Advanced Vitrification Techniques that will Reduce Multiple Pregnancy rates

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Chicago
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Interest in blastocyst vitrification

- Large full service IVF facility
- Median patient age is 38
- Large donor oocyte program
- Blastocyst transfers
- Cryopreservation on D5 and D6

Blastocyst preservation
Scope of service

- 2008: 902 retrievals
- 453 (50%) had embryos frozen
- Average no. of embryos vitrified = 4.4
- 355 patients had FET's (28% of cases)

Blastocyst transfers
Who had a day 5 ET in 2009?

As a rule, patients with more good quality embryos on D3 than they plan to transfer, go to D5

Patient age	< 35	35-37	38-40	41-42	> 42		Donor
Total Number of Cycles	170	144	175	89	45		173
D5 ET (%)	117 (69)	81 (56)	66 (38)	25 (28)	10 (22)		155 (90)

Overall, 57% of patients had a D5 ET

Blastocyst transfers
Fresh clinical outcomes 2009

Patient age	< 35	35-37	38-40	41-42	> 42		Donor
Number of Cycles	117	81	66	25	10		155
Number of Transfers	117	81	66	25	10		155
Pregnancies (ongoing)	48%	52%	30%	28%	10%		68%
Embryos transferred	1.61	2.05	2.58	2.84	2.50		1.4

Blastocyst transfers in 2009
Who has embryos frozen?

Patient age	< 35	35-37	38-40	41-42	> 42		Donor
Number of Cycles	117	81	66	25	10		155
Number patients with embryos to freeze (%)	94 (80)	56 (69)	32 (48)	15 (60)	2 (20)		139 (90)
Average number of embryos frozen	4.3	3.7	3.6	4.5	1.0		6.0
Usable blastocysts	6.0	5.8	6.2	7.3	3.5		7.4

Overall 338/454 with embryos to freeze (74%)
1,630 Blastocysts frozen

Implementation (2007)

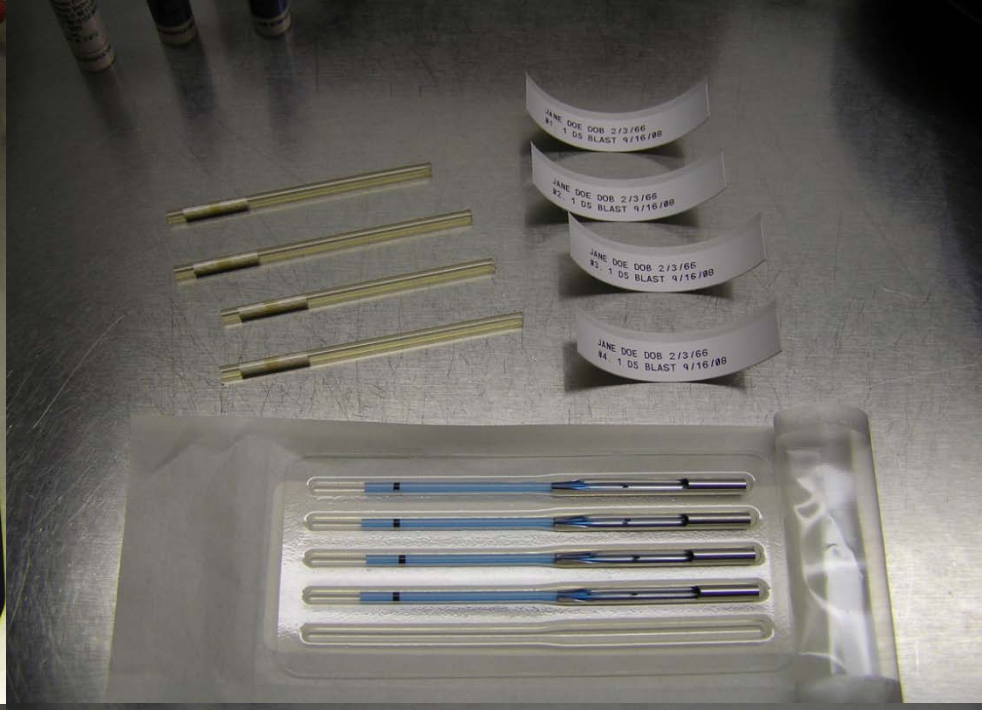
- Well trained staff
- Practice
- Vitrify good quality embryos
- Artificial collapse?
- Assisted hatching?

Methods

- Vit Kit and CryoTip
- Straws labeled carefully
- One blastocyst per straw
- Room temp.

Tip 1 : Examine straw carefully before starting


Vitrification
freeze your future
BRADY
LABPAL
PRINT
FUNCTION
SPACE


JANE DOE DOB 2/3/66
#1 1 D5 BLAST 9/16/08
JANE DOE DOB 2/3/66
#2 1 D5 BLAST 9/16/08
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#3 1 D5 BLAST 9/16/08
JANE DOE DOB 2/3/66
#4 1 D5 BLAST 9/16/08


JANE DOE DOB 2/3/66
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#1. 1 D5 BLAST 9/16/08
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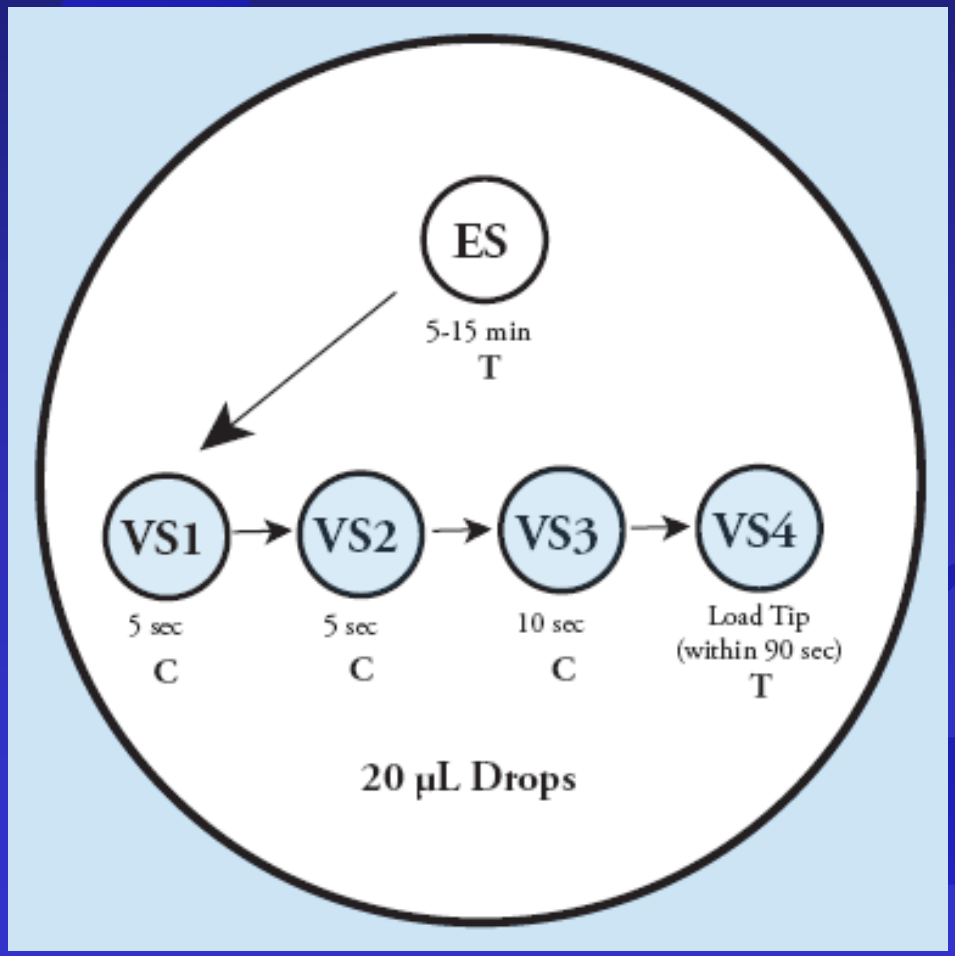
#3. 1 D5 BLAST 9/16/08
#4. 1 D5 BLAST 9/16/08

Materials and Methods

Vitrification Methods – Cooling procedure



Key:

ES= Equilibration Solution
VS= Vitrification Solution
→ = Transfer specimen to next drop
C = Center of drop
T = Top of drop

We have settled on 8 mins in ES for all blastocysts

Materials and Methods

90133-DES Blastocyst Vitrification Freeze Kit

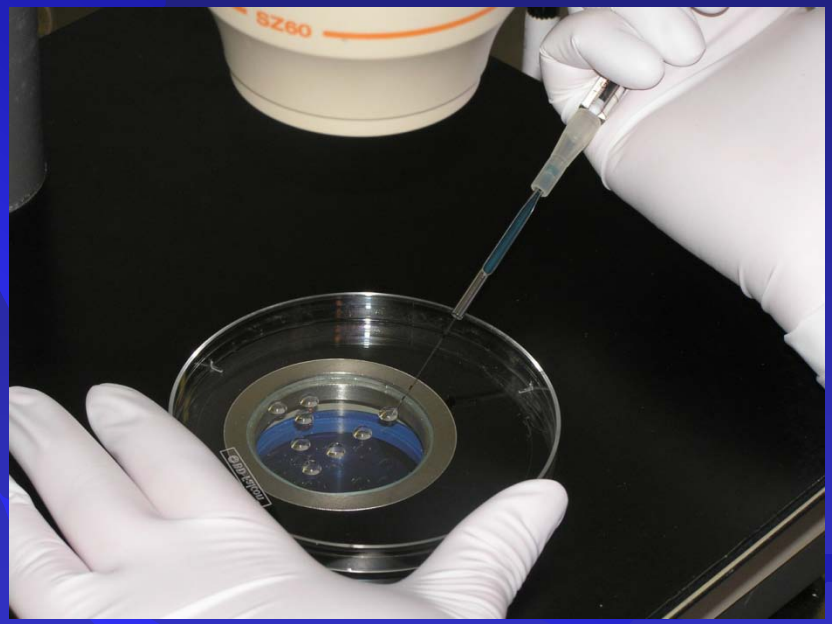
Vit Kit® - Freeze Starter
Each Kit Contains:
liquid, ready-to-use solutions
does not require CO_2 incubation

1 Vial of ES (Equilibration Solution)
* Contains 7.5% DMSO
* Contains 7.5% ethylene glycol
* Contains 20% DSS
* Contains gentamicin
* In a M-199 HEPES Buffered Medium

2 Vials of VS (Vitrification Solution)
* Contains 15% DMSO
* Contains 15% ethylene glycol
* Contains 20% DSS
* Contains 0.5 M sucrose
* Contains gentamicin
* In a M-199 HEPES Buffered Medium

Materials and Methods

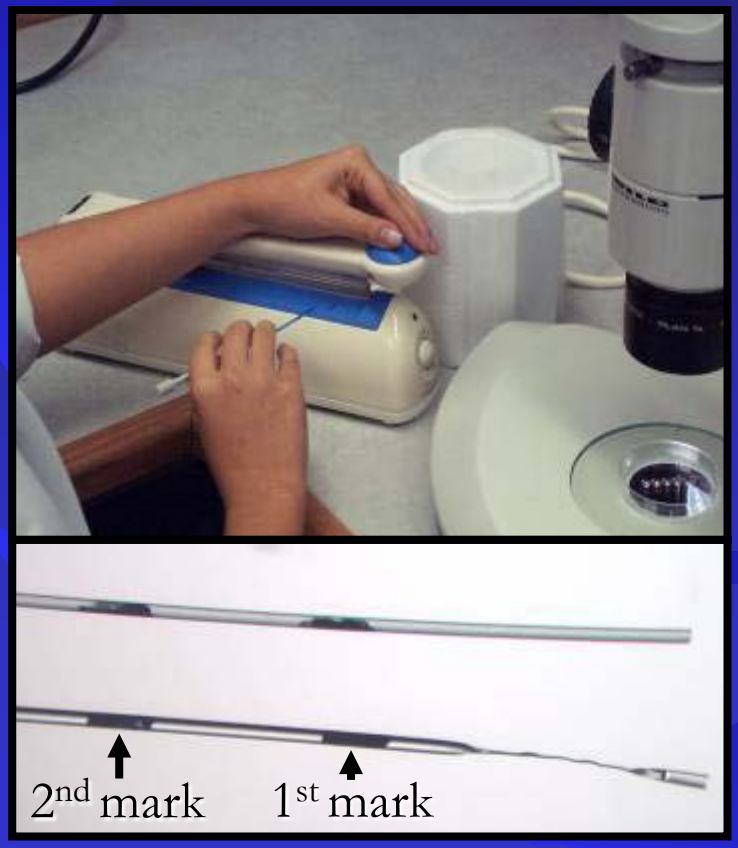
Vitrification Methods – Straw loading



1. Begin loading immediately after embryos in last drop
2. Medium to first mark, then embryo to 2nd mark
3. Continue loading medium to 3rd mark

Materials and Methods

Vitrification Methods – Straw sealing



1. Seal small end and check carefully
2. Seal large end and check carefully
3. If not sure about seal, reseal or load embryo into a new straw

Tip 2: Examine straw carefully after sealing

Materials and Methods

Vitrification Methods – Storage

Must keep embryos in N2 at all times

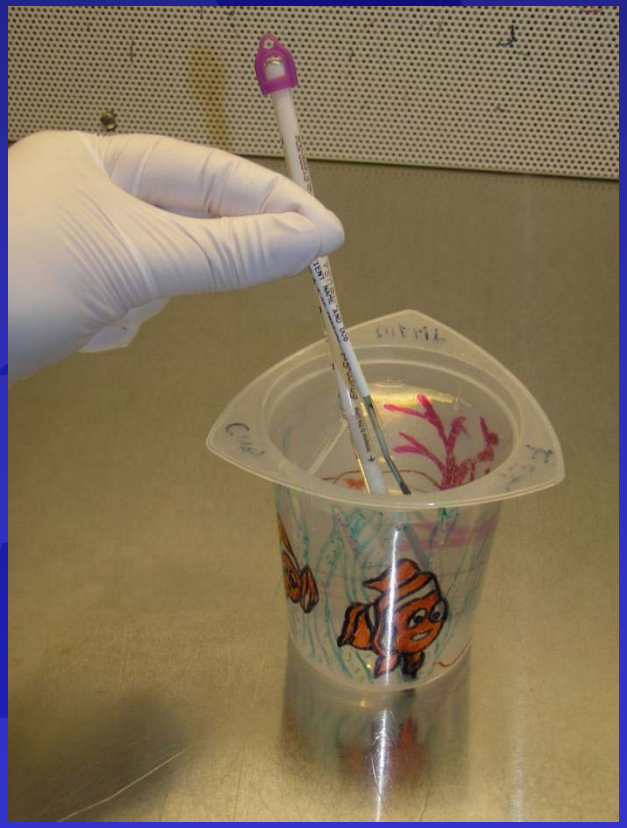
Have system where label can be read easily

Avoid shipping embryos if possible

Methods continued

Straw warming

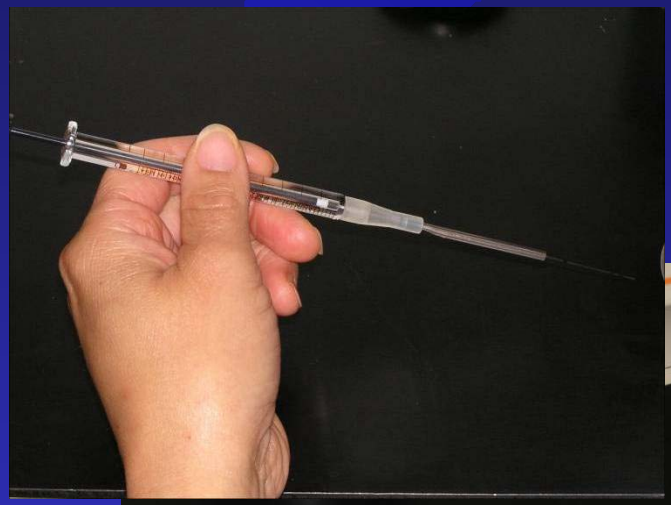
1. Check straw label while keeping straw submerged
2. Quickly transfer straw to water bath (37^0C)



Tip 3: Make sure you use a large water bath. Stir straw in.

Methods continued

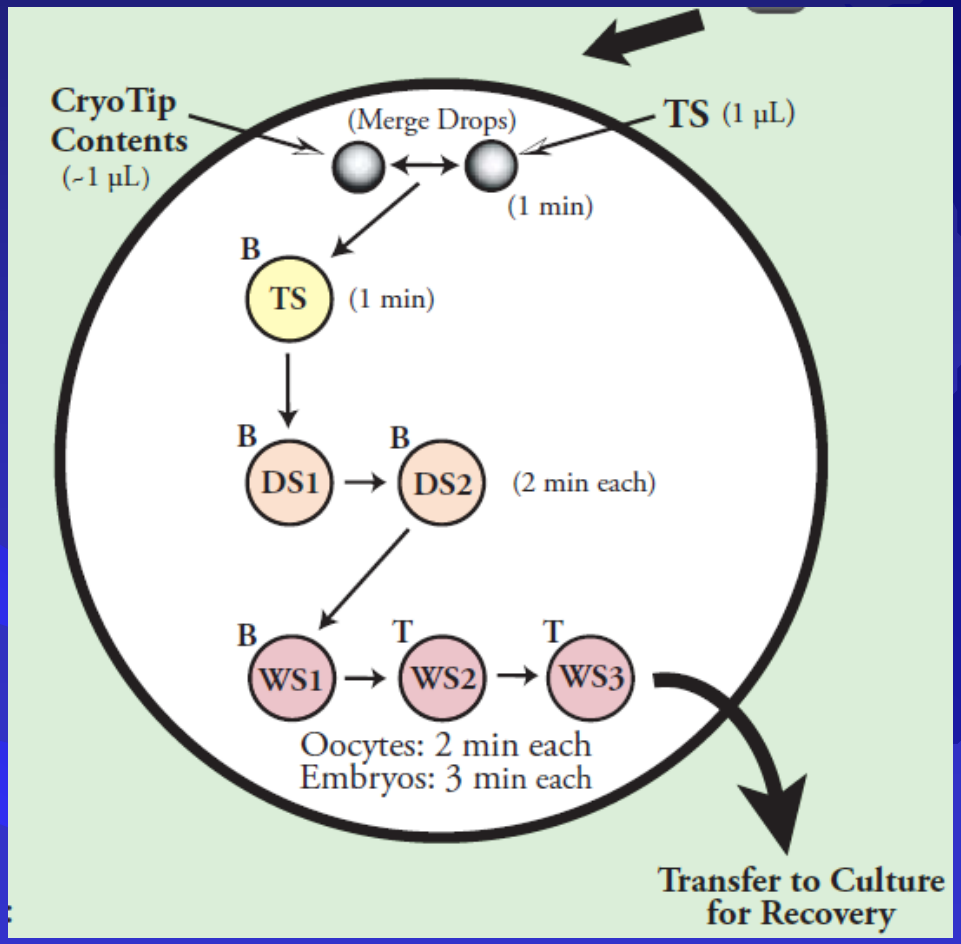
Straw unloading



1. Cut large end of straw and gently attach Hamilton syringe
2. Dry off fine end and cut just above seal with fine scissors
3. Gently expel contents onto plate surface

Materials and Methods

Vitrification Methods – Warming Procedures



Materials and Methods

Vitrification Methods – Warming Procedures

90137	**Vitrification Thaw Kit**
	Vit Kit® - Thaw for oocytes, embryos and blastocysts. Each Kit Contains: liquid, ready-to-use solutions does not require CO_2 incubation 2 Vials of TS (Thawing Solution) * Contains 1 M sucrose * Contains 20% DSS * Contains gentamicin * In a M-199 HEPES Buffered Medium 2 Vials of DS (Dilution Solution) * Contains 0.5 M sucrose * Contains 20% DSS * Contains gentamicin * In a M-199 HEPES Buffered Medium 2 Vials of WS (Washing Solution) * Contains 20% DSS * Contains gentamicin * In a M-199 HEPES Buffered Medium

Tip 4: Culture in medium with 20% DSS post warming

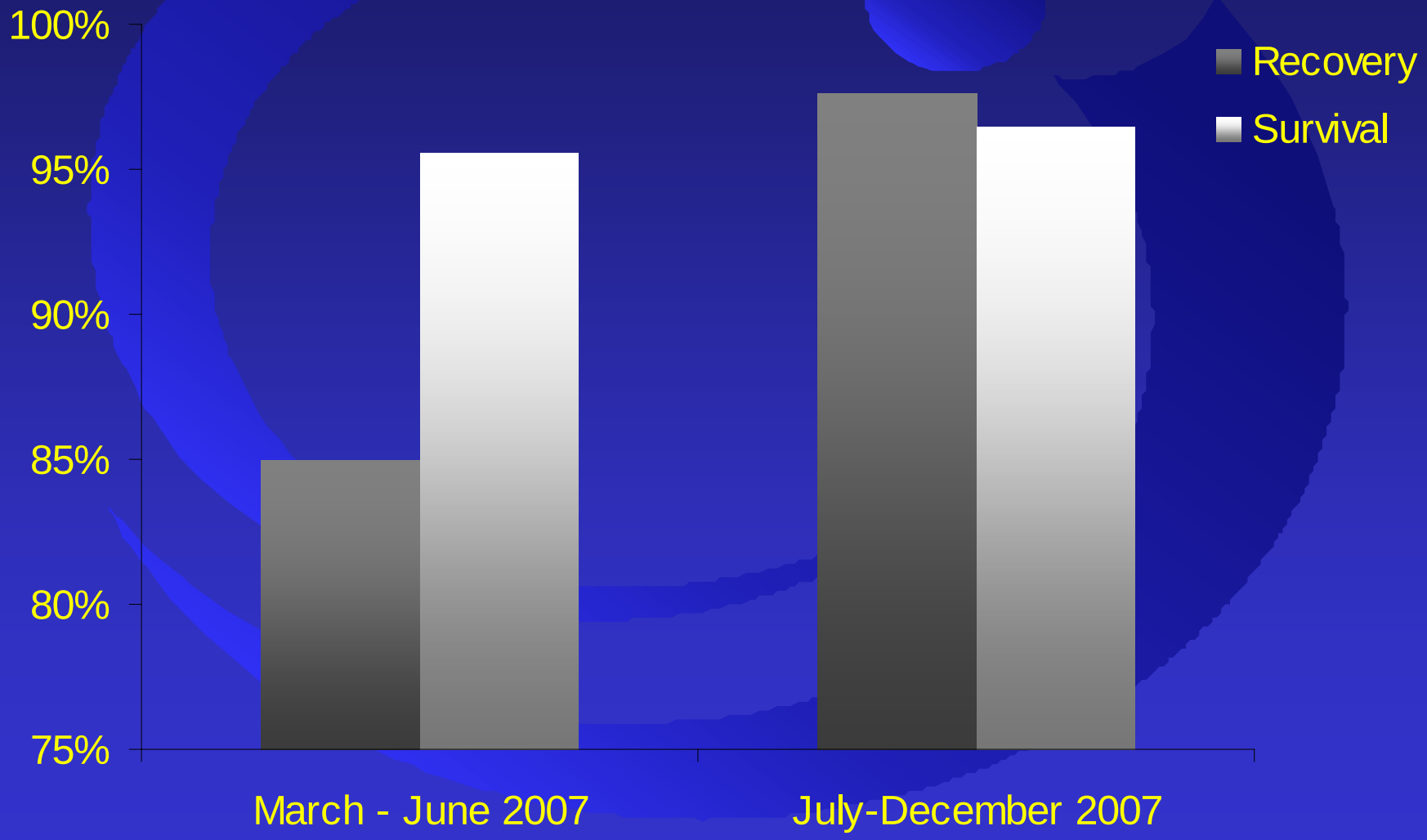
When to warm and transfer

Natural cycle	hCG	Day -1	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Controlled cycle		P4 Day 1	P4 Day 2	P4 Day 3	P4 Day 4	P4 Day 5	P4 Day 6	P4 Day 7
Day or date	Mon	Tue	Wed	Thur	Fri	Sat	Sun	Mon
2PN's				Thaw		ET		
D3's						Thaw/ ET		
D5 or D6							Warm/ ET	

Warming results: laboratory

Cycles	558
Embryos Warmed (mean)	1171 (2.09)
Embryos recovered	1116 (95%)
Embryos survived	1032 (92%)
Embryos transferred	1021 (1.8)

Early Results



Warming results: clinical

Cycles	558
Embryos Transferred (mean)	1,021 (1.8)
Clinical Pregnancies	243 (44%)
Sacs	312 (31%)
Twin Pregnancies	55 (23%)
Triplet Pregnancies	7 (3%)

Warming results: Clinical

Patient age	<35	35-37	38-40	>40	OD
Cycles	164	80	76	21	217
Pregnancies	90	31	27	7	88
Pregnancy rate	55%	39%	36%	33%	41%
Emb. Transferred (mean)	295 (1.8)	145 (1.8)	150 (2.0)	51 (2.4)	380 (1.7)
Sacs	112	35	35	10	120
Implantation rate	38%	24%	23%	20%	32%

Blastocyst transfers

Patient age	< 35	35-37	38-40	>40		Donor
Fresh Transfers	117	81	66	35		155
Pregnancies (ongoing)	48%	52%	30%	23%		68%[a]
Embryos transferred	1.6	2.05	2.58	2.7		1.4

Patient age	< 35	35-37	38-40	>40		Donor
Frozen Transfers	164	80	76	21		217
Pregnancies (ongoing)	55%	39%	36%	33%		41%[a]
Embryos transferred	1.8	1.8	2.0	2.4		1.8

a difference is significant ($p<0.0001$). Differences between other age groups are not significant

eSET 2008 and 2009

- 43% of donor oocyte recipients in 2008
- 60% of donor oocyte recipients in 2009
- 18% of < 35patients 2008
- 40% of < 35patients 2009

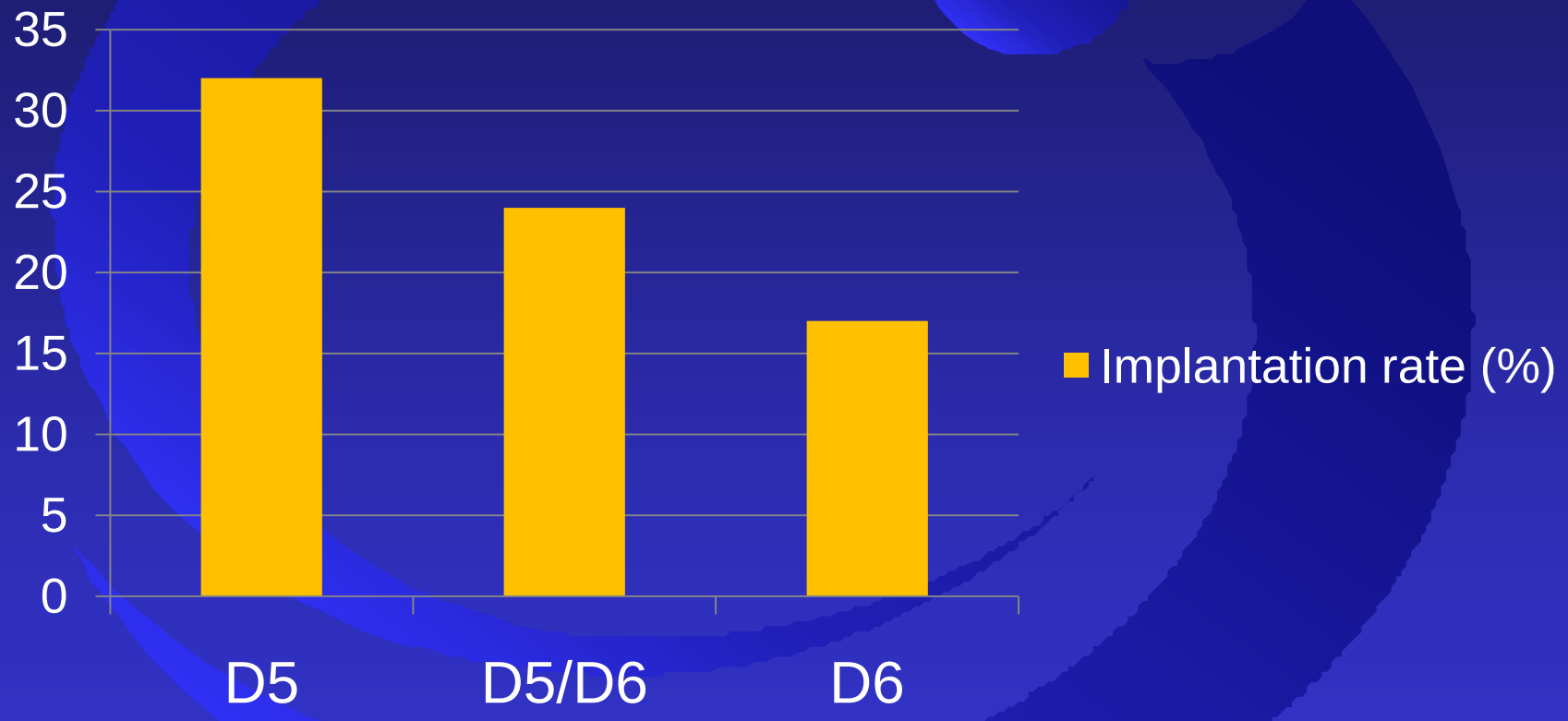
FET's: average number of embryos transferred in young patients:

1.9 in 2008

1.8 in 2009

D5 and D6 differences (OD)

Mean age = 43, n=177

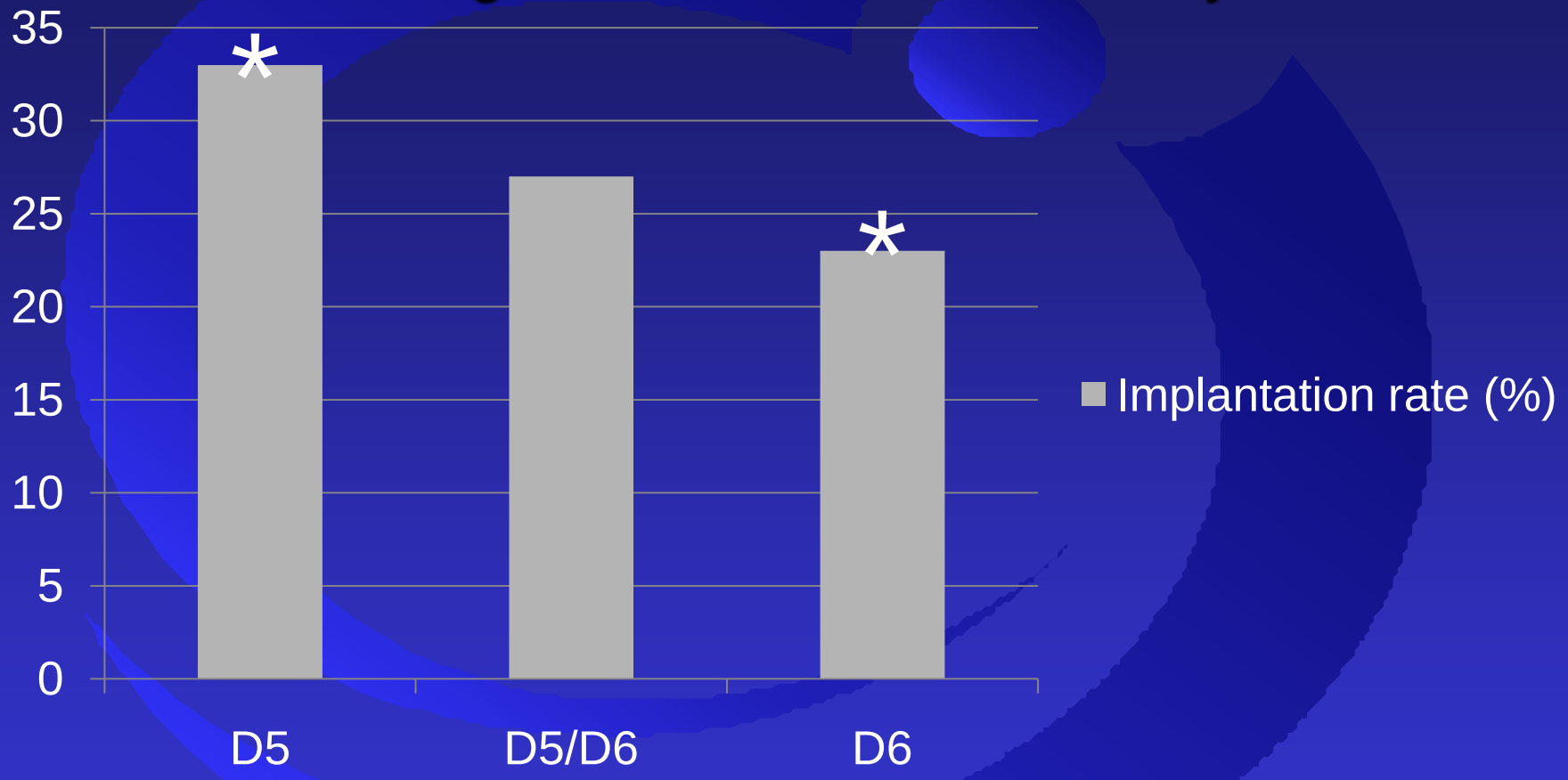


	D5 only	D5+D6	D6 only
Implantation/transfer	80/251 (32%)	8/33 (24%)	6/36 (17%)

Differences not significant (D5 vs. D6: p=0.07)

D5 and D6 differences

Mean age = 33, n=290, own oocytes



	D5 only	D5+D6	D6 only
Implantation/transfer	104/318 (33%)	29/107 (27%)	29/128 (23%)

* p= 0.03

D5 embryos do better

For patients <35 using own oocytes:

PR = 58% (51/88)

Mean of 1.8 embryos/FET

IR = 41% (62/153)

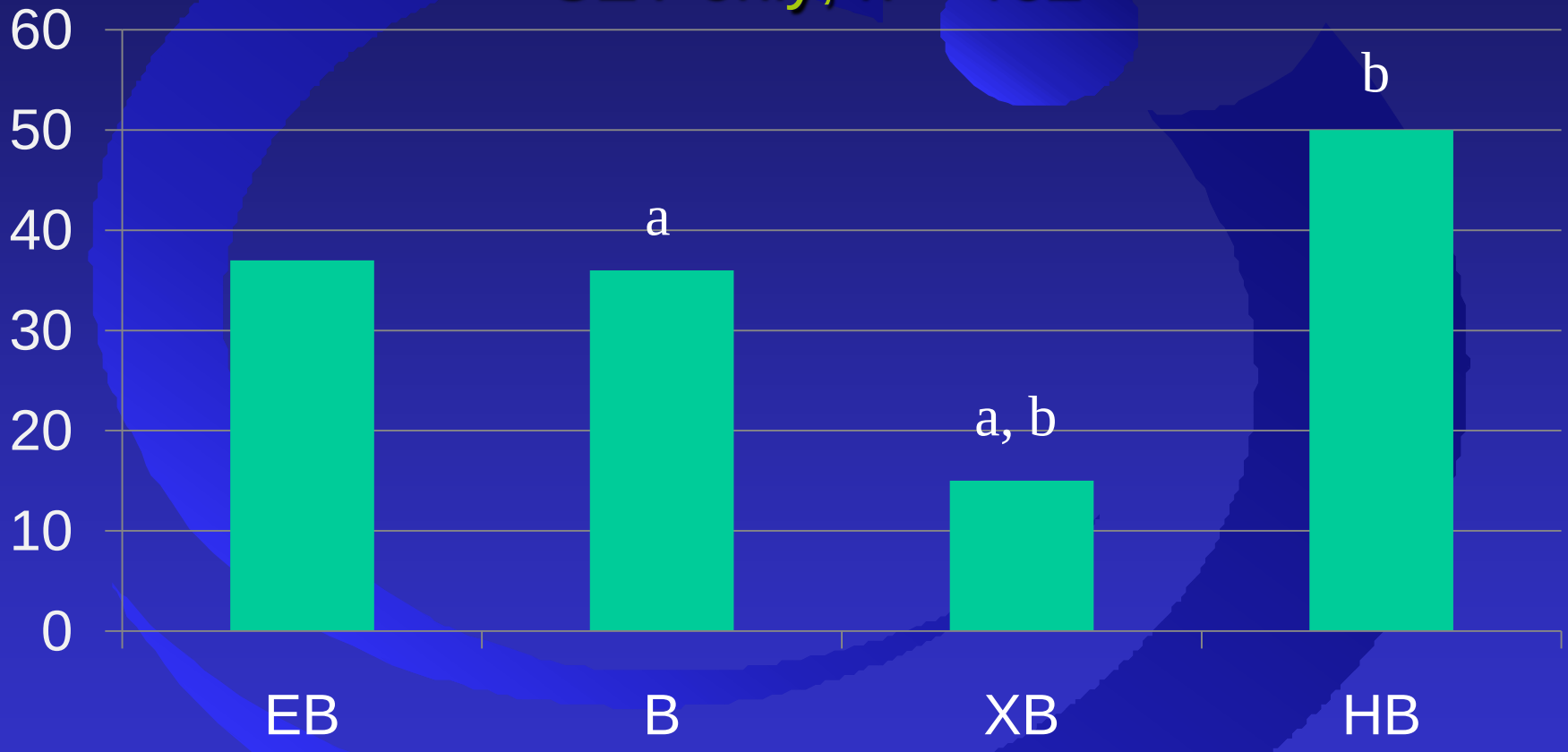
9 x twin, 1 x triplet (20% multiples)

Blastocyst survival

- Blastocysts look very nice during and immediately after warming.
- Most ET's done within 1 hour of warming
- Culture and ET in 20% SSS

Implantation by stage

SET only, n = 182

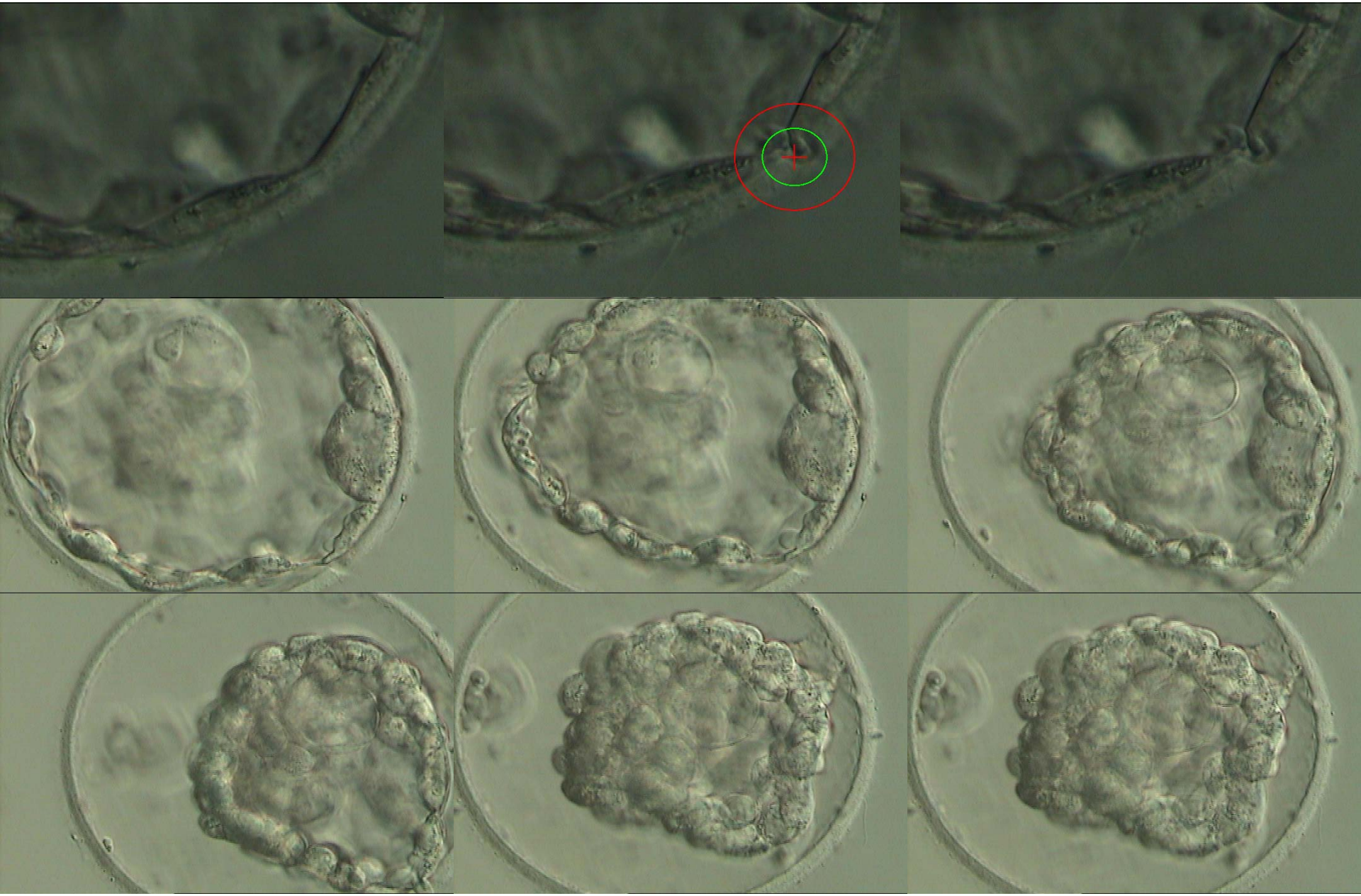


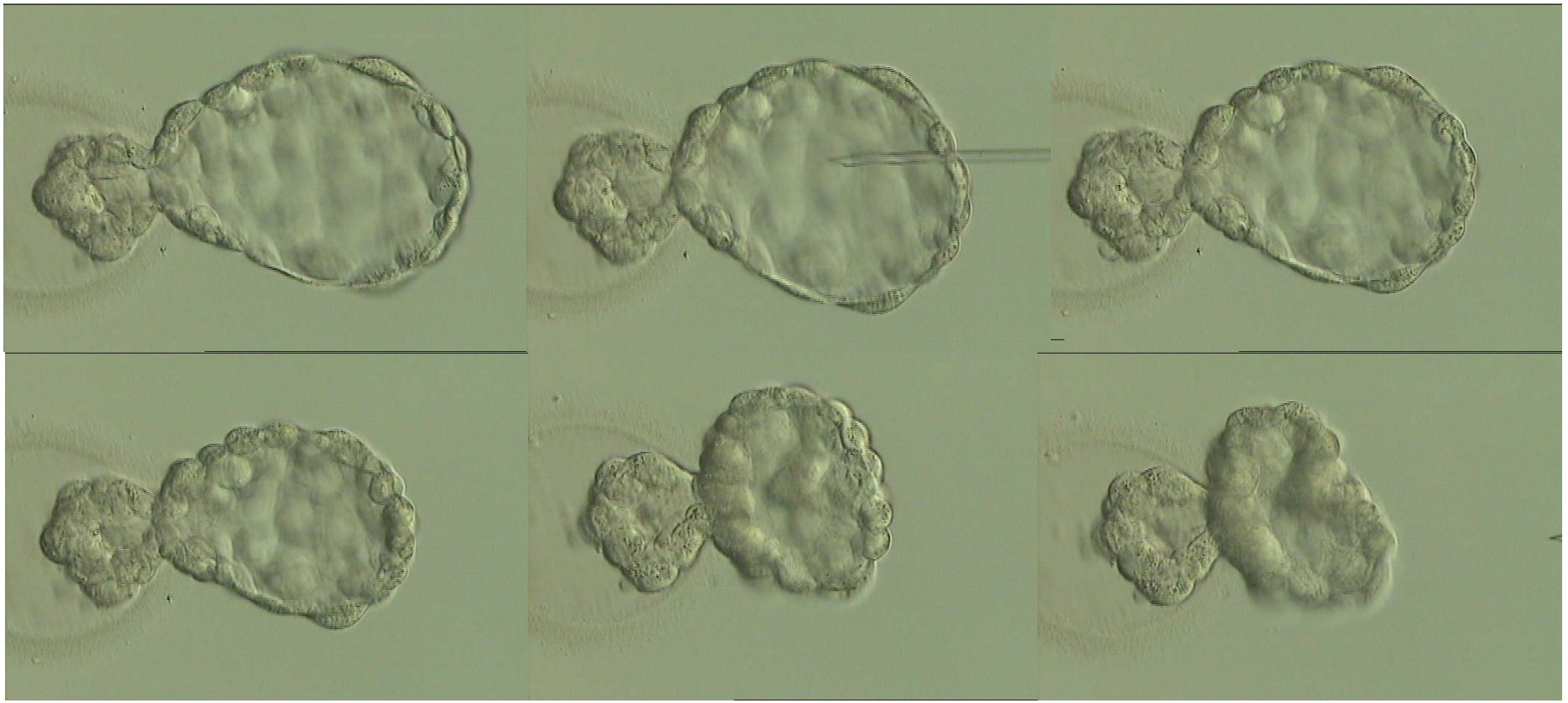
Early Blastocyst	Blastocyst	Expanded	Hatching
7/19	43/120	4/27	8/16

a, p = 0.04 and b, p = 0.03

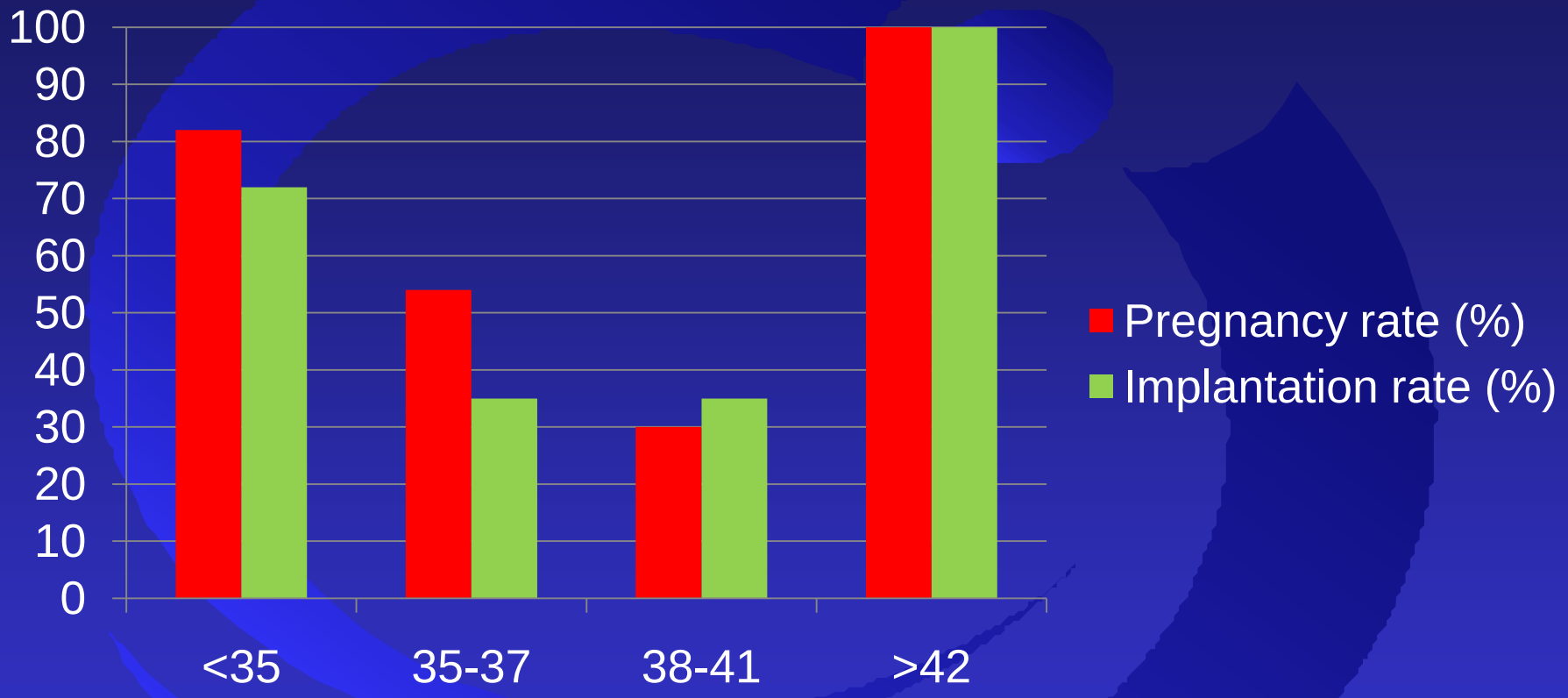
Artificial collapse of blastocysts

- Blastocysts that do not survive warming usually come out of straw fully expanded
- Collapsing will eliminate this problem
- Implemented summer 2009





Artificial Collapse



	<35	35-37	38-41	≥42
Pregnancy	14/17	7/13	3/10	1/1
Implantation	15/20	7/20	7/20	1/1
Av. transferred	1.2	1.5	2	1

The story so far

1. The CryoTip and VitKit provide a reliable method for blastocyst preservation
2. With 558 cycles completed, we have a 44% pregnancy and 31% implantation rate
3. Collapsing results look promising
4. We are doing a lot of eSET's

Where are we in 2010

1. 3 years experience
2. All 6 embryologists vitrifying and warming
3. Very loose on what we will vitrify
4. Collapsing most embryos
5. Still reducing the number of embryos transferred

Blastocyst transfers
Frozen cycle outcomes 2010

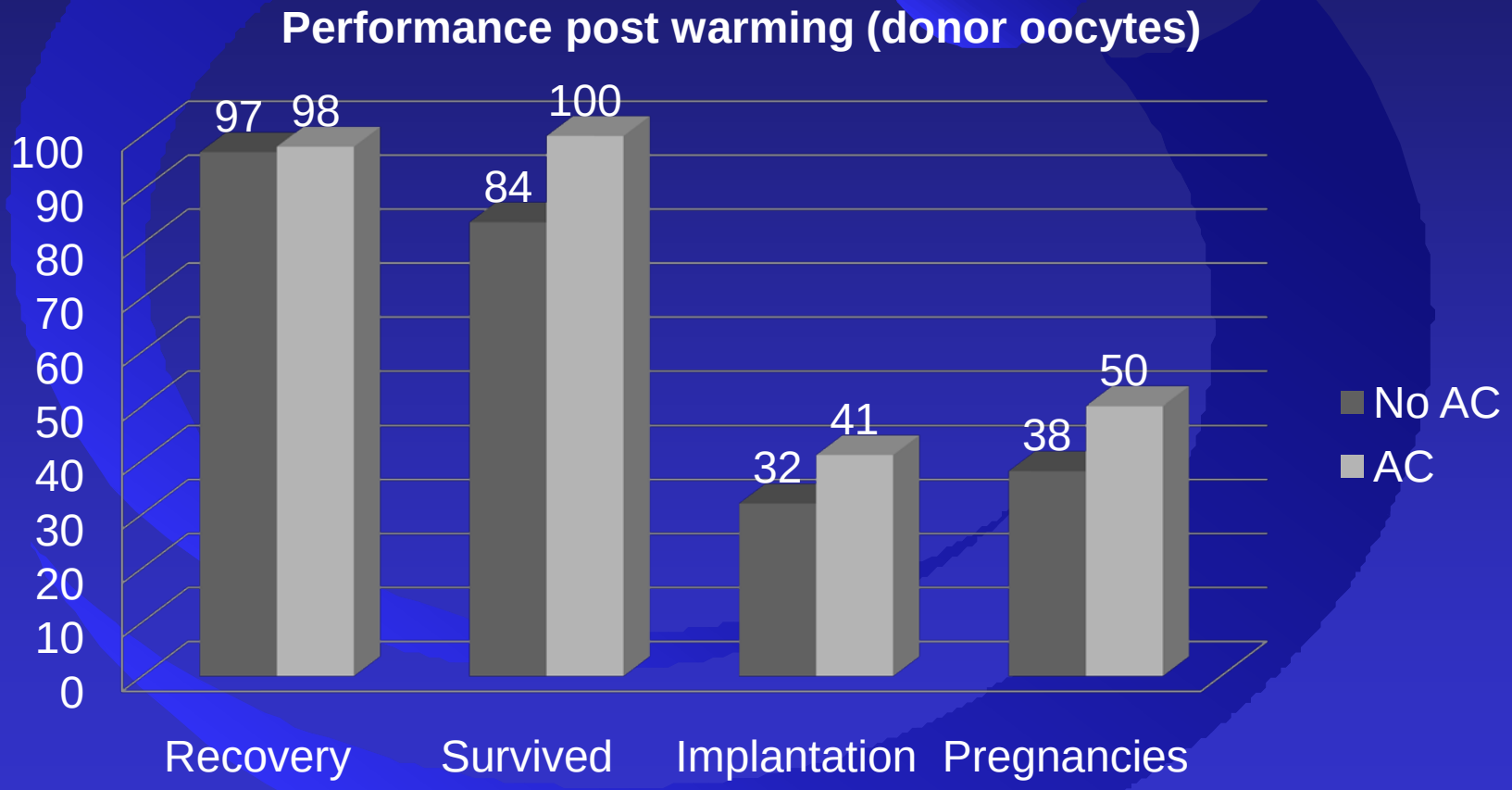
FET Cycles	158	
Embryos warmed	274	1.7 per pt.
Recovered	269	98%
Survived	245	91%
Transferred	244	1.5 per pt.
Implanted	131	38%

Much more aggressive about warming another embryo when 1st one has some dead cells

158 Blastocyst Transfers
Frozen cycle outcomes 2010

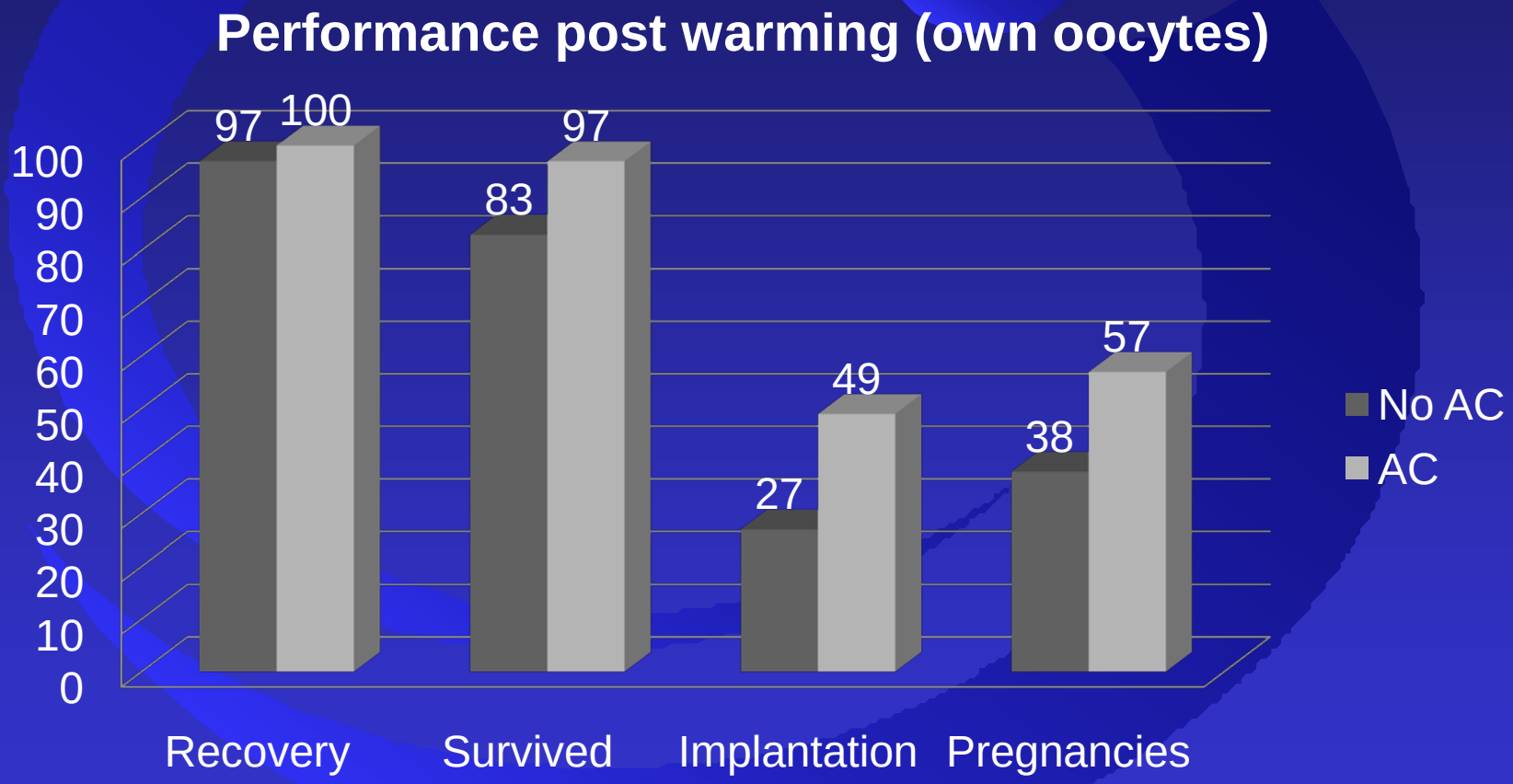
Patient age	< 35	35-37	38-40	> 40		Donor
Number of Transfers	46	23	16	5		68
Pregnancies (clinical)	59%	57%	25%	20%		44%
Embryos transferred	1.4	1.5	1.5	1.8		1.4

Does assisted collapse help?
Frozen cycle outcomes 2010



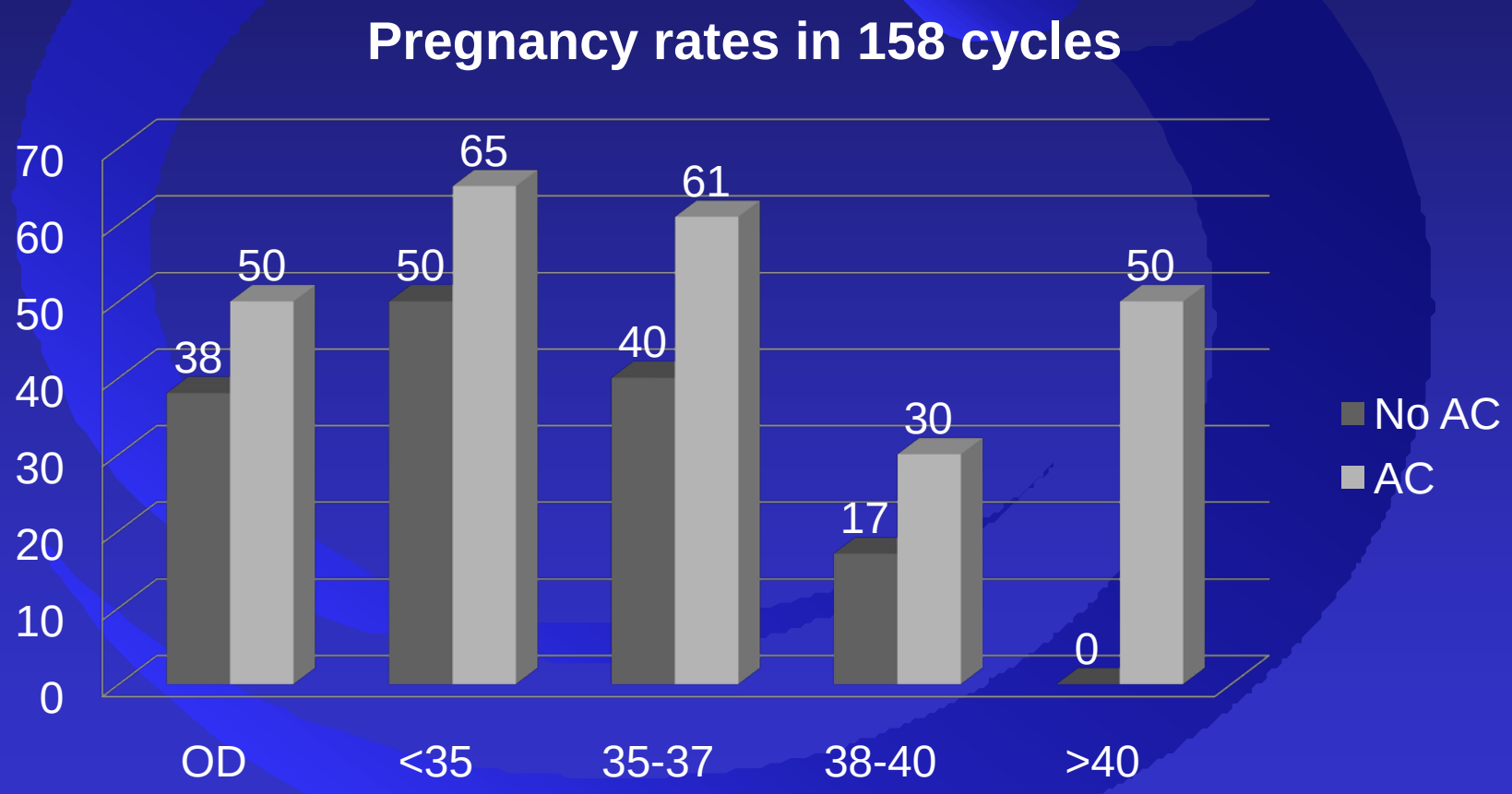
n = 58 for “no AC” group and 52 for “AC” group

Does assisted collapse help?
Frozen cycle outcomes 2010



n = 71 for “no AC” group and 93 for “AC” group

Clinical FET outcomes 2010



Game plan: Freezing

1. Aggressively vitrifying early blastocysts
2. Fairly “loose” in what we will vitrify
3. Collapsing any blastocysts that we can
4. Only one embryo/straw
5. Results continuing to improve

Game plan: Thawing

1. Aim is to thaw and transfer 1
2. Young patients, D5 embryos, collapsed
3. Thaw 30-60 mins prior to FET
4. Culture and transfer in 20% SSS
5. Type of cycle not a concern

Outcomes of natural cycles vs. programmed cycles for 1677 frozen embryo transfers." Givens CR, Markun LC, Ryan IP, Chenette PC, Herbert CM, and Schriock ED. Reprod Biomed Online, 2009 Sept, 19(3): 380-384

Discussion-tips and tricks

- Examine straws carefully
- One embryo per straw
- Consider collapsing exp. blastocysts
- Use >500 ml water bath for warming
- 20% SSS in post warm culture media
- Warm/ET on day 4/5 (P4 day 6/7)