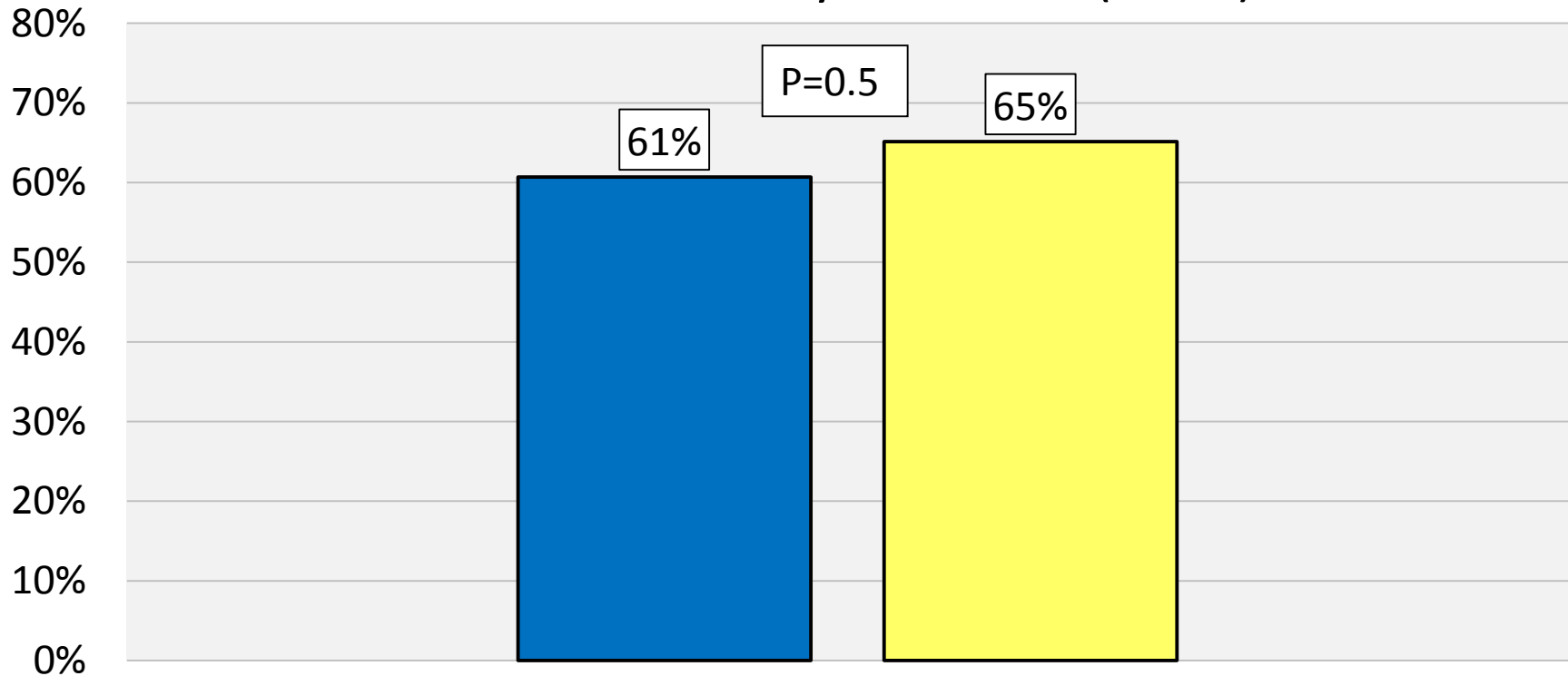


Implantation = cardiac activity at time of discharge to obstetrical care (~9 weeks)

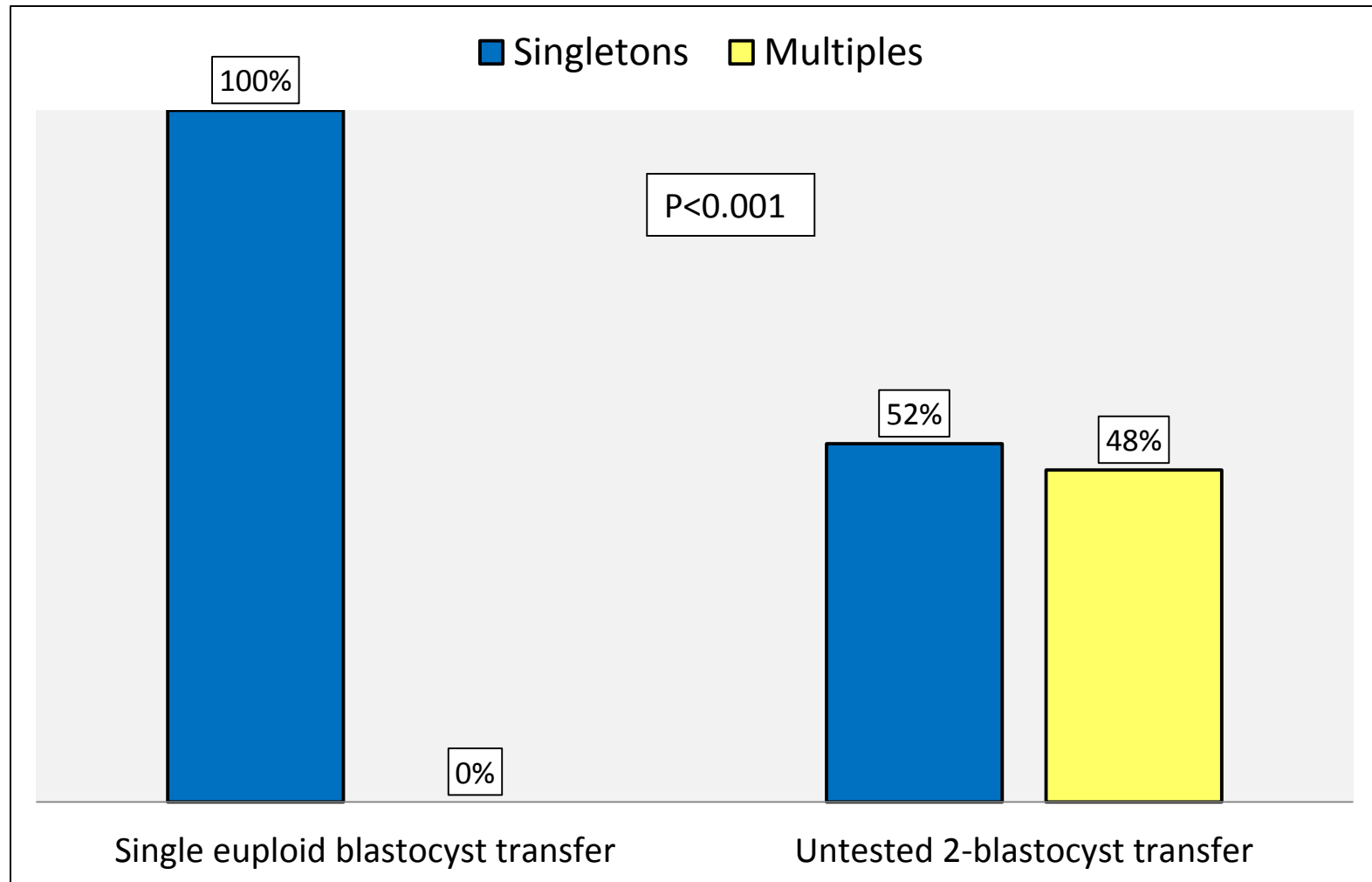
# Same Delivery Rate: Randomized Controlled Trial

## Delivery Rate Per Patient (n=175)

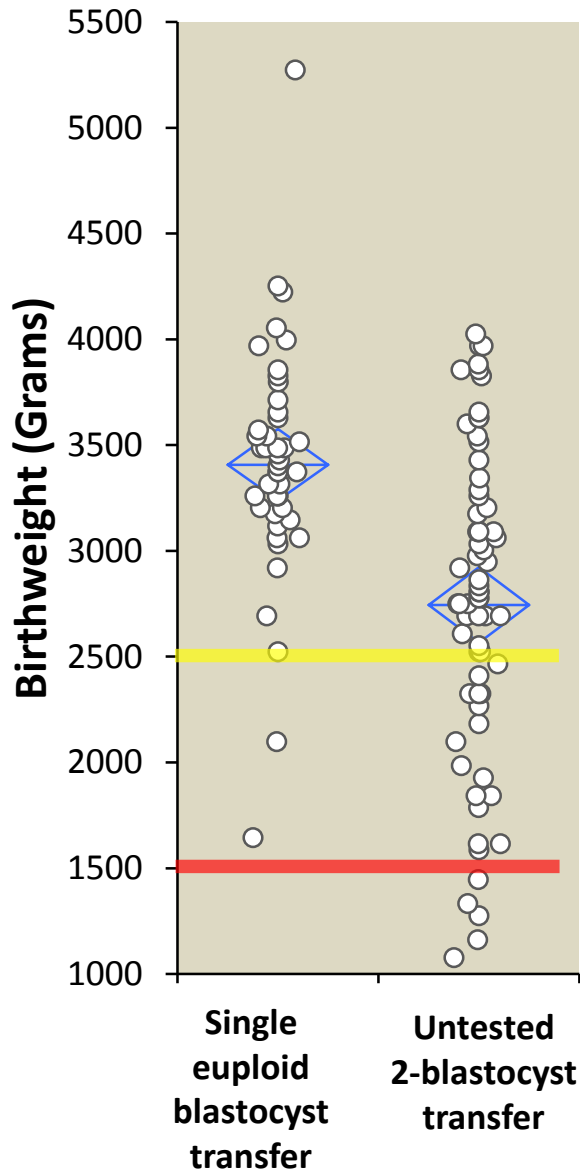
- Single euploid blastocyst transfer (N=89)
- Untested 2-blastocyst transfer (N=86)



# Eliminates Multiples

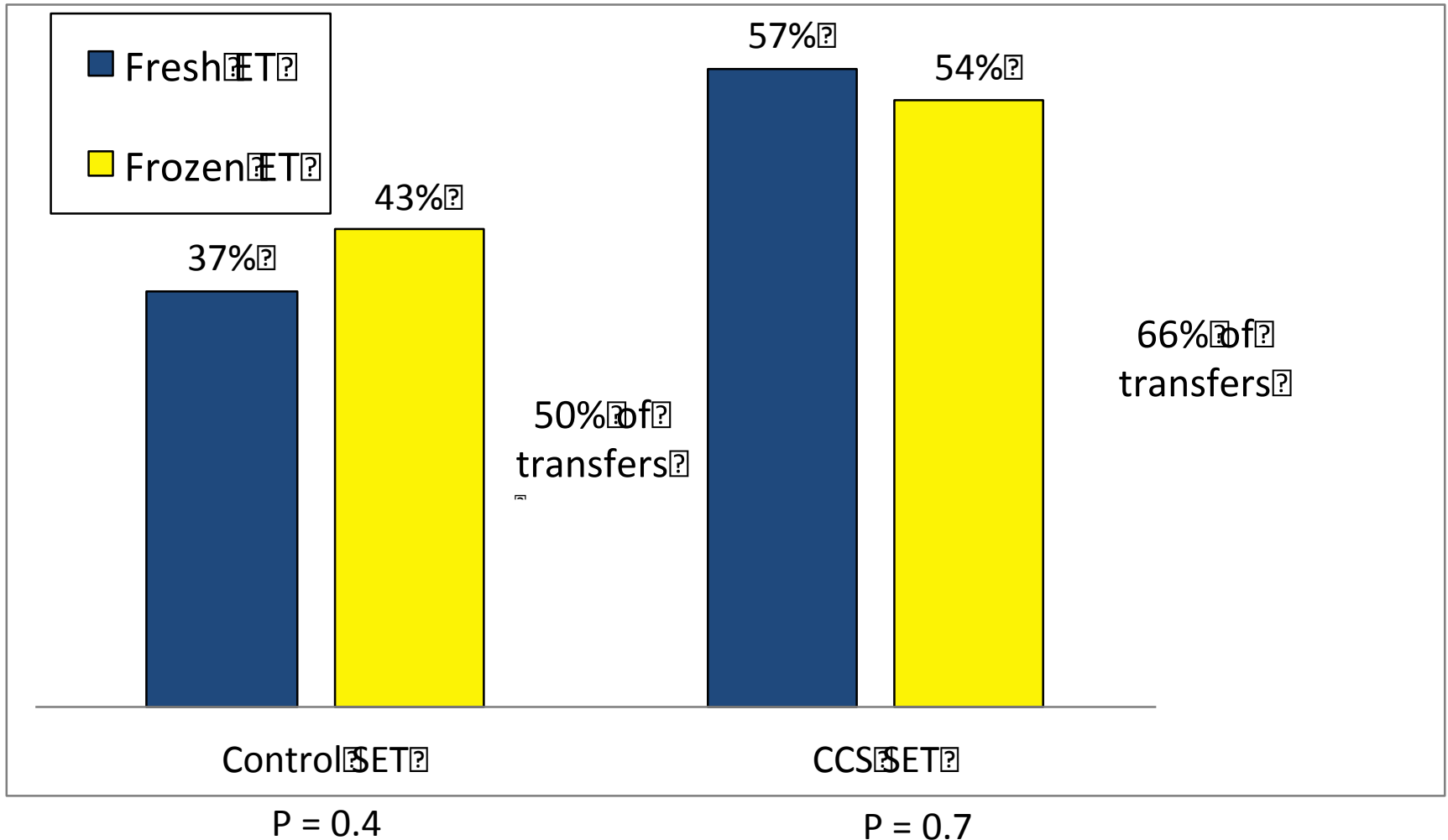


# Better Obstetrical Outcomes are Attained CCS/eSET than Conventional Two Embryo Transfer



- Mean Birthweight:  
3408 ± 562g – Single euploid  
2745 ± 743g – 2-Blastocyst  
(P<0.001)
- Low birthweight (<2,500g):  
4.4% (2/45) – Single Euploid  
31.9% (22/69) – 2-Blastocyst  
(P<0.001)
- Very low birthweight (<1,500g):  
0% (0/45) – Single Euploid  
7.2% (5/69) – 2-Blastocyst (P=0.06)

# Ongoing Pregnancy Rates Fresh vs. Frozen Transfers





# Obstetrical Costs for 100 Patients

Current Standard Of Care

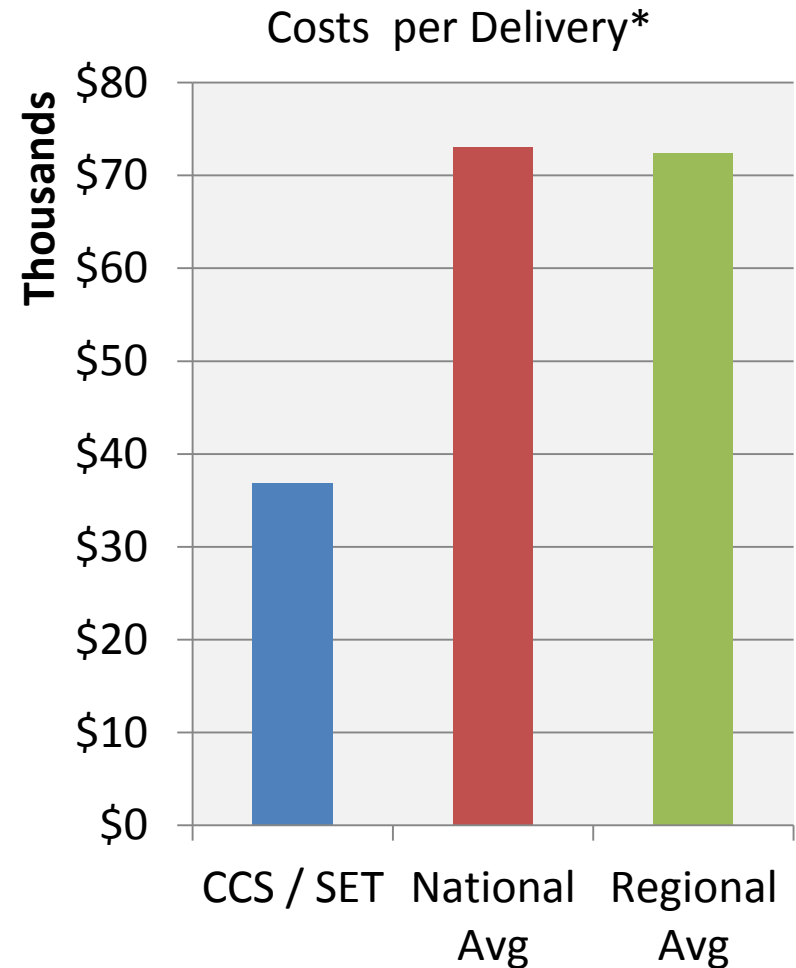
Costs per Delivery*	
Singleton	\$21,458
Twins	\$104,831
Triplets	\$407,199

## + Does not include:

- + Pediatric costs after 28 days of age
- + Disability costs during bed rest
- + Loss of productivity in the work place

# Overall Cost to Provide Care CCS with SET versus Conventional Treatment

- Use actual cost data
- Inclusive of all IVF costs including
  - IVF cycle costs
  - CCS costs
  - Medication costs
- Delivery costs and subsequent hospital stay through 28 days of life



*Do we ever recommend two  
embryo transfers?*



*Yes – but with caution...*

CLINICAL RESEARCH OPPORTUNITY

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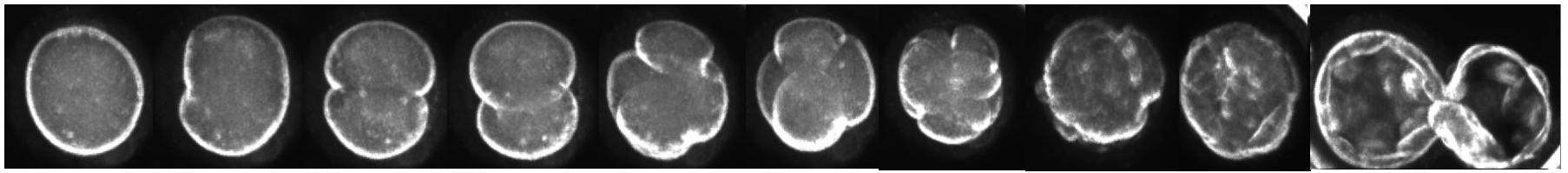
# SOLAIRE

SCREENING OF LOW RESPONDERS  
FOR ANEUPLOIDY TO IMPROVE  
REPRODUCTIVE EFFICIENCY

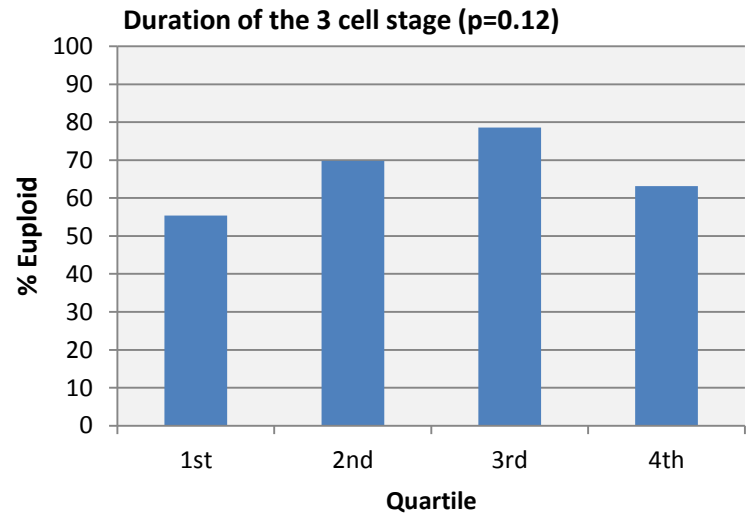
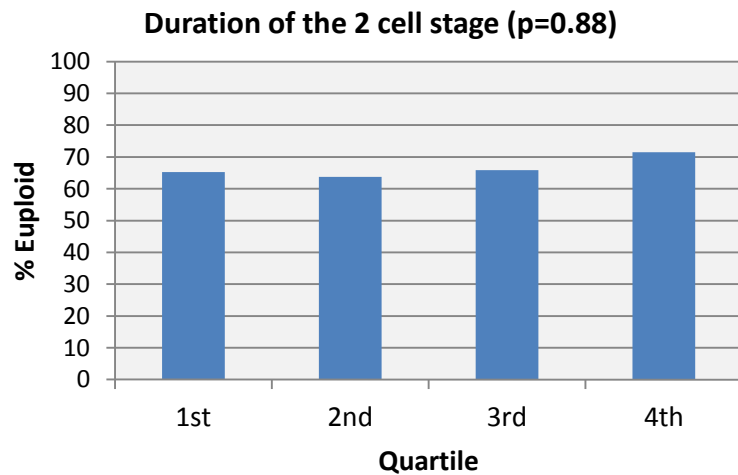
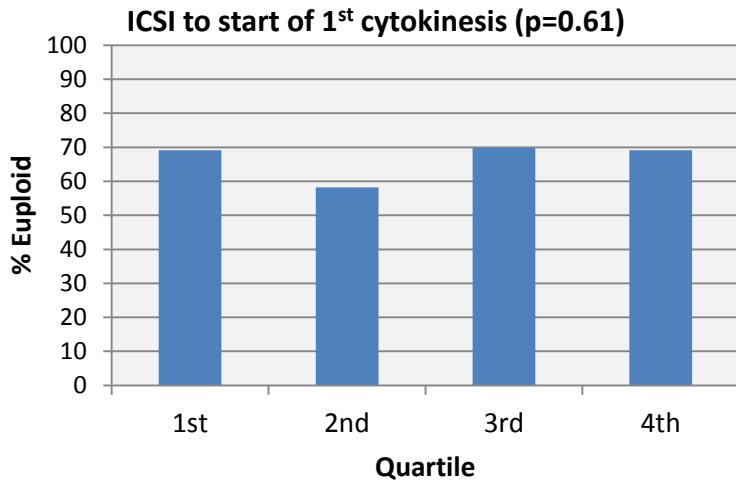
# Time Lapse Observations in the Embryology Laboratory

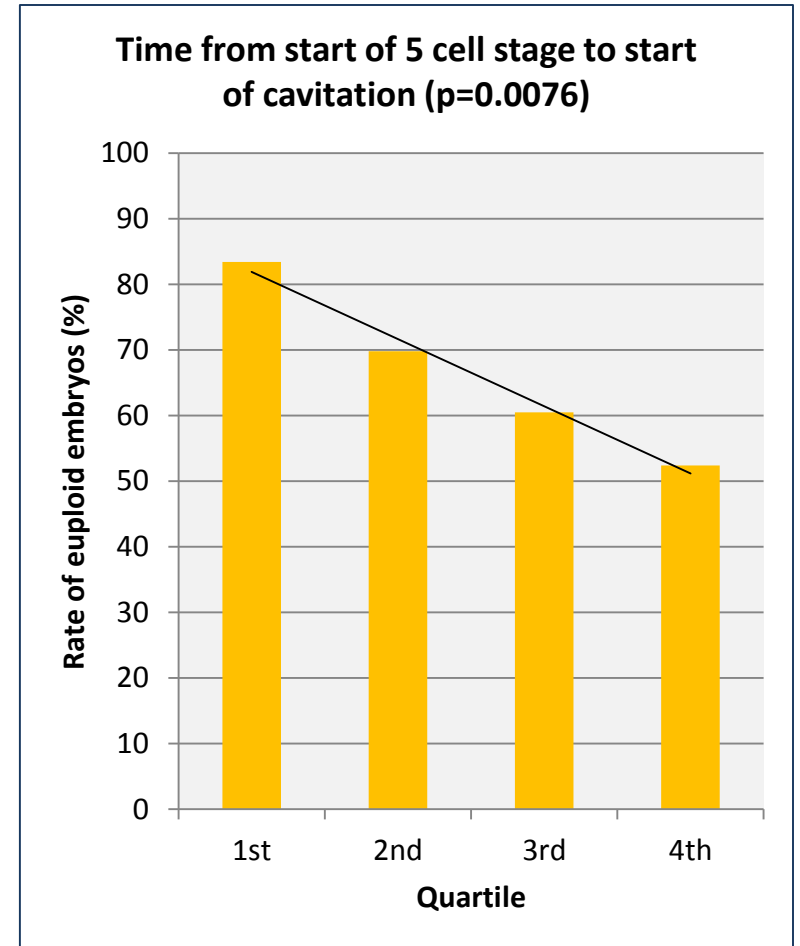
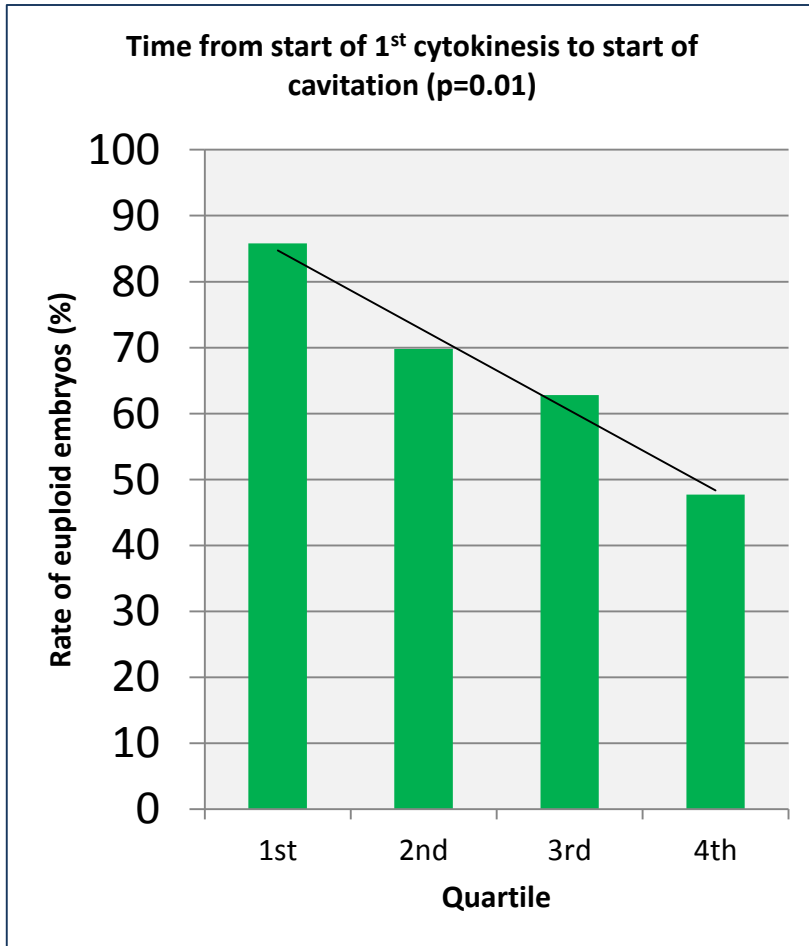
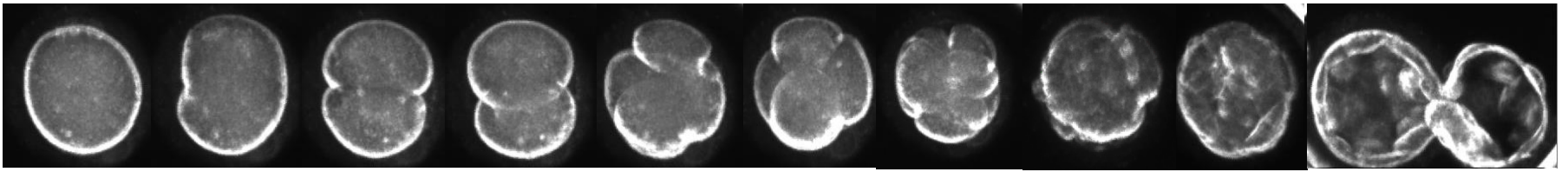


And others.....



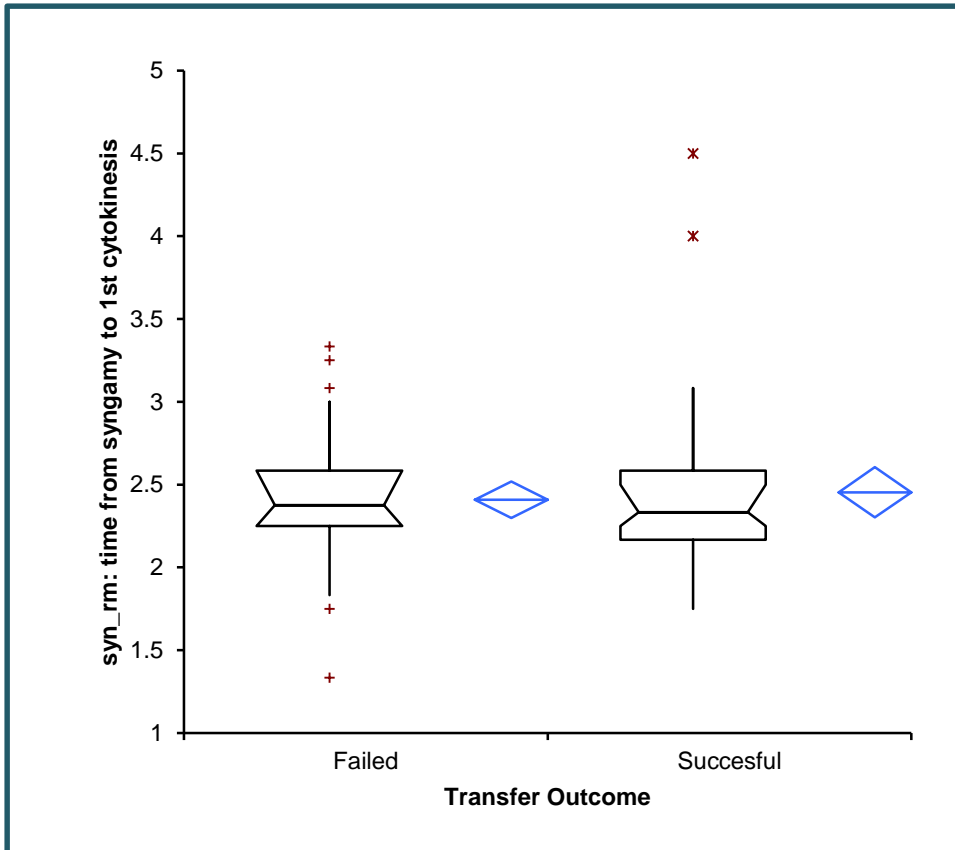
# Time Lapse and Aneuploidy Traditional Markers







# Can Time Lapse Help Distinguish Which Euploid Blasts will Deliver from those Destined to Fail?



Temporal data evaluated:

- 5 conventional endpoints through cleavage stage
- Additional temporal endpoints from extended culture:
  - First compaction
  - Morula formation
  - First cavitation
  - Blastocyst Expansion
  - First contraction

**NO:** None of the 5 traditional parameters or 5 additional blast related parameters prognosticate outcome



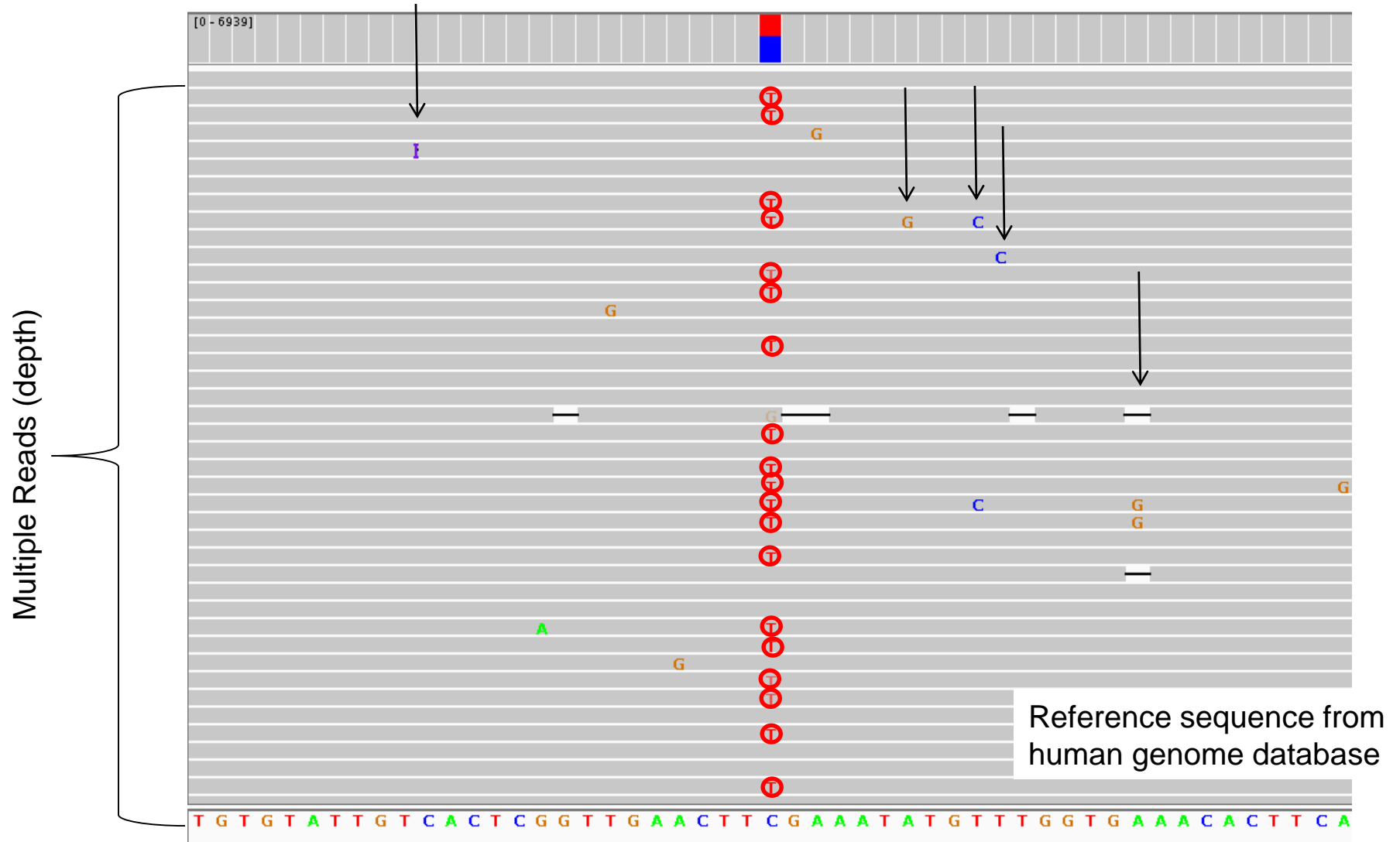


ne<sup>x</sup>gen

NEXT GENERATION SEQUENCING  
FOR EMBRYO SELECTION

# Next Generation Sequencing

## *Aligned Results*



# *The Economics of NextGen*

## *A Major Factor for Accuracy*



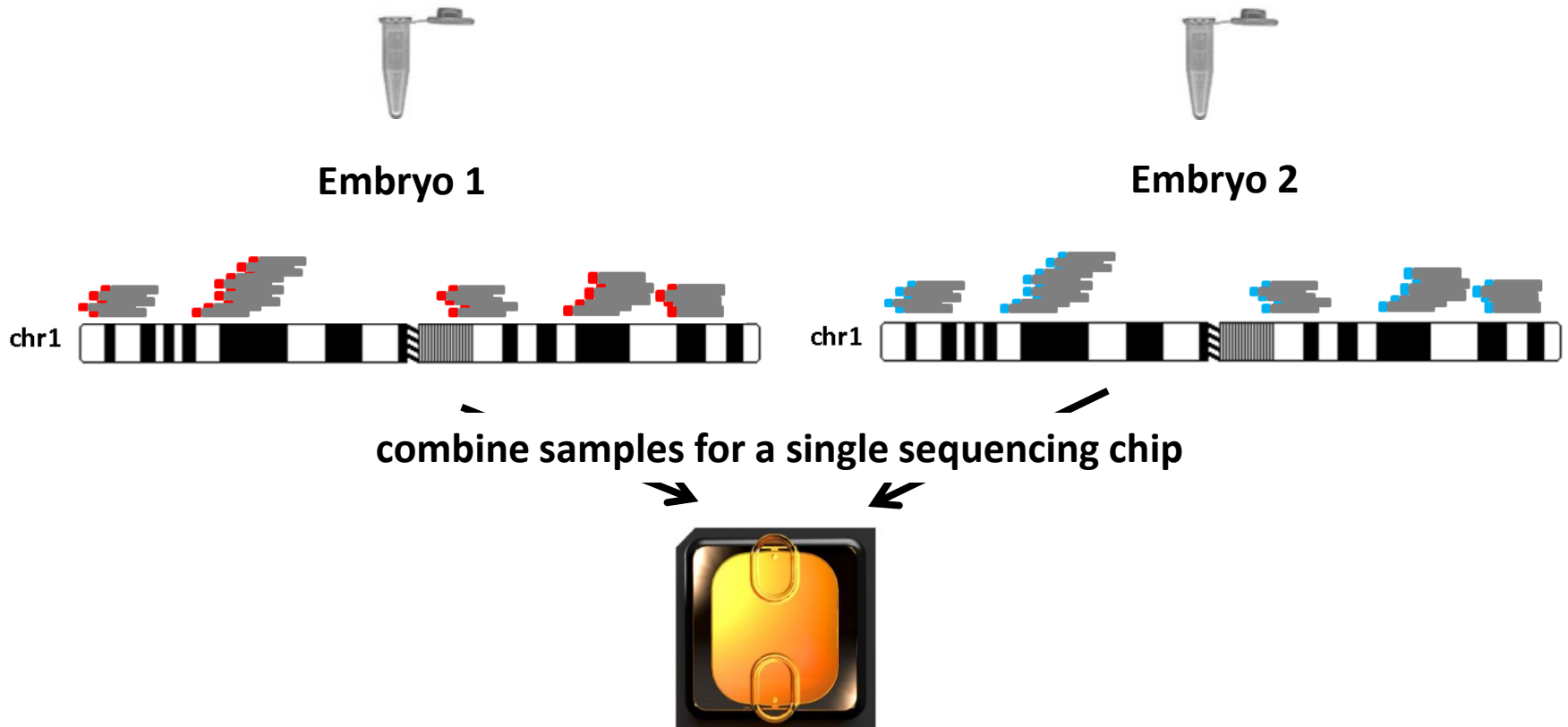
NextGen Sequencing Chip





\$\$\$\$\$\$

# NextGen Molecular Barcoding

→ Reduced Costs



Barcode 1	CTAAGGTAAC	
Barcode 2	TAAGGAGAAC	

# *The Economics of NextGen*

## *A Major Factor for Accuracy*



NextGen Sequencing Chip



\$\$\$\$\$\$/2

# *The Economics of NextGen*

## *A Major Factor for Accuracy*



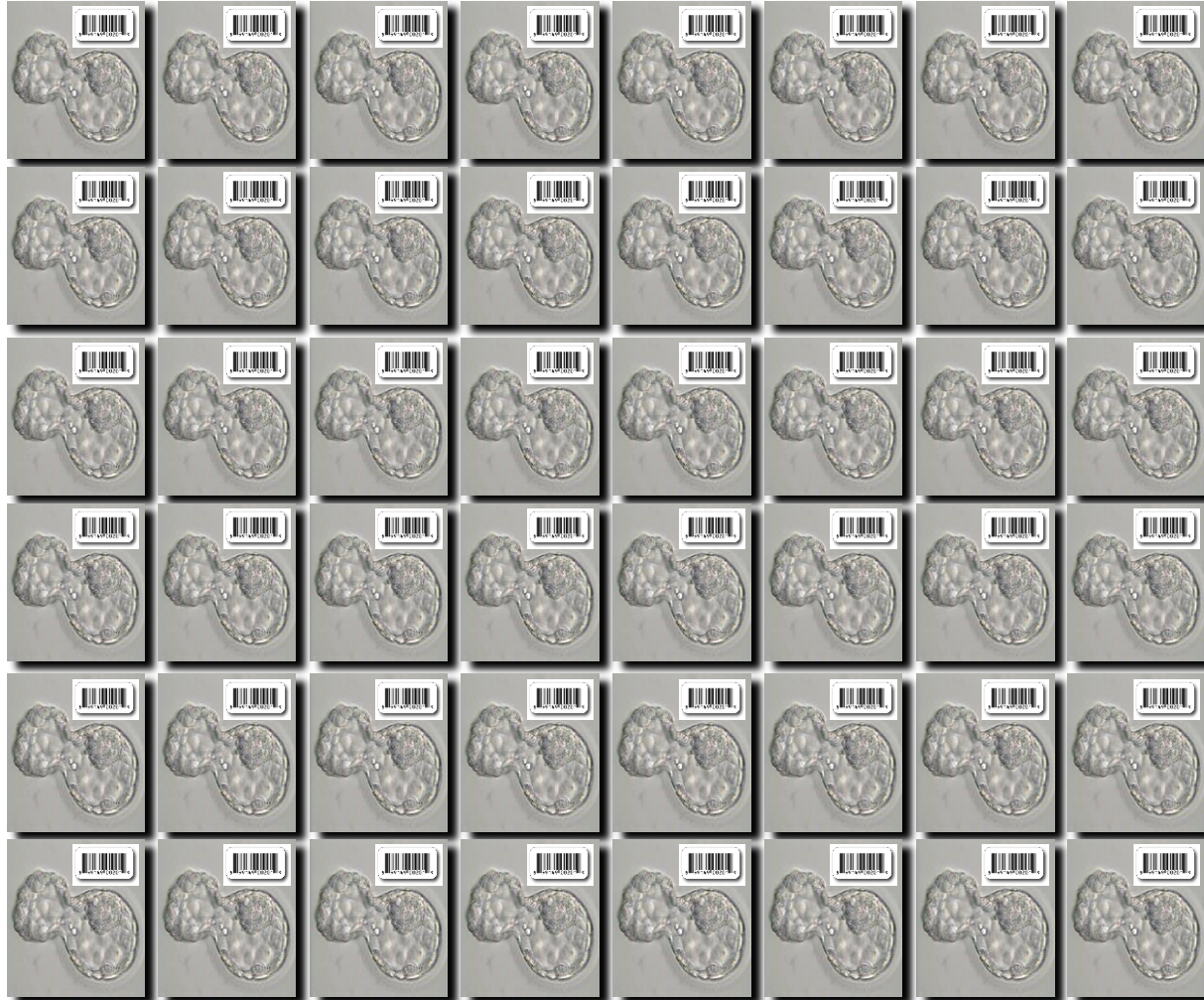
NextGen Sequencing Chip



\$\$\$\$\$\$/4

# *The Economics of NextGen*

## *A Major Factor for Accuracy*



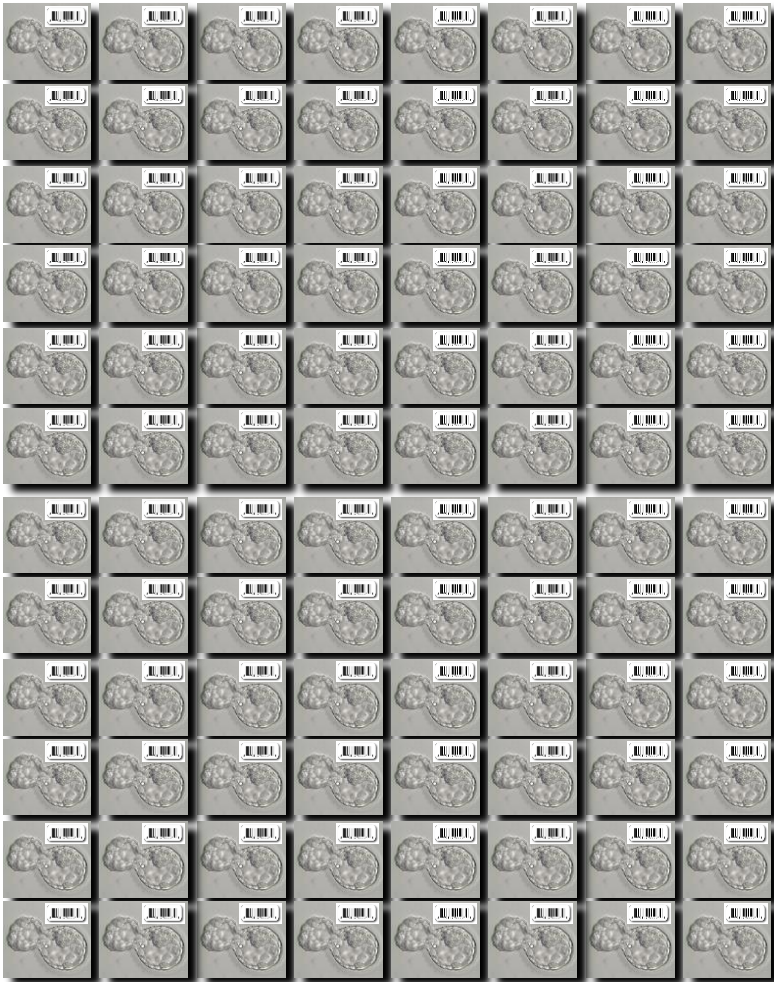
NextGen Sequencing Chip



\$\$\$\$\$\$/48

# *The Economics of NextGen*

## *A Major Factor for Accuracy*



*96 or more...*

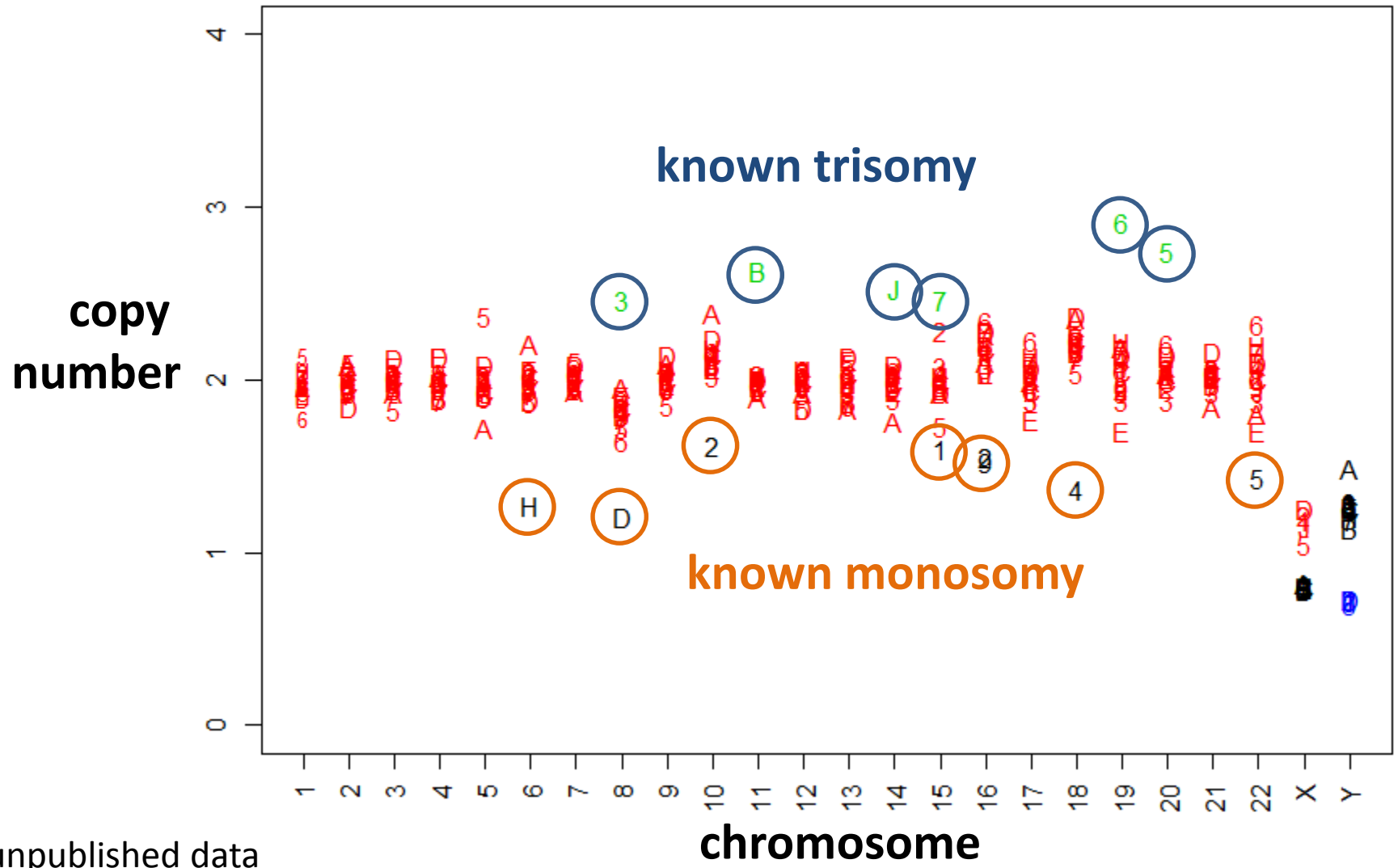
NextGen Sequencing Chip



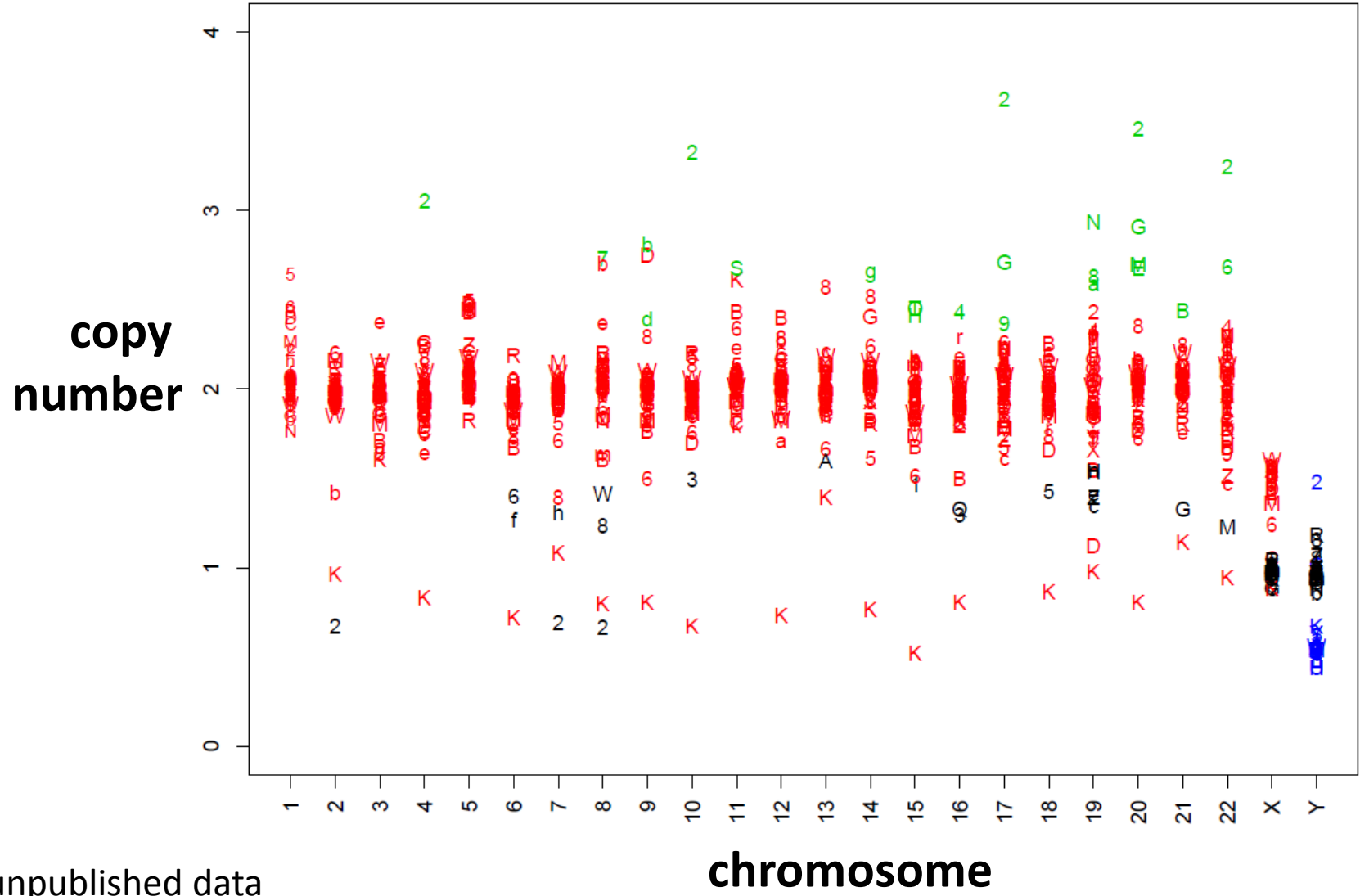
\$\$\$\$\$\$/96



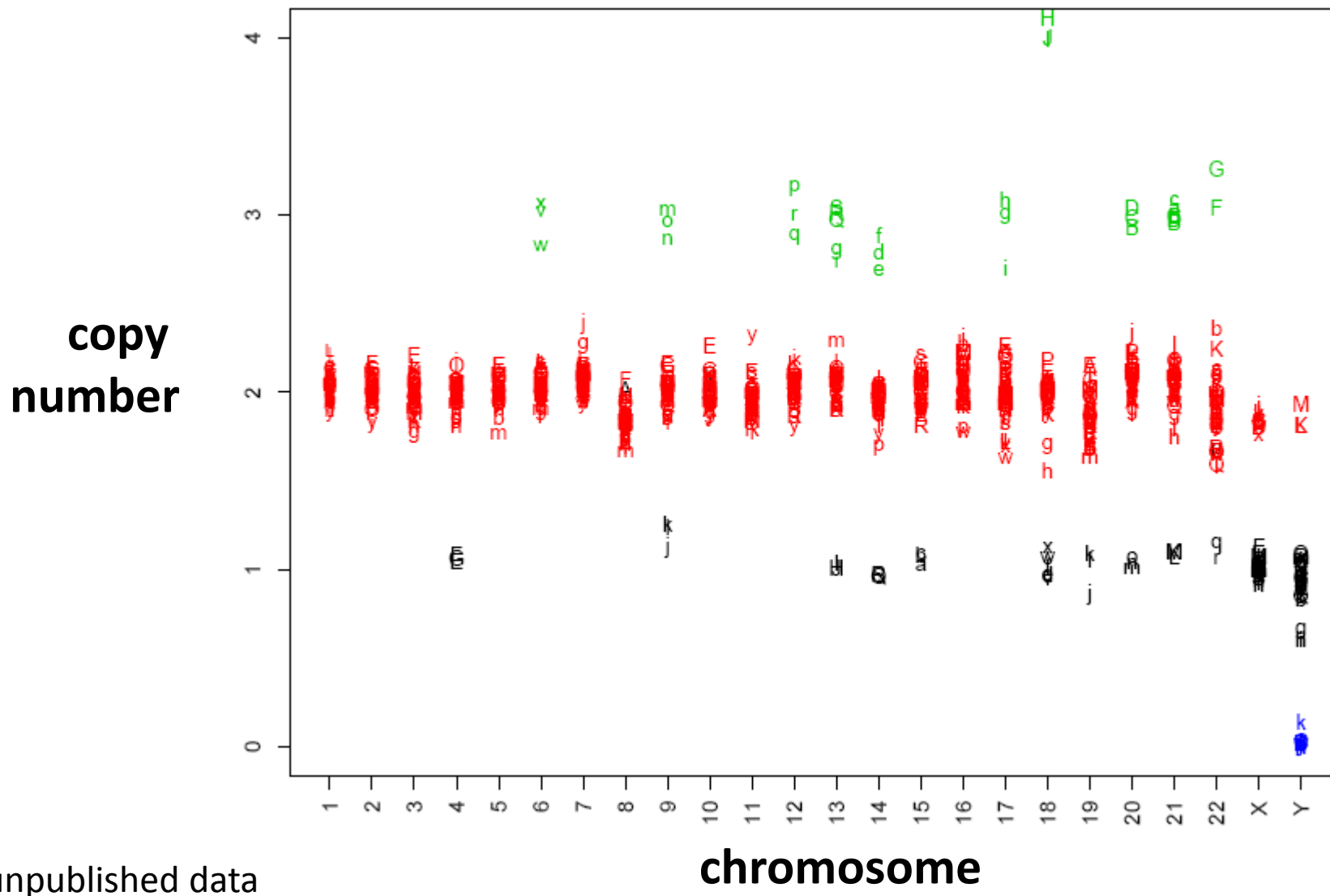
# WGS (16 per chip)



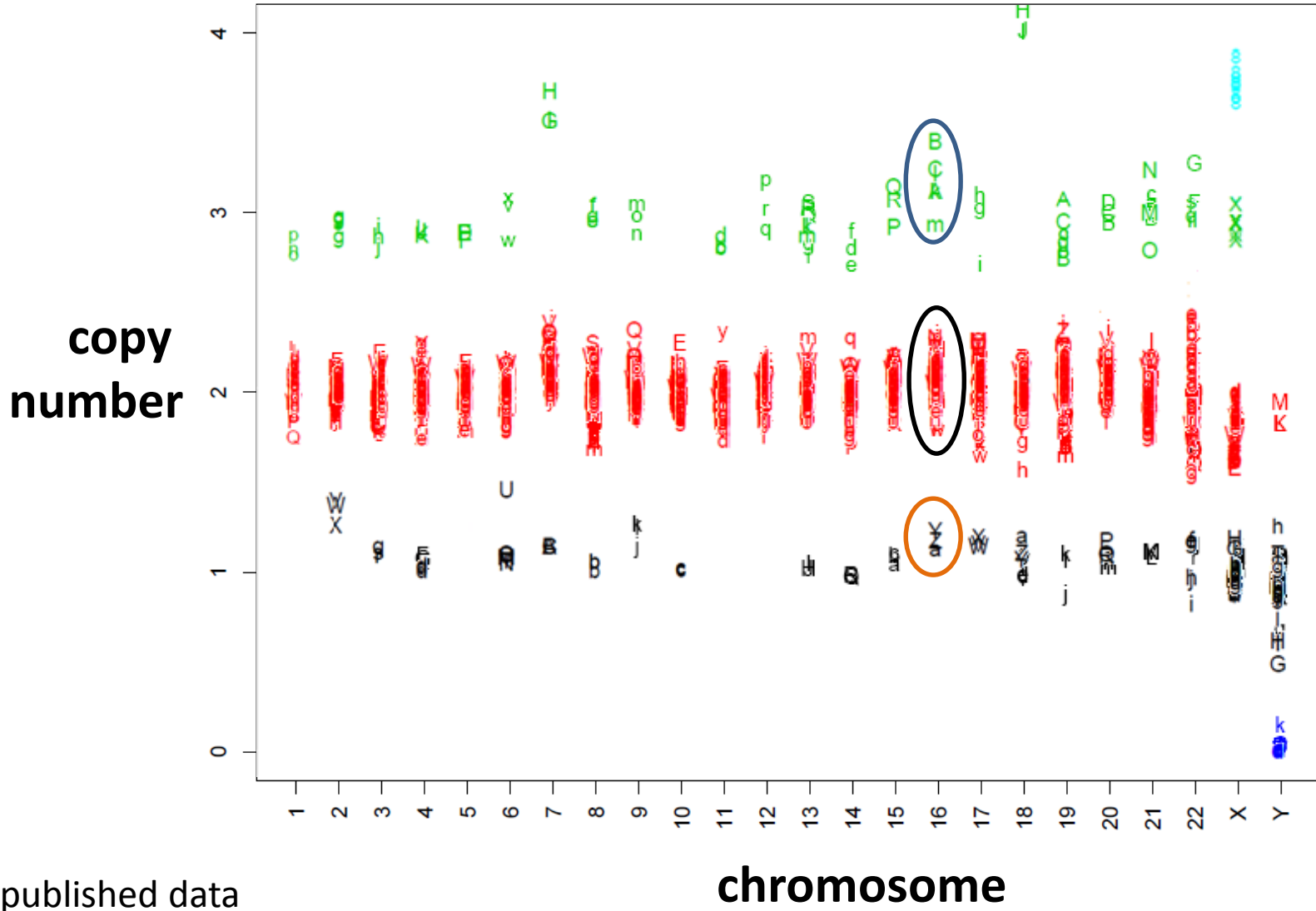
# WGS (48 per chip)



# Targeted NGS (96 per chip)



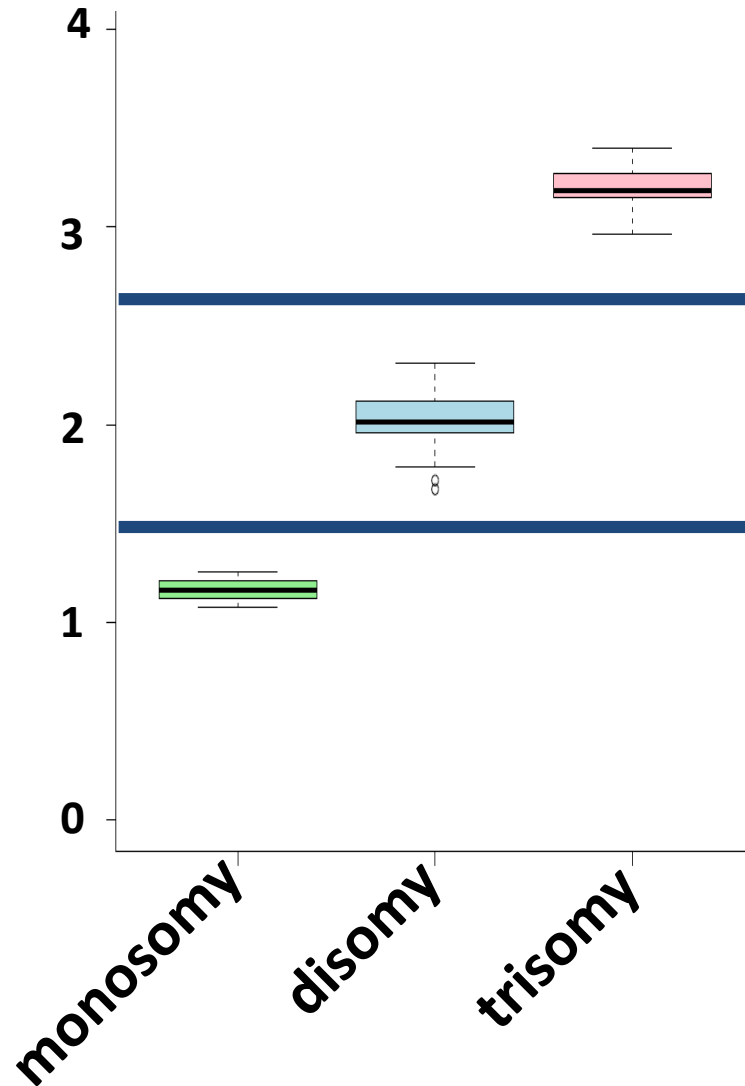
# Embryo calibration results



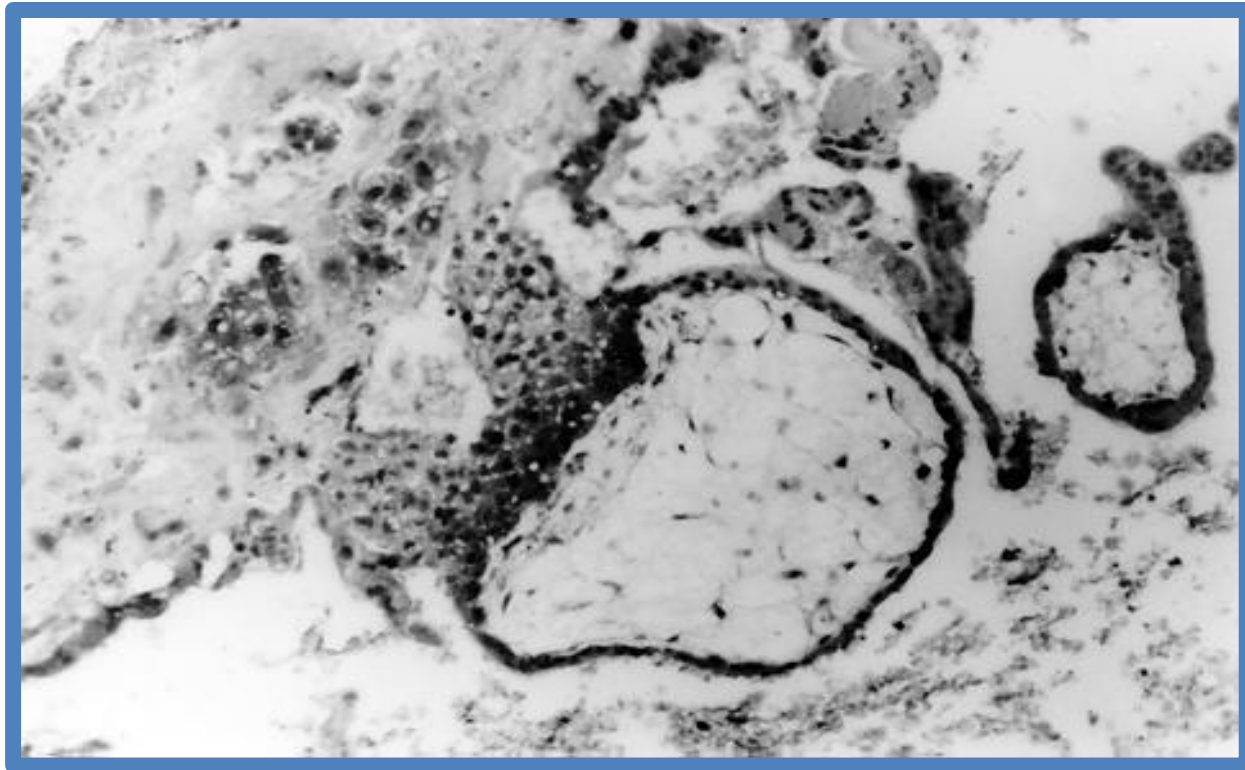
# Chromosome specific cutoffs

NGS based  
copy number  
on chr16

chr16 specific  
cutoffs

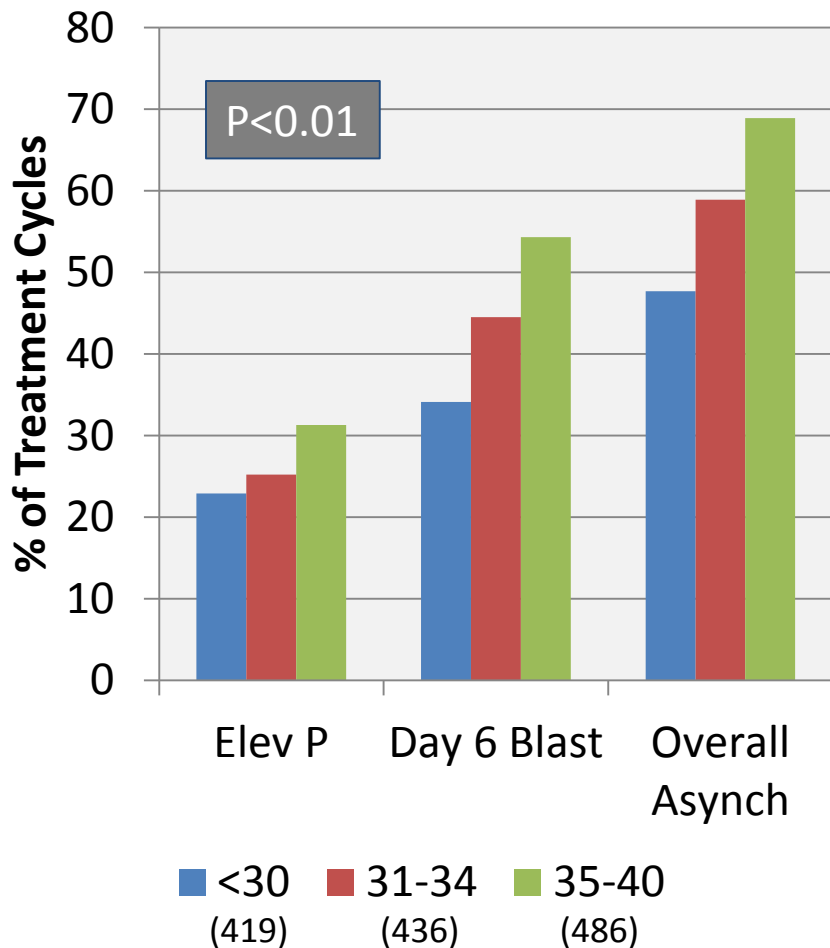


# Embryonic Endometrial Synchrony



*It take two.....*

# Embryonic-Endometrial Asynchrony Increases with Maternal Age

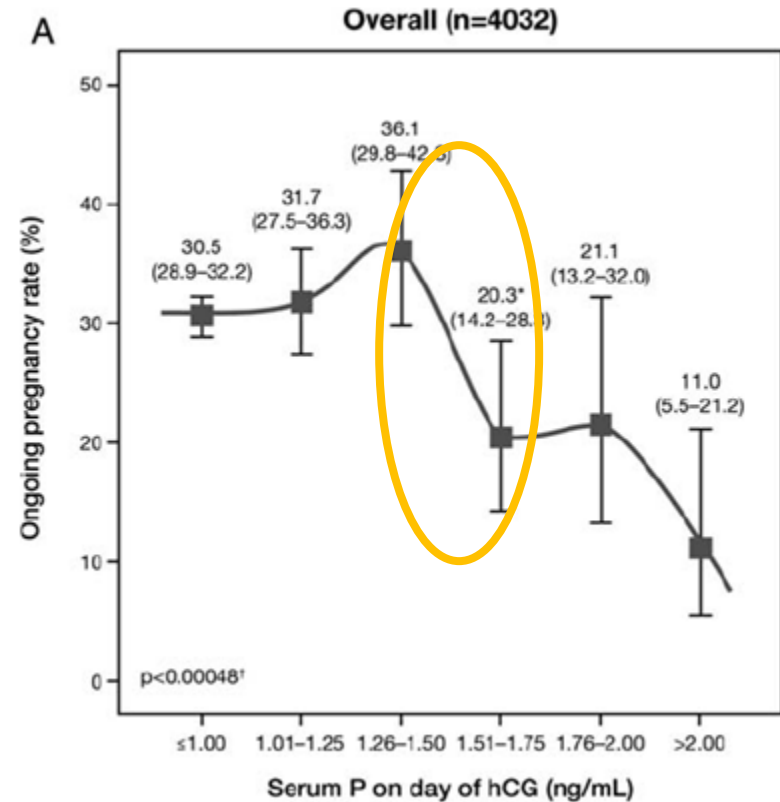


- Retrospective
- 1,341 IVF cycles
- Thresholds for Asynchrony (either)
  - $P > 1.5$  mg/mL on day of hCG
  - No blastulation prior to day 6
- Risk for asynchrony increases with maternal age
- Live birth predicted
  - Day 5 blastulation ( $P < 0.0001$ )
  - $P < 1.5$  ng/mL ( $P = 0.0002$ )

Is it asynchrony or an  
intrinsic diminution in quality?

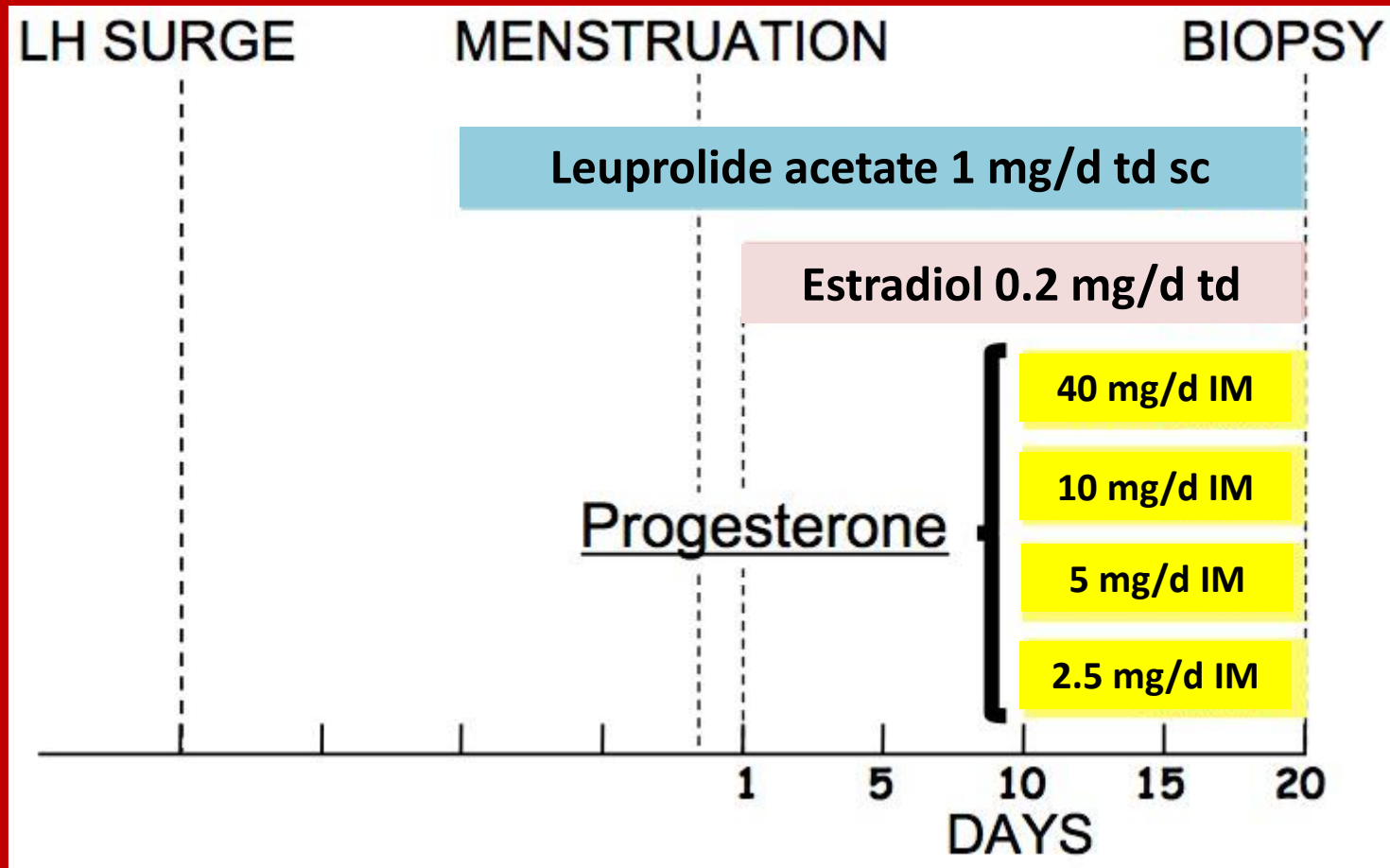
# Late follicular rise in progesterone

- Retrospective study
- 4032 patients
- $P_4 \geq 1.5 \text{ ng/mL}$  associated with lower ongoing pregnancy rates

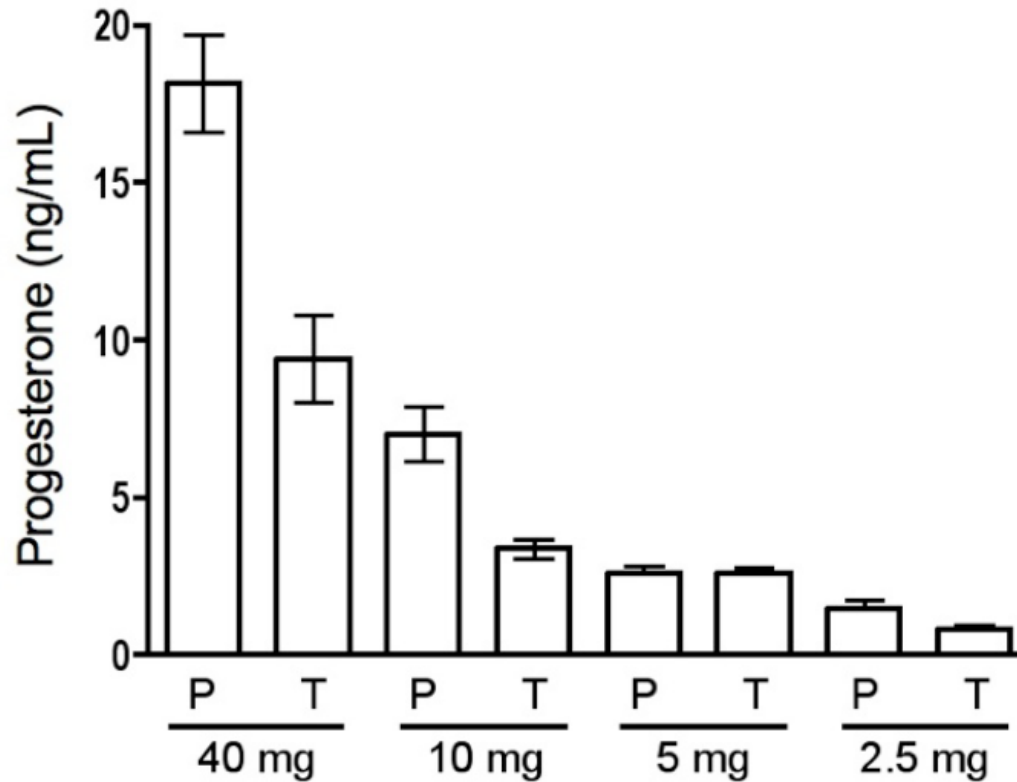




# Progesterone and the Endometrial Transcriptome



# Progesterone Pharmacokinetics



*Usadi RS, et al. JCEM 2008 & Young Lab, Unpublished*

# Progesterone and the Endometrial Transcriptome

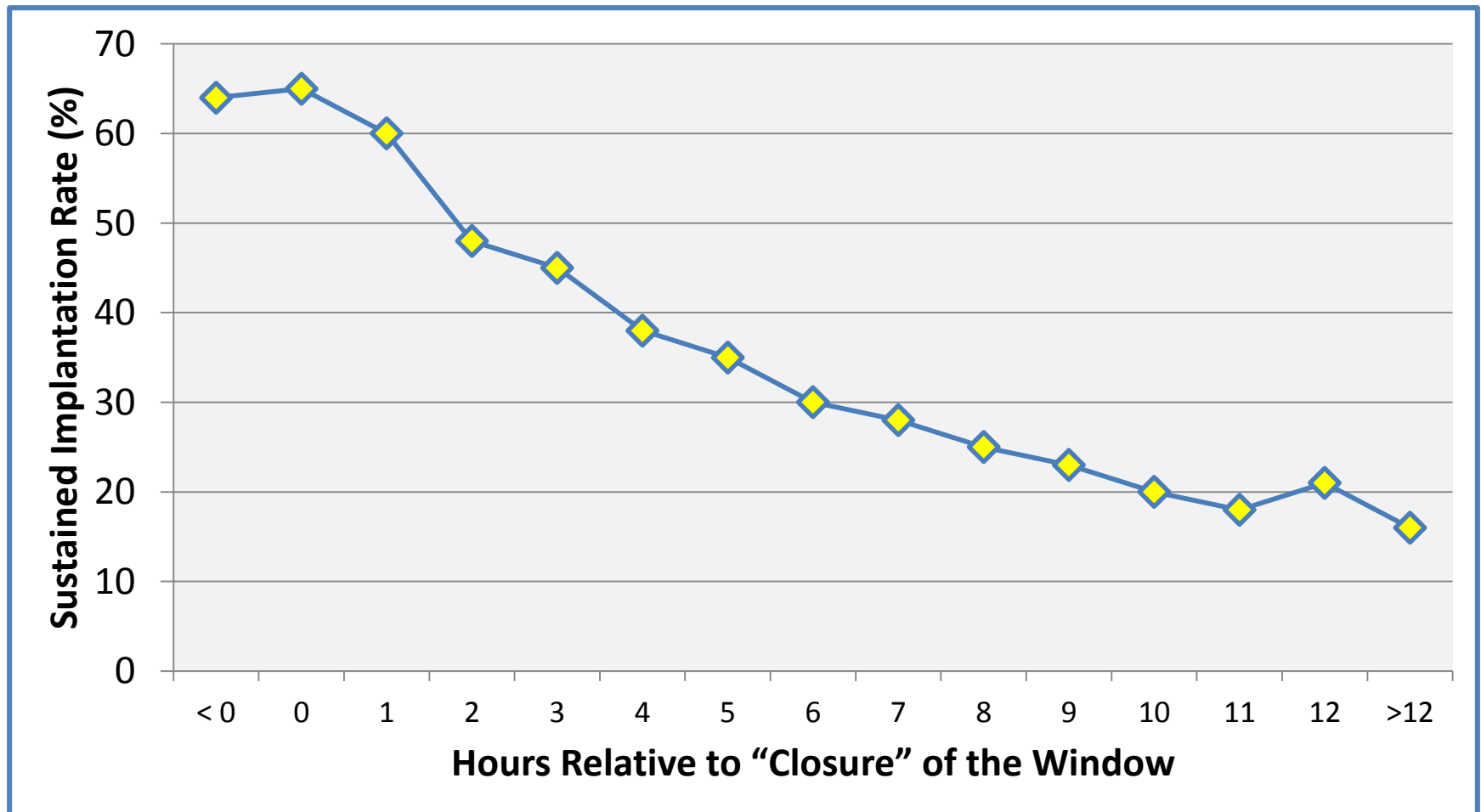
Number of genes differentially expressed vs. 40 mg P

	Natural Cycle	10 mg P	5 mg P	2.5 mg P
$\geq 2$ -fold change	0	0	70	236
$\geq 1.5$ -fold change	0	0	605	1186

*Young Lab, Unpublished*

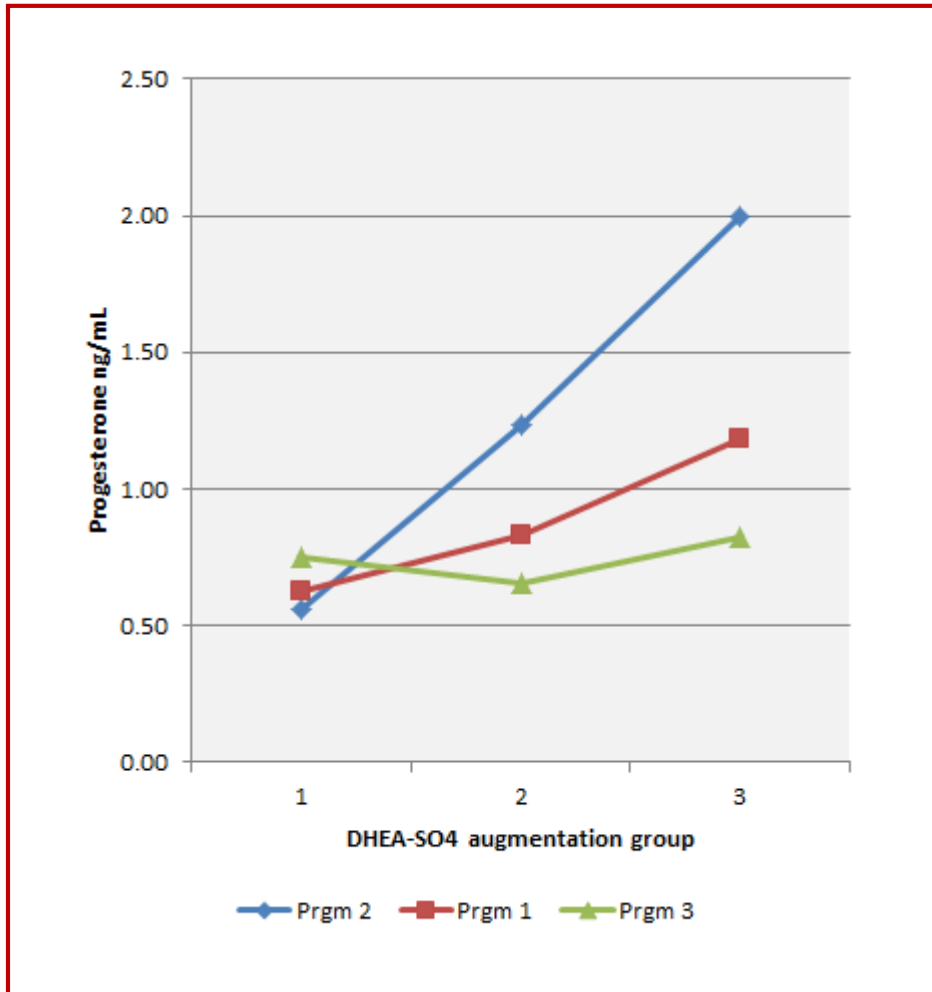
*Adapted from S. Young, MD, PhD*

# Progesterone and Impaired Implantation: A Pilot Study of Euploid Embryos



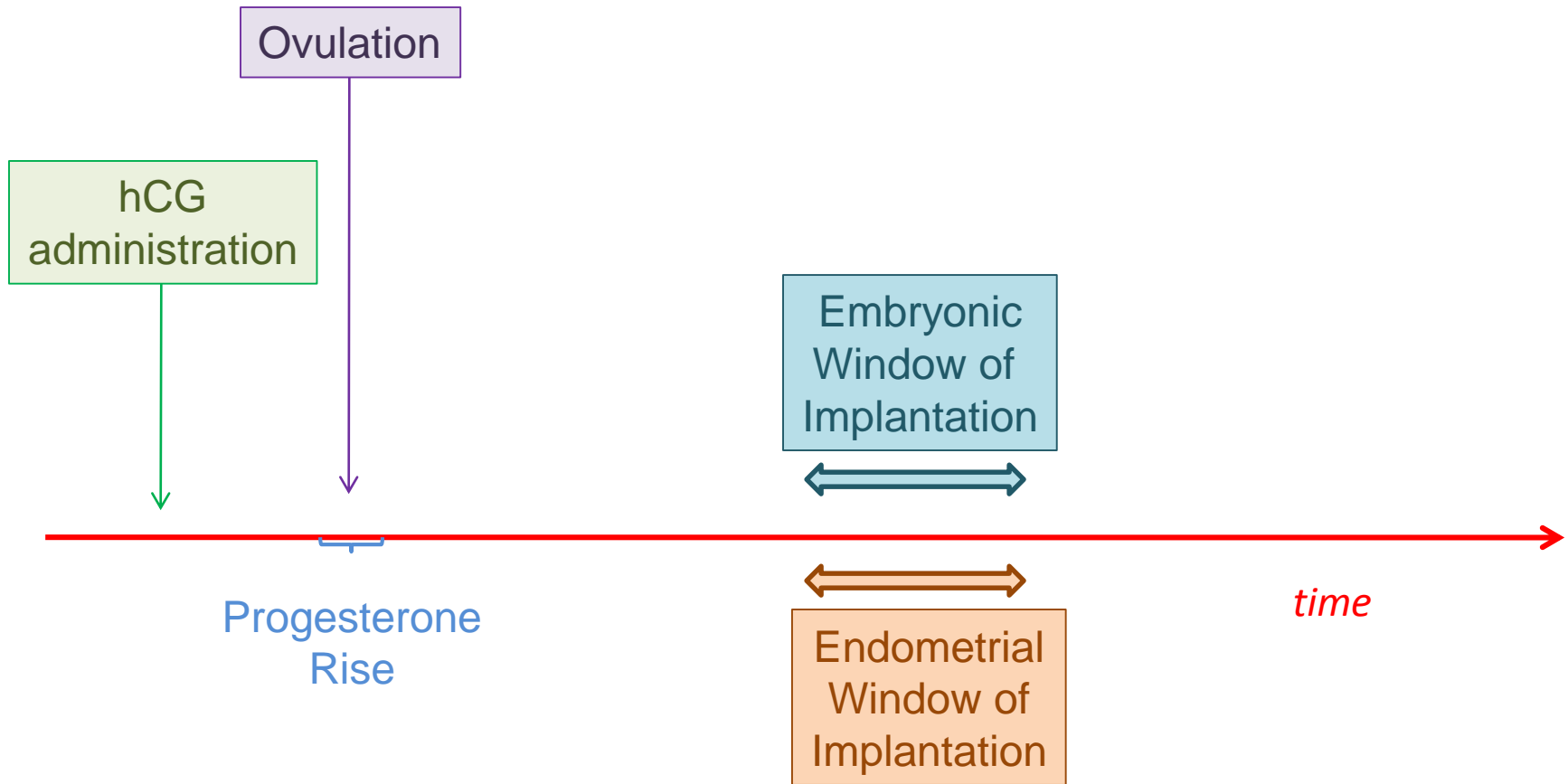
*All patients had normal P levels prior to the administration of hCG*

# Beware of Interference in your P Assay

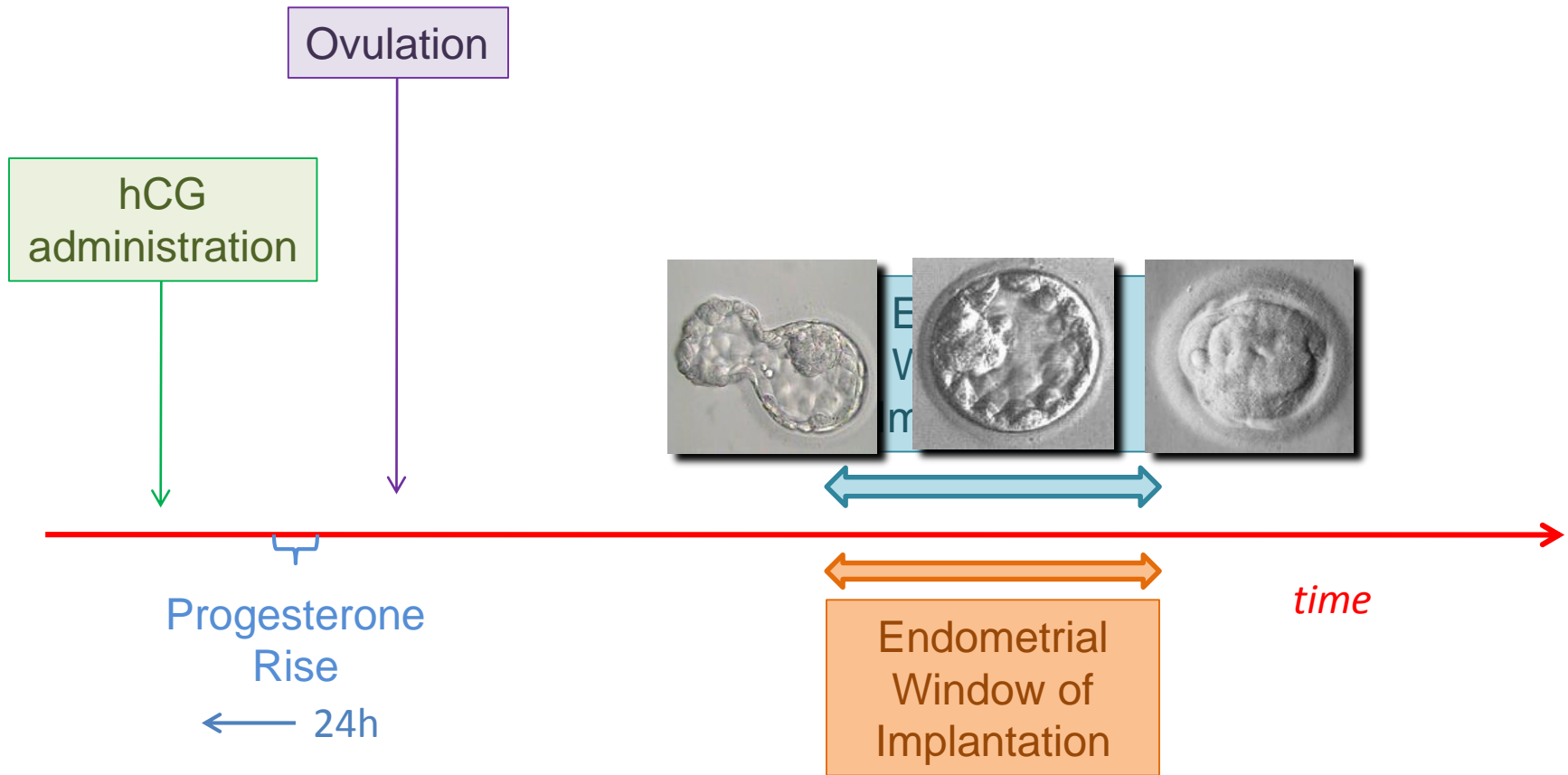


- Patients receiving DHEA have elevated DHEA-SO<sub>4</sub> levels
- These levels may falsely elevate P levels
- Assay dependent

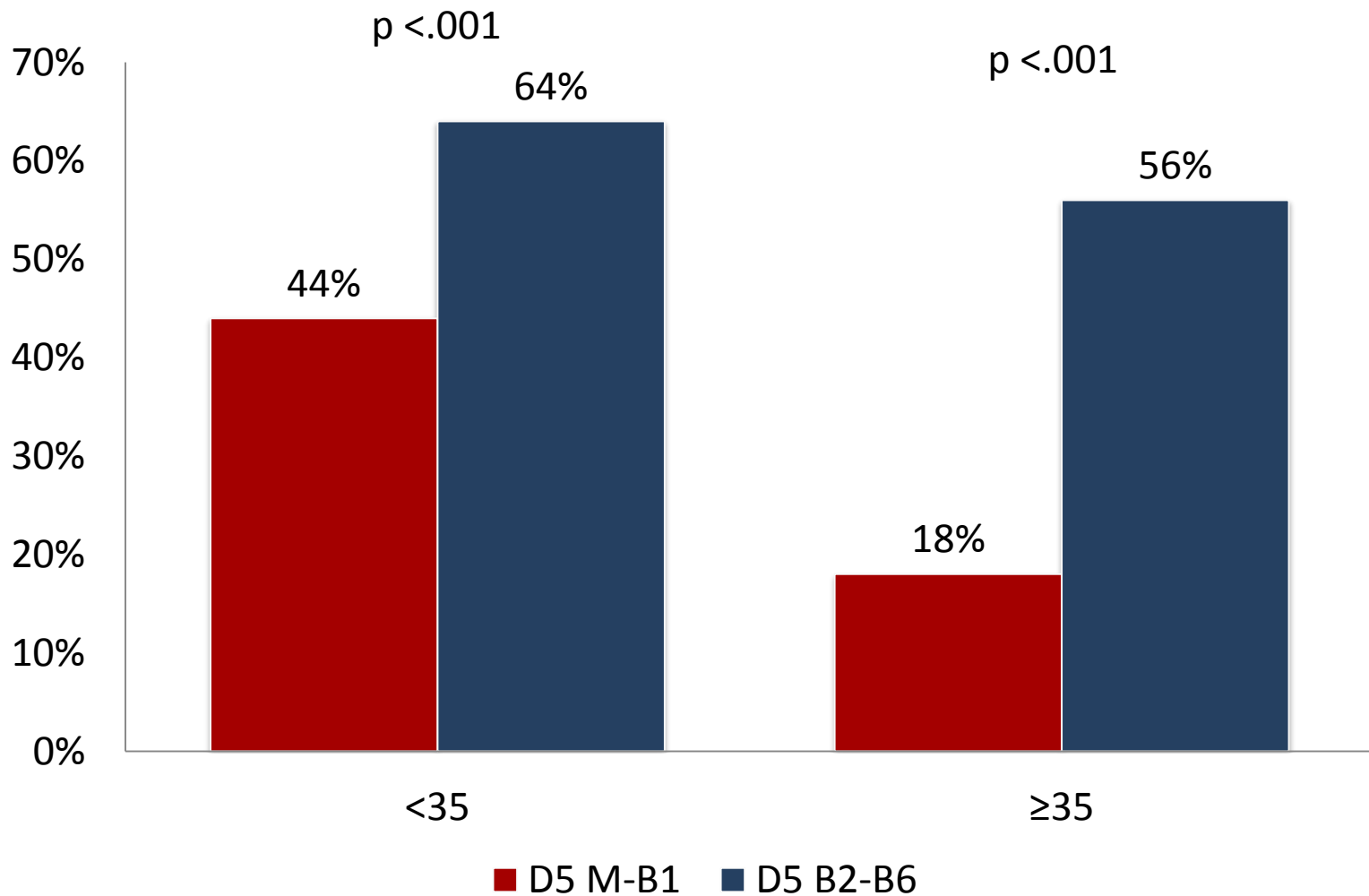
# Natural Cycle



# embryo and endometrium synchrony - *revisited*

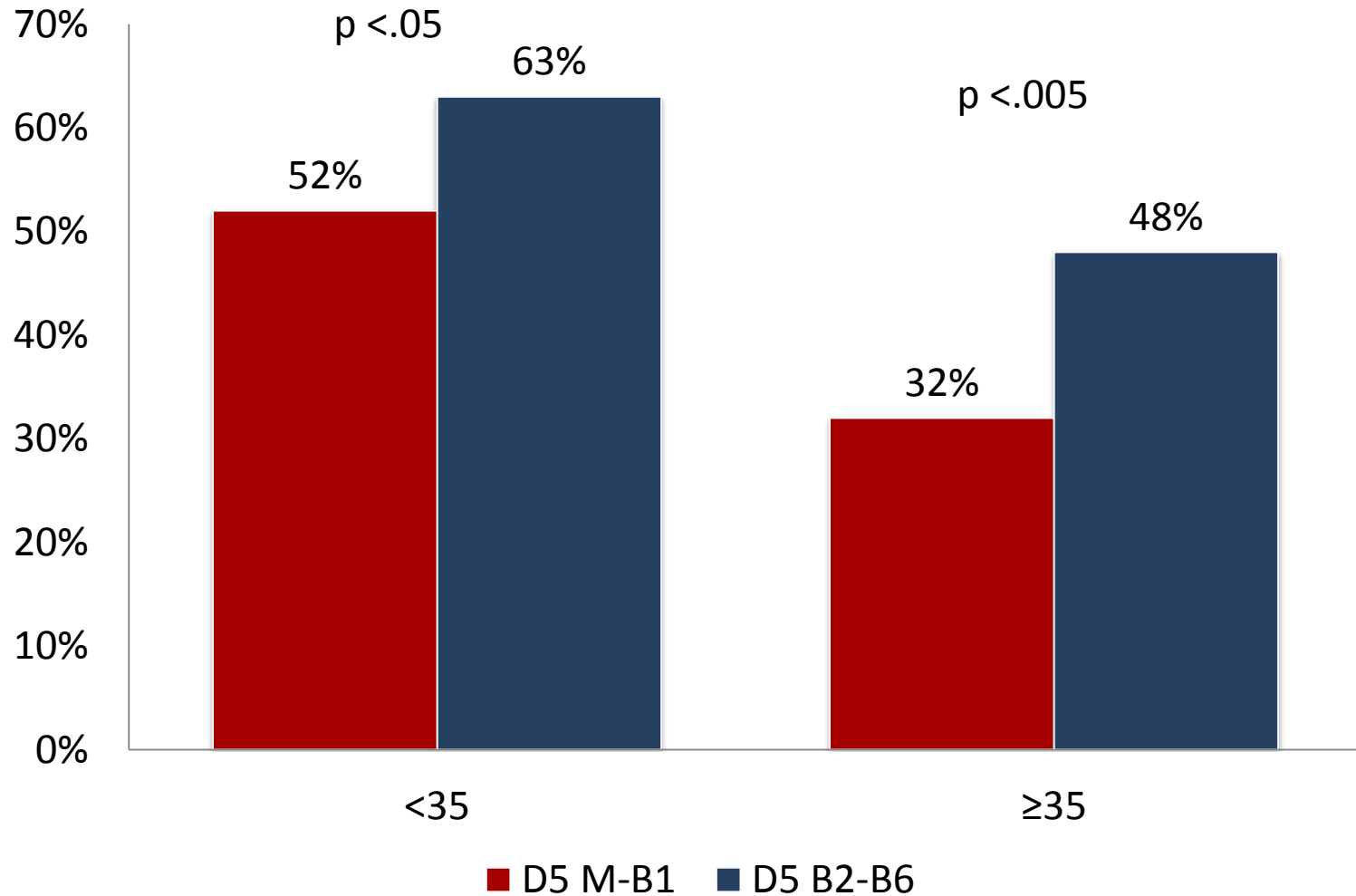


# Fresh day 5 embryo transfer

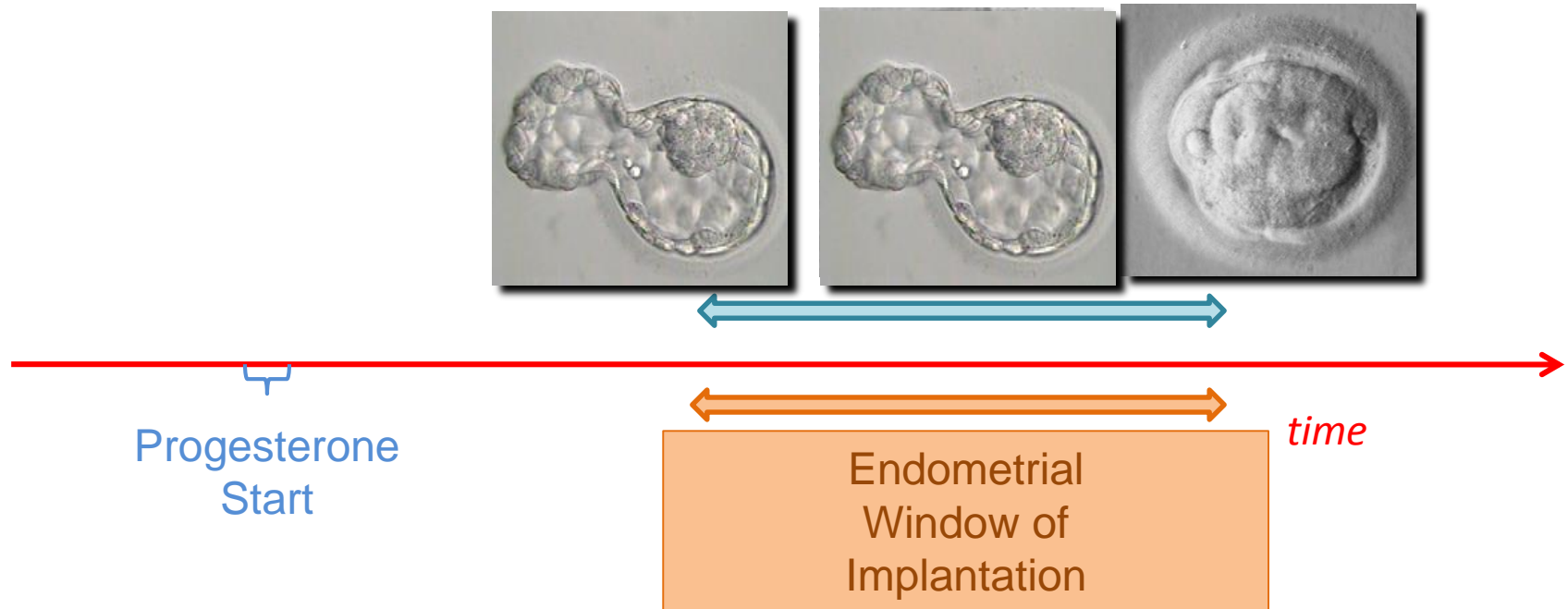




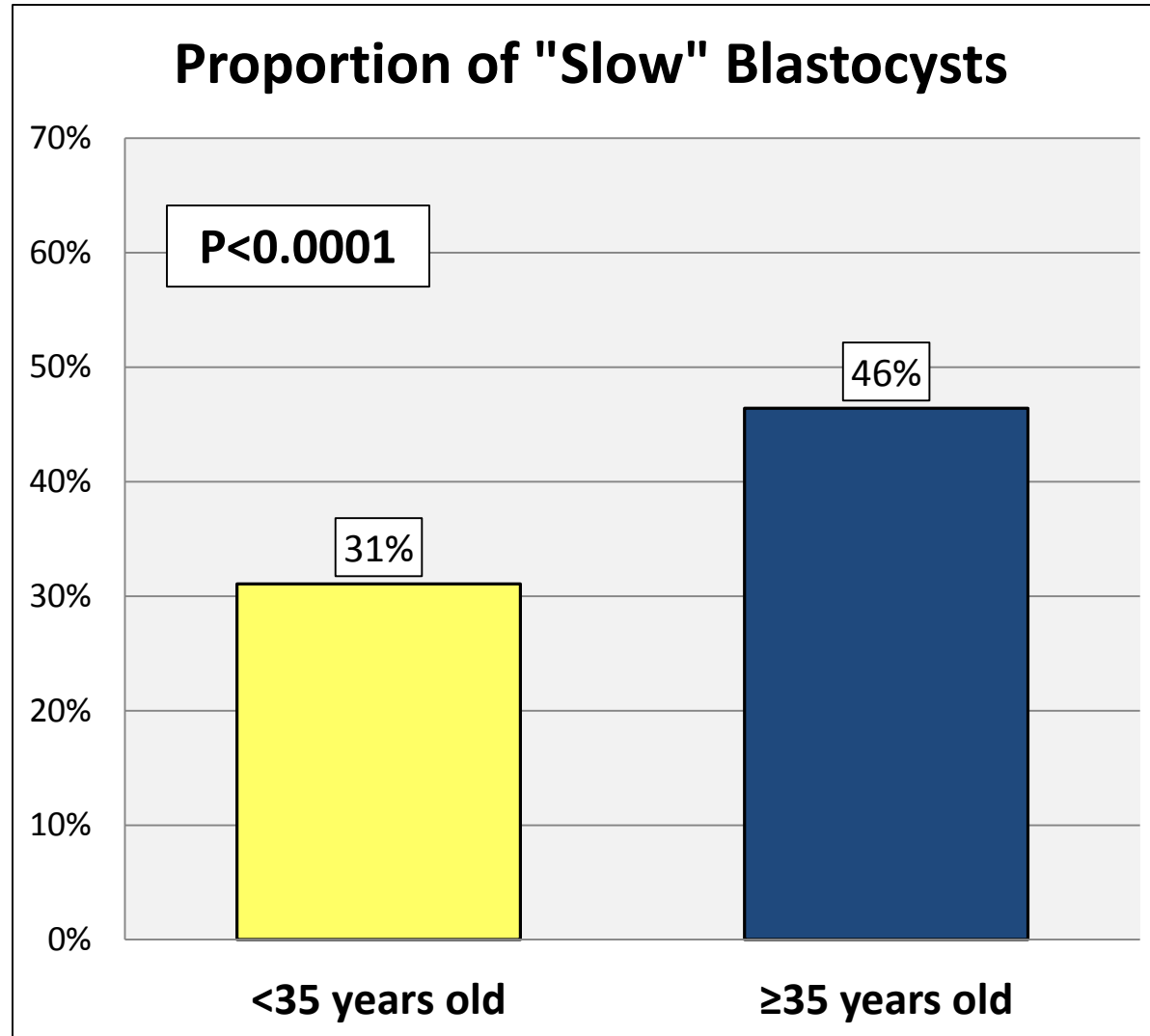
# Fresh day 6 embryo transfer



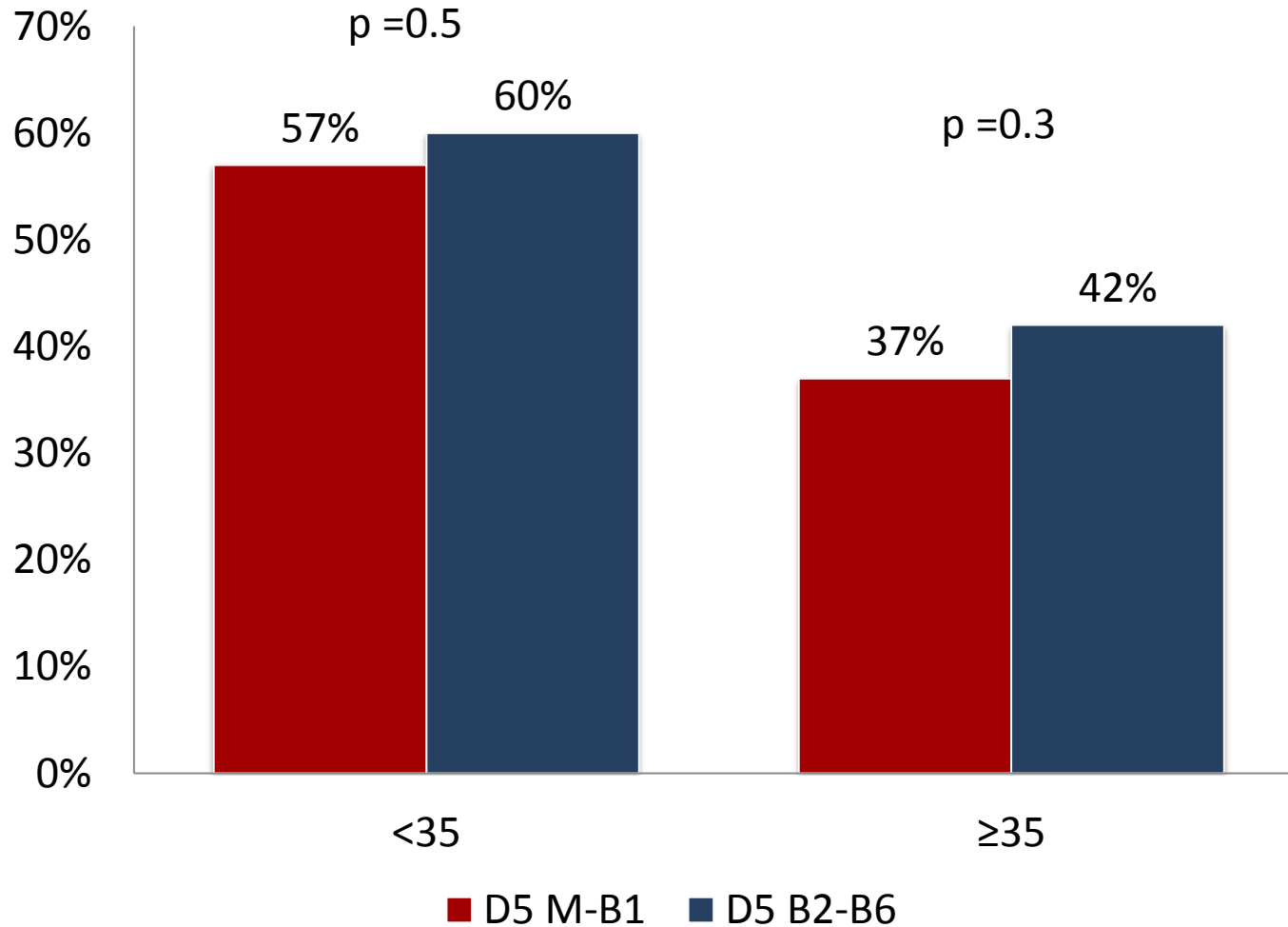
# Frozen synchronous cycle



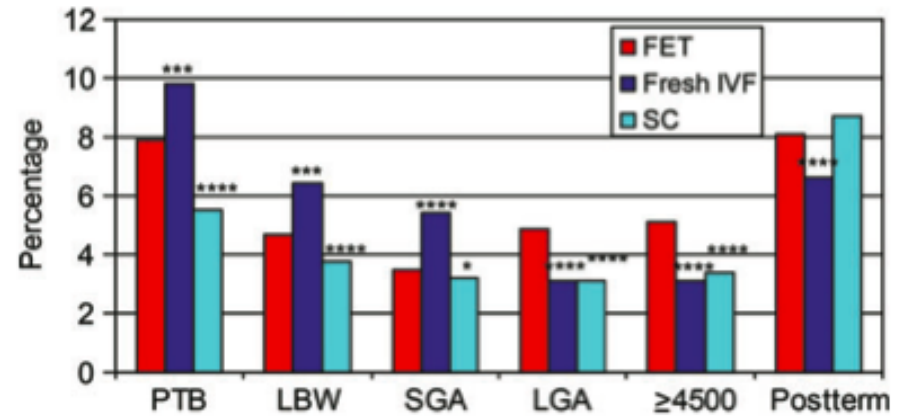
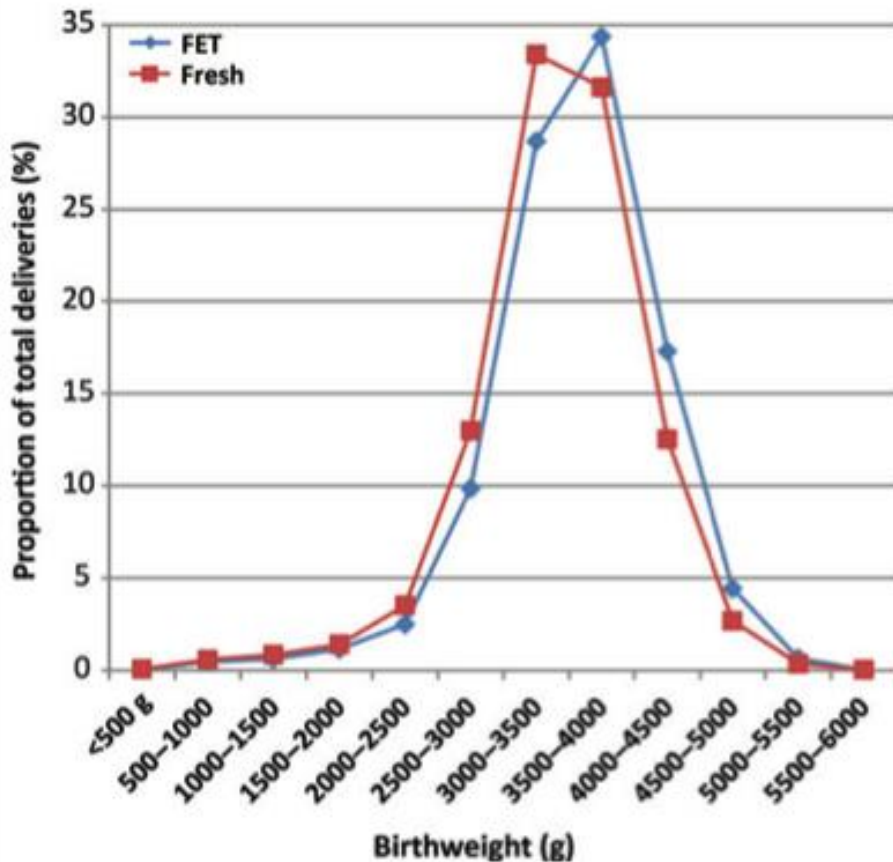
# Older patients are more likely to have "slow" embryos



# Frozen day 6 embryo transfer

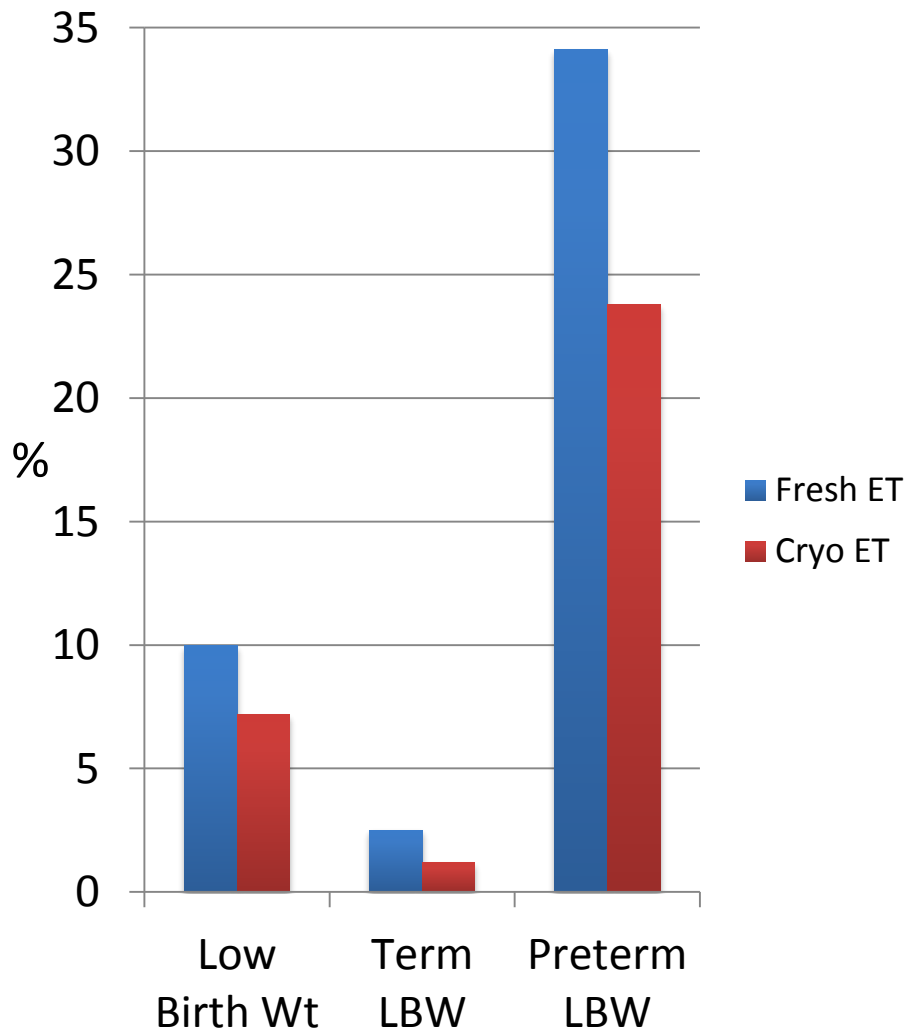


# Obstetrical Outcomes Following Fresh versus Cryopreserved Embryo Transfer



- Fresh embryos at increased risk for
  - Preterm birth
  - Low birth weight
  - Small for gestational age

# The supraphysiologic milieu which accompanies superovulation impact low birth weight risk



- Retrospective review of SART data
- 2004-2006
- 56,792 neonates
- Fresh embryo transfer at increased risk for LBW

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