

# Advantages and practical applications of human oocytes cryopreservation



- Ethical, moral, legal reasons
- Risk of premature ovarian failure due to:
  - Antineoplastic therapies
  - Endometriosis
  - Ovarian surgery
  - Genetic predisposition
- Flexibility of IVF routine
  - Failure to produce semen sample
  - Failure to retrieve sperms
  - Possibility of doing IVF in the physical absence of patients
  - Risks of developing OHSS
- Oocyte donation
- Family planning



1983

FIRST PREGNANCY FROM FROZEN  
EMBRYOS

1986

FIRST PREGNANCY FROM FROZEN  
OOCYTES

# HUMAN OOCYTE CRYOPRESERVATION



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AUTHORS	YEAR	BIRTHS
Chen	1986	Twin (IVF)
(Adelaide, Australia)	1987	Single (IVF)
van Uem	1987	Single (IVF)
(Erlangen, Germany)		
Porcu		
(Bologna, Italy)	1997	Single (ICSI)

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Chen, 1986-87

Egg survival: 76%

Fertilization: 71%

Cleavage: 85%

Pregnancy: 29%



**1988-1996**

**NO BIRTHS FROM**

**CRYOPRESERVED HUMAN OOCYTES**



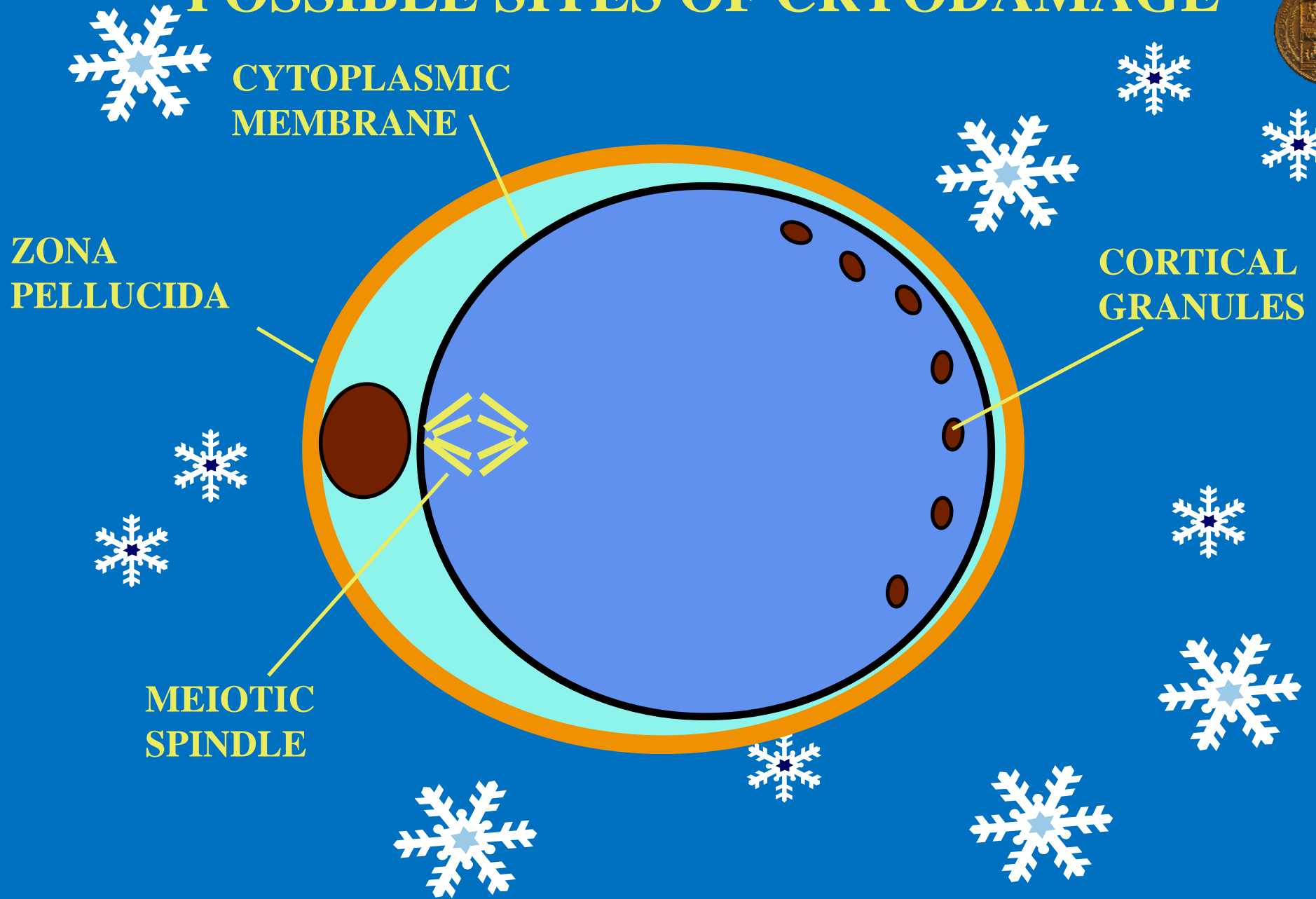
# Human egg freezing

<b>Survival</b>	<b>0-90%</b>
<b>Fertilization</b>	<b>0-70%</b>
<b>Cleavage</b>	<b>0-90%</b>
<b>Implantation</b>	<b>0-50%</b>



**INEFFICIENT**  
**UNRELIABLE**  
**UNSAFE**

# POSSIBLE SITES OF CRYODAMAGE





# CRYODAMAGE: MEIOTIC SPINDLE



- Present in Metaphase II oocytes
- Bounds 23 dichromatidic chromosomes
- Highly sensitive to ice crystals
- Disruption can lead to loss of chromosomes

# CRYODAMAGE: MEIOTIC SPINDLE



*Gook, Hum Reprod, 1993*

- Human and mouse oocytes cryopreserved with PROH
- Slow freeze, rapid thaw method
- Result: mouse and human oocytes cryopreserved by the same method show opposing results
  - mouse oocytes: 4% survival rate
  - human oocytes: 64% survival rate \*

\* 60% of the surviving oocytes had a normal spindle and chromosome configuration

Fertilization of human oocytes following cryopreservation; normal karyotypes and absence of stray chromosomes



*Gook, Hum Reprod, 1994*

- Fresh and aged human oocytes cryopreserved with PROH
- Oocytes examined for chromosomal loss
- No *stray chromosomes* observed in 137 cryopreserved oocytes
- In the cryopreserved oocytes which had undergone normal fertilization, normal sets of 23 chromosomes were observed



# Abnormal chromosomes in cryopreserved human oocytes

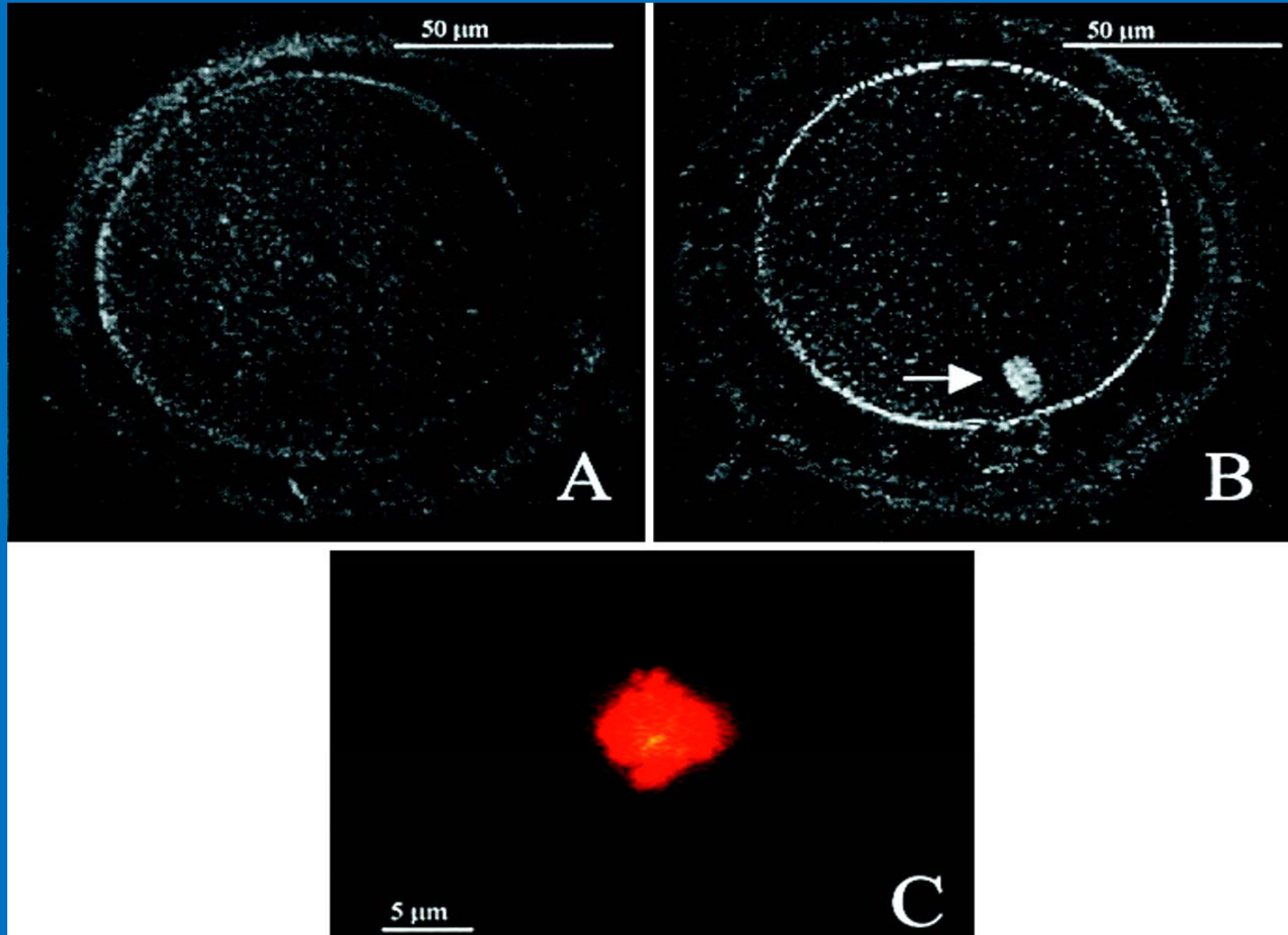
*Cobo et al., Fertil Steril 2001*

28,6% in frozen eggs

26% in controls

The technique is, *nevertheless*, safe

Sequential Polscope images of a metaphase II human oocyte in which the meiotic spindle was undetectable immediately after thawing in thawing solution 1 (A) and re-appeared after cryoprotectant removal during incubation at 37{degrees}C in culture medium (B)



# CRYODAMAGE



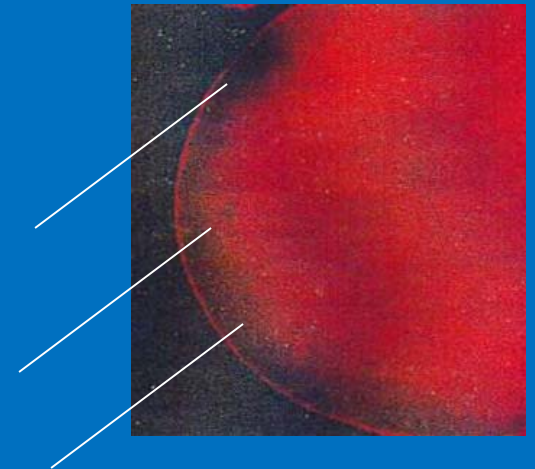
Zona pellucida

Cortical granules



# CRYODAMAGE: CORTICAL GRANULES

- Present in Metaphase II oocytes
- Located at the periphery of the oocyte
- At fertilization the exocytosis of the cortical granules (zona reaction) prevents the entry of more than one spermatozoon
- Loss can lead to polyspermy
- **Gook et al., 1993:**  
*abundant cortical granules in cryopreserved eggs*





# EGG FREEZING

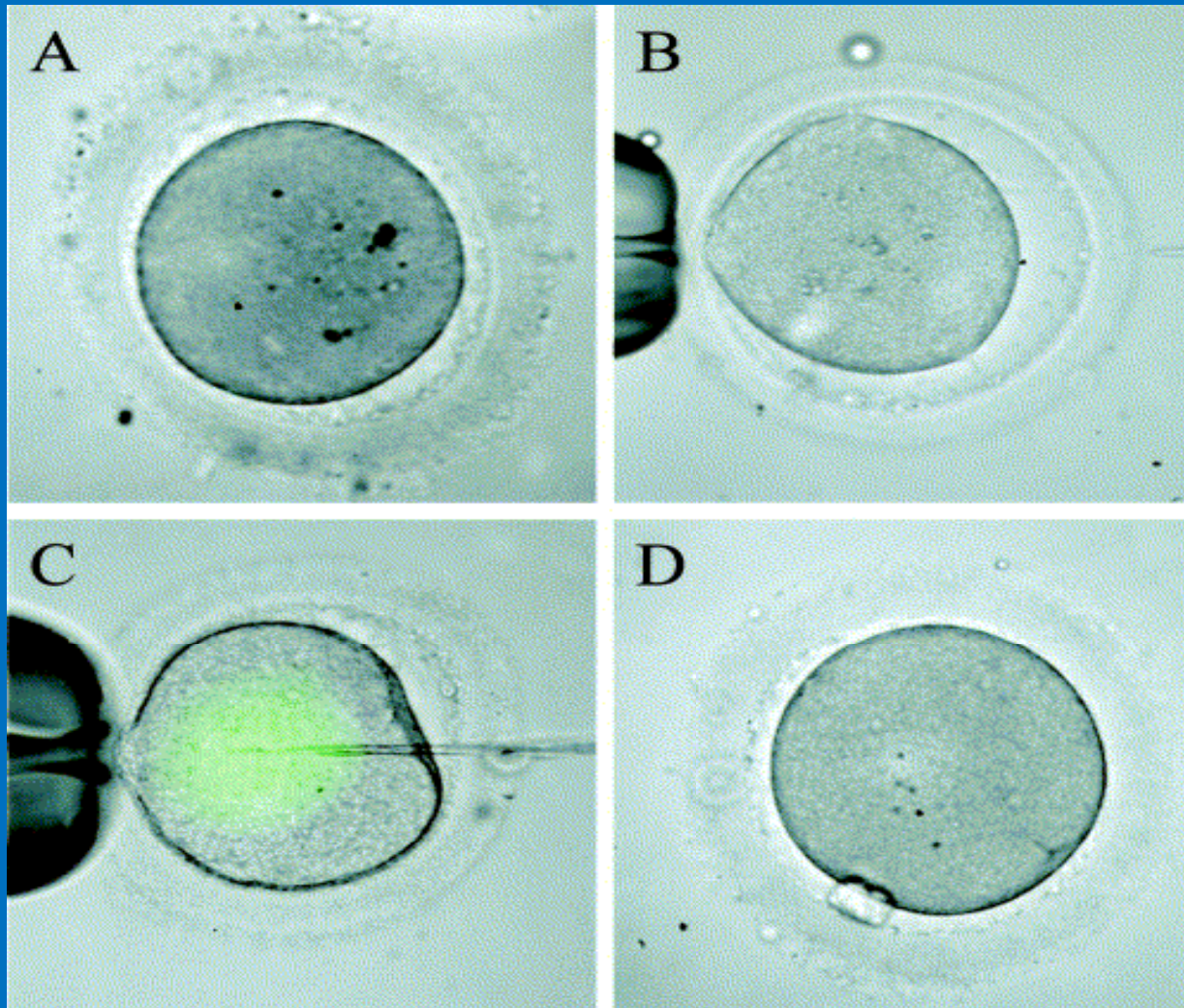
## TECNICAL VARIABLES

Cryoprotectants	DMSO, PROH, Gly, EtilGly, Sucrose, Threalose
Freezing-thawing rate	Slow, rapid, ultrarapid
	Vitrification
Seeding temperature	-4,5-8 °C
Medium components	Low sodium content
	Choline
Egg maturity	GV-MII
Insemination	IVF-ICSI



# MICROINJECTION OF THREALOSE

*Eroglu, Fertil Steril, 2002*



# LOW SODIUM IN THE MEDIUM



*Goud, Fertil Steril, 2002*

- The cryopreservation medium plays a critical role in preventing cellular injury during freeze-thaw
- During freezing, water leaves the cell and solute concentrations raise
- High solute concentrations may be harmful to oocytes
- Partial replacement of sodium with choline was found to be beneficial for mouse and human oocytes

- UNEFFICIENT**
- UNRELIABLE**
- UNSAFE**



# WHY?

- LOW NUMBER OF OOCYTES**
- BAD QUALITY OF OOCYTES**
- NO APPROPRIATE ANIMAL MODEL**

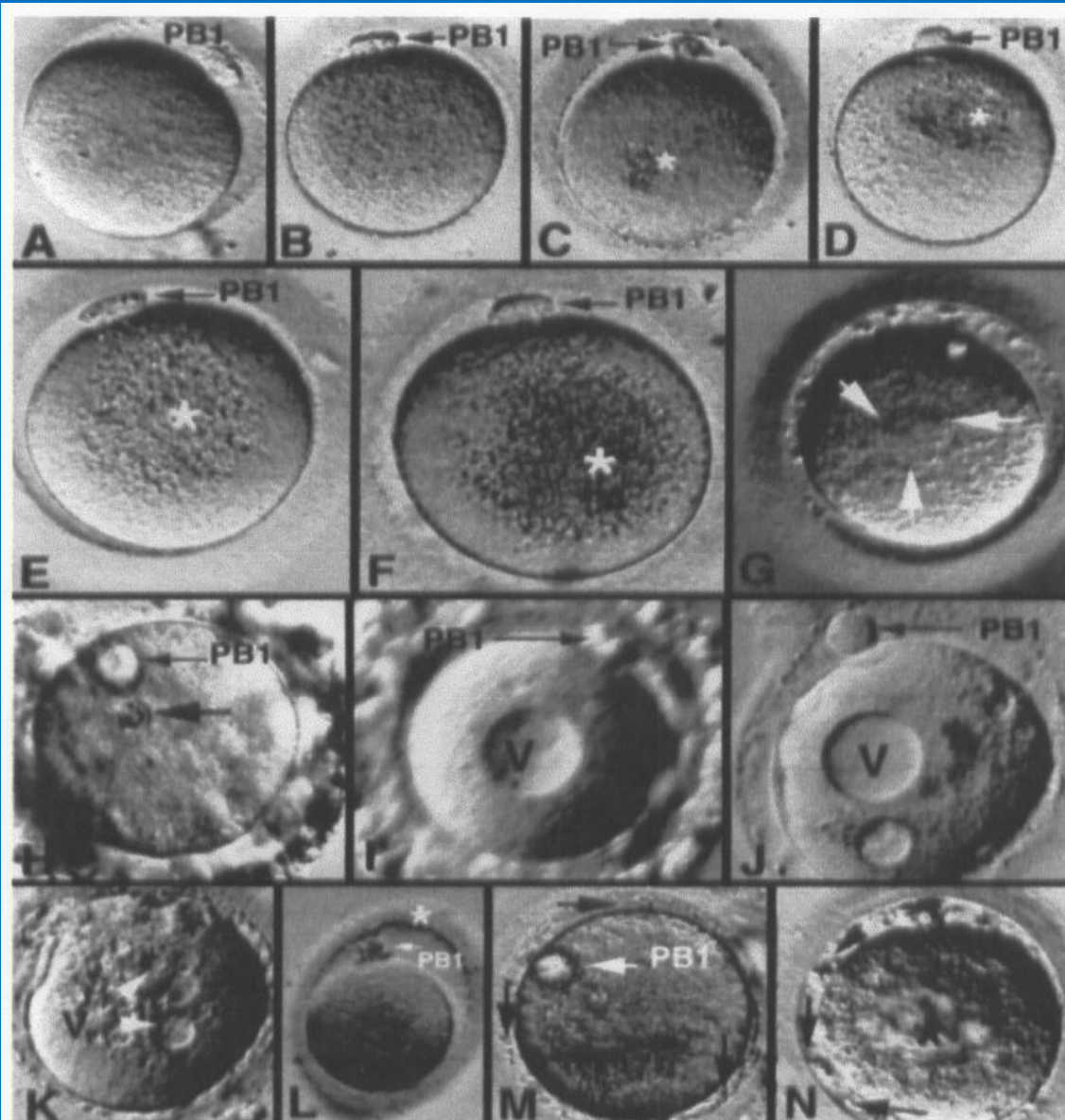
# "Ideal oocyte"

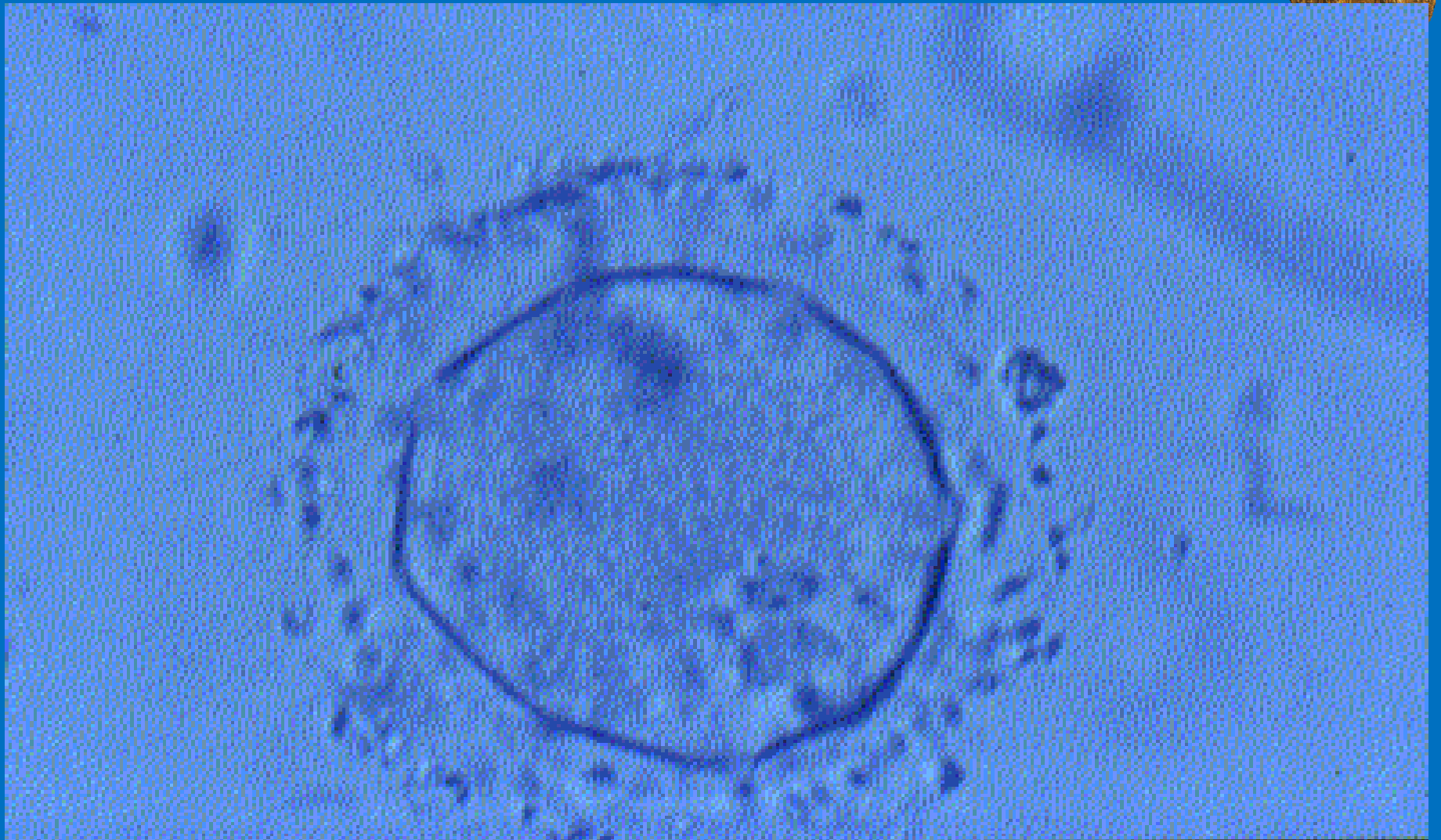






# Oocyte anomalies





Porcu et al., 1998

# HUMAN OOCYTES CRYOPRESERVATION

## clinical experimental design



### Patients

- **Age: < 38 years**
- Tubal infertility
- Normal seminal parameters
- No previous IVF failures

### Oocytes

- At least ten mature oocytes retrieved
- All the good quality oocytes frozen
- Oocytes thawed inseminated with ICSI and embryo replacement in subsequent cycle



# **SLOW FREEZING RAPID THAWING PROTOCOL**

**PROH 1.5 M solution + 0.2 M Sucrose**

**INSEMINATION WITH ICSI**





RECENTLY **ICSI** HAS BEEN PROPOSED AS  
A SOLUTION FOR CRYOPRESERVATION  
DAMAGES INVOLVING:

- **ZONA PELLUCIDA**
- **CORTICAL GRANULES**

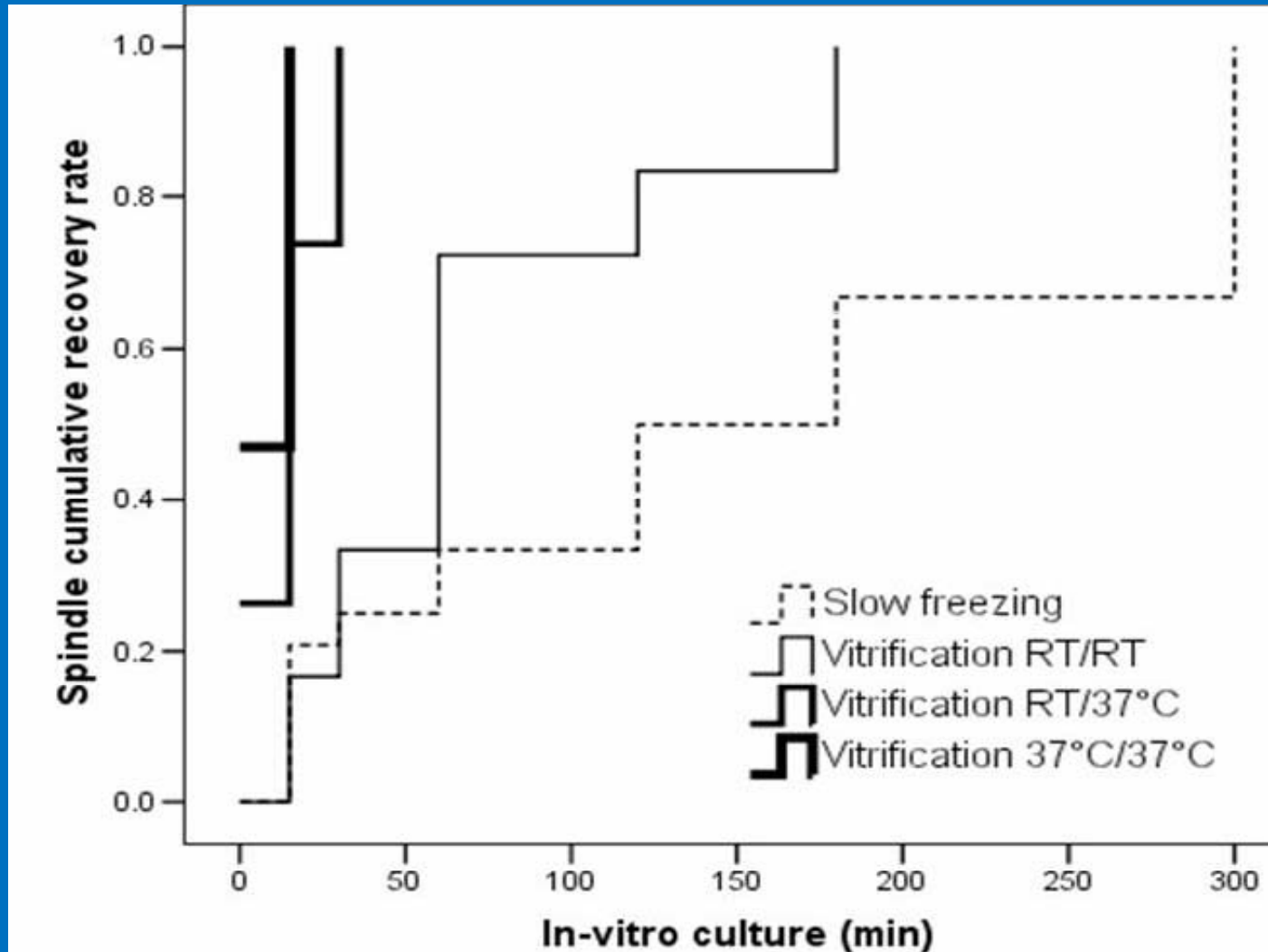
**ICSI** SHOULD:

- **INCREASE FERTILIZATION RATE**
- **AVOID POLYSPERMIC FERTILIZATION**



Ciotti et al 2004

Spindle cumulative recovery rate during *in vitro* culture in the four groups: slow freezing; vitrification RT/RT, RT/37°C and 37°C/37°C.





**CRYOPRESERVATION OF OOCYTES IN PCO  
TO RESCUE TREATMENT CYCLE AND AVOID  
SEVERE OHSS**

# OOCYTES CRYOPRESERVATION IN PCO PATIENTS

Infertility and IVF Center - University of Bologna



	n	%
Patients	51	
Thawing cycles	62	
Transfer	62	
Pregnancies	15	
Sacs	18	
Deliveries	10	
Children	13	
Ongoing	2	
Miscarriages	3	
Pregnancies rate/cycle		24
Pregnancies rate/patient		29
Implantation rate		10
Abortion rate		20

# HUMAN OOCYTES CRYOPRESERVATION



## Efficiency

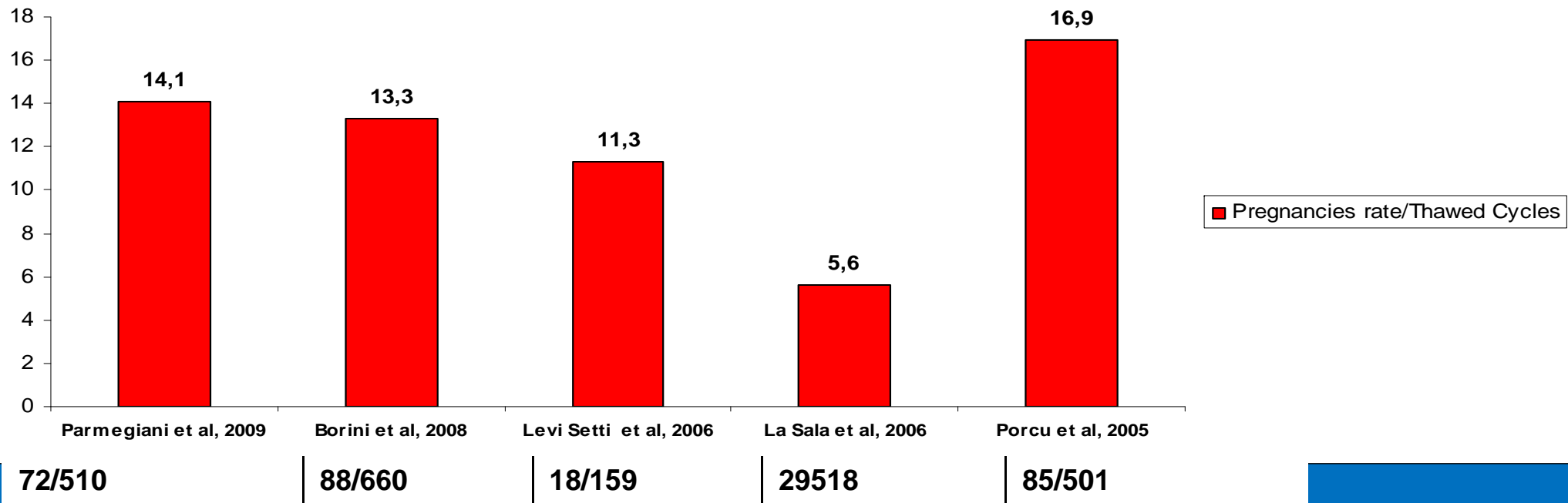
**Until recently, oocyte cryopreservation was considered a low efficiency technique because of low survival, fertilization and cleavage rates.**

**With the improvement of the freezing protocol, the survival rate is presently satisfactory.**

**With the introduction of ICSI, the results, in terms of fertilization, embryo cleavage and implantation, approach those obtained with fresh oocytes.**



Pregnancies rate/Thawed Cycles



# HUMAN OOCYTES CRYOPRESERVATION



## Safety

- 1- Safety of oocyte cryopreservation has been extensively debated and the main concern is related to the possible damage of the meiotic spindle and the induction of aneuploidy.**
- 2- However, Gook investigations showed normal karyotypes and absence of stray chromosomes in cryopreserved oocytes.**
- 3- In addition, cryopreservation processes expose oocytes to a rigid selection: probably only the strongest cells can survive.**
- 4- At present, children born from frozen oocytes are normal and healthy .**





WITH MORE THAN 900 OOCYTE  
CRYOPRESERVATION BORN,  
CONGENITAL DEFECTS DO NOT  
APPEAR MORE FREQUENT THAN IN  
NATURAL CONCEPTION

*Noyes, Porcu and Borini,  
RBM OnLine, 2009.*



## EGGS OR EMBRYO STORAGE?

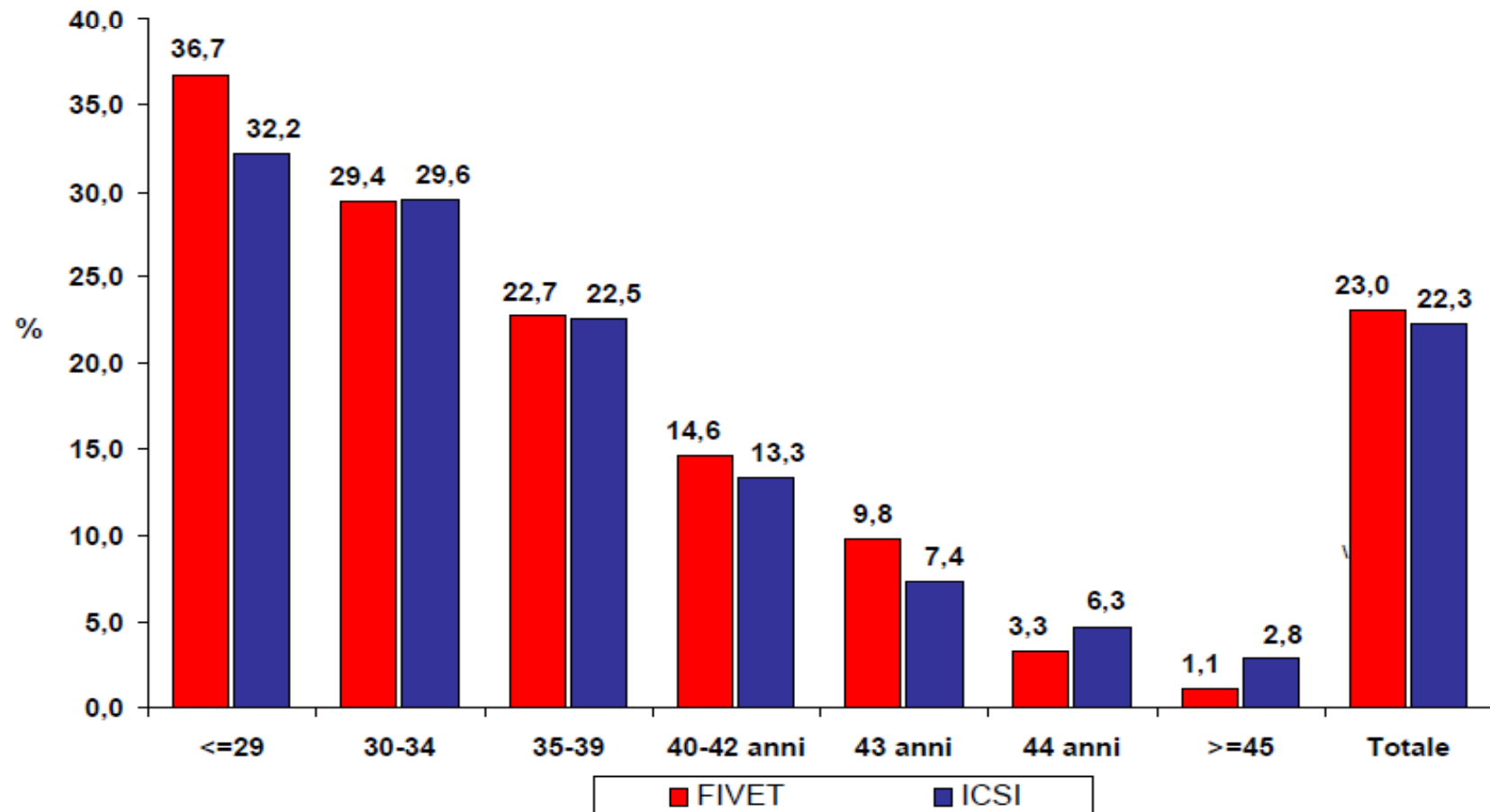
	eggs	embryos
Thawing cycles	19	23
Transfers	17	22
Thawed	95	69
Survived	75(79%)	56(82%)
Tran.embryos	45	56
Preg(tran.)	3(17.7)	4(18.2)

Porcu et al., Fertil Steril, 2002

## Italian IVF fresh cycles results



Figura 3.24 Percentuali di gravidanza sui prelievi da tecniche a fresco nell'anno 2008 per classi di età delle pazienti.



*(Italian Ministry of Health, 2008)*



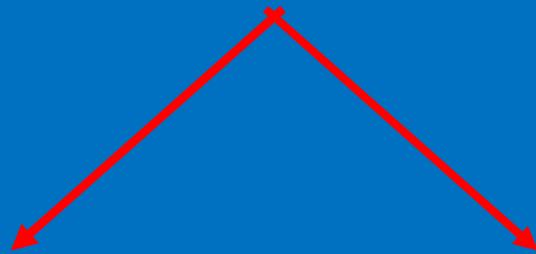
IVF cycles: 117.318

ICSI cycles: 232.844

*ESHRE Registry, 2010*



Pregnancy rate / Transfer



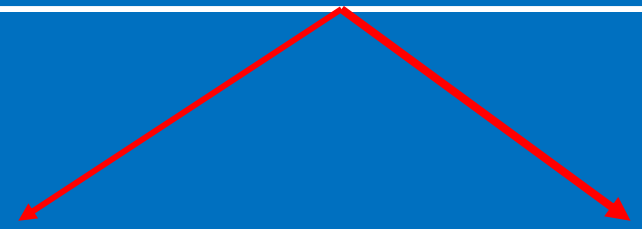
32.4 %

33.0%

IVF

ICSI

Pregnancy rate/egg retrieval



29.0 %

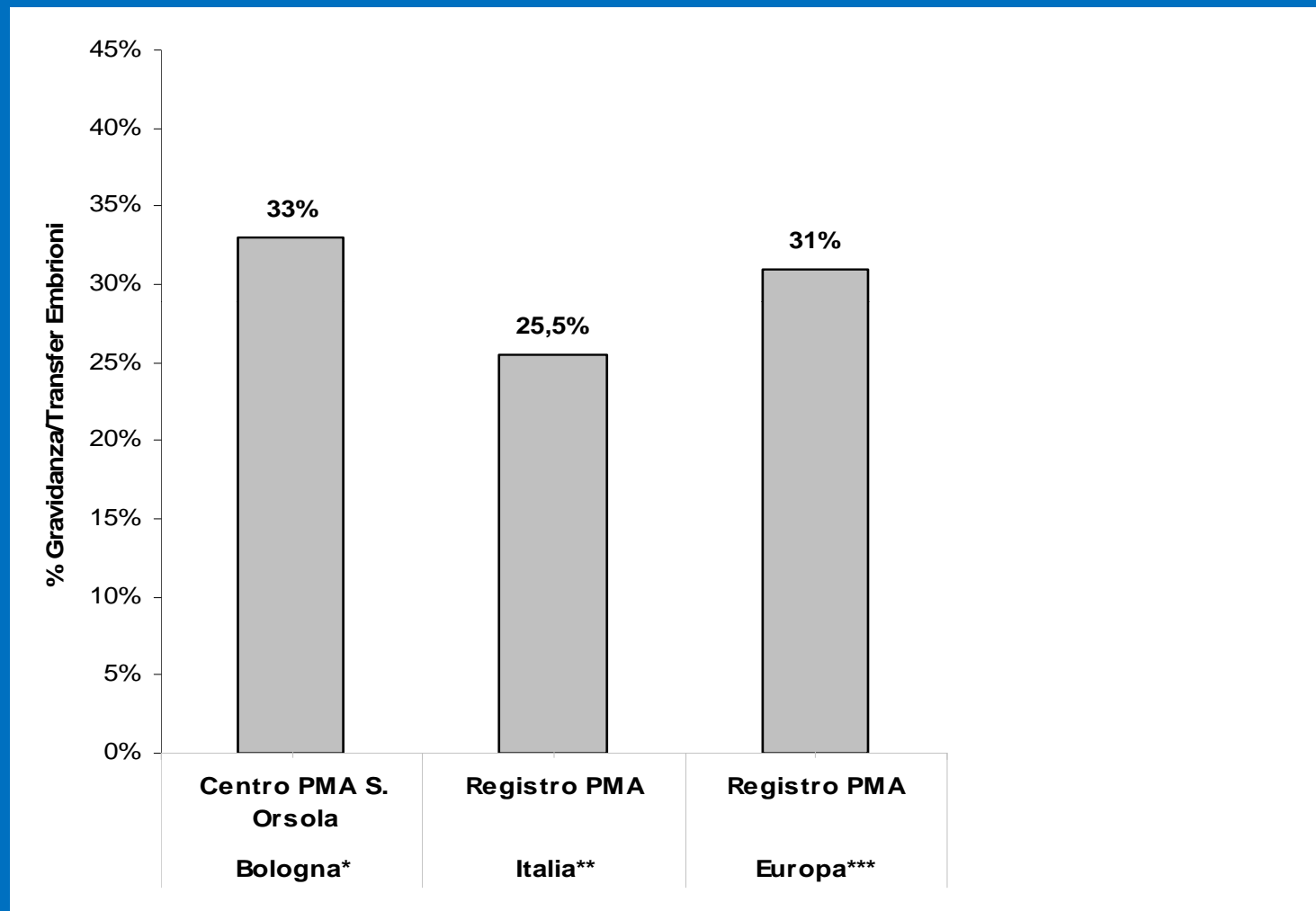
29.9%

IVF

ICSI

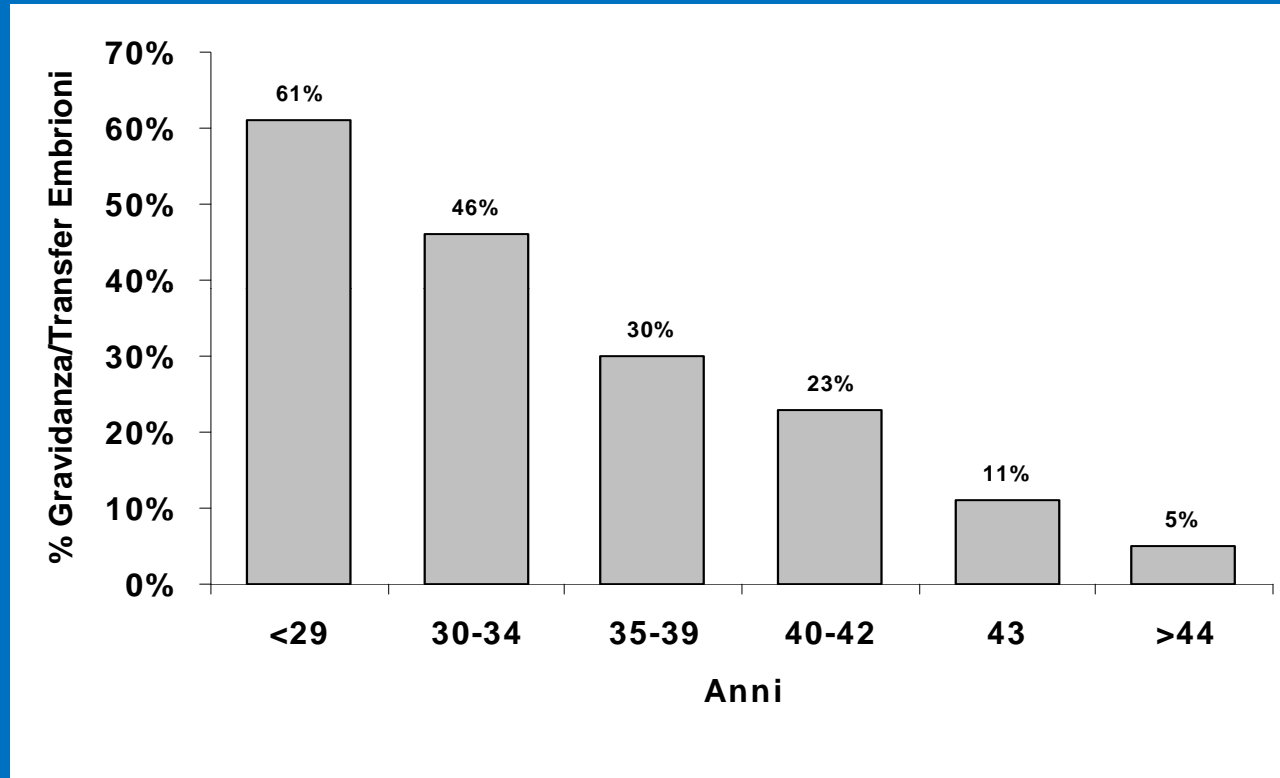
*ESHRE Registry, 2010*

## Pregnancy rate per embryo transfer



Porcu et al., 2010

# Pregnancy rate/transfer in fresh cycles, according to female age ( year 2008 )



Risultati clinici a confronto tra il gruppo di pazienti che ha scelto elettivamente di inseminare solo 3,4 ovociti (Study group) , il gruppo di pazienti che ha prodotto pochi ovociti (Poor prognostic) ed il gruppo che ha inseminato tutti gli ovociti disponibili (Good prognostic)



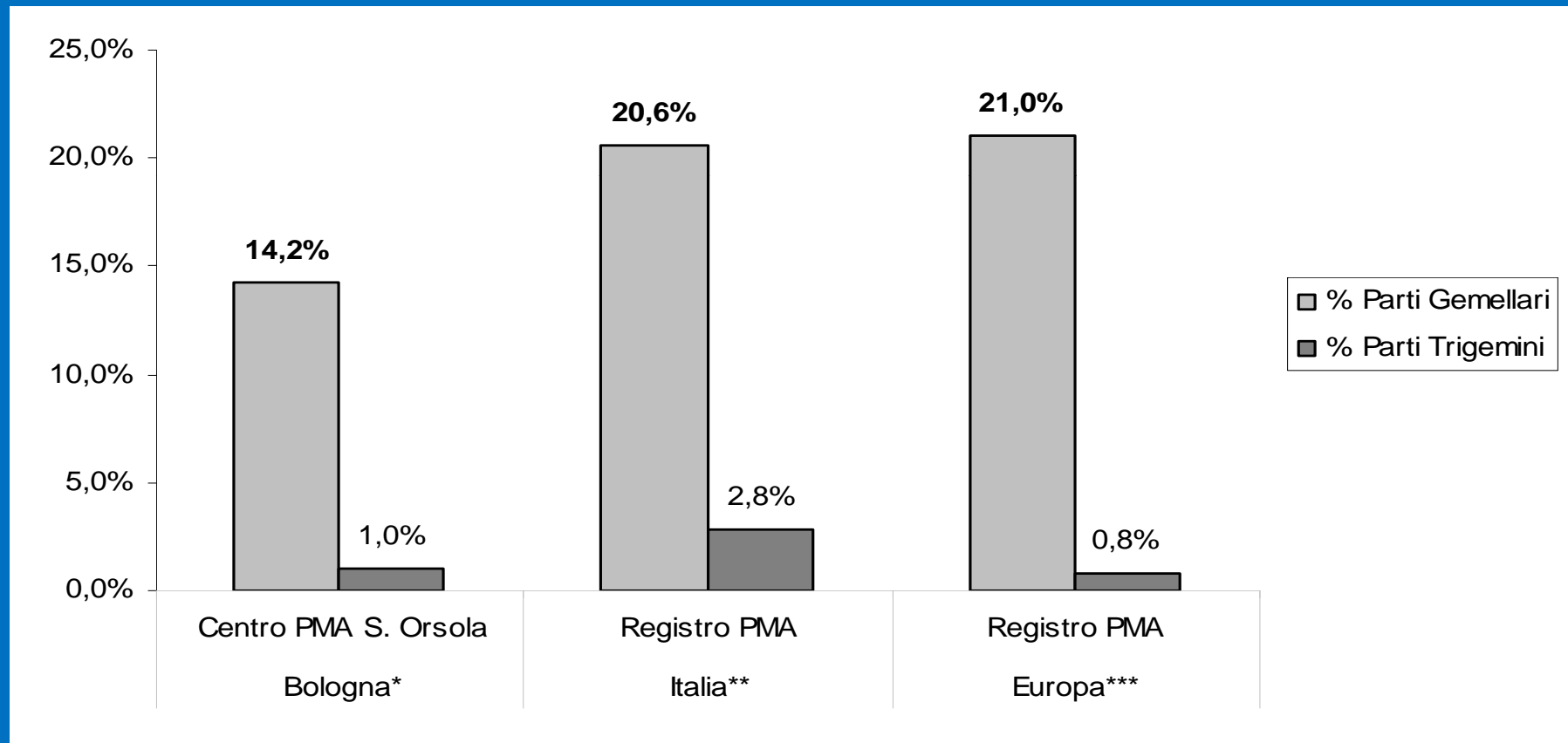
Outcome of IVF treatment

Parameter	Study group	Poor-prognostic Group	Good-prognostic group
Implantation rate, % (n)	35.7 (15/42)	21.7 (15/69)	47.5 (151/318)
Clinical pregnancy rate, % (n)	62.5 (10/16)	29.7 (11/37) <sup>a</sup>	64 (96/150)
Ongoing pregnancy rate, % (n)	56.3 (9/16)	24.3 (9/37) <sup>a</sup>	60.7 (91/150)
Ongoing multiple pregnancy rate, % (n)	44.4 (4/9)	33.3 (3/9)	42.8 (39/91)

<sup>a</sup>  $P_{.05}$  when compared with the study and good-prognostic groups.

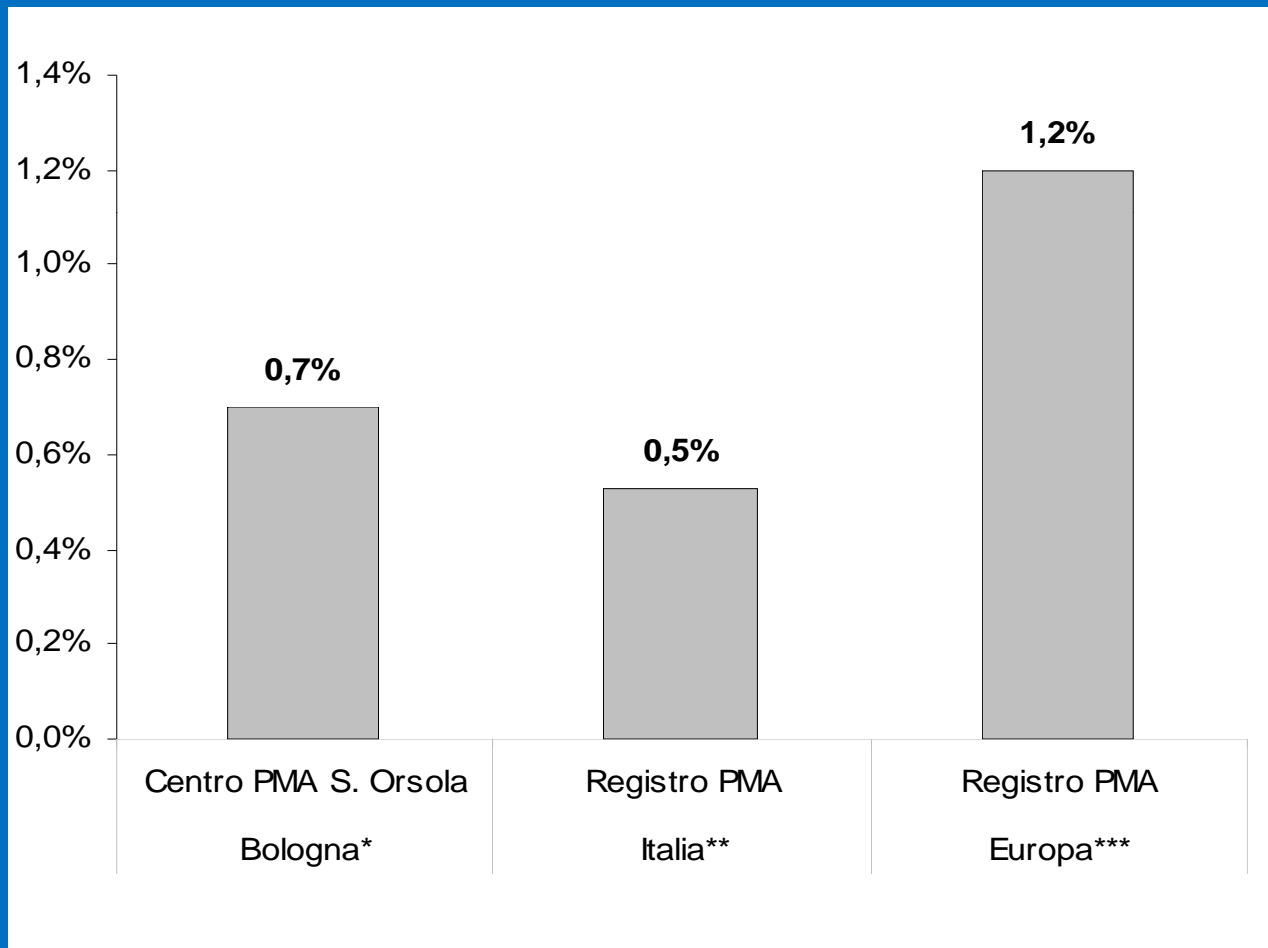


## Twins and triplets deliveries



Porcu et al., 2010

# Ovarian Hyperstimulation Syndrome



Porcu et al., 2010

## Risultati Clinici da Ovociti Crioconservati



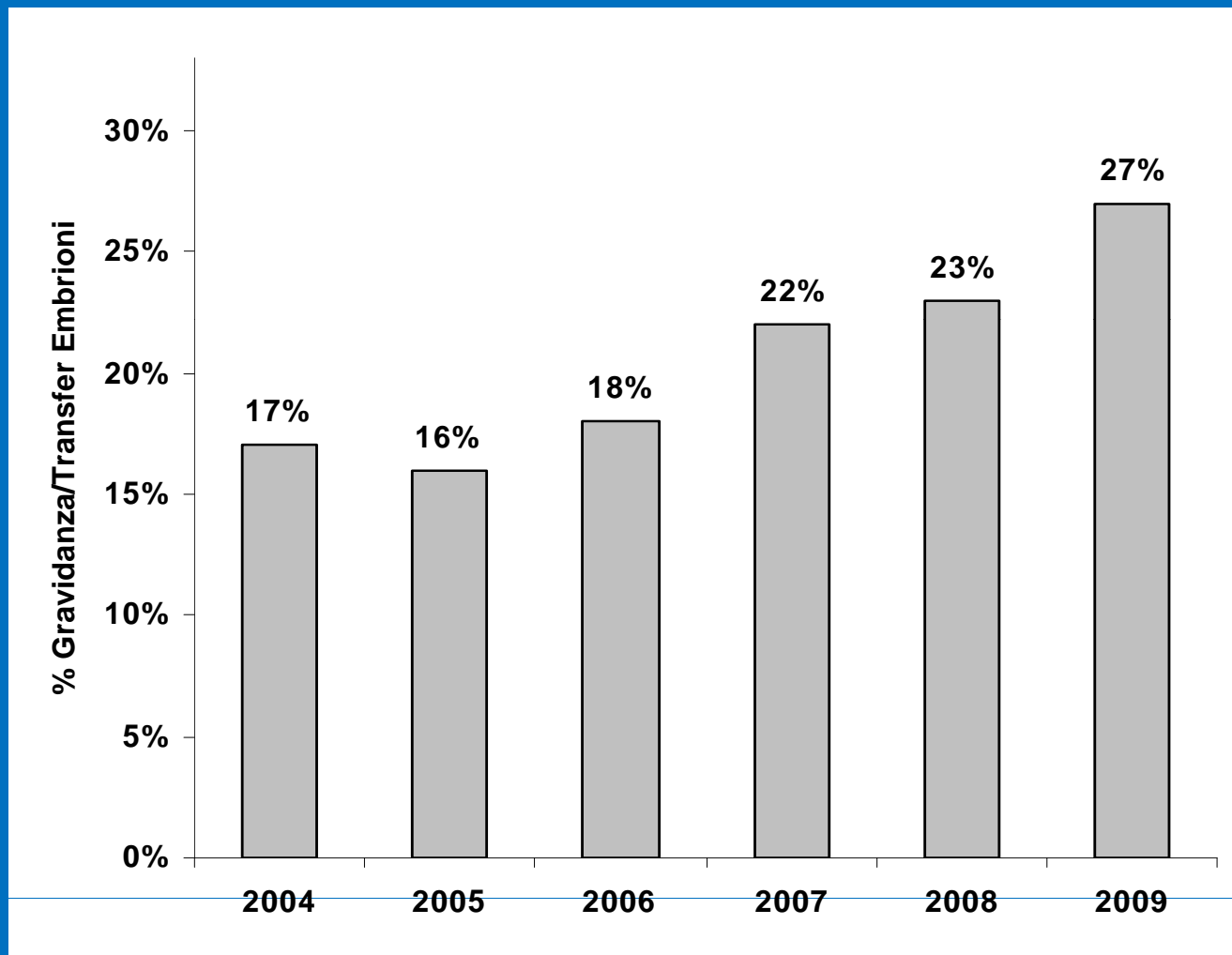
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Pazienti	360
Cicli scongelamento ovociti	501
Transfers embrionali	482
Gravidanze	85
Camere gestazionali	103
Parti	64
Bambini	70
Aborti	21
gravidanza /ciclo	17.0 %
gravidanza /transfer	17.6 %
gravidanza /paziente	23.6 %
Percentuale di impianto	10.1 %
Percentuale di aborto	25.0 %

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Porcu., 2005

## Pregnancy rate / transfer from oocyte cryopreservation



# Cumulative pregnancy rate from fresh and cryopreserved oocyte cycles



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No. patients	749
No. pregnancies	355
No. gestational sacs	458
Cumulative pregnancy rate/transfer	47.4% (355/749)
Cumulative implantation rate	15.5% (458/2947)

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European Journal of Obstetrics & Gynecology and  
Reproductive Biology 113S (2004) S14–S16

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## Oocyte cryopreservation in oncological patients

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# Oocyte cryopreservation in oncological patients

Porcu et al., Eur J Obstet Gynaecol, 2004



Patients	Neoplasia	age	Frozen eggs	Stimulation days	E <sub>2</sub>	FSH ampoules
1	CML	26	22	12	450	33
2	CML	27	16	9	1200	30
3	Craniofaringioma	14	18	8	750	28
4	Medulloblastoma	15	25	13	630	36
5	Mielofibrosi Idiopatica	18	28	12	2820	31
6	Trombocitemia Essenziale	22	17	11	1150	38
7	Linfoma di Hodking	17	21	12	950	36
8	CML	24	12	10	820	29
9	Multiple Sclerosis	27	17	10	350	34
10	CML	21	12	9	630	32
11	Ewing Sarcoma	16	6	11	820	33
12	Wilms	19	15	13	615	39
13	CML	14	19	11	840	37
14	Hodking	15	11	11	1230	34
15	CML	24	12	14	780	42
16	Ewing	18	7	13	1200	38
17	Hodking	23	9	9	1900	28
18	CML	17	12	12	480	36
		19±4	15±6	11±2	978±558	34±4



RBM Online - Vol 17 No 2. 2008 265-267 *Reproductive BioMedicine Online*; [www.rbmonline.com/Article/3364](http://www.rbmonline.com/Article/3364) on web 26 June 2008

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## Case report

Healthy twins delivered after oocyte cryopreservation and bilateral ovariectomy for ovarian cancer

**Porcu et al, 2008**





# Change the way you do IVF !

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