POOR OVARIAN RESPONSE (POR)

IS OOCYTE CRYOPRESERVATION A PREVENTIVE TOOL FOR PATIENT AT RISK OF POR?

L. Gianaroli, S. Resta, M.C. Magli, A.P. Ferraretti

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Oocyte cryopreservation – Clinical applications

entails potential advantages for human IVF

→ It is a less ethically disputable alternative to embryo cryopreservation

→ It could solve the dilemma of abandoned frozen embryos in the IVF laboratory

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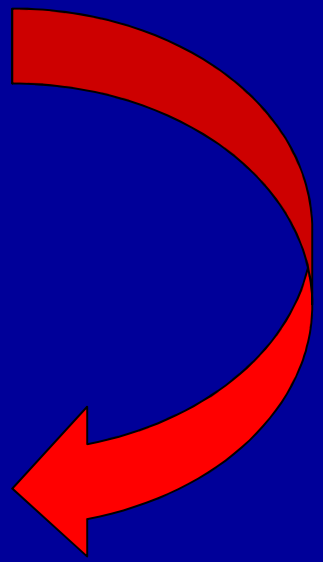
Oocyte cryopreservation – Clinical applications

It gives an opportunity for fertility preservation



to women at risk of premature ovarian failure

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Oocyte cryopreservation
Indications to fertility preservation

- Tumors
- Genetic factors (fragile X)
- Autoimmune diseases
- Chromosomal abnormalities (deletions, Turner's syndrome)
- Endometriosis
- Recurrent ovarian cysts

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PGD FOR ANEUPLOIDY

508 patients
Normal karyotype → 714 cycles → 13 16 18 21 22 X Y 15

Indication to PGD	No. cycles	Mean age	Previous cycles
Maternal age (≥ 36 years)	567	39.9±2.6	2.5±2.5
Repeated cycles (≥ 3)	128	32.7±2.1	4.1±1.6
Recurrent abortions (≥ 3)	19	31.9±1.9	1.9±1.9

Updated from: Gianaroli et al. (2000) Gonadal activity and chromosomal constitution of in vitro generated embryos.
Molec Cell Endocrinol 161, 111-116

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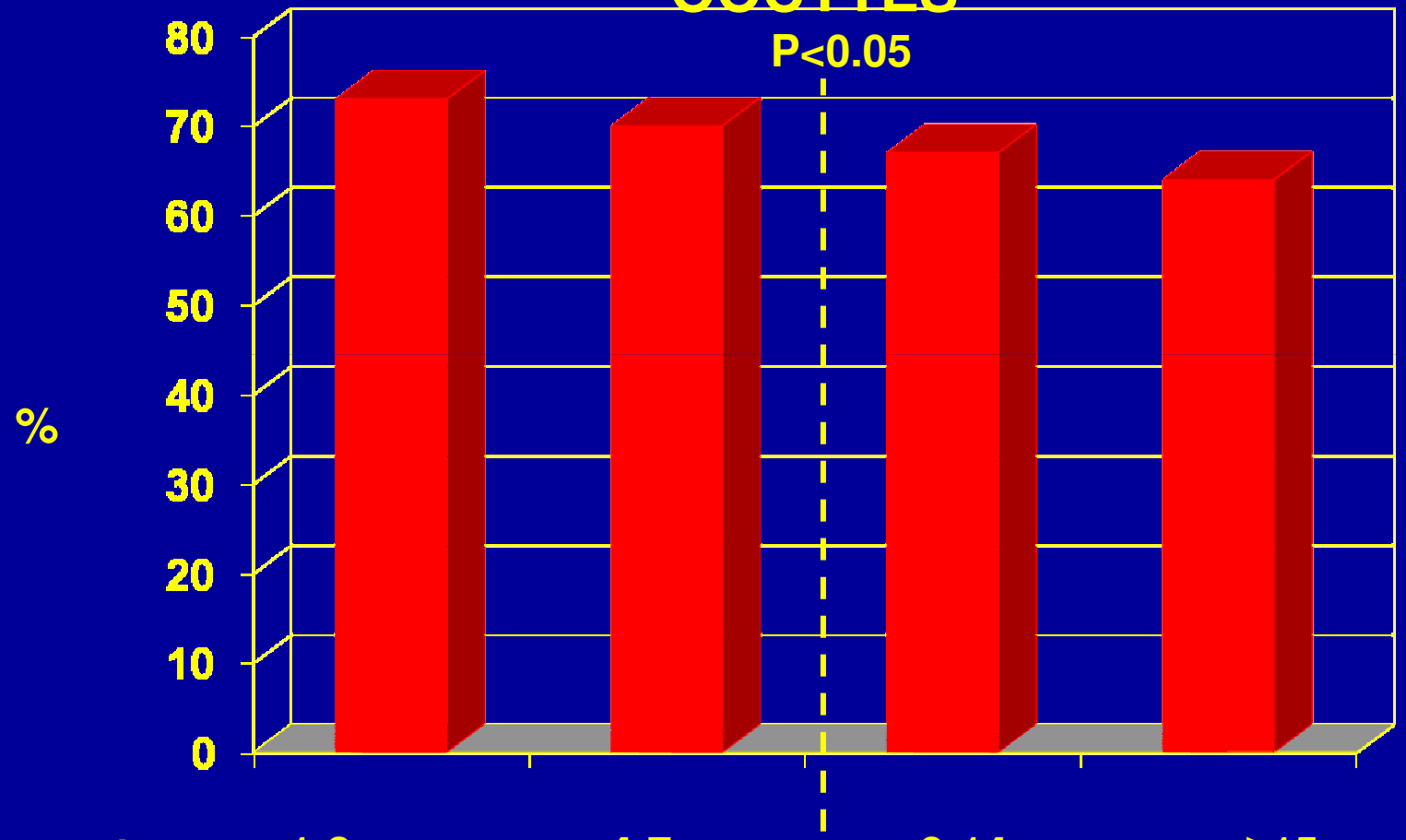
PGD FOR ANEUPLOIDY

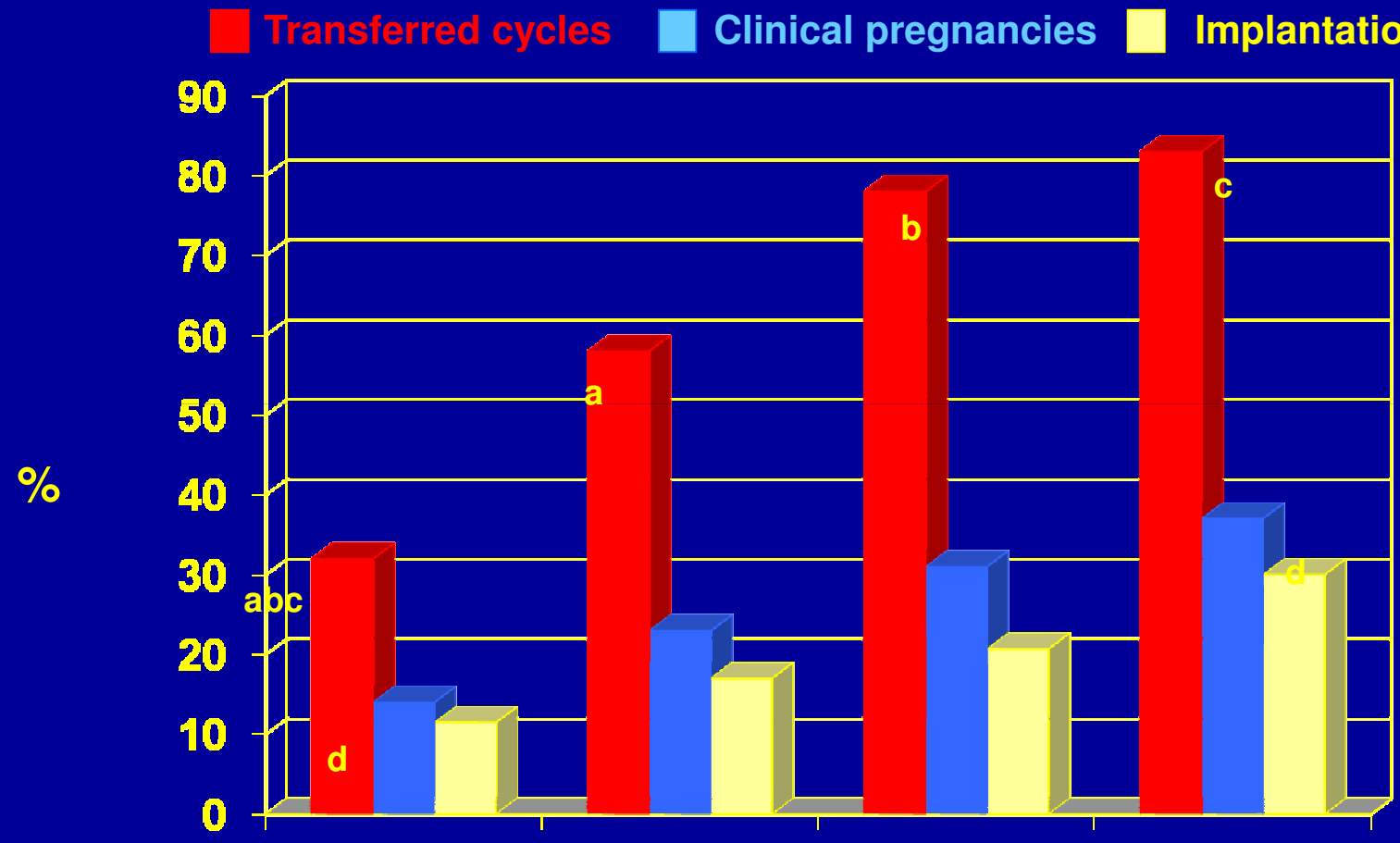
CHROMOSOMALLY ABNORMAL EMBRYOS ACCORDING TO THE NUMBER OF COLLECTED OOCYTES



No. oocytes	1-3	4-7	8-14	≥15
Maternal age	41.4±3.7[abc]	38.4±3.7[a]	38.1±3.7[b]	37.8±4.1[c]

[abc]P<0.001

PGD FOR ANEUPLOIDY
CLINICAL OUTCOME ACCORDING TO THE NUMBER OF COLLECTED OOCYTES



$^{abc}P<0.001$

$^{d}P=0.03$

No. oocytes	1-3	4-7	8-14	≥15
Maternal age	41.4 ± 3.7^{abc}	38.4 ± 3.7^{a}	38.1 ± 3.7^{b}	37.8 ± 4.1^{c}

$^{abc}P<0.001$

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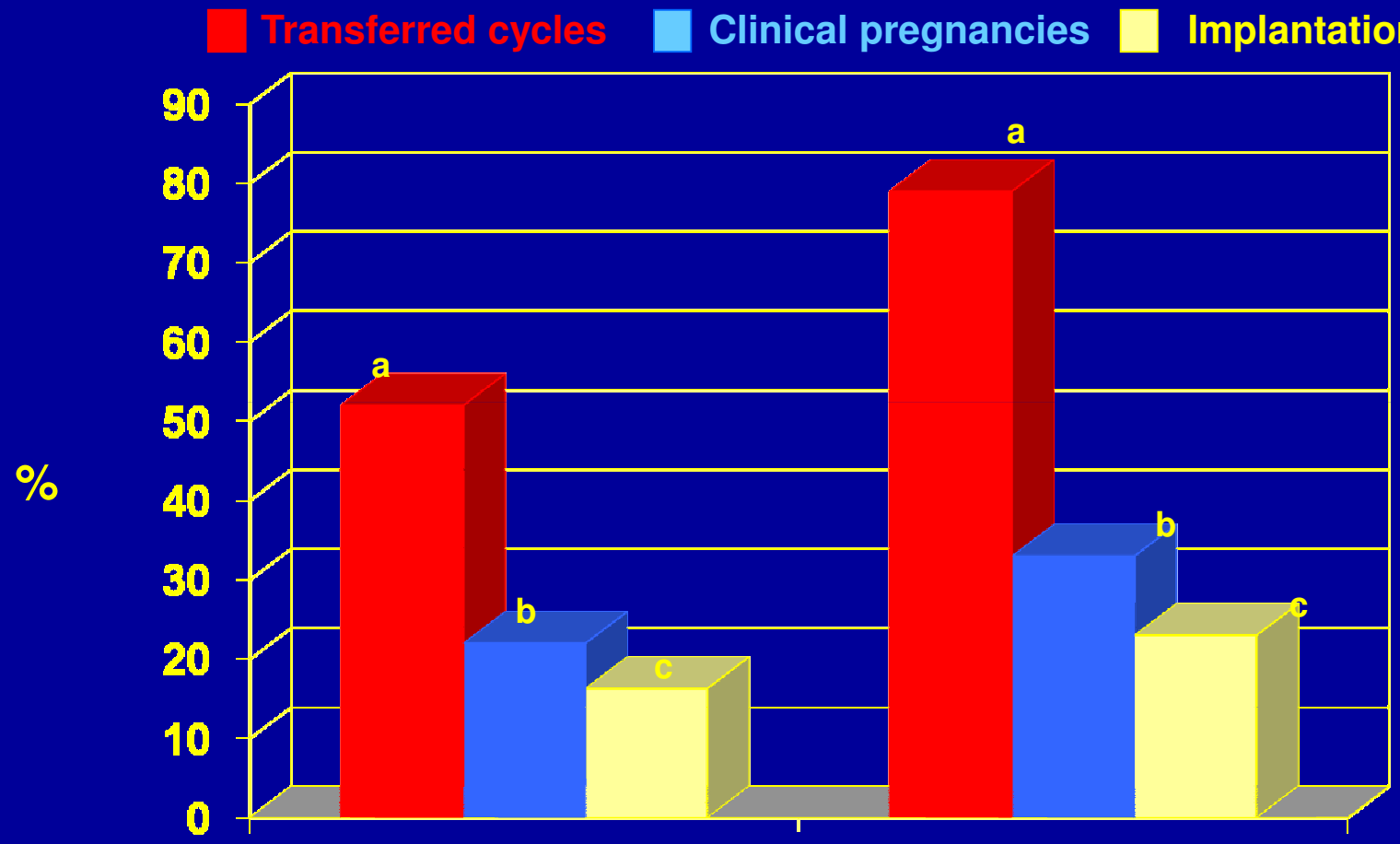
PGD FOR ANEUPLOIDY
CLINICAL OUTCOME ACCORDING TO THE NUMBER OF COLLECTED OOCYTES



$^{a}P<0.001$

$^{b}P<0.01$

$^{c}P=0.05$

No. oocytes	1-7		8≥15	
Maternal age	41.4 ± 3.7^{abc}	38.4 ± 3.7^{a}	38.1 ± 3.7^{b}	37.8 ± 4.1^{c}

$^{abc}P<0.001$

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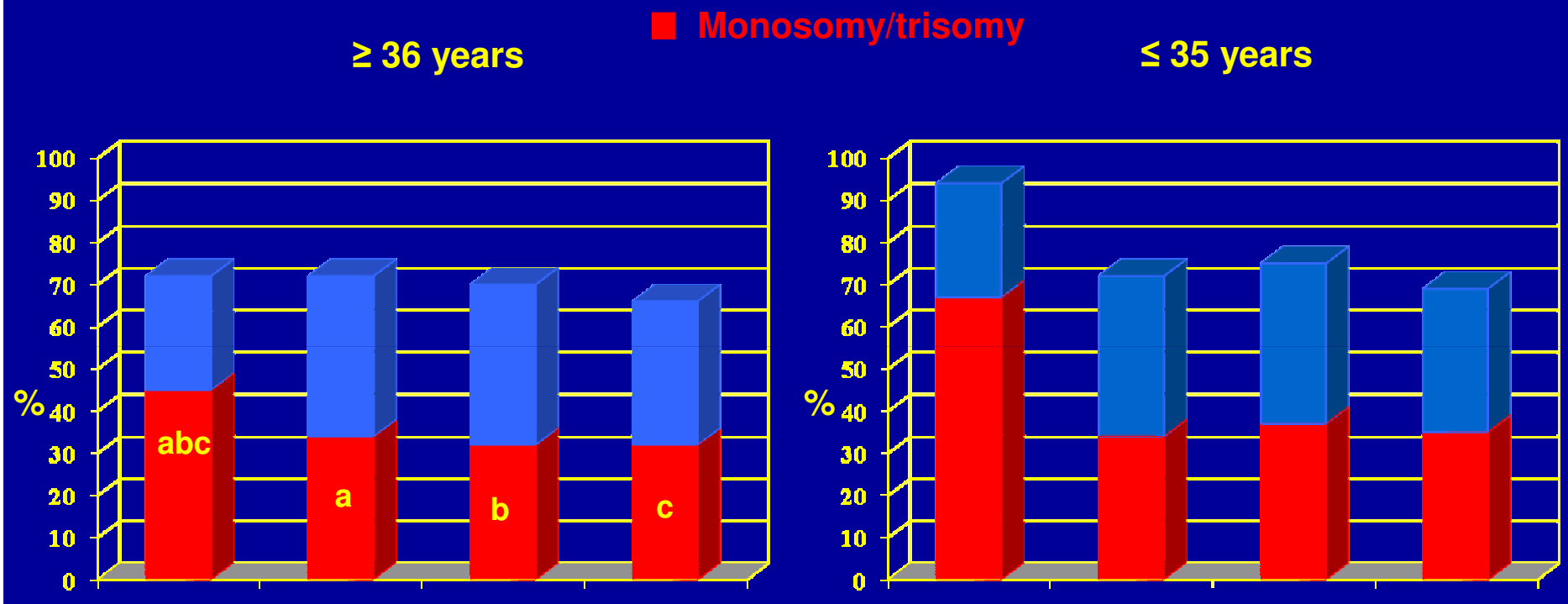
PGD FOR ANEUPLOIDY

CHROMOSOMALLY ABNORMAL EMBRYOS ACCORDING TO THE NUMBER OF COLLECTED OOCYTES AND AGE



No. oocytes	1-3	4-7	8-14	≥15
No. cycles	62	163	273	69

No. oocytes	1-3	4-7	8-14	≥15
No. cycles	4	38	75	30

[a]P<0.05 [b]P<0.005 [c]P<0.01

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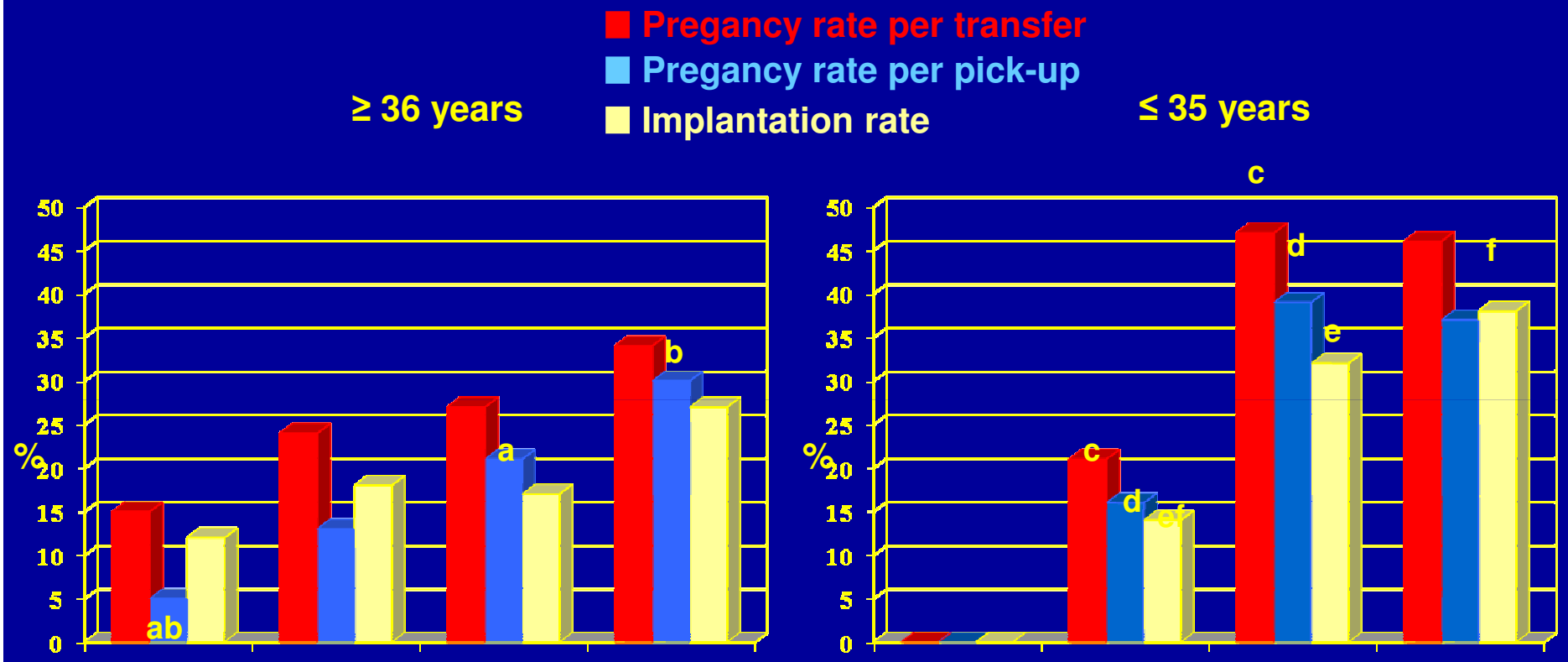
PGD FOR ANEUPLOIDY

CLINICAL OUTCOME ACCORDING TO THE NUMBER OF COLLECTED OOCYTES AND AGE



	1-3	4-7	8-14	≥15
No. oocytes				
No. cycles	62	163	273	69

	1-3	4-7	8-14	≥15
No. oocytes				
No. cycles	4	38	75	30

[a]P<0.005 [b]P<0.001 [c]P<0.05 [d]<P0.025 [ef]P=0.005

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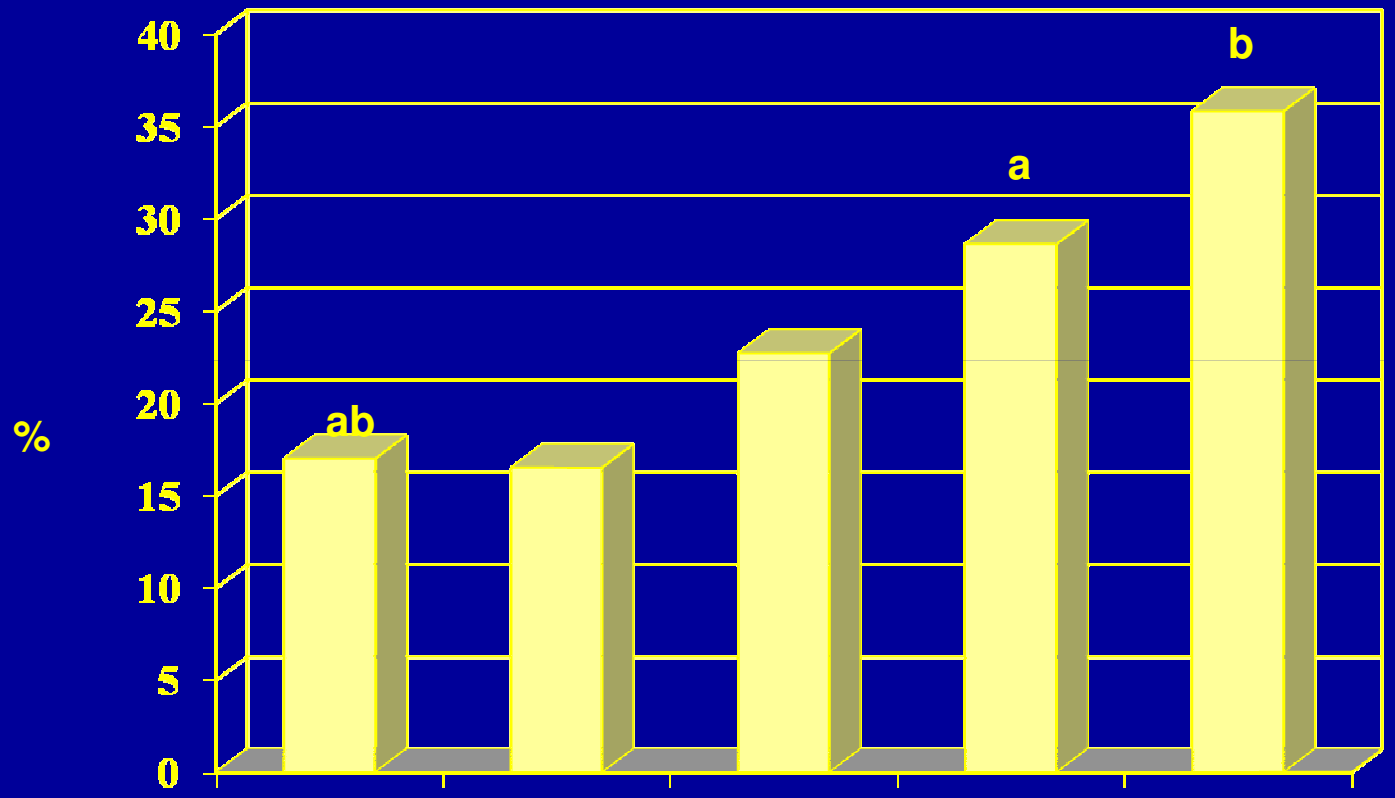
PGD FOR ANEUPLOIDY

IMPLANTATION RATE ACCORDING TO THE NUMBER OF EUPLOID EMBRYOS



[a]P<0.05 [b]P<0.005

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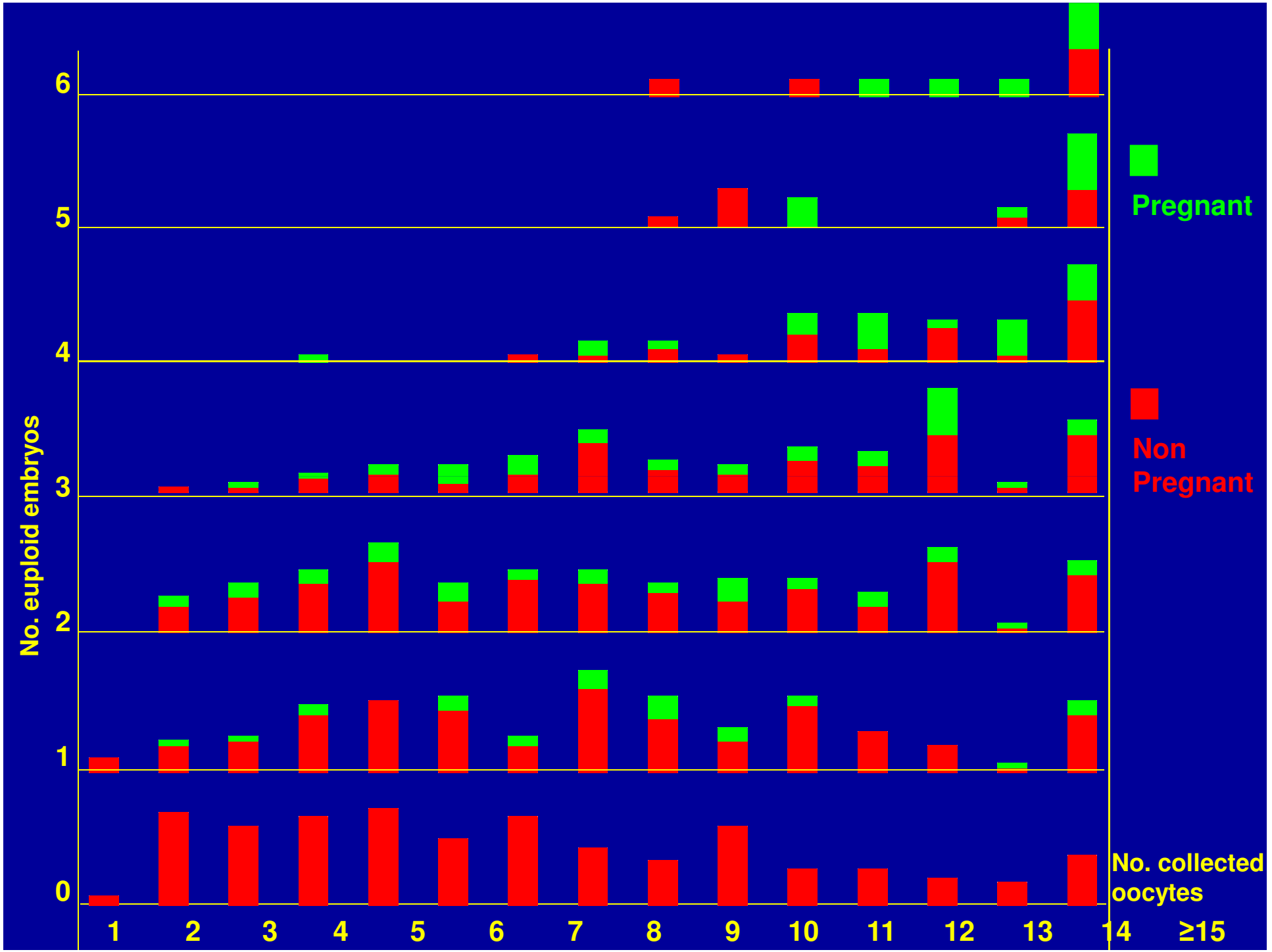
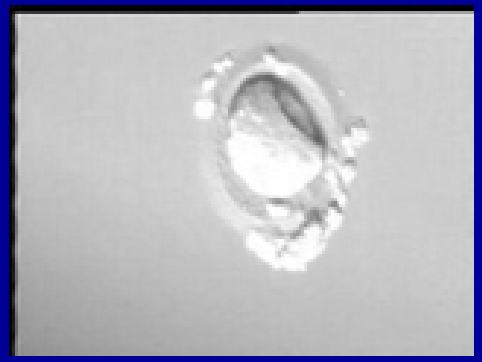
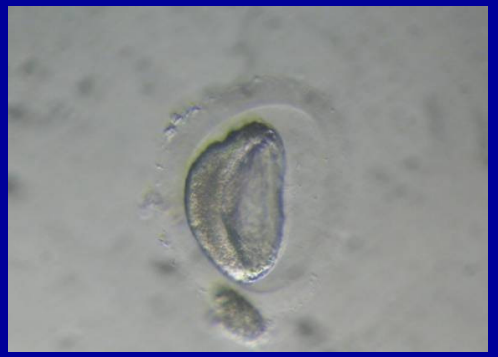

No. euploid embryos
6
5
4
3
2
1
0
Pregnant
Non Pregnant
No. collected oocytes
1
2
3
4
5
6
7
8
9
10
11
12
13
14
≥15

Oocyte Cryopreservation Methods:

– Slow Freezing



– Ultrarapid Freezing (Vitrification)



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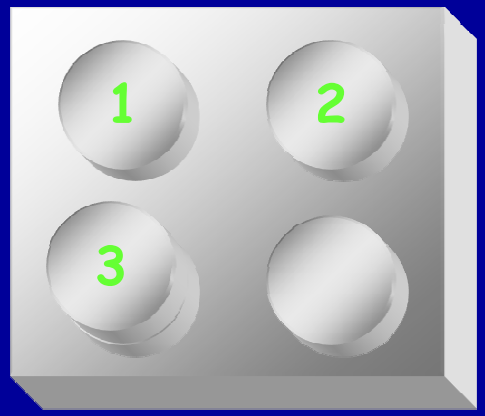
Slow-freezing/Rapid thawing



4-well dish

1 = Washing solution

PBS + 30% Plasmanate

2 = Equilibration solution (10 min)

1.5 M PROH + 30% Plasmanate

3 = Loading solution

1.5 M PROH + 0.3 M SUC + 30 % Plasmanate

Thawing solutions consist in a gradually decreasing concentration of PROH and a constant 0.3 M sucrose concentration:

SOLUTION 1: 1.0 M PROH + 0.3 M SUC + 30% Plasm. (5 min)

SOLUTION 2 : 0.5 M PROH + 0.3 M SUC + 30% Plasm. (5 min)

SOLUTION 3 : 0.3 M SUC + 30 % Plasm. (10 min)

SOLUTION 4 : PBS + 30 % Plasm. (10 min + 10 min at 37°C)

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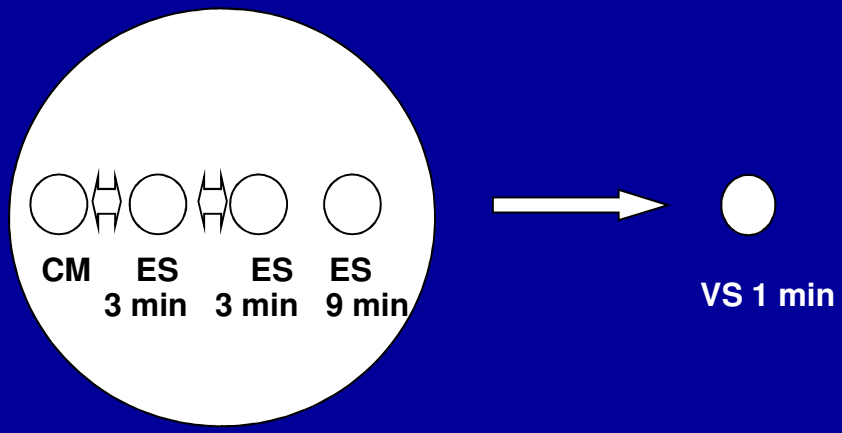
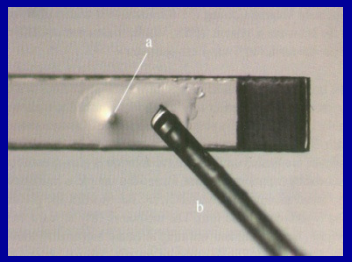
Vitrification (Kuwayama method)



Equilibration Solution (ES):
7,5% DMSO
7,5% ETHYLENE GLYCOL
10% HSA

Vitrification Solution (VS)
15% DMSO
15% ETHYLENE GLYCOL
10% HSA
0.5 M Sucrose



Re-warming

Thawing Solution (TS): 1 M Sucrose + 10 % HSA at 37°C for 1 min

Dilution Solution 1 (DS1): 0.5 M Sucrose + 10 % HSA for 3 min

Dilution Solution 2 (DS2): 0.25 M Sucrose + 10 % HSA for 3 min

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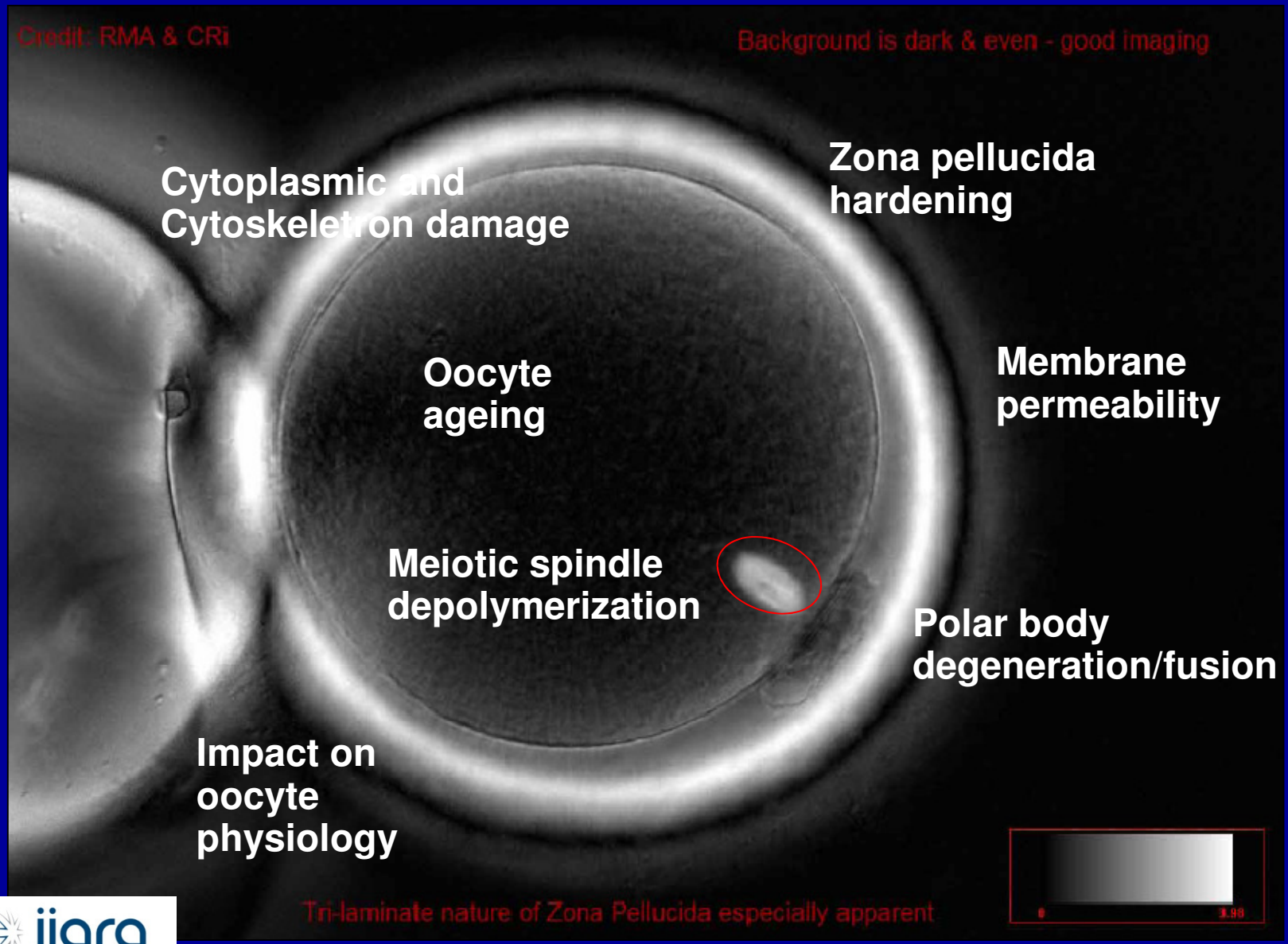
Oocyte cryopreservation procedures

	Slow freezing	Vitrification
CPA concentration	1.5 M	3.0-5.0 M
Volume	0.3-1.0 mL	<1 µL
Cooling rate	~0.5°C/min	~25000-50000°C/min
Reduced osmotic injury	No	Yes
Seeding	Yes	No need
Procedure	Time consuming	Simple
Freezing machine	Required	No need
Costs	High	Less??? (no freezing machine needed, but expensive handling devices)
Liquid nitrogen	High amount	Low amount
Risk of contamination	Close systems	Open systems

Oocyte cryopreservation – Possible injuries



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Is oocyte cryopreservation efficient enough to be employed as a method to preserve female fertility?

In the last few years advances in cryopreservation methodologies have dramatically improved the efficiency of oocyte cryopreservation.

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Slow-freezing
(1.5 M PROH + 0.3 M Sucrose)

Authors	Oocytes		Pregnancies/ ET (%)
	N° Survived/ N° Thawed (%)	Fertilization Rate %	
Chen et al., Hum. Rep. (2002)	8/8 (100)	57	1/1 (100)
Fosas et al., Hum. Rep. (2003)	79/98 (90)	73	4/7 (57)
Porcu , Hum. Rep.(2005)	1914/2750 (69.6)	73.9	85/501 (17)
Bianchi et al., RBM Online (2007)	306/403 (75.9)	76.2	17/80 (21.3)
Magli et al., Fertil. Steril. (2010)	726/997 (73)	73	37/203 (18)

Vitrification

Authors	Oocytes		Pregnancies/ ET (%)
	N° survived/ N° Thawed (%)	Fertilization Rate %	
Kuleshova et al., Hum. Rep. (1999)	11/17 (65)	45	1/3 (33)
Katayama et al., Fertil. Steril. (2003)	42/46 (94)	91	2/6 (33)
Yoon et al., Fertil. Steril. (2003)	325/474 (69)	72	6/28 (21)
Kuwayama et al., RBM Online (2005)	58/64 (91)	90	12/29 (41)
Selman et al., Fertil. Steril. (2006)	18/24 (75)	78	2/6 (33)
Antinori et al., RBM Online (2007)	328/330 (99.4)	92.9	39/120 (32.5)

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Oocyte cryopreservation – SISMER experience

1999 : research on vitrification as a valid alternative to slow-freezing

Human Reproduction, Vol. 14, No. 12, 3077-3079, December 1999

Birth following vitrification of a small number of human oocytes: Case Report

Lilia Kuleshova[1], Luca Gianaroli[2], Cristina Magli[2], Anna Ferraretti[2] and Alan Trounson[1,3]

[1] Centre for Early Human Development, Monash Institute of Reproduction and development, Monash University, Wright Street, Clayton, Victoria, Australia and [2] SISMER srl , Bologna, Italy

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Oocyte cryopreservation – SISMER experience

March 2004 – Introduction of the Italian law that limits to three the number of oocytes to be inseminated:

the sudden need to cryopreserve oocytes adviced to choose slow-freezing as the most reliable method.

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Oocyte cryopreservation – SISMER experience

A cautious approach to vitrification was decided by applying this technique only when appropriately verified. The program provided the use of

1) Discharged oocytes
2) Sybling oocytes in patients with more than 10 oocytes
3) Alternating patients

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Oocyte cryopreservation – SISMER experience

	TOTAL March 2004 - December 2009
No. cycles	812
No. Transferred cycles (%)	665 (82)
No. Clinical pregnancies (%)	106 (15.9)
Implantation rate (%)	122/1317 (9.2)
Abortions (%)	26 (24.5)
LBR (%)	80 (9.9)

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Oocyte cryopreservation – SISMER experience

	Slow-freezing	Vitrification
No. cycles	771	41
Age	35.5 ± 4.1	36.4 ± 4.2
Survival Rate (%)	2566/3731 (68.8)	142/201 (70.6)
Fertilization Rate (%)	1494/2015 (74.1)	81/111 (73)
Cleavage rate (%)	1283/1494 (85.9)	69/81 (85.2)
4C1 (+2)	218/1283 (17)*	23/69 (33.3)*
8C1 (+3)	64/680 (9.4)**	12/36 (33.3)**
No. Transferred cycles (%)	631 (81.8)	34 (82.9)

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* P<0.001 **P<0.001

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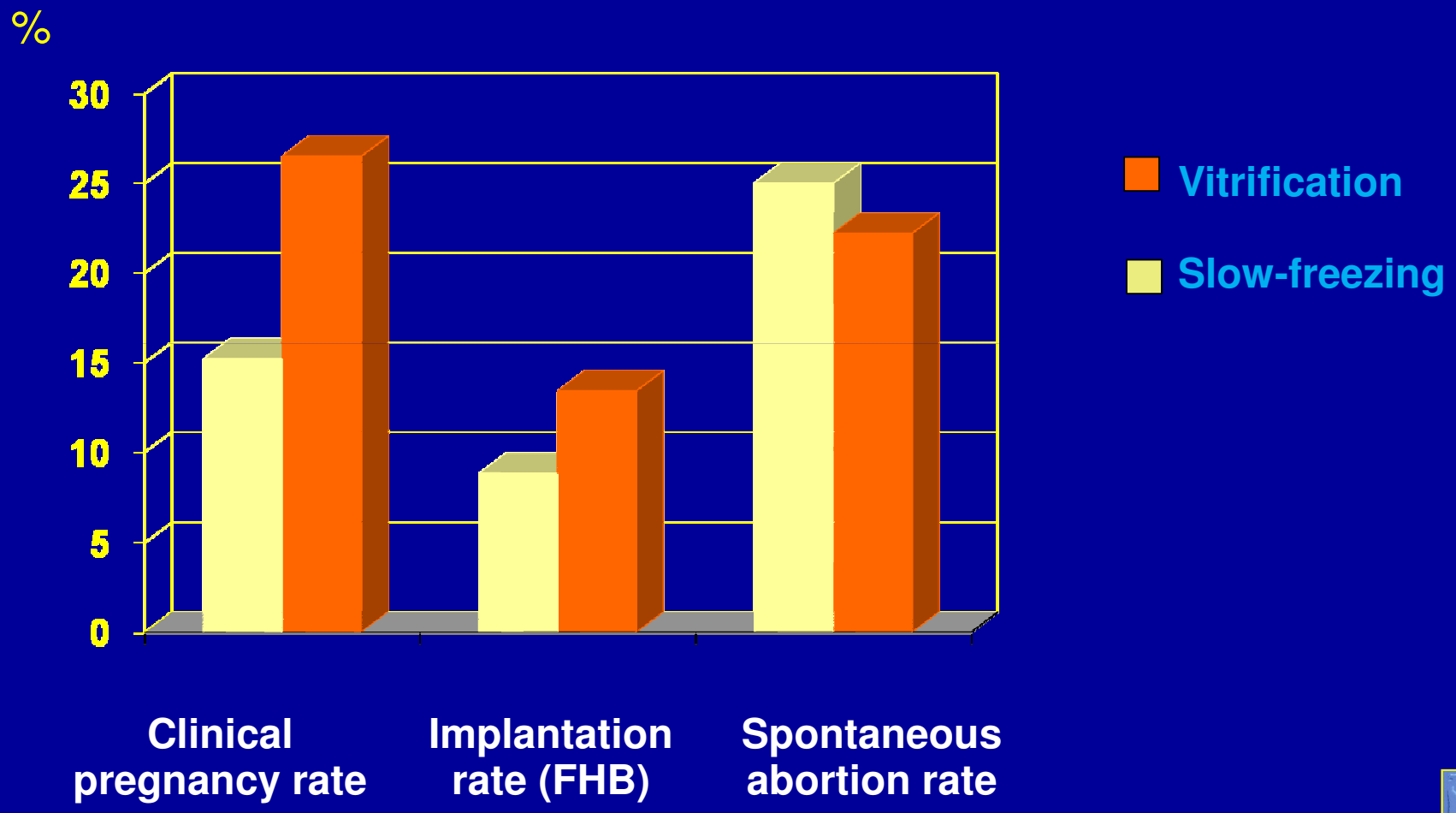
Slow-freezing Vs Vitrification

SISMER EXPERIENCE (2004-2009)



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Is embryo development comparable between slow-freezing and vitrification?

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Oocyte cryopreservation – SISMER experience
March 2008 - November 2009

	Slow freezing	Vitrification
N° cycles	77	41
Age	35.4 ± 3.7	36.5 ± 4.2
Survival Rate (%)	248/383 (64.8)	142/201 (70.6)
Fertilization Rate (%)	160/217 (73.7)	81/111 (73)
Cleavage rate (%)	125/160 (78.1)	69/81 (85.2)
4C1 (+2)	20/125 (16)[a]	23/69 (33.3)[a]
8C1 (+3)	9/72 (12.5)[b]	12/36 (33.3)[b]
PR/ET (%)	10/64 (15.6)	9/34 (26.5)
IR (%)	11/138 (8)	10/67 (14.9)

a) P<0.005
b) P<0.01

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Slow-freezing and embryo development

The complexity in freezing human oocytes is due to their high temperature sensitivity and despite the recent improvements the implantation rate per thawed oocyte remains extremely low implying that the efficiency of slow-freezing is still far from being optimal.

→ Does oocyte slow-freezing have an impact on embryo development?

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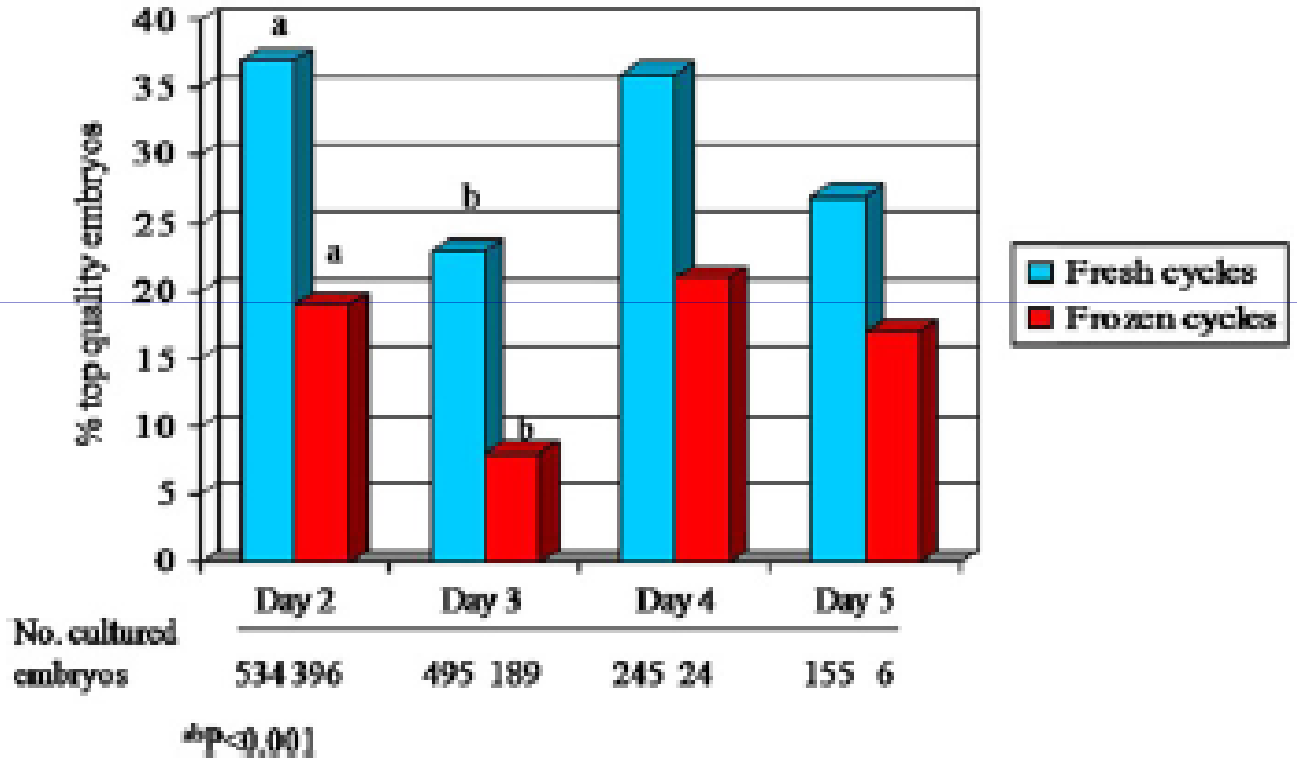
FIGURE 2

Top-quality embryo development after culture to day 2, day 3, day 4, and day 5. Values with same superscripts are significantly different.



Magli. Embryo development from thawed oocytes. Fertil Steril 2010.

Magli et al., (2010) Embryo development from thawed oocytes. Fertil. Steril. 93: 510-516

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TABLE 2

Fertilization and embryo development in fresh and frozen cycles according to maternal age.

	≤35 y		≥36 y	
	Fresh cycles	Frozen cycles	Fresh cycles	Frozen cycles
No. cycles	117	120	117	136
Age (mean ± SD) (y)	31.9 ± 4.6	31.7 ± 4.1	38.5 ± 4.6	38.5 ± 4.2
No. inseminated oocytes	351	303	351	316
No. fertilized oocytes (%)	287 (82)[a]	219 (72)[a]	298 (85)[h]	231 (73)[h]
No. zygotes with the configurations A1α, A2α, A1β, and A2β (%)	164 (57)[c]	101 (46)[c]	190 (64)[d]	124 (54)[d]
No. embryos (%)	259 (90)	189 (86)	275 (92)	207 (90)
Day 2—top-quality embryos (%)	91 (35)[e]	33 (17)[e]	106 (39)[f]	41 (20)[f]
Day 3—top-quality embryos (%)	58/255 (23)[g]	10/102 (10)[g]	57/240 (24)[h]	5/87 (6)[h]
Day 4—top-quality embryos (%)	43/128 (34)	4/16 (25)	44/117 (38)	1/8 (13)
Day 5—top-quality embryos (%)	22/78 (28)	1/4 (25)	25/77 (32)	0/2
No. transferred cycles (%)	108 (92)[i]	93 (78)[i]	110 (94)[j]	110 (81)[j]

[a] $P < .01$, fresh vs. frozen.
[b] $P < .001$, fresh vs. frozen.
[c] $P < .025$, fresh vs. frozen.
[d] $P < .025$, fresh vs. frozen.
[e] $P < .001$, fresh vs. frozen.
[f] $P < .001$, fresh vs. frozen.
[g] $P < .01$, fresh vs. frozen.
[h] $P < .001$, fresh vs. frozen.
[i] $P < .005$, fresh vs. frozen.
[j] $P < .005$, fresh vs. frozen.

Magli. Embryo development from thawed oocytes. Fertil Steril 2010.

Magli et al., (2010) Embryo development from thawed oocytes. Fertil. Steril. 93: 510-516

The mean number of top quality embryos that were transferred was significantly lower in thawed cycles than in fresh cycles, in both young and old patients.

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Oocyte cryopreservation and “social freezing”

The approach to oocyte cryopreservation and, consequently, the patient expectation depends on the purpose:

- Medical reason
- Non-medical reason (postponed parenthood, donation of oocytes)

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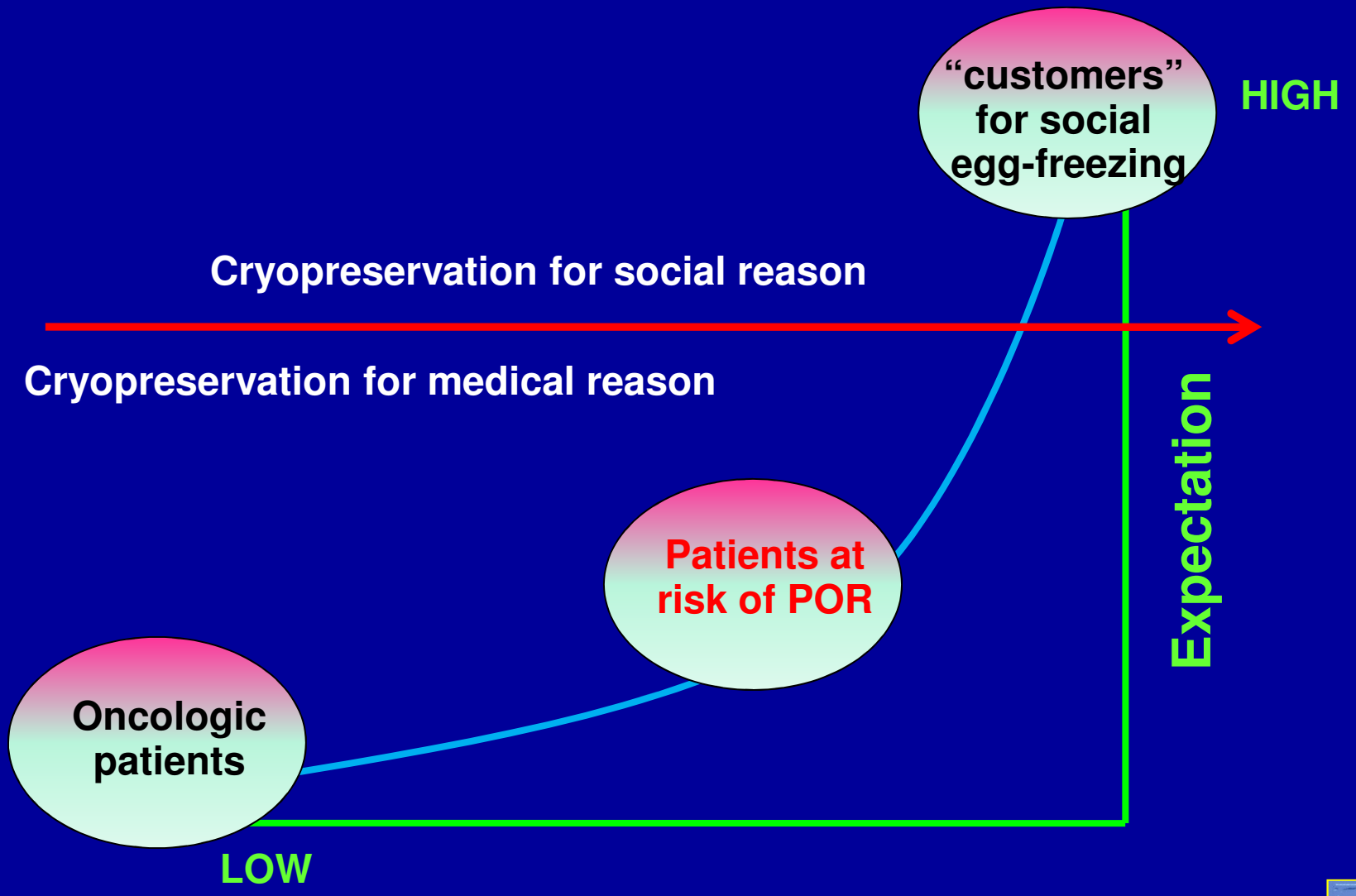
Oocyte cryopreservation – Patient expectations



According to Jim Catt, director of embryology,-Monash IVF- Victoria, Australia at www.ivf.net

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The purpose of oocyte cryopreservation for fertility preservation is based on data showed in recent literature demonstrating that outcomes from cryopreserved oocytes are comparable to these from fresh cycles.

→ Are vitrified oocytes performing as well as fresh oocytes?

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Fresh vs vitrified oocytes

Table III Primary and secondary outcomes measures: fertilization, pronuclear morphology, embryo development and embryo morphology of fresh and vitrified sibling oocytes

	Fresh ICSI	Vitrified/Warmed ICSI (%)	Absolute difference (%) (95% CI)	OR (95% CI)	P
Fertilization (2PN) per sibling oocyte	100/120 (83.3)[b]	95/124 (76.6)[a]	−6.73 (−16.6 to 3.39)	0.65 (0.33 to 1.29)	0.20
Fertilization (2PN) per injected oocyte	100/120 (83.3)[b]	95/120 (79.2)[b]	−4.17 (−14.0 to 5.7)	0.76 (0.37 to 1.53)	0.50
Normal 2PN morphology	96/100 (96.0)[c]	86/95 (90.5)[c]	−5.47 (−13.4 to 1.84)	0.39 (0.08 to 1.49)	0.16
1PN oocytes	3/120 (2.5)[b]	6/120 (5.0)[b]	2.5 (−2.82 to 8.22)	2.05 (0.42 to 12.9)	0.50
3PN	1/120 (0.83)[b]	2/120 (1.66)[b]	0.83 (−3.09 to 5.1)	2.01 (0.10 to 119.9)	1
Degenerated oocytes post-ICSI	1/120 (0.83)[b]	4/120 (3.34)[b]	2.51 (−1.75 to 7.47)	4.08 (0.39 to 203.5)	0.37
Day 2 embryo development	100/100 (100)[c]	93/95 (97.9)[c]	−2.11 (−7.3 to 1.9)	0.0 (0.00 to 0.23)	0.24
Excellent quality embryos	52/100 (52.0)[d]	49/95 (51.6)[d]	−0.43 (−14.2 to 13.3)	0.98 (0.53 to 1.79)	0.90
Good quality embryos	38/100 (38.0)[d]	41/95 (43.2)[d]	5.16 (−8.49 to 18.6)	1.24 (0.67 to 2.28)	0.47
Fair/poor quality embryos	10/100 (10.0)[d]	3/95 (3.16)[d]	−6.84 (−14.6 to 0.42)	0.29 (0.05 to 1.19)	0.10

[a]Percentages, expressed per warmed oocyte.
[b]Percentages, expressed per inseminated oocyte.
[c]Percentages, expressed per 2PN fertilized oocyte.
[d]Percentages, expressed per cleaved oocyte.

Rienzi et al., (2010) Embryo development of fresh 'versus'vitrified metaphase II oocytes after ICSI: a prospective randomized sibling-oocyte study. Hum.Rep., 25: 66–73

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Fresh vs vitrified oocytes

Table II Clinical outcomes of cycles performed with vitrified/warmed oocytes

	Patients included ($N = 40$)
Number of warmed oocytes (mean ± SD)	3.1 ± 0.30
Number of embryos transferred (mean ± SD)	2.3 ± 0.88
Number of embryo transfer performed (%)	39/40 (97.5)
Clinical pregnancy rate per cycle (%)	15/40 (37.5)
Clinical pregnancy rate per transfer (%)	15/39 (38.5)
Ongoing pregnancy rate per cycle (%)	12/40 (30.0)
Ongoing pregnancy rate per transfer (%)	12/39 (30.8)
Implantation rate (%)	19/93 (20.4)
Ongoing implantation rate (%)	16/93 (17.2)

Rienzi et al., (2010) Embryo development of fresh 'versus'vitrified metaphase II oocytes after ICSI: a prospective randomized sibling-oocyte study. Hum.Rep., 25: 66–73

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Fresh vs vitrified oocytes
(vitrification egg donation program)

TABLE 2

Oocyte distribution, survival, and fertilization.

	Vitrified	Fresh	*P* value
MII oocytes No. (%)	231 (87.2)	219 (89.7)	.363
MI oocytes No. (%)	19 (7.2)	11 (4.5)	.203
GV oocytes No. (%)	15 (5.7)	14 (5.7)	.974
Survival No. (%)	224/231 (96.9)	—	
No. of injected oocytes	224	219	
Normal fertilization No. (%)	171 (76.3)	180 (82.2)	.128
Abnormal fertilization No. (%)	9 (4.0)	12 (5.4)	.469
Degenerated oocytes No. (%)	7 (3.1)	6 (2.7)	.809

TABLE 3

Embryo quality.

	Vitrified	Fresh	*P* value
Cleavage rate day 2 embryos (%)	161/171 (94.2)	176/180 (97.8)	.083
No. of cell day 2 embryos (mean ± SD)	3.8 ± 1.1	3.9 ± 1.5	.567
Good quality day 2 embryos (%)	136/161 (84.4)	126/176 (71.5)	.005
Cleavage rate day 3 embryos (%)	125/161 (77.6)	149/176 (84.6)	.098
No. of cell day 3 embryos (mean ± SD)	6.9 ± 2.3	6.9 ± 2.7	.558
Good quality day 3 embryos (%)	101/125 (80.8)	120/149 (80.5)	.956
No. of embryo undergoing extended culture	78	143	
Blastocyst rate No. (%)	38/78 (48.7)	68/143 (47.5)	.869
Good quality blastocysts No. (%)	24/32 (81.1)	42/60 (70)	.612

Cobo. Clinical outcome of oocyte vitrification. Fertil Steril 2008.

Cobo et al., (2008) Clinical outcome of oocyte vitrification. Fertil. Steril. 89:1657-1664

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Fresh vs vitrified oocytes
(vitrification egg donation program)

TABLE 4

Clinical results.

	Vitrified	Fresh	Mixed
No. of transfers	23	1	4
No. of embryos transferred (mean ± SD)	49 (2.1 ± 1.2)	2 (2 ± 0)	8 (2.1 ± 0.1)
Pregnancy rate per transfer	15/23 (65.2)	1 (100)	2 (50)
Implantation rate (No. of sacs/ No. of embryos transferred)	20/49 (40.8)	2/2 (100)	2/8 (25)
Multiple pregnancy rate (twin)	5/15 (23.8)	1 (100)	0
Miscarriage rate	3/15 (20)	0	0
Biochemical pregnancy rate	1/15 (6.6)	0	0
Ongoing pregnancy rate	11/23 (47.8)	1 (100)	2 (100)

Note: Numbers in parentheses are percentages.

Cobo et al., (2008) Clinical outcome of oocyte vitrification. Fertil. Steril. 89:1657-1664

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How many eggs do we need to get a pregnancy?

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Table I Patient's baseline characteristics and fresh cycle parameters

	Patients included (*N* = 40)
Female age (mean years ± SD)	35.5 ± 4.8
Baseline FSH (mean mU/ml ± SD)	6.44 ± 3.1
Previous IVF attempts (mean ± SD)	0.58 ± 1.0
GnRH-agonist long protocol (%)	31/40 (77.5)
Antagonist protocol (%)	9/40 (22.5)
Days of stimulation (mean ± SD)	10.8 ± 1.95
Total gonadotrophin amount IU (mean ± SD)	2201.65 ± 765.7
Number of COCs retrieved (mean ± SD)	13.3 ± 4.5
Number of MII oocytes (mean ± SD)	10.7 ± 3.6
Number of MII oocytes vitrified (mean ± SD)	6.3 ± 2.8

COC, cumulus corona oocyte complex; MII, metaphase II.

Table II Clinical outcomes of cycles performed with vitrified/warmed oocytes

	Patients included (*N* = 40)
Number of warmed oocytes (mean ± SD)	3.1 ± 0.30
Number of embryos transferred (mean ± SD)	2.3 ± 0.88
Number of embryo transfer performed (%)	39/40 (97.5)
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Is oocyte cryopreservation still experimental?

In a recent ASRM publication (June 2008), the Society defined an "experimental" procedure, indicating that one should be designed as such until "there is adequate scientific evidence of safety and efficacy from appropriately designed peer-reviewed published studies by different investigator groups".

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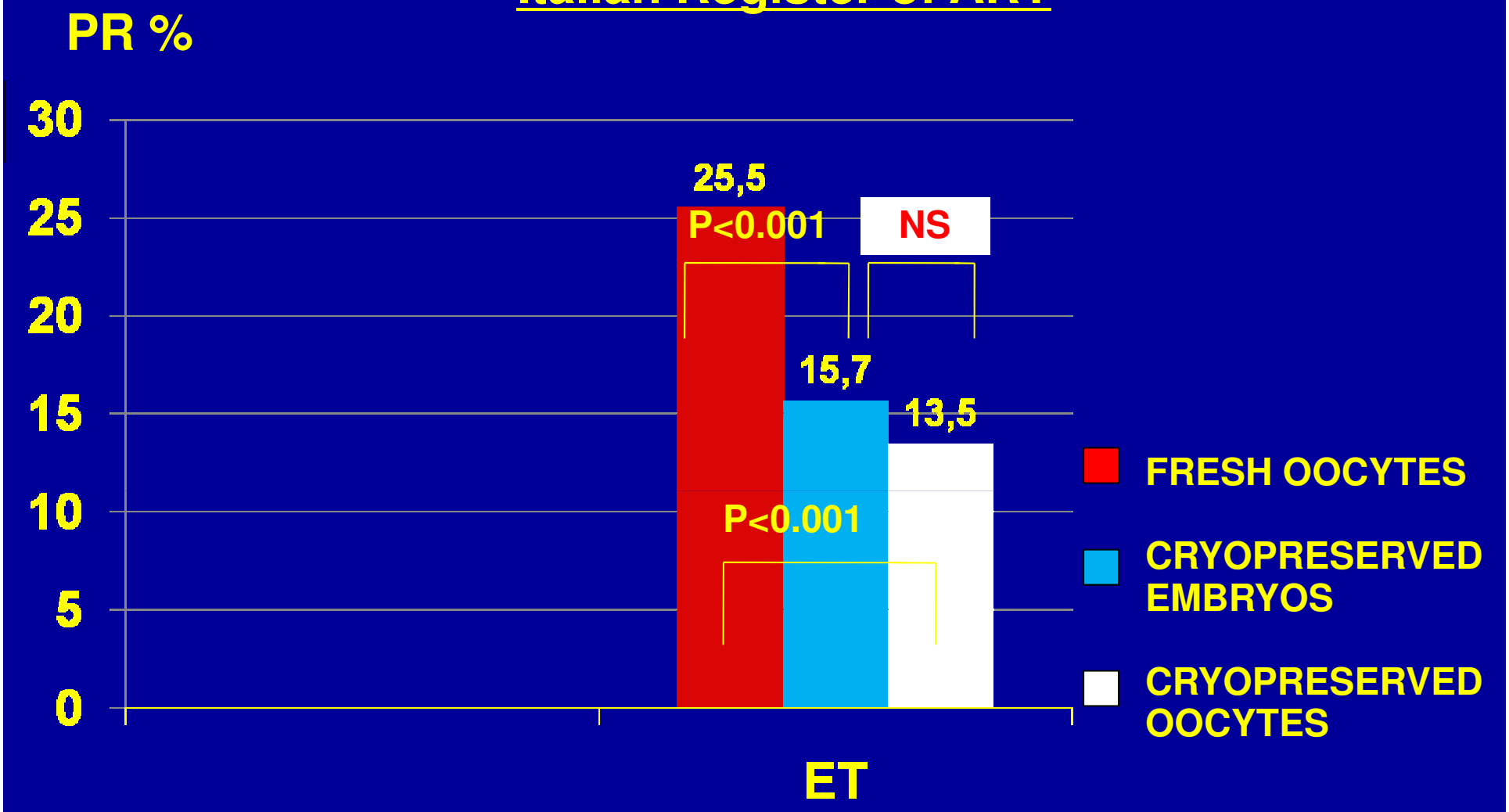
The most recent ASRM Practice Commitee statement acknowledges that oocyte cryopreservation offers “great promise for application in oocyte donation and fertility preservation”.

ASRM Practice Commitee (2009).

Italian Register of ART



Pregn.	7847	104	327
Cycles	30780	661	2428

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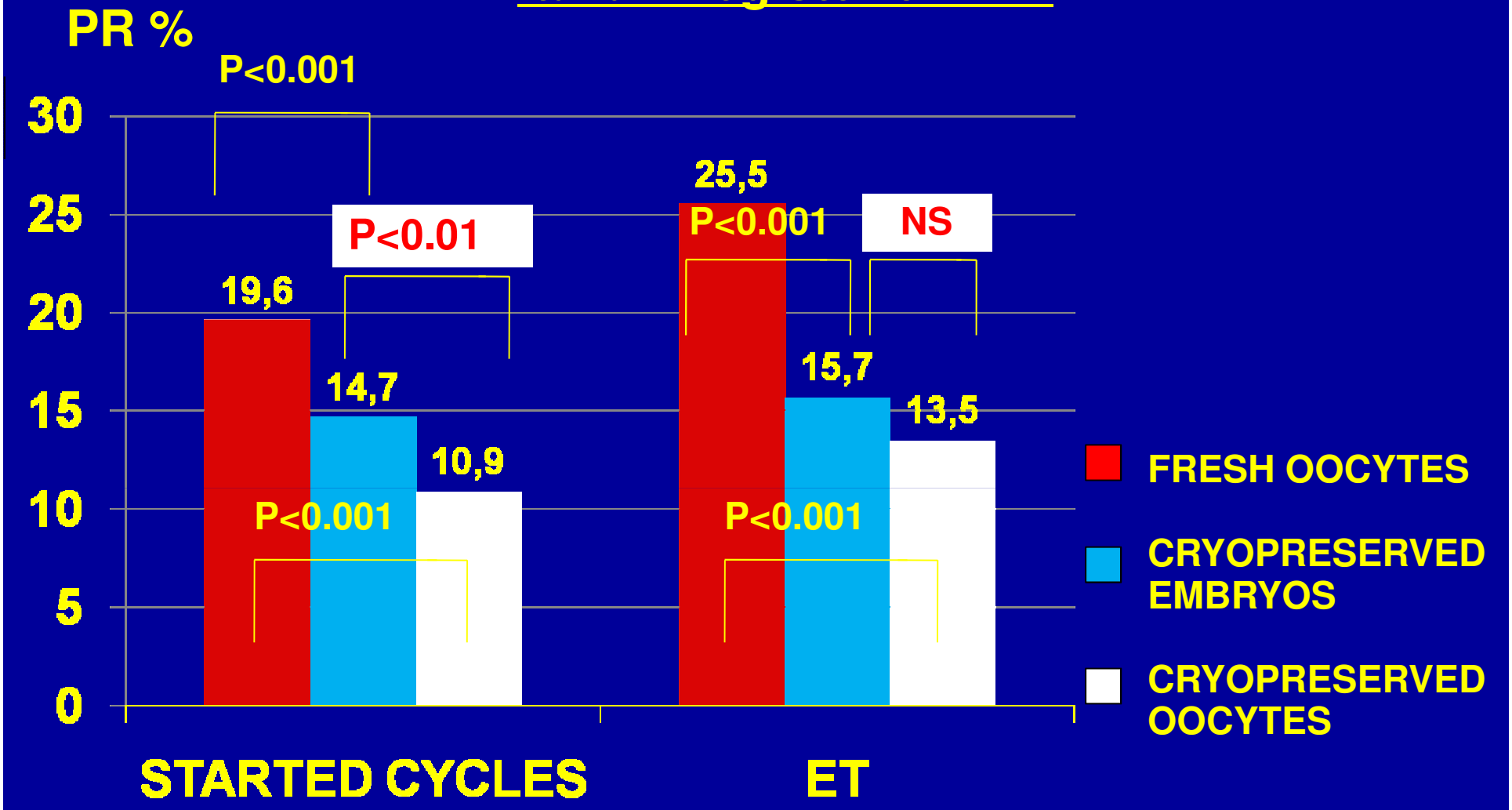
Italian Register of ART



Pregn.	7847	104	327	7847	104	327
Cycles	40005	709	2994	30780	661	2428

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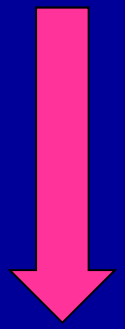
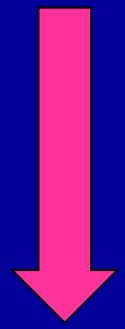
CRYOPRESERVED OOCYTES



IR 2.6 %

CRYOPRESERVED EMBRYOS



IR 6.3 %

P<0.001

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Slow-freezing or vitrification?

Vitrification seems to be a very promising method, but we have to keep in mind:

- **Longer learning curve**
- **Inter-operator variability**
- **Possible Liquid N_2 contamination using open-sistems (performance with closed systems is not as good as using open systems)**

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Slow-freezing or vitrification?

- The most exciting data in literature are on young or fertile patients

- Pregnancy Follow up (5% malformation rate according to Cobo at "Updates in infertility treatment" – January 2010 – Seville, Spain)

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Conclusions

✓ Oocyte cryopreservation is a promising method expecially using vitrification procedures

✓ To determine efficacy and safety of oocyte cryopreservation there is still the need

- ➢ to verify the performance on infertile patients in all age categories (both young and old patients)
- ➢ to verify the pregnancy follow up.

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Conclusions

Despite these queries, oocyte cryopreservation

→ is a valid option that will have a significant impact on the practice of human IVF in the near future, in particular in the case of medical indication.

→ maybe is not a preventive tool for patients at risk of POR (Poor Ovarian Response) or DOR (Desperate Ovarian Response) because the number of oocytes needed to get a pregnancy is still too high.

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POOR OVARIAN RESPONSE (POR)

IS OOCYTE CRYOPRESERVATION A PREVENTIVE TOOL FOR PATIENT AT RISK OF POR?

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