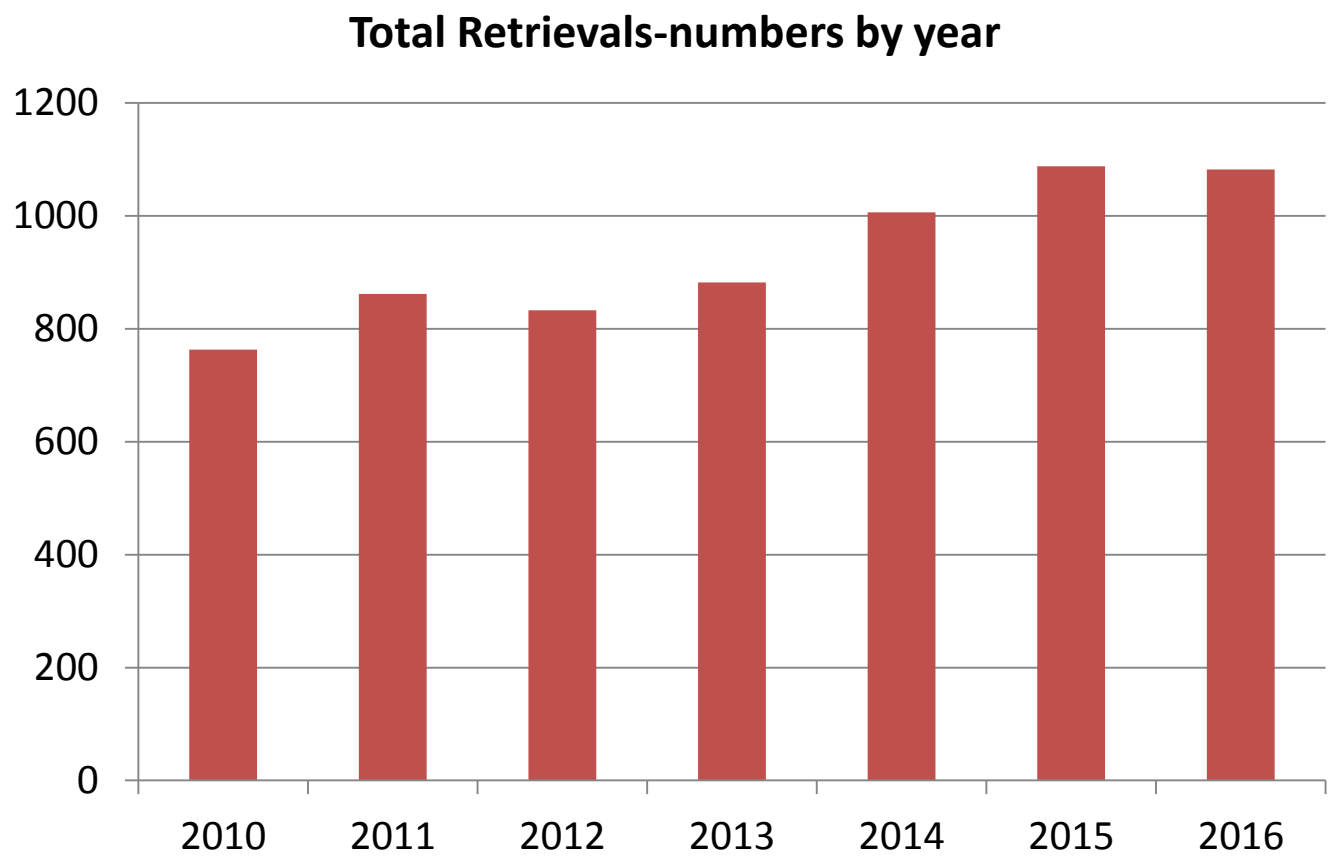
Extended embryo culture in the era of Time-Lapse, PGS and Vitrification

Joe Conaghan, PhD, Pacific Fertility Center, San Francisco

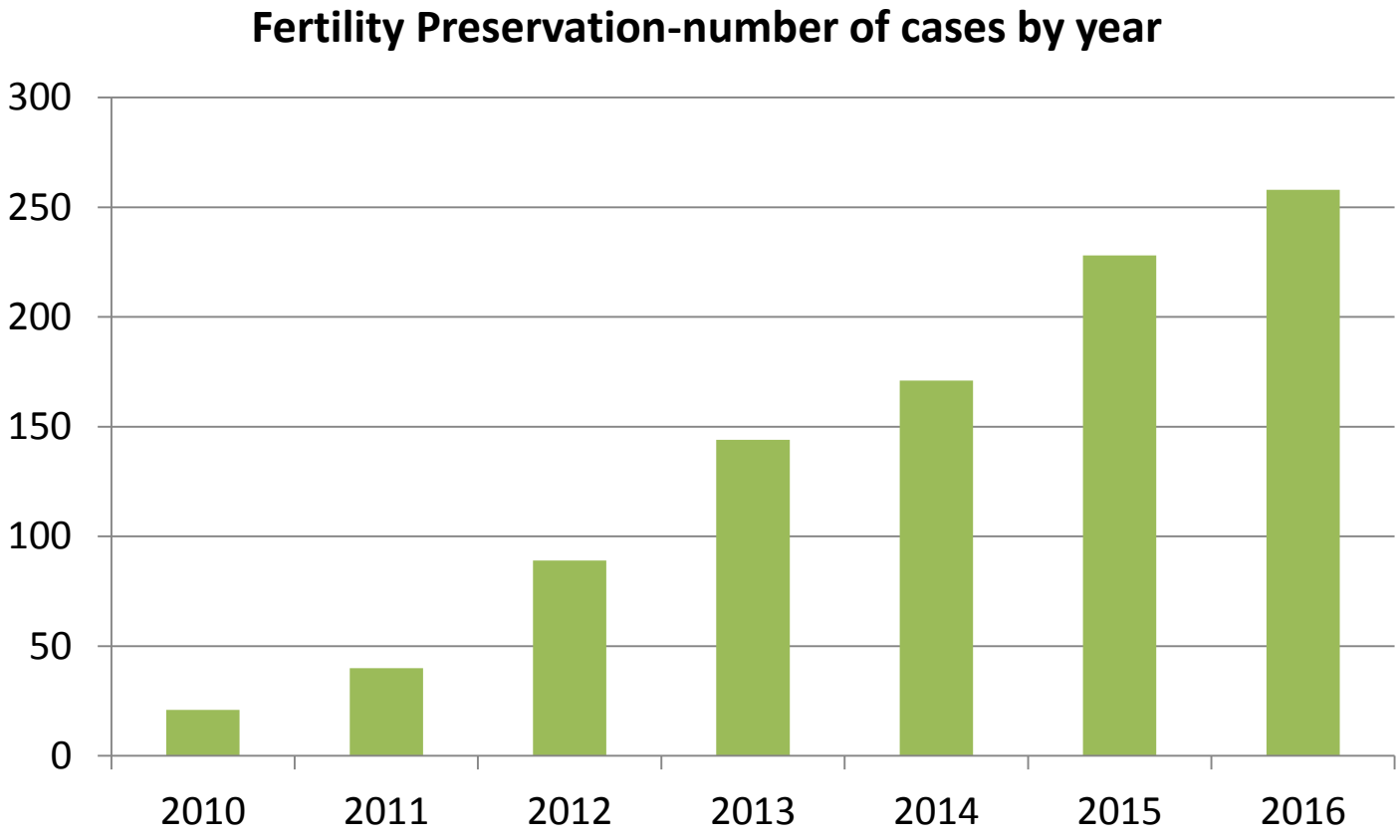
Question: How often are you culturing embryos to D7 for your patients?

a. Never (0%)
b. Occasional patients depending on circumstances
c. Most patients
d. All (100%)

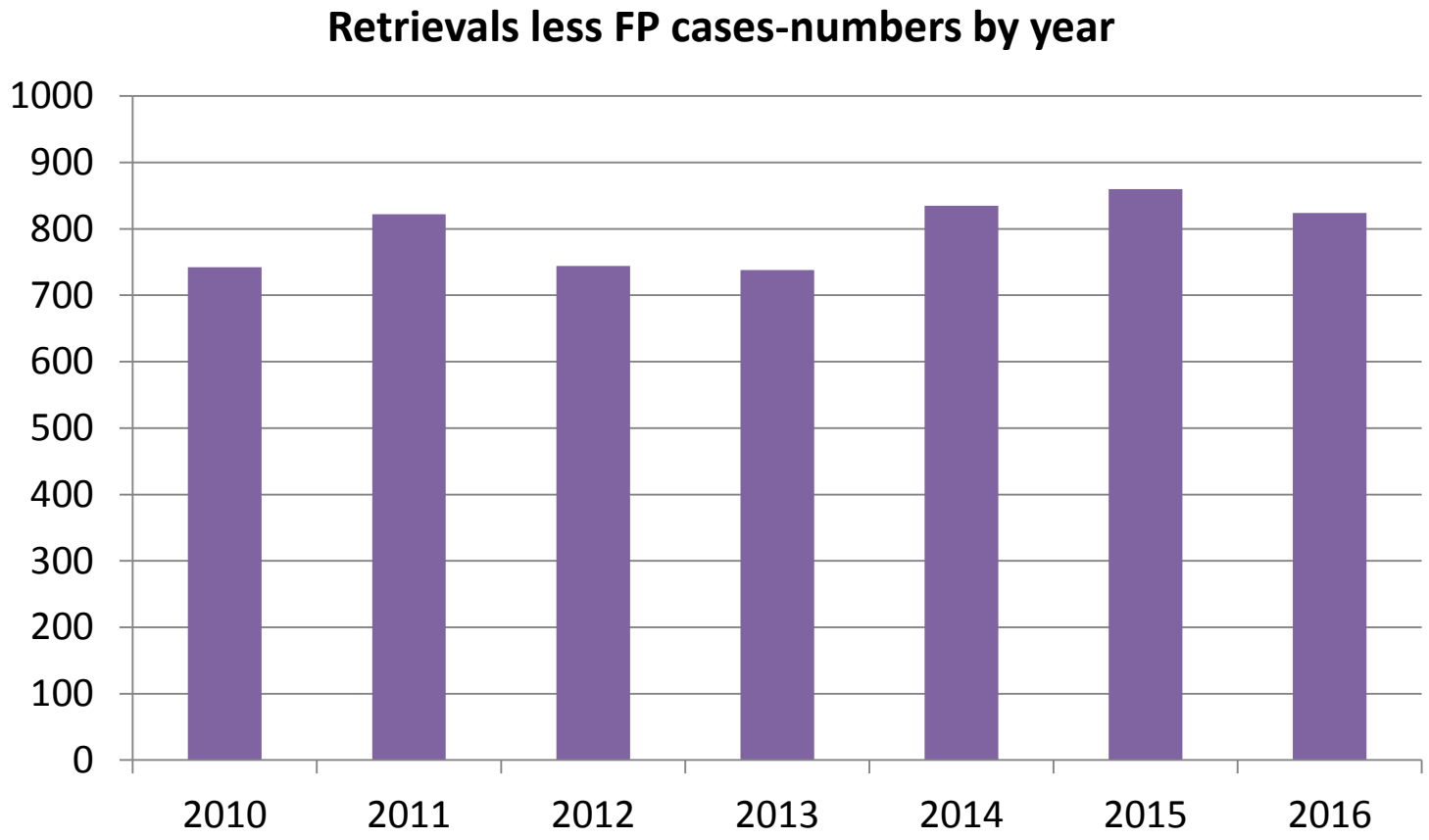
Procedure stats 2010-2016



Procedure stats 2010-2016



Procedure stats 2010-2016



Fertility Preservation

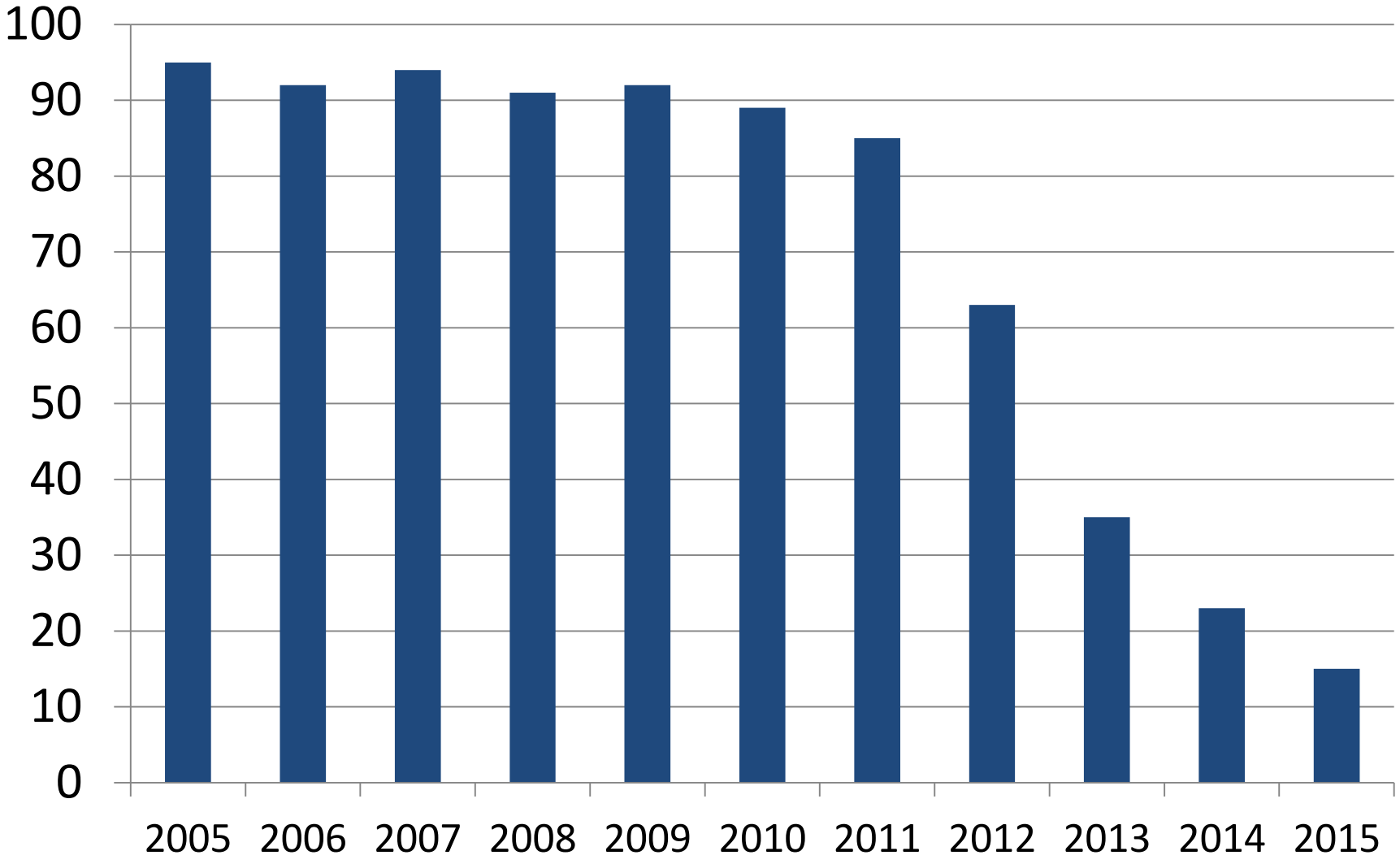
Apple®

facebook

facebook

Google

Percentage of retrievals with ET



Question: What percentage of your patients have a fresh ET?

a. <25
b. 26-50
c. 51-74
d. >75

The changing face of IVF

Fertil Steril. 2010 Oct;94(5):1700-6. doi: 10.1016/j.fertnstert.2009.10.015. Epub 2009 Nov 25.

Clinical application of comprehensive chromosomal screening at the blastocyst stage.

Schoolcraft WB[1], Fragouli E, Stevens J, Munne S, Katz-Jaffe MG, Wells D.

⊕ Author information

Abstract

OBJECTIVE: To evaluate a new strategy for comprehensive chromosome screening at the blastocyst stage.

DESIGN: Clinical research study.

SETTING: An IVF clinic and a specialist preimplantation genetic diagnosis laboratory.

PATIENT(S): Forty-five infertile couples participated in the study. The mean maternal age was 37.7 years, and most couples had at least one previous unsuccessful IVF treatment cycle (mean 2.4).

INTERVENTION(S): This study used a novel chromosome screening approach, combining biopsy of several trophectoderm cells on day 5 after fertilization and detailed analysis of all 24 types of chromosome using comparative genomic hybridization.

MAIN OUTCOME MEASURE(S): Proportion of embryos yielding a diagnostic result, aneuploidy rate, implantation rate, and pregnancy rate.

RESULT(S): A diagnosis was obtained from 93.7% of embryos tested. The aneuploidy rate was 51.3%. The probability of an individual transferred embryo forming a pregnancy reaching the third trimester/birth was 68.9%, an implantation rate 50% higher than contemporary cycles from the same clinic. The pregnancy rate was 82.2%.

CONCLUSION(S): The comprehensive chromosome screening method described overcomes many of the problems that limited earlier aneuploidy screening techniques and may finally allow preimplantation genetic screening to achieve the benefits predicted by theory. The high embryo implantation rate achieved is particularly encouraging and, if confirmed in subsequent studies, will be of great significance for IVF clinics attempting to reduce the number of embryos transferred or to implement single embryo transfer.

The changing face of IVF

Fertil Steril. 2013 Sep;100(3):697-703. doi: 10.1016/j.fertnstert.2013.04.035. Epub 2013 Jun 1.

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

Scott RT Jr[1], Upham KM, Forman EJ, Hong KH, Scott KL, Taylor D, Tao X, Treff NR.

⊕ **Author information**

Abstract

OBJECTIVE: To determine whether blastocyst biopsy and rapid quantitative real-time polymerase chain reaction (qPCR)-based comprehensive chromosome screening (CCS) improves in vitro fertilization (IVF) implantation and delivery rates.

DESIGN: Randomized controlled trial.

SETTING: Academic reproductive medicine center.

PATIENT(S): Infertile couples in whom the female partner (or oocyte donor) is between the ages of 21 and 42 years who are attempting conception through IVF.

INTERVENTION(S): Embryonic aneuploidy screening.

MAIN OUTCOME MEASURE(S): Sustained implantation and delivery rates.

RESULT(S): We transferred 134 blastocysts to 72 patients in the study (CCS) group and 163 blastocysts to 83 patients in the routine care (control) group. Sustained implantation rates (probability that an embryo will implant and progress to delivery) were statistically significantly higher in the CCS group (89 of 134; 66.4%) compared with those from the control group (78 of 163; 47.9%). Delivery rates per cycle were also statistically significantly higher in the CCS group. Sixty one of 72 treatment cycles using CCS led to delivery (84.7%), and 56 of 83 (67.5%) control cycles ultimately delivered. Outcomes were excellent in both groups, but use of CCS clearly improved patient outcomes.

CONCLUSION(S): Blastocyst biopsy with rapid qPCR-based comprehensive chromosomal screening results in statistically significantly improved IVF outcomes, as evidenced by meaningful increases in sustained implantation and delivery rates.

Multiple Pregnancies

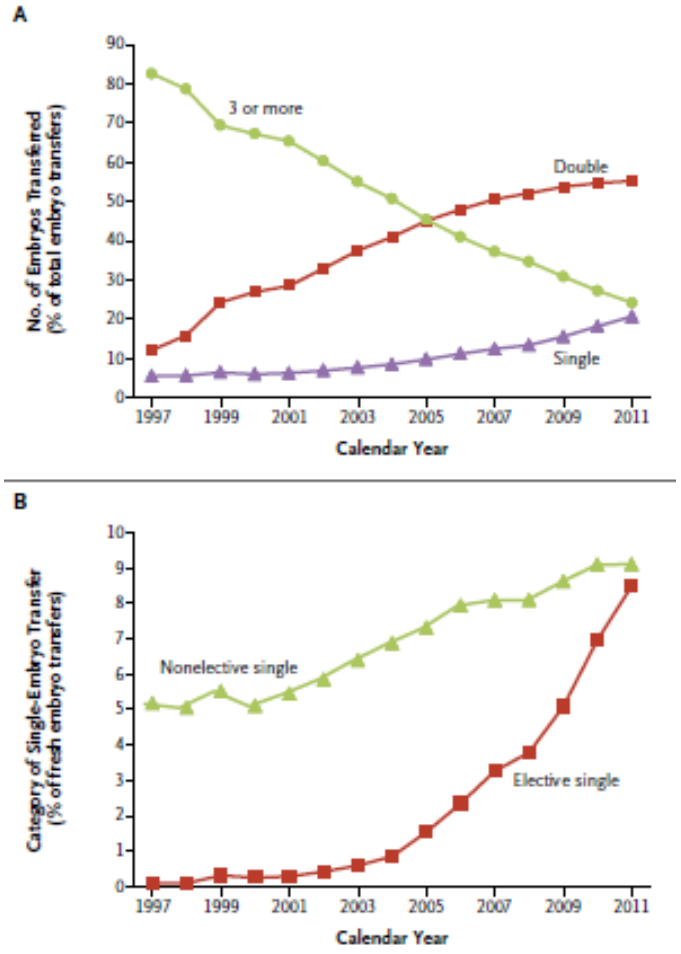
Multiple pregnancies are still the largest complication in IVF

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Fertility Treatments and Multiple Births in the United States

Aniket D. Kulkarni, M.B., B.S., M.P.H., Denise J. Jamieson, M.D., M.P.H., Howard W. Jones, Jr., M.D., Dmitry M. Kissin, M.D., M.P.H., Maria F. Gallo, Ph.D., Maurizio Macaluso, M.D., Dr.P.H., and Eli Y. Adashi, M.D.



Multiple Pregnancies

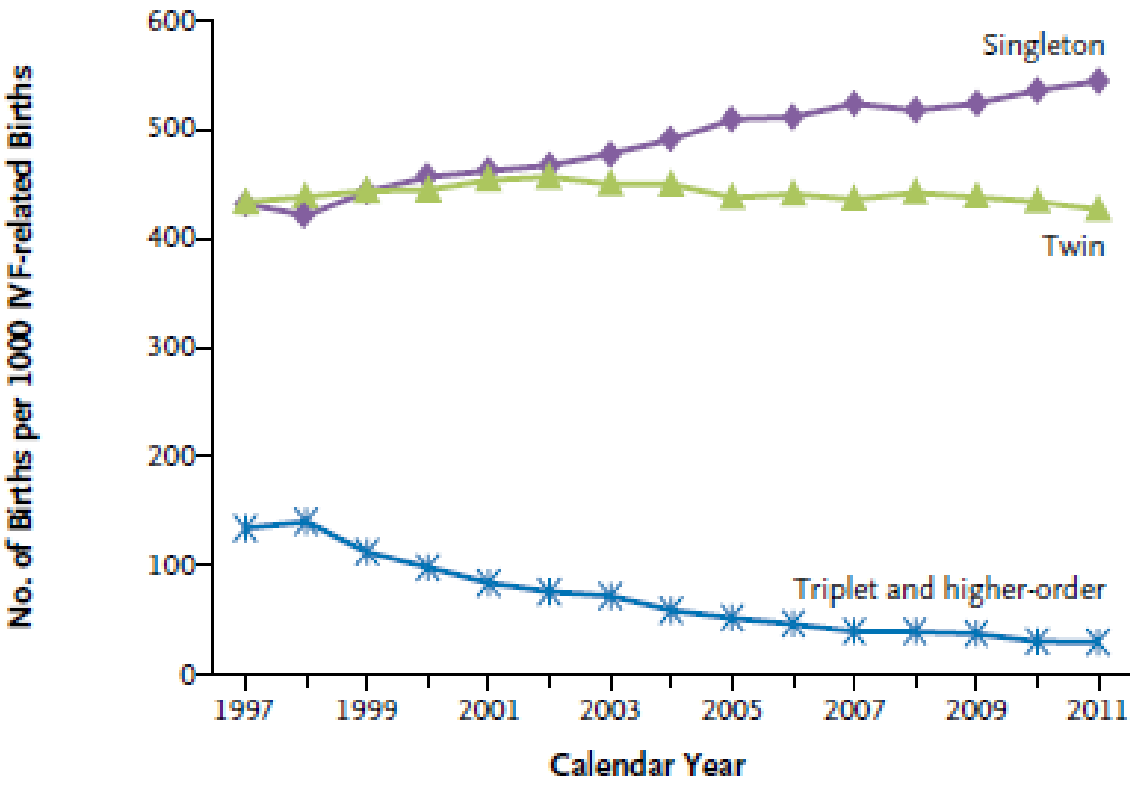
Multiple pregnancies are still the largest complication in IVF

The NEW ENGLAND JOURNAL

ORIGINAL ARTICI

Fertility Treatments and M
in the United S

Aniket D. Kulkarni, M.B., B.S., M.P.H., Denise
Howard W. Jones, Jr., M.D., Dmitry M. Kissin, M.D.
Maurizio Macaluso, M.D., Dr.P.H., and



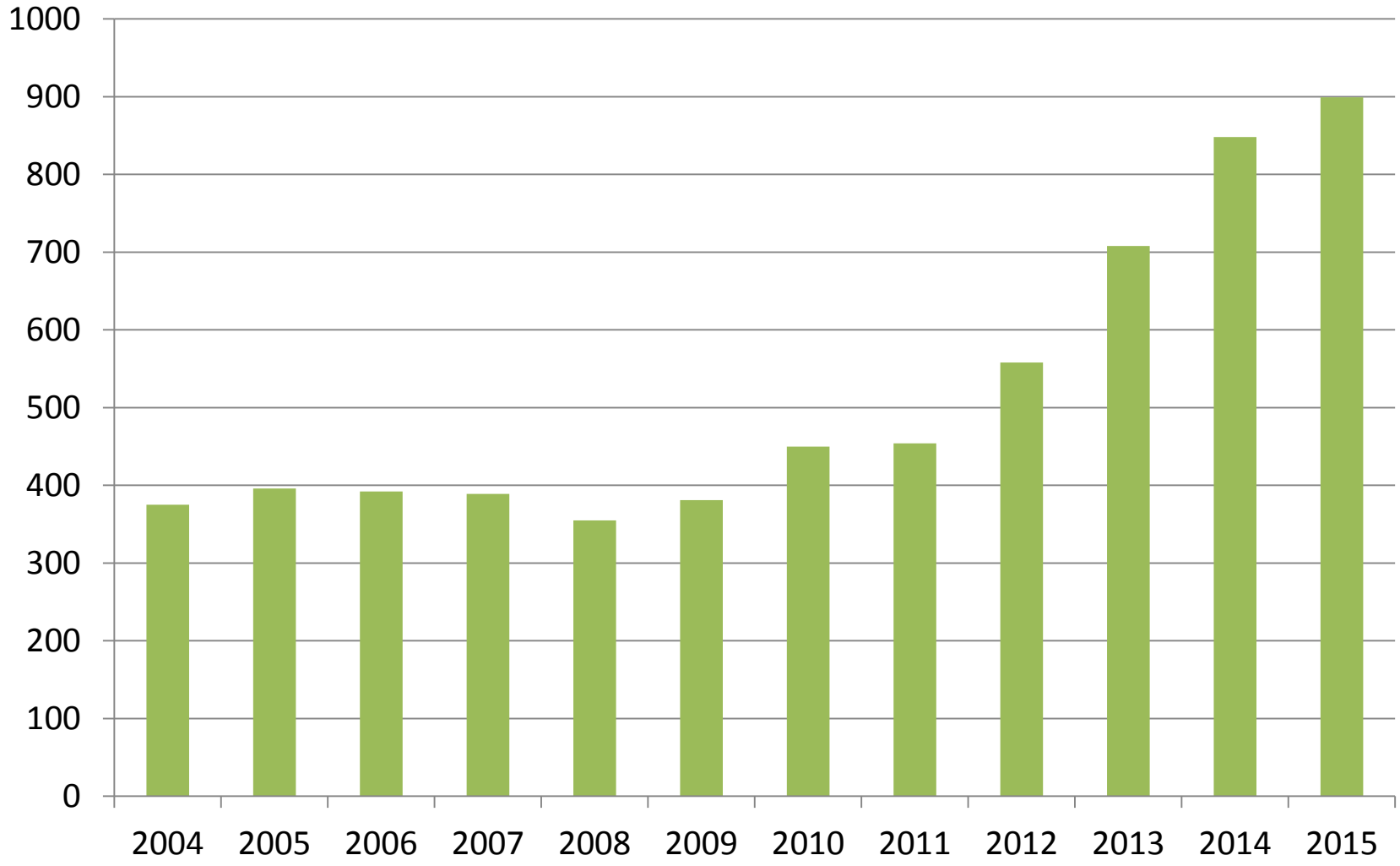
There Really Are So Many More Twins Now

A **million** more, roughly, when compared to the pre-1980 twin rates. So what changed?

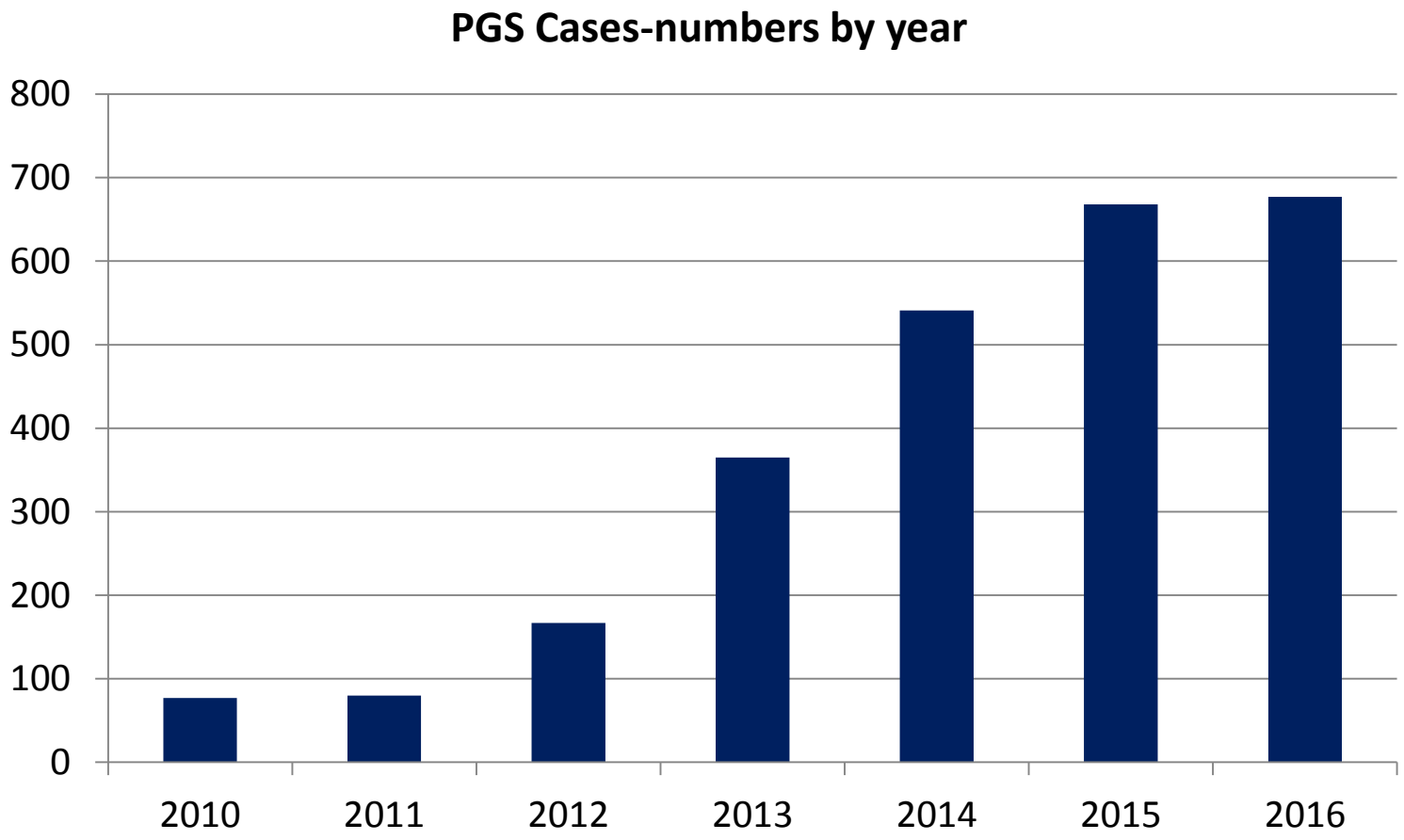
Computed from CDC data (Alexis Madrigal)

The Atlantic

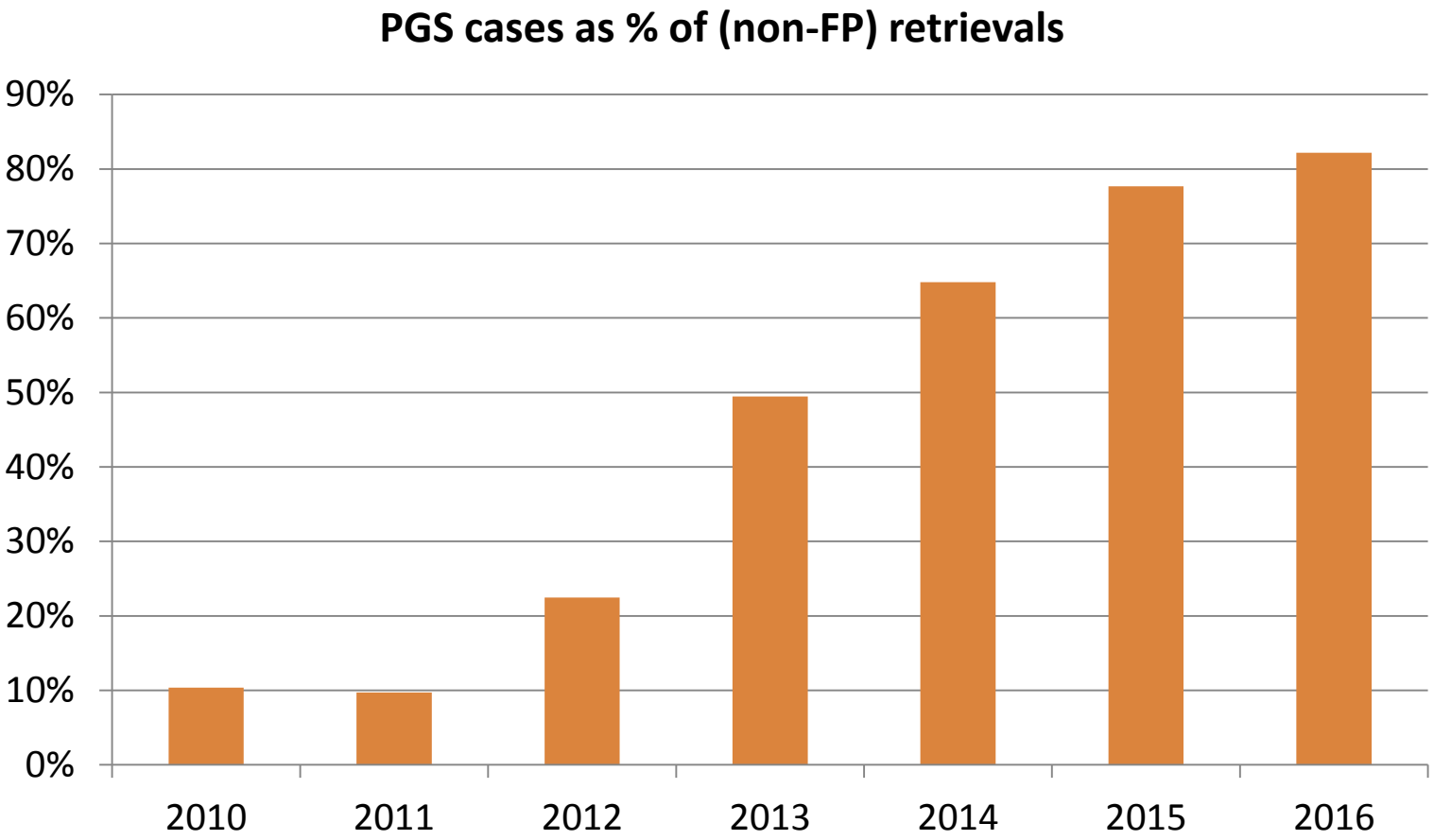
Number of FET's



Procedure stats 2010-2016



Procedure stats 2010-2016



Procedure stats 2010-2016

Year	Retrievals	Fertility Preservation	Retrievals less FP	PGS Cases	PGS cases as %
2010	763	21	742	77	10%
2011	862	40	822	80	10%
2012	833	89	744	167	22%
2013	882	144	738	365	49%
2014	1006	171	835	541	65%
2015	1088	228	860	668	78%
2016	1082	258	824	677	82%

Fresh transfers: Jan 2015-August 2016

1373 cycles inseminated

277 fresh transfers (15%)

Day of transfer	Patients	Percentage	
D2	34	12	45%
D3	91	33	
D5	148	53	54%
D6	4	1	

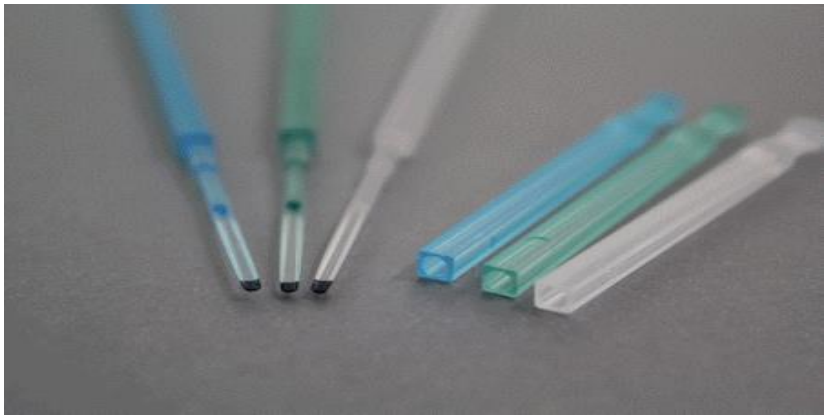
Fresh transfers: Jan 2015-August 2016

Day of transfer and pregnancy outcome (277 patients)

Number of embryos transferred	Percentage of patients
1	64
2	25
>2	10

Gestation	Percentage of patients
Singleton	89
Twin	11
High order	0

Freezing: Jan 2015-August 2016



Day of freezing	Embryos	Percentage	
D2	1	<1	<1%
D3	23	<1	
D5	1860	27	>99%
D6	4181	61	
D7	861	12	

Frozen transfers: Jan 2015-August 2016

Transfer and pregnancy outcome (1399 patients)

Number of embryos transferred	Percentage of patients
1	91*
2	9
>2	0

Gestation	Percentage of patients
Singleton	92
Twin	8
High order	0

*1249 patients had 1 embryo transferred. 899 (72%) were true eSET's
13 patients (1%) had no ET (6 no survival + 7 PGS)

We're doing a lot of freezing

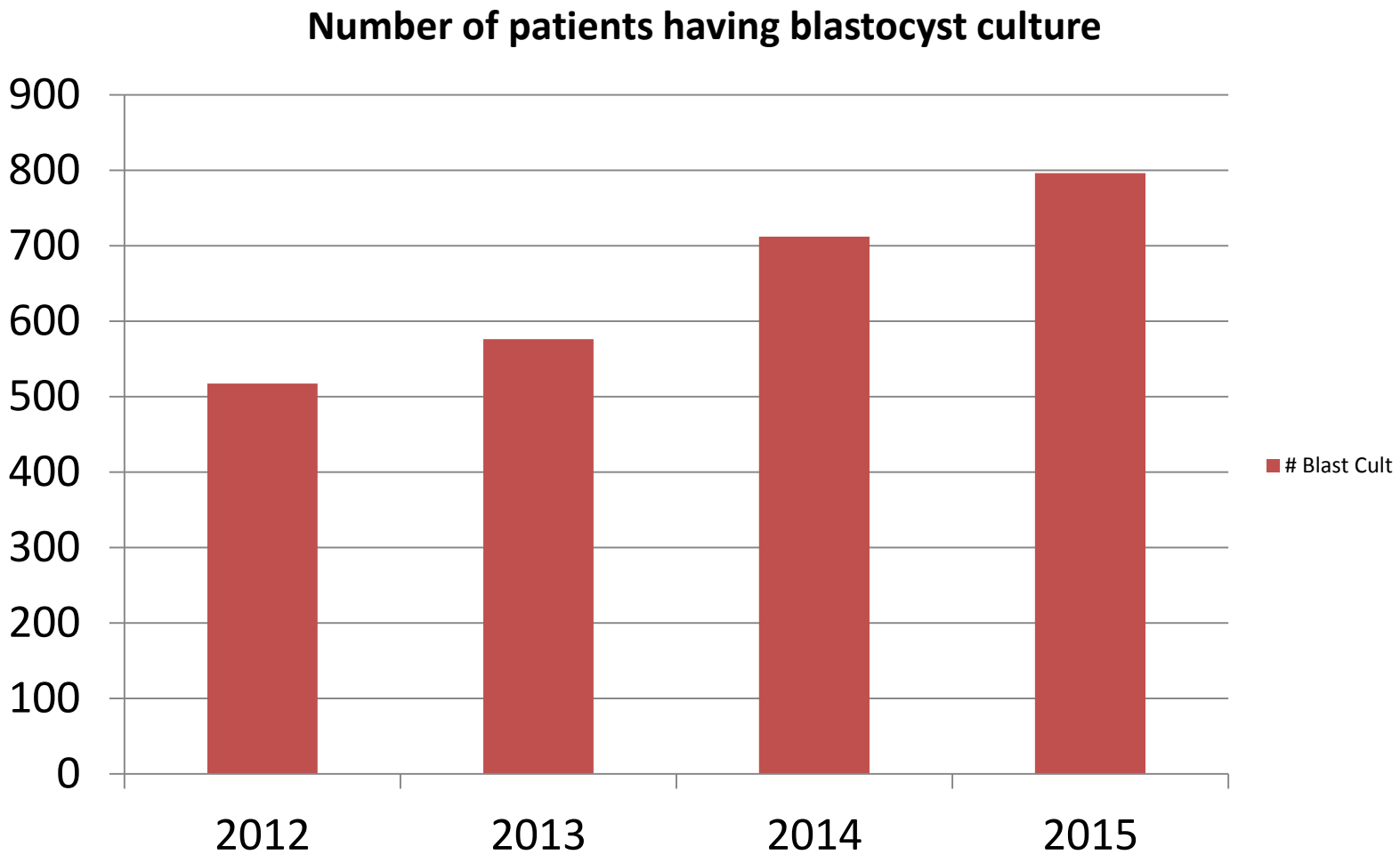
Jan 2015-August 2016

Cycle type	Number of cycles	Percentage
Oocyte freezing	438	24
Fresh ET	277	15
PGS	1009	73

Oocytes vitrified	6386
Embryos vitrified	6926

Note: All blastocysts are collapsed before vitrification

More PGS means more blastocyst culture

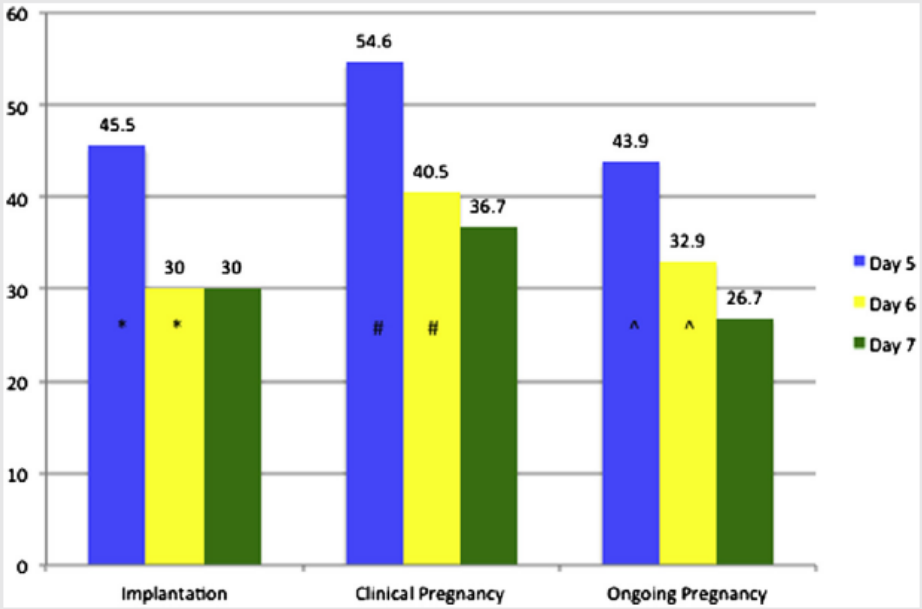


We're culturing all embryos to D7!

Should embryos developing to blastocysts on day 7 be cryop an an impla

George Kovalevsk
Adrienne B. Neith
Reproductive Associ

FIGURE 1



Pregnancy and implantation rates according to day of embryo cryopreservation. Only statistically significant *P* values are reported: **P*<.001, #*P*=.002, ^*P*=.01.

Kovalevsky. Frozen day 7 blastocyst PRs. Fertil Steril 2013.

Started D7 PGS January 2014

Total PGS cases (2.5 months)

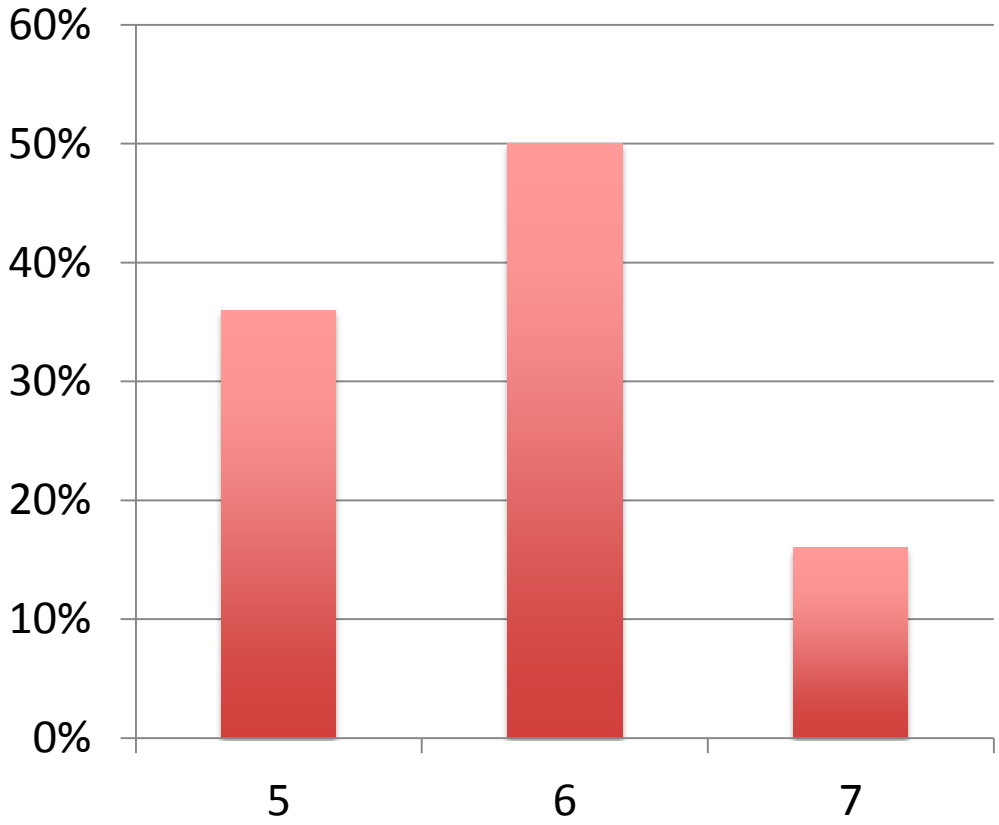
PGS cases	D5 Biopsy	D6 Biopsy	D7 Biopsy
66	21 (32%)	62 (94%)	27 (41%)

Euploid embryos

Embryos*	D5	D6	D7
157 (5.8/pt.)	17/27 (62%)	52/87 (60%)	19/43 (44%)

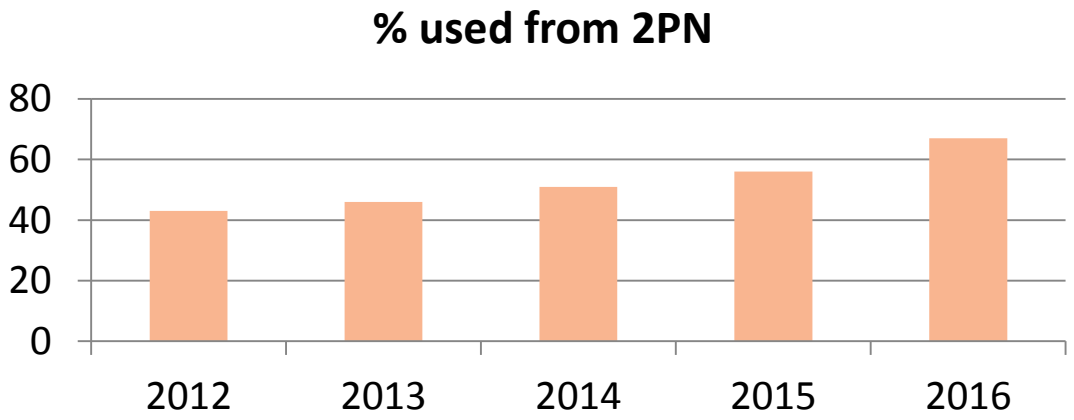
*D7 Biopsy patients only

Blastocyst yield by Day



- We don’t freeze early blastocysts
- D7 blastocysts need to be really nice

We are generating more embryos

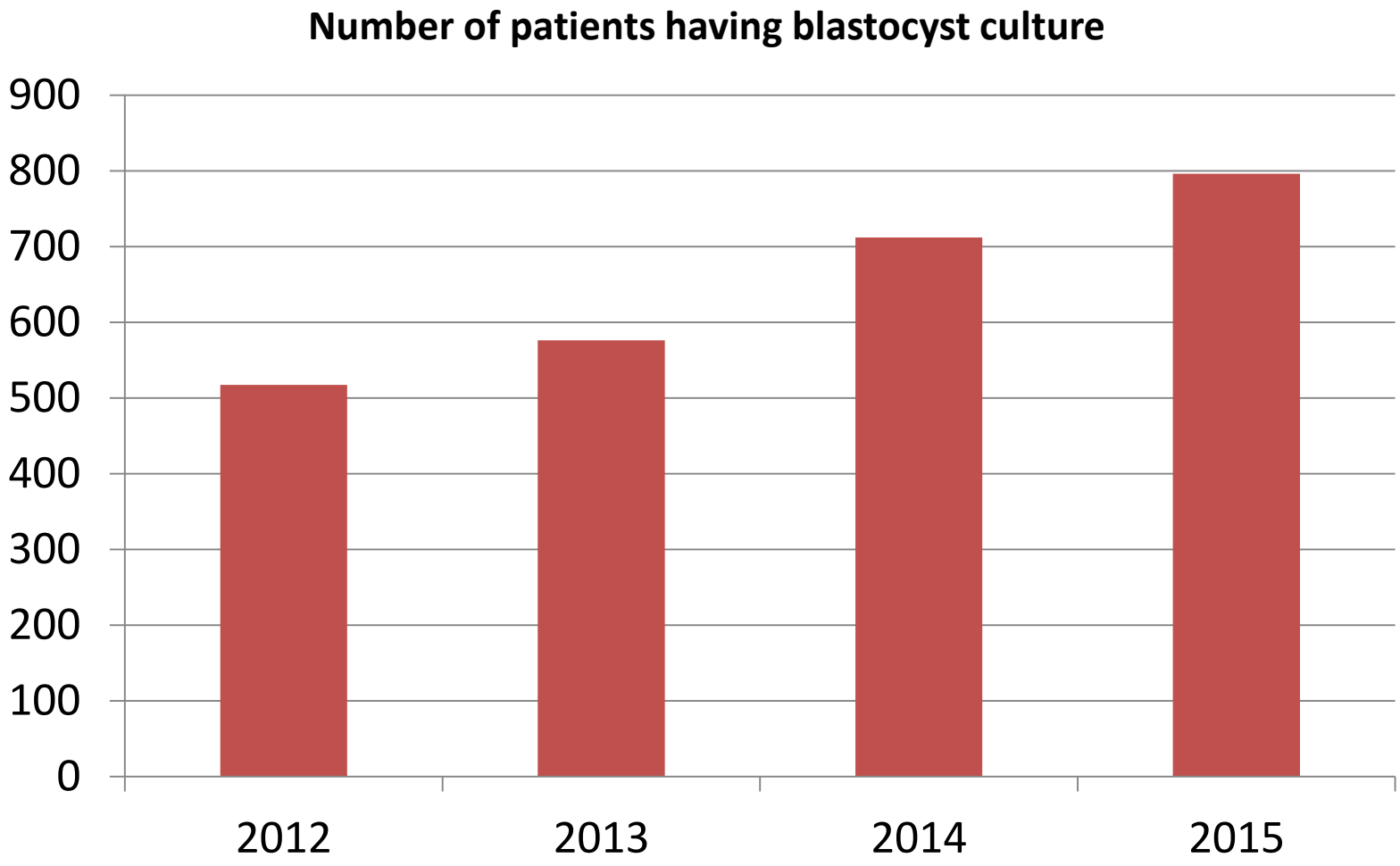


Year	2012	2013	2014	2015	2016
Average 2PN/Patient	14.6	16.7	16	16.7	18
% used from 2PN	43	46	51	56	67
Average used/retrieval	6.3	7.6	8.2	9.4	12.1

Changing Landscape

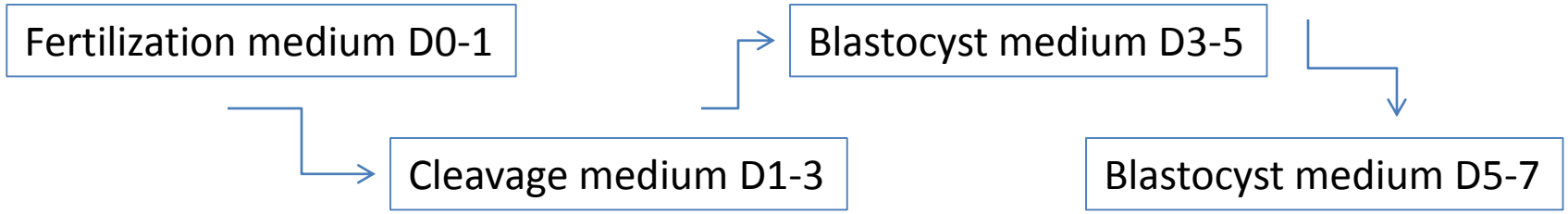
- More patients for blastocyst culture
- More PGS/CCS
- Increased volume of vitrification
- Increasing volume of FET's
- More D3 AH
- Increased dish prep
- Higher equipment usage

Goal: Can we simplify the culture system

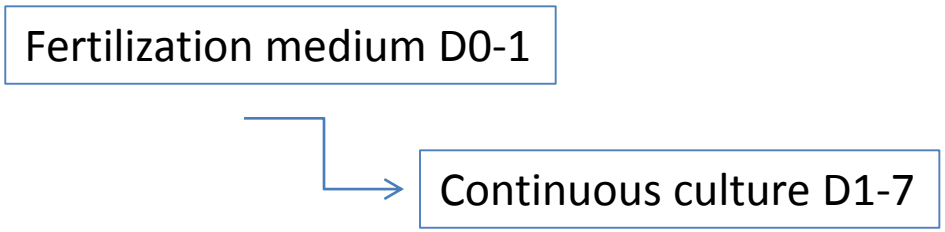


2015 Changes

2014 Sequential culture system



2015 Continuous culture system



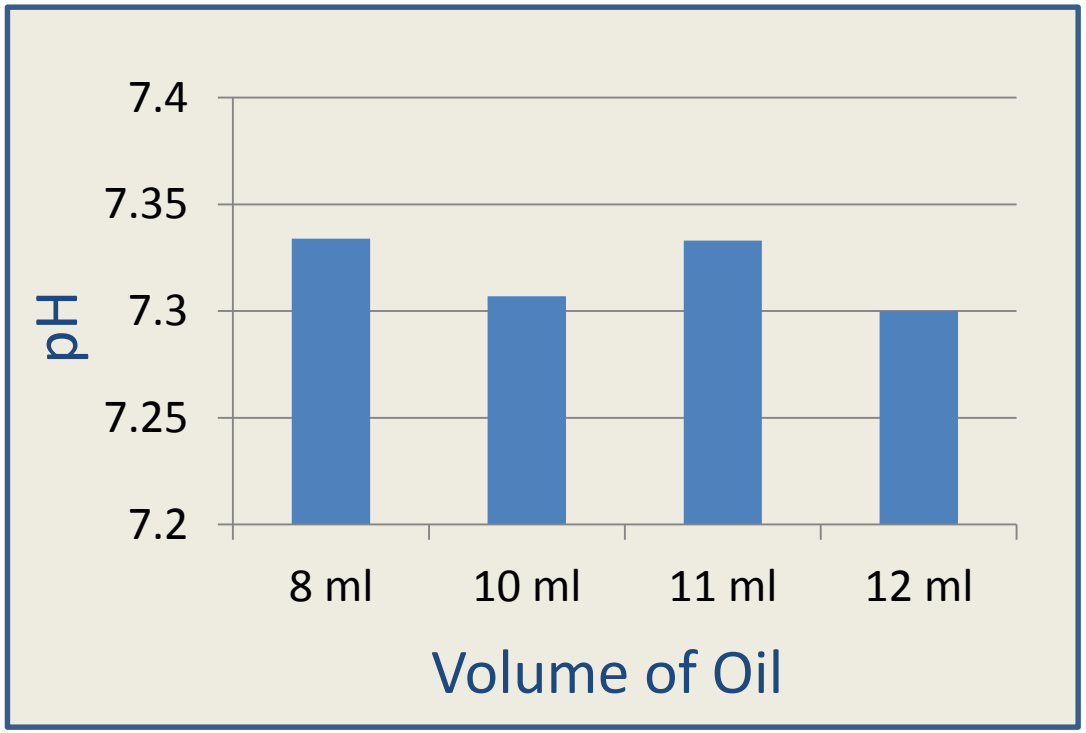
Incubator Questions

- Ideal number of embryos/drop?
- Medium/embryo/day?
- Embryo sorting?
- Is changing medium necessary?

Moving to dry benchtop incubators

- Verify gas concentrations
- Measure pH and osmolality
- Blood gas analyzer
- Be aware that chamber is dry

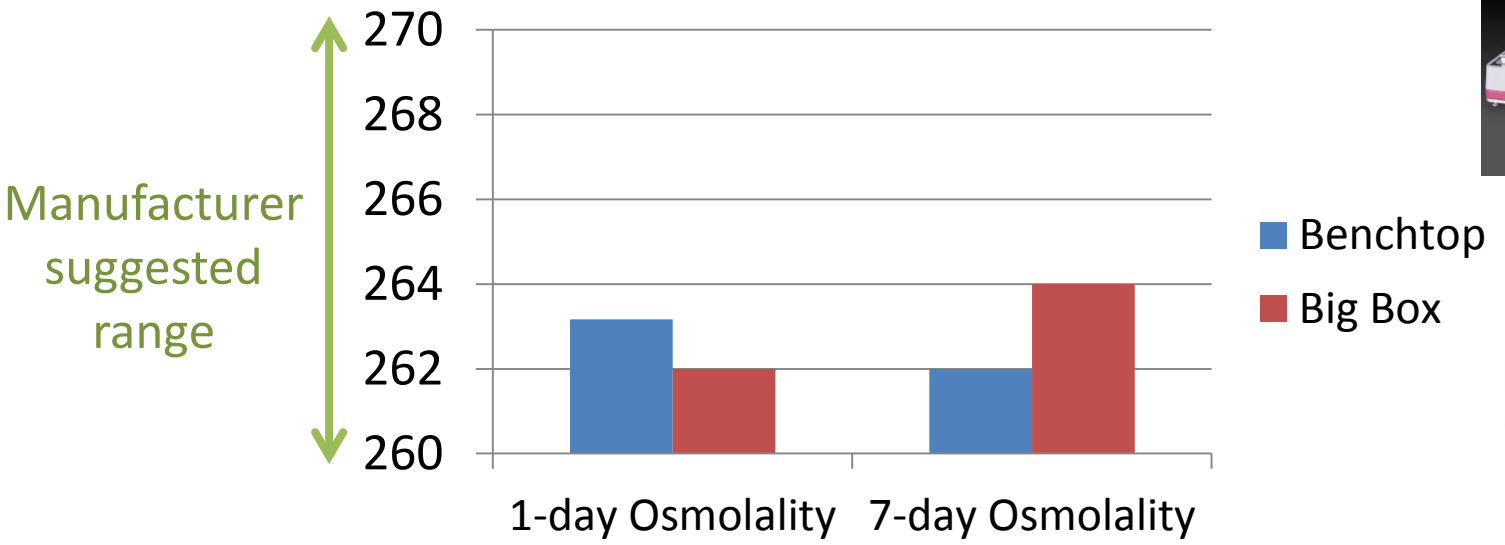
Media pH in a dry benchtop incubator



- 60ul of medium under oil
- Equilibrated overnight (16 hours)
- pH is average of 4 measurements

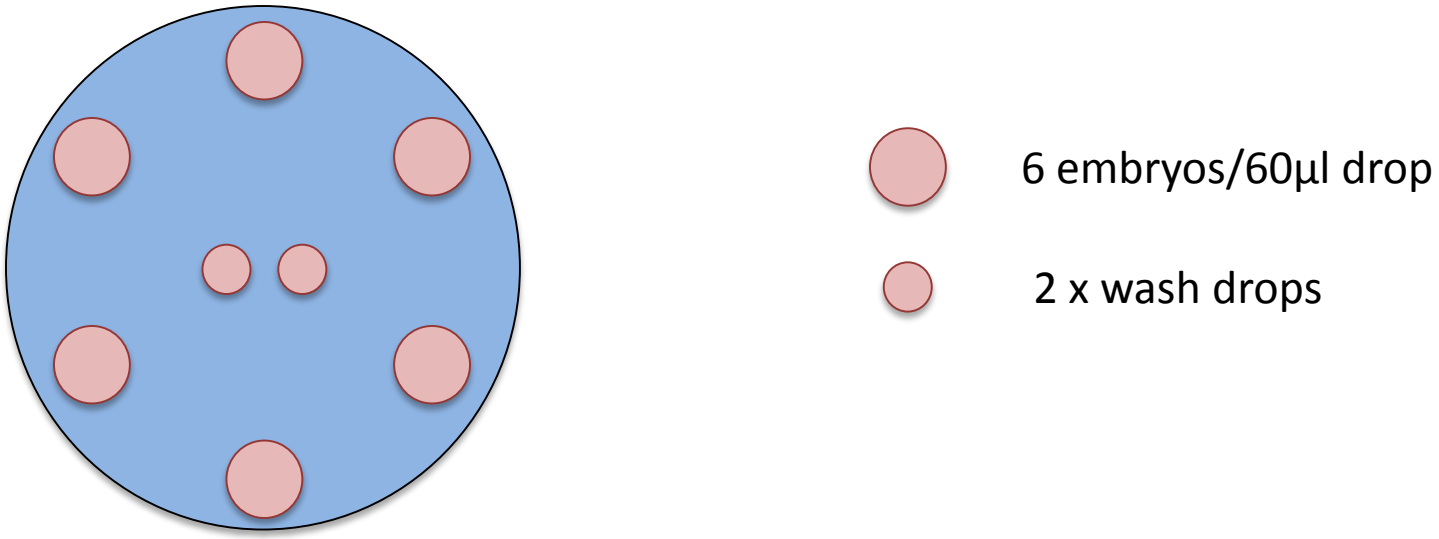
Does osmolality of media change after 7 days in the incubator?

Osmometer callibration
100 mOsmol/Kg
290 mOsmol/Kg
1000 mOsmol/Kg



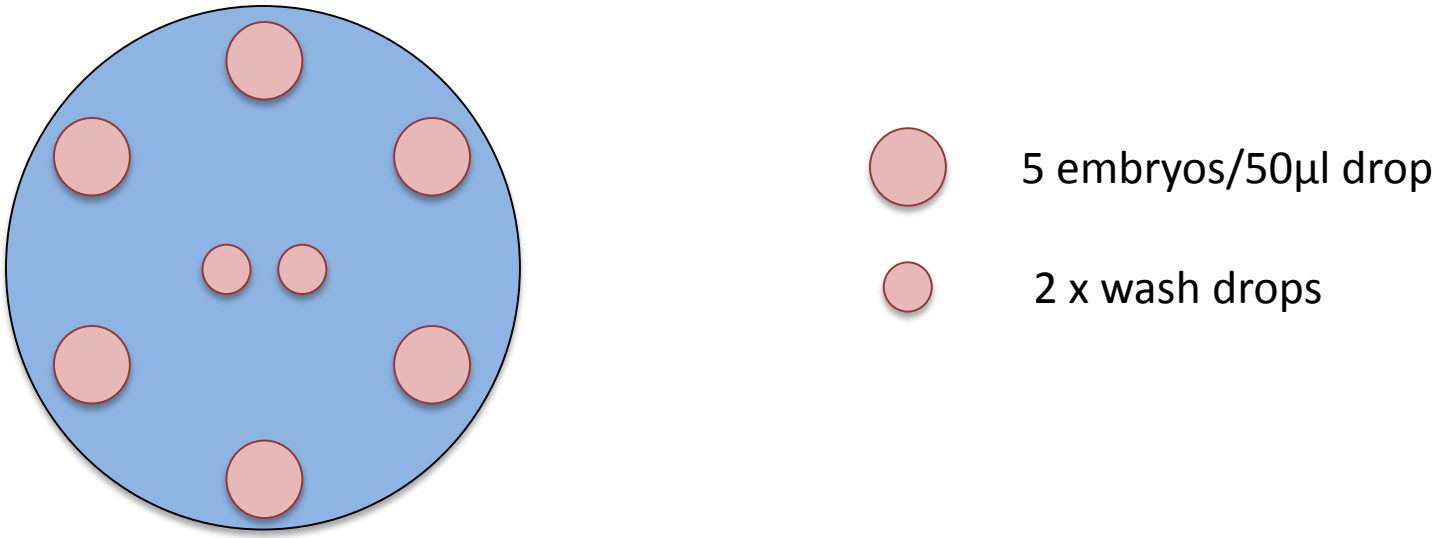
Suggested system

- Six embryos per 60µl droplet CSCM + 10% SSS
- Culture from D1 up to D7 (2PN to Blastocyst)
- Undisturbed culture (no D3 check)

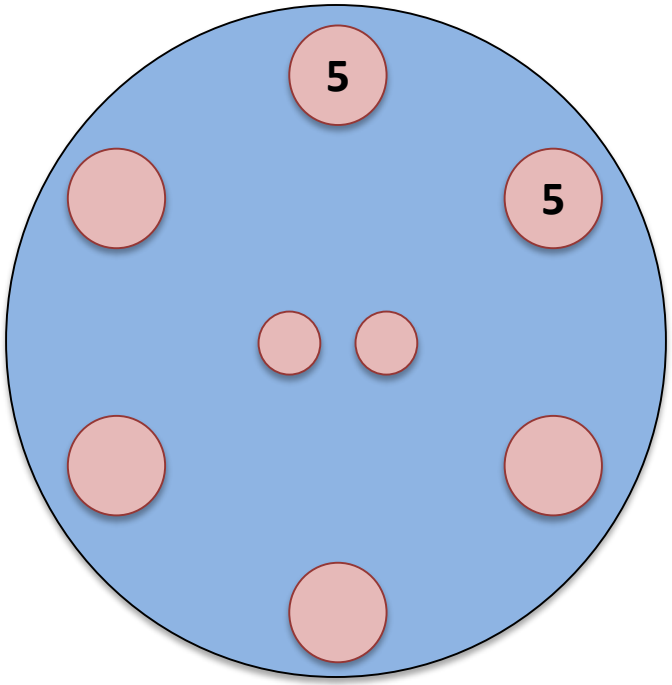


Established system

- Five embryos per 50µl droplet CSCM + 10% SSS
- Culture from D1 up to D7 (2PN to Blastocyst)
- Undisturbed culture (no D3 check)



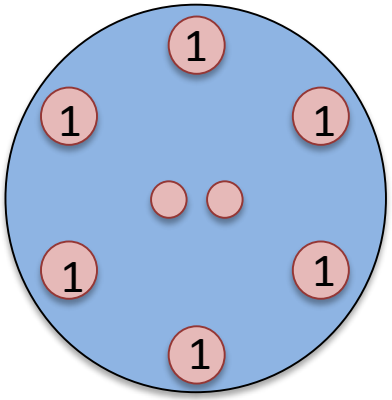
Established system



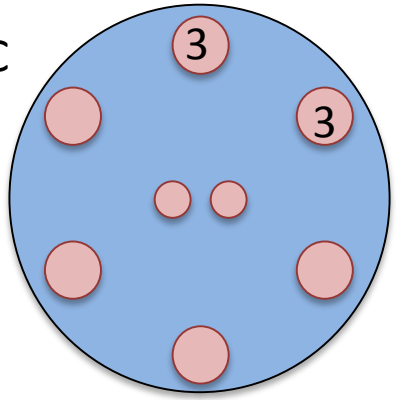
Example 1: Patient has 10 x 2PN

Question: Patient has 6 x 2PN. How do we divide embryos among culture drops?

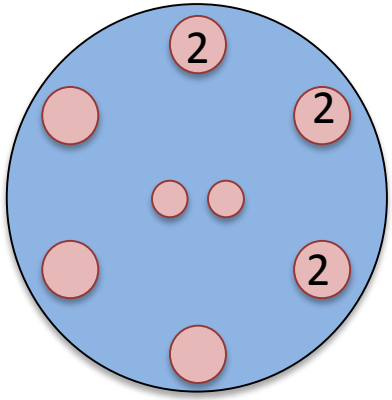
A



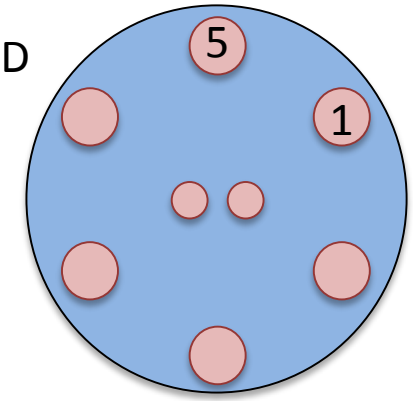
C



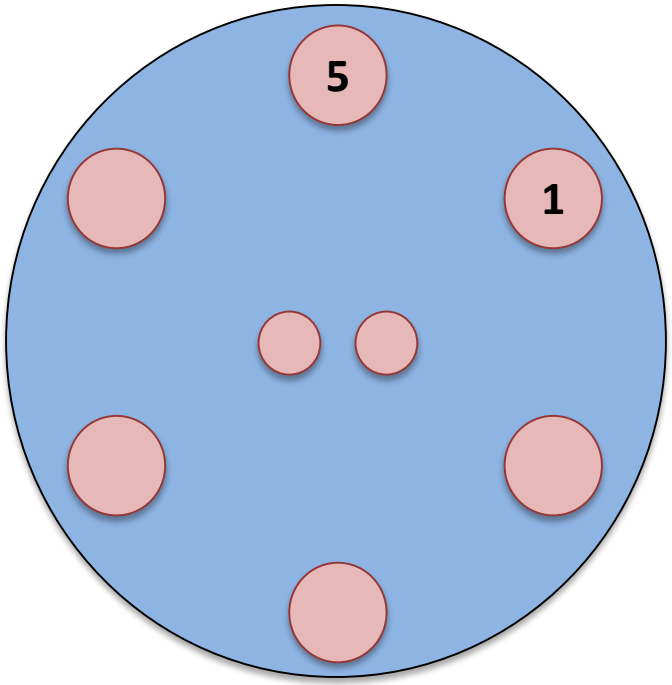
B



D

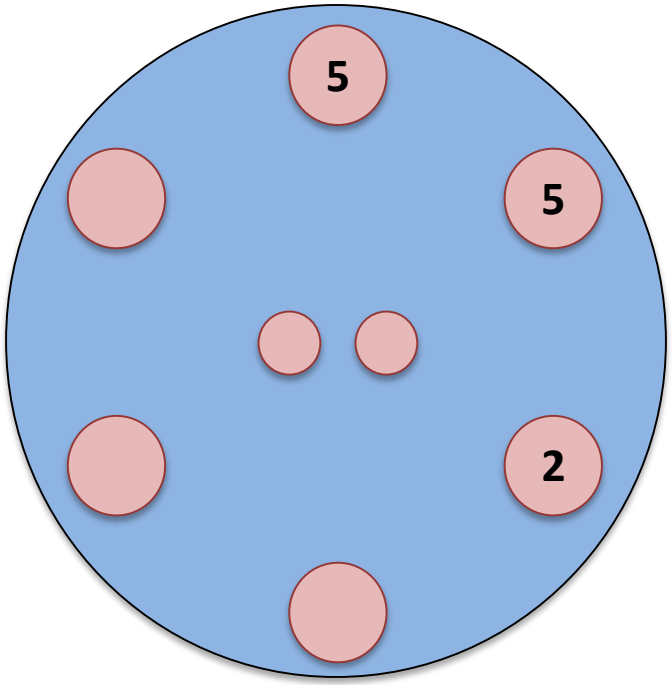


Established system



Example 2: Patient has 6 x 2PN

Established system



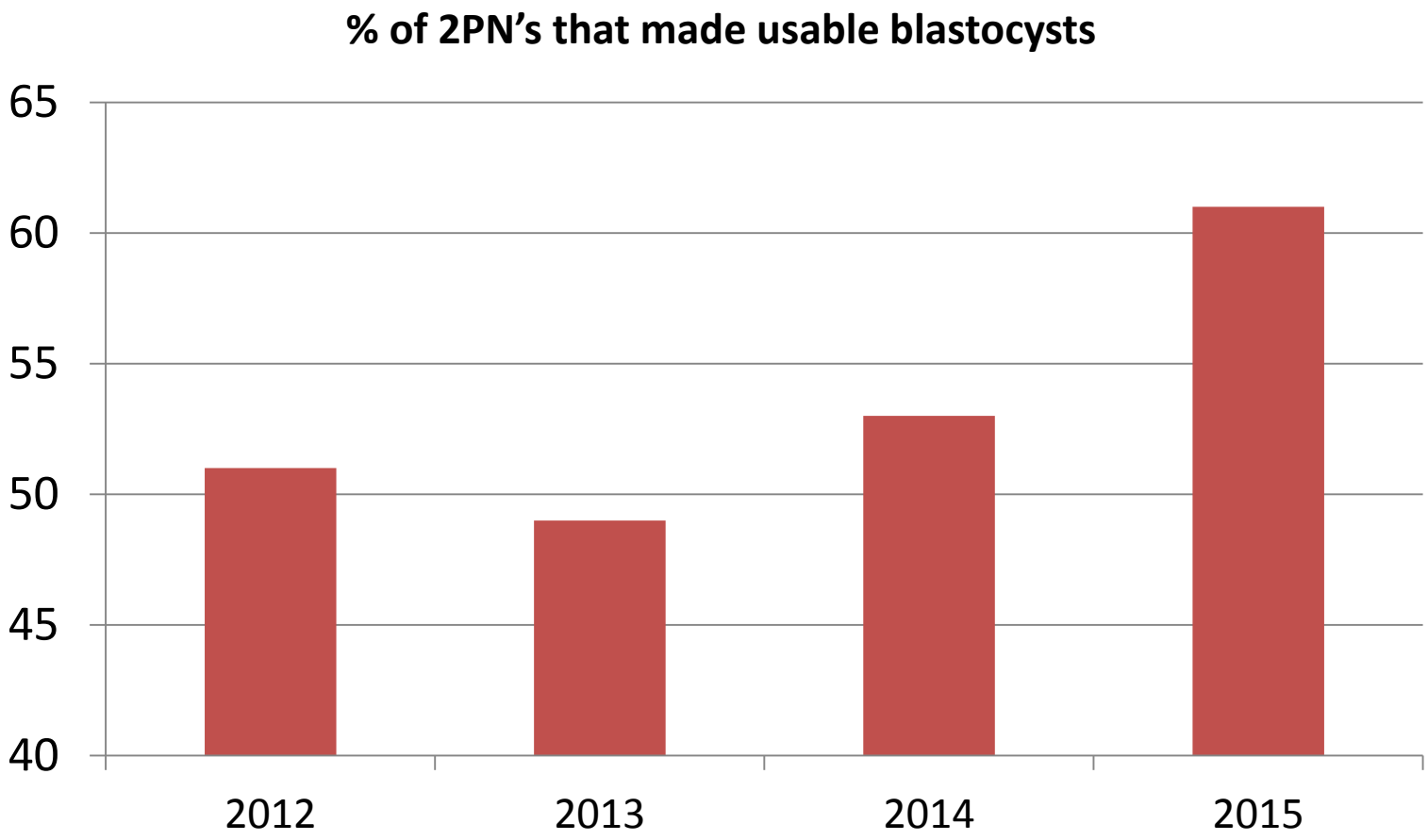
Example 3: Patient has 12 x 2PN

Tiny Incubators

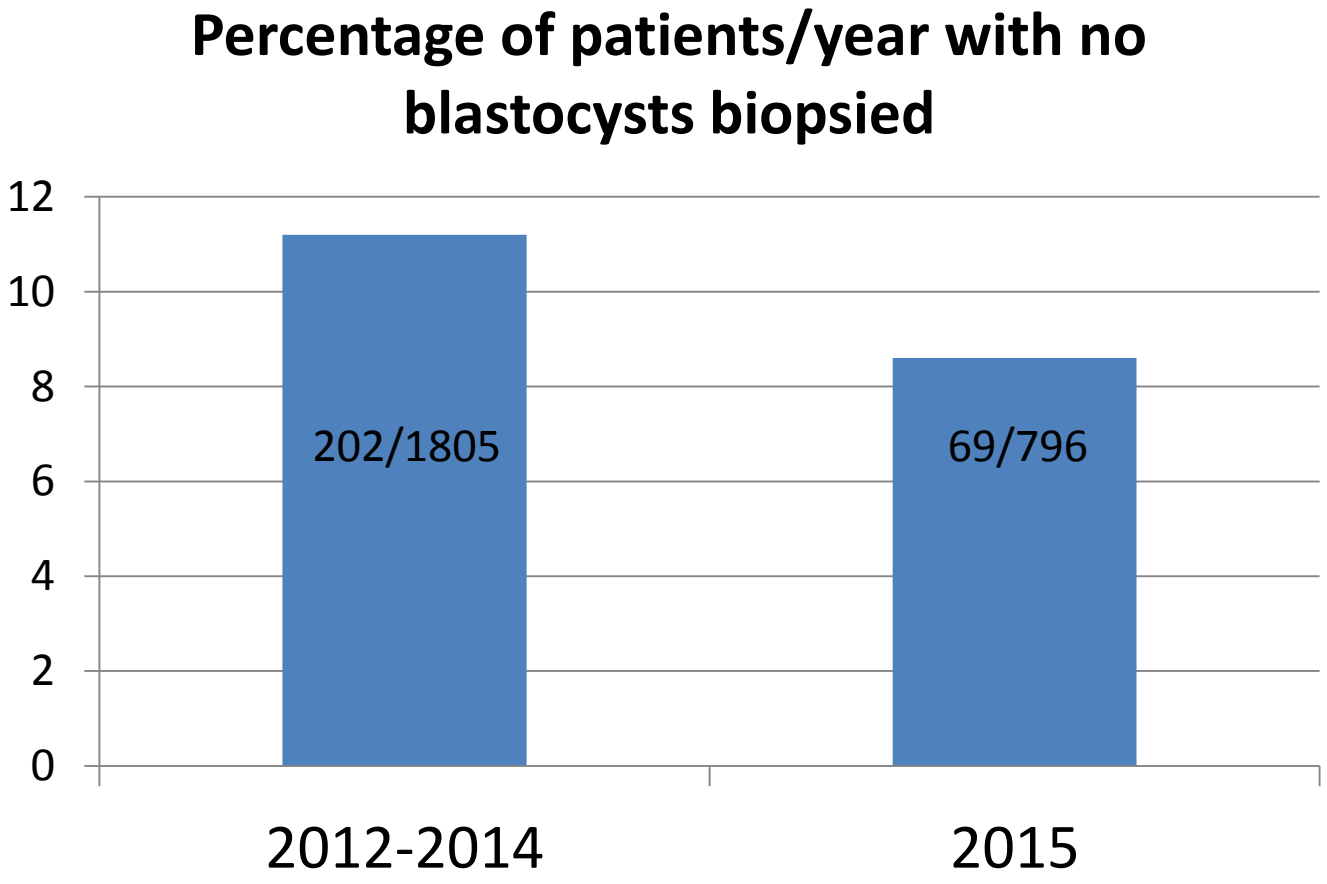
- Group
- Autocrine effects
- Nutrient depletion
- Waste build-up

- Single
- Less waste
- More nutrients
- Loss of synergestic effects

Are we making more blastocysts?



How many patients have no blastocysts to biopsy?

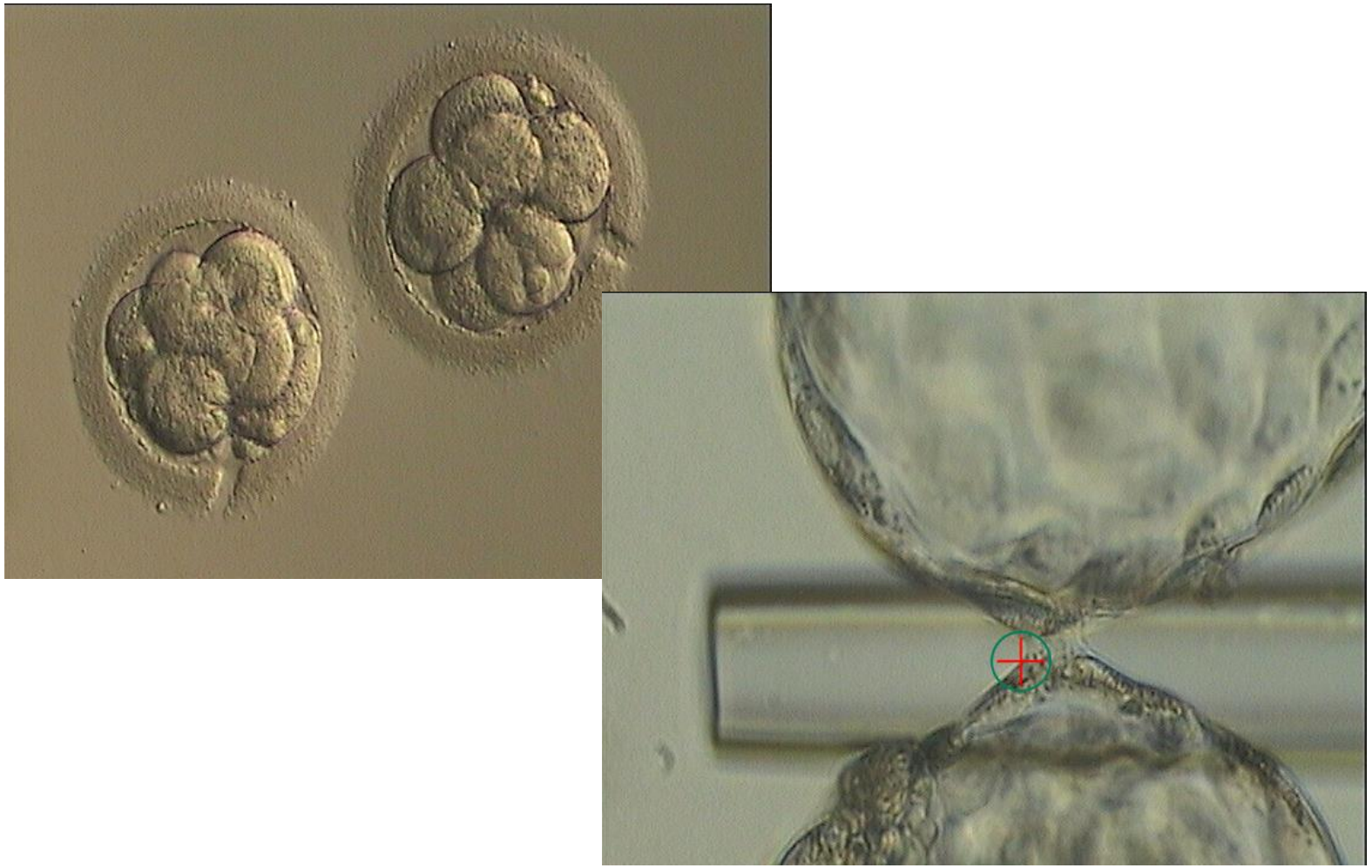


How we made more blastocysts

- Media change
- Continuous culture from D1-D7
- Undisturbed culture*
- Group culture. 5 embryos/50µl droplet

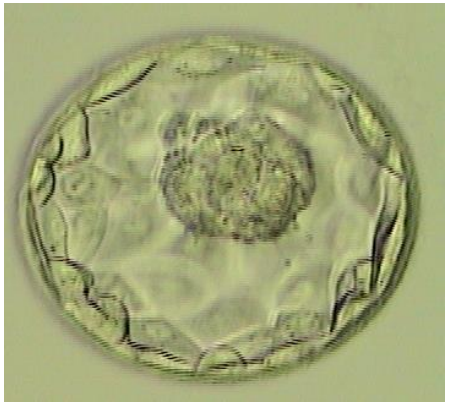
*Embryos may have AH on D3

Can development be too good?



Are there benefits

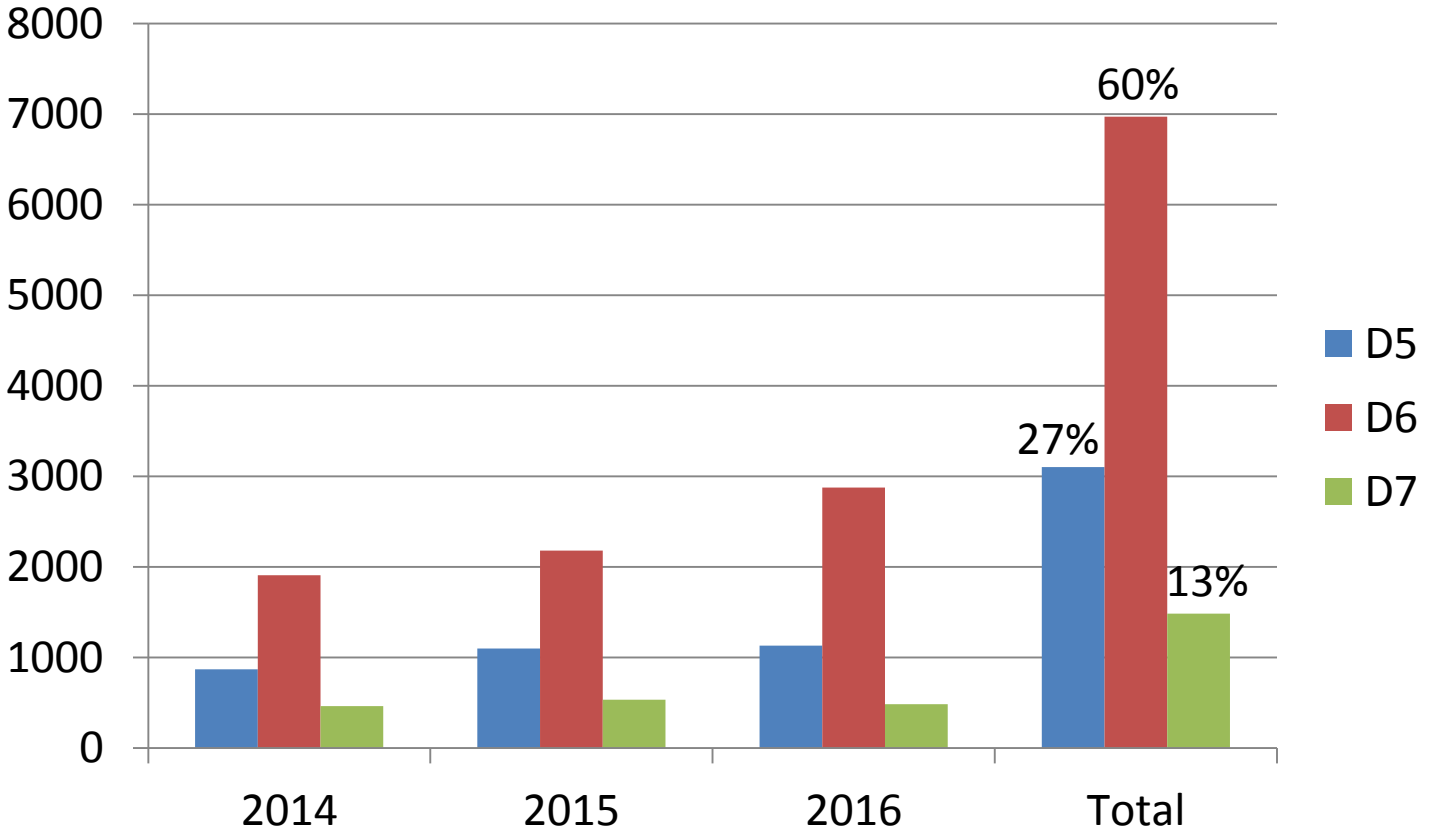
- Increased pregnancy rate
- Decreased miscarriage rate
- Faster turnaround for patients
- Lower numbers of embryos transferred
- Reduced costs
- Increased efficiency



Clinical PGS outcomes 2014

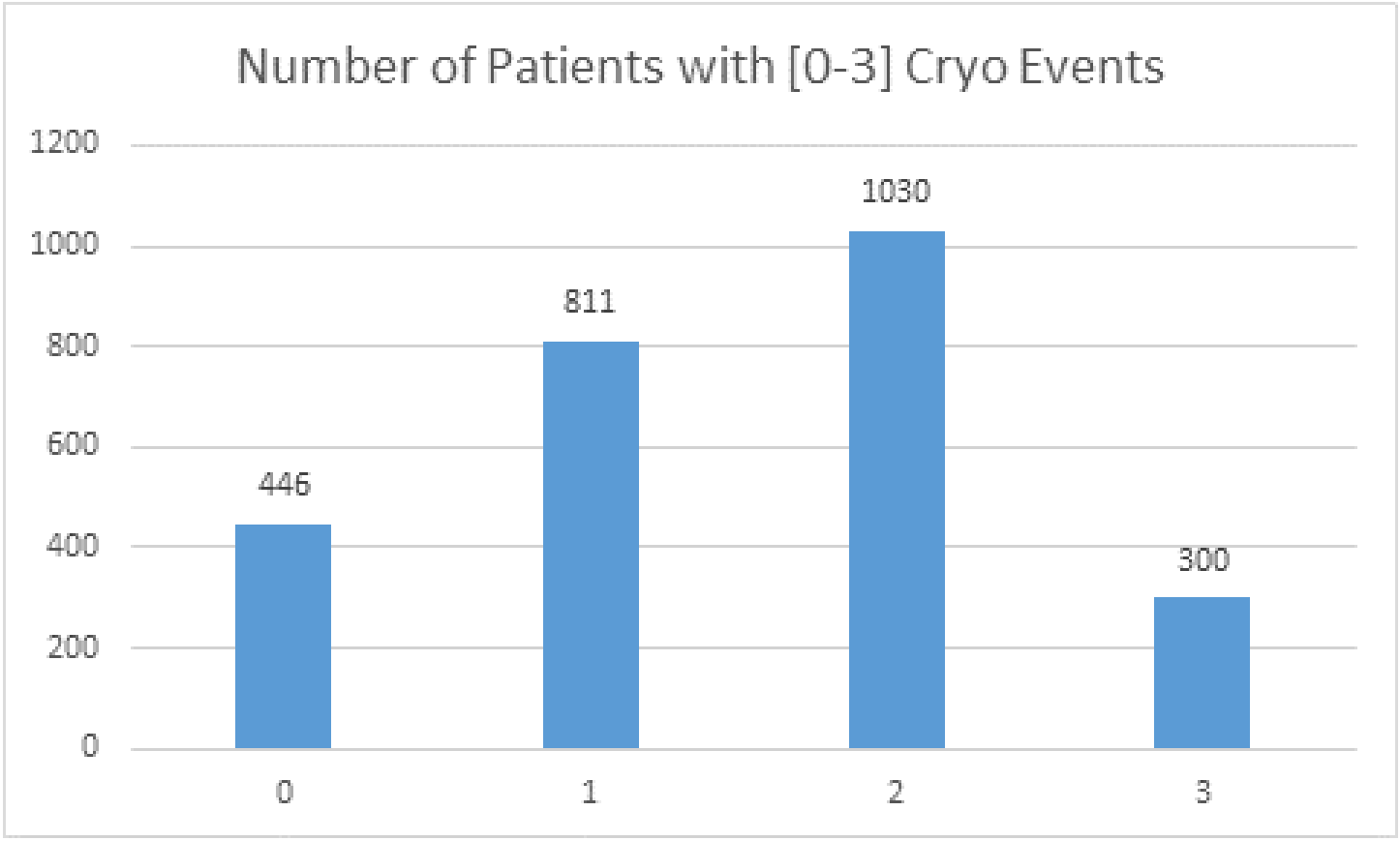
Age	ET's	# transferred	Clinical	Sacs	Live Birth cycles	# babies born	Clinical Preg. Rate	Implantation Rate	Live Birth Rate/cycle
<35	83	88	56	60	52	55	67.5%	68.2%	62.7%
35-37	99	103	60	63	53	56	60.6%	61.2%	53.5%
38-40	89	89	59	61	48	49	66.3%	68.5%	53.9%
41-42	29	30	17	18	14	15	58.6%	60.0%	48.3%
>42	6	6	6	6	4	4	100.0%	100.0%	66.7%
All own eggs	306	316	198	208	171	179	64.7%	65.8%	55.9%

How many embryos have we vitrified (2014-6)?



	D5	D6	D7
2014	870	1911	466
2015	1102	2183	534
2016	1132	2879	487
Total	3104	6973	1487

How many times are we vitrifying per cycle?



0	1	2	3	Total
446	811	1030	300	2587
17%	31%	40%	12%	100%

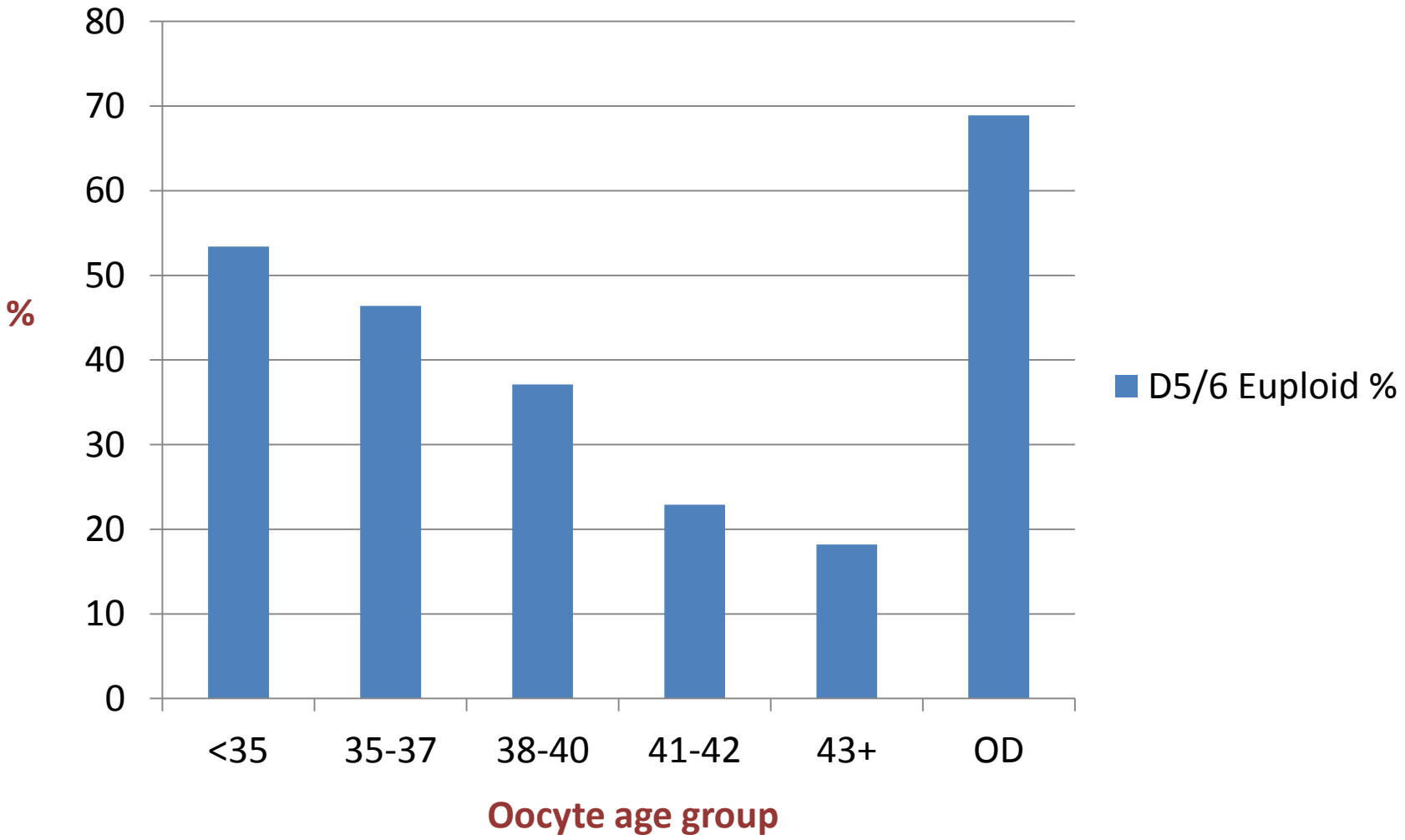
Day 7 PGS FET's

D7 embryos only	n =	%
FET's	81	
Embryos transferred	86	
Average transferred	1.06	
Clinical pregnancies	25	30.9
Sacs	27	31.4
Live births	21	25.9
Babies born	22	

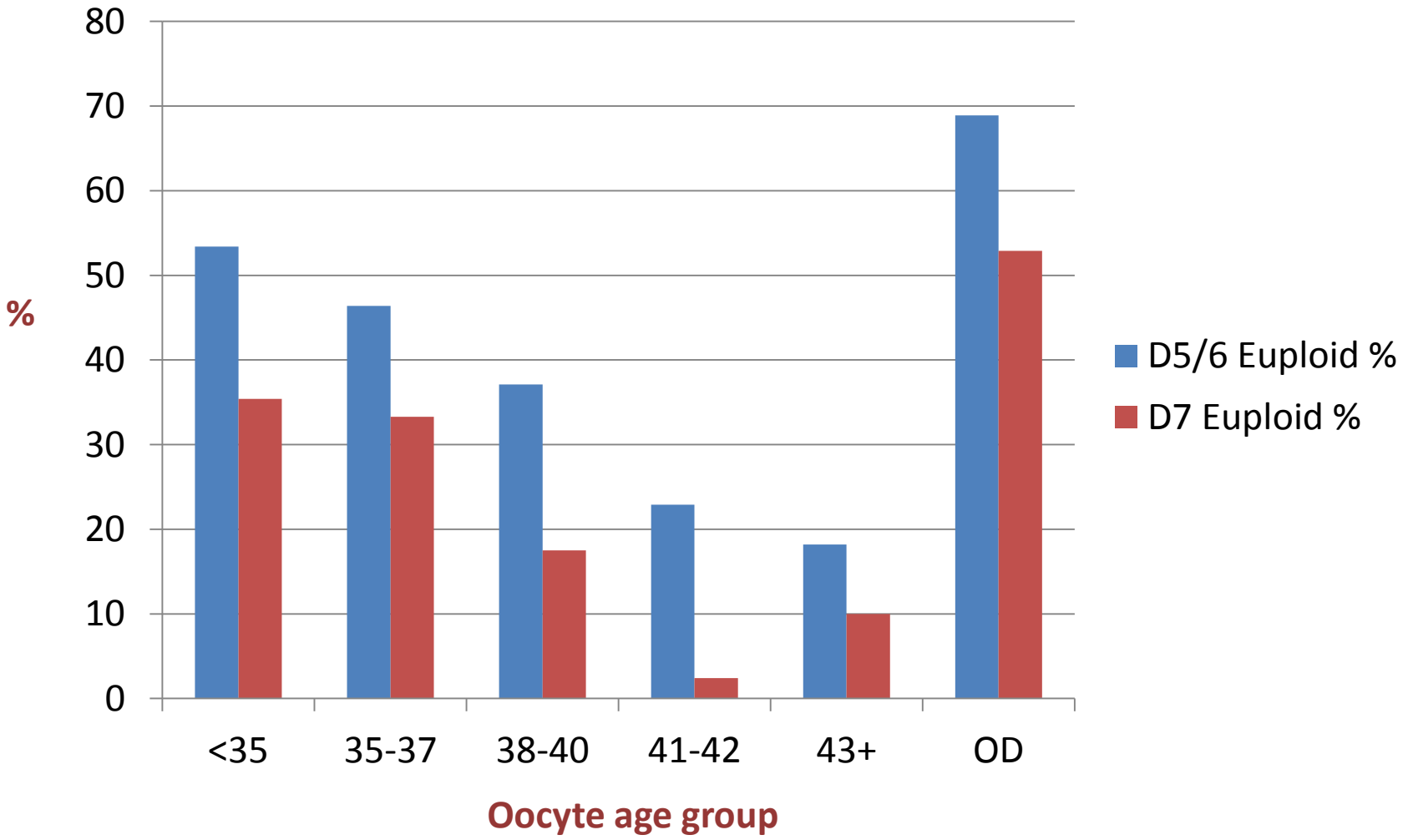
2016 PGS Cases (n = 633)

Age group	<35	35-37	38-40	41-42	43+	OD
Retrievals	121	114	187	100	49	92
# blastocysts biopsied	960	634	881	303	108	898
% of retrievals with embryo biopsy	98	95	94	82	39	99
% of retrievals with euploid embryo(s)	93	84	66	38	22	98

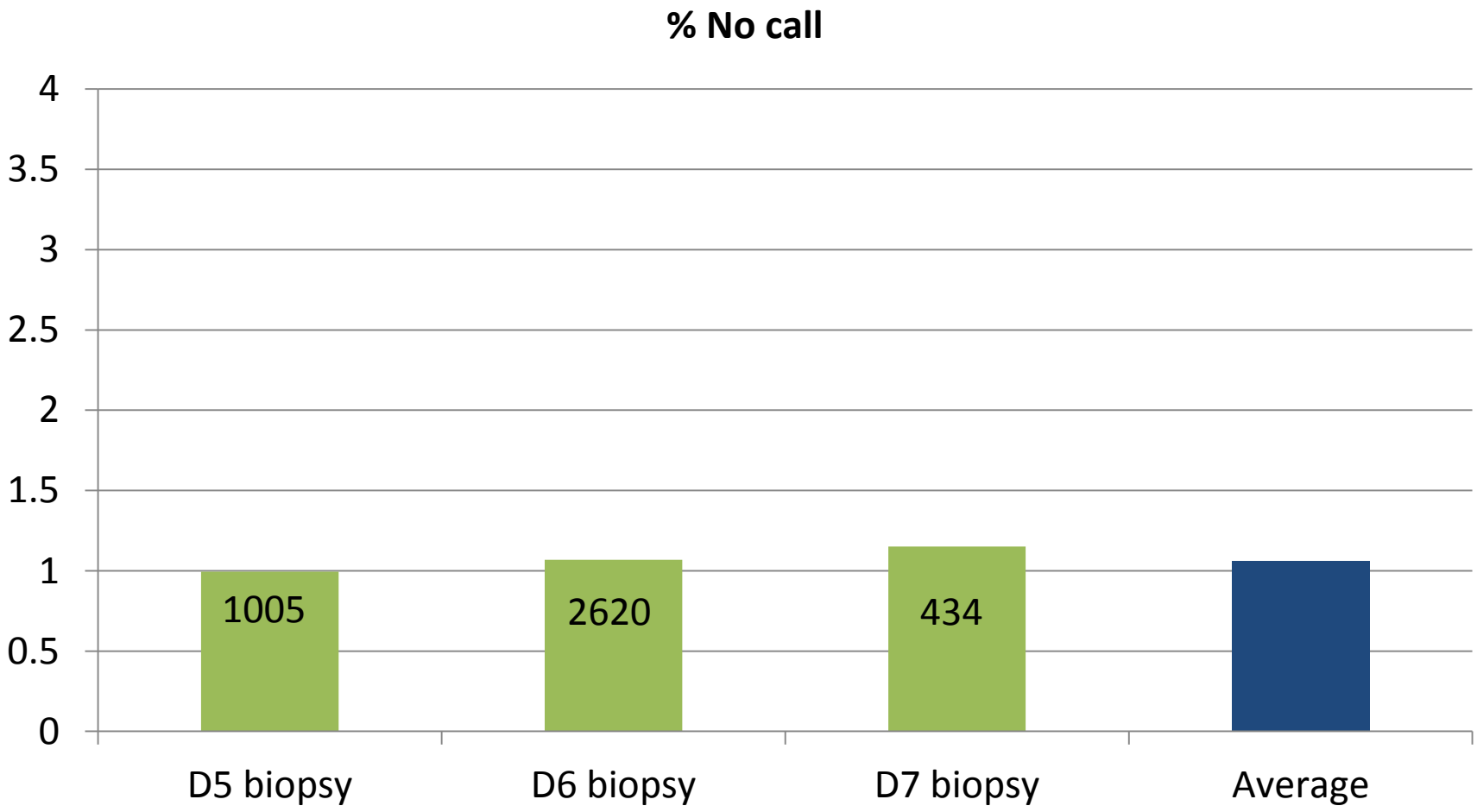
Euploidy rate in D5/6 blastocysts



Euploidy Rate in D7 blastocysts



No call rate by day of Biopsy (2016)



Is D7 worth the effort: 3 years of outcomes

- 2,707 patients cultured 1 more day
- 818 patients with D7 vitrification
- 89 patients with only D7 vitrification
- 1487 more embryos vitrified
- 451 more normal embryos
- 818 extra freeze events
- 76 new FET's
- 22 children born

Summary

- Effort to make more blastocysts has paid off
- Increased use of PGS/PGS
- Culturing all embryos to D7
- Simplified group culture system
- Making the transition to benchtop incubators
- Is culture to D7 worth the effort?

joe@pacificfertility.com

Question: Is embryo culture to D7 worth the effort?

a. No
b. For a small subset of patients
c. For all patients