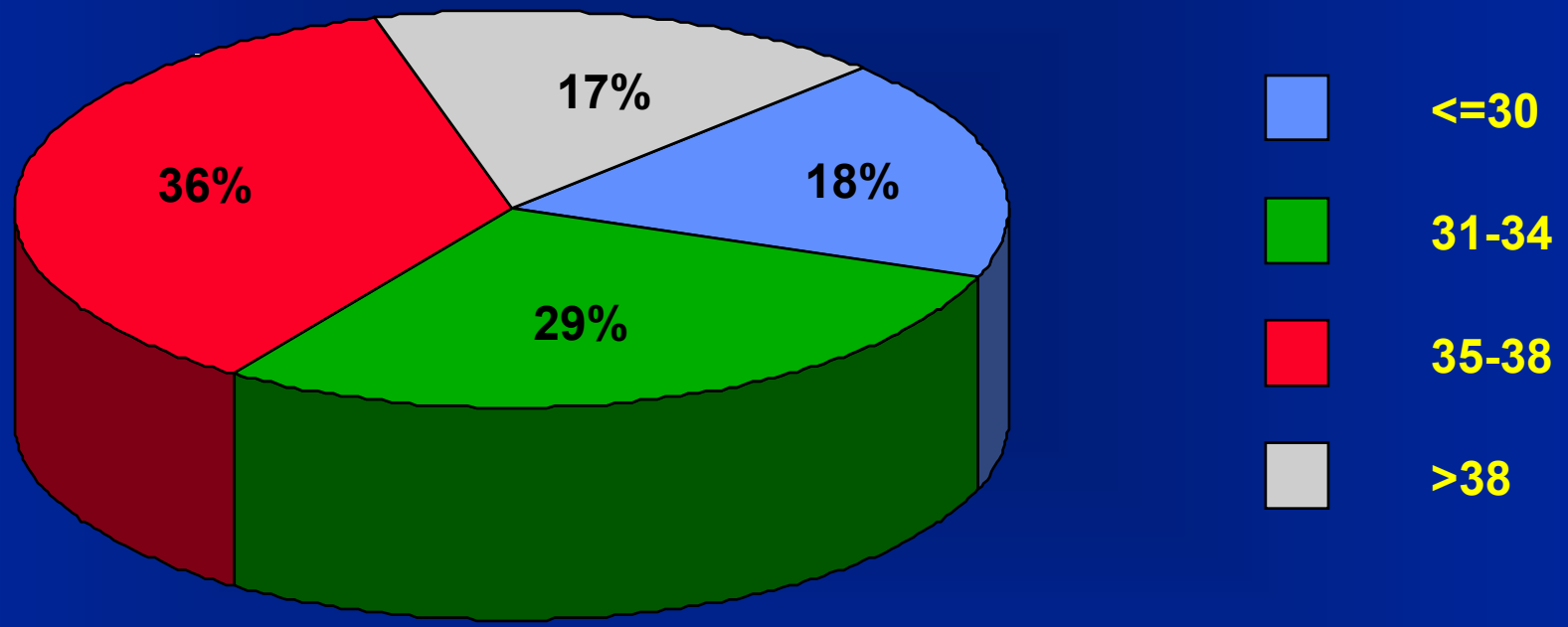
PROGNOSTIC FACTORS FOR IVF OUTCOME

Aldo Campana, Diaa El-Mowafi, Hervé Lucas, and Didier Chardonnens

- Female age as a prognostic factor.
- Early cleavage of in vitro fertilized human embryos to the 2-cell stage
- Development of spare embryos to the blastocyst stage
- Impact of used media on outcome.
- Assisted hatching.

Age Distribution in Our Patients



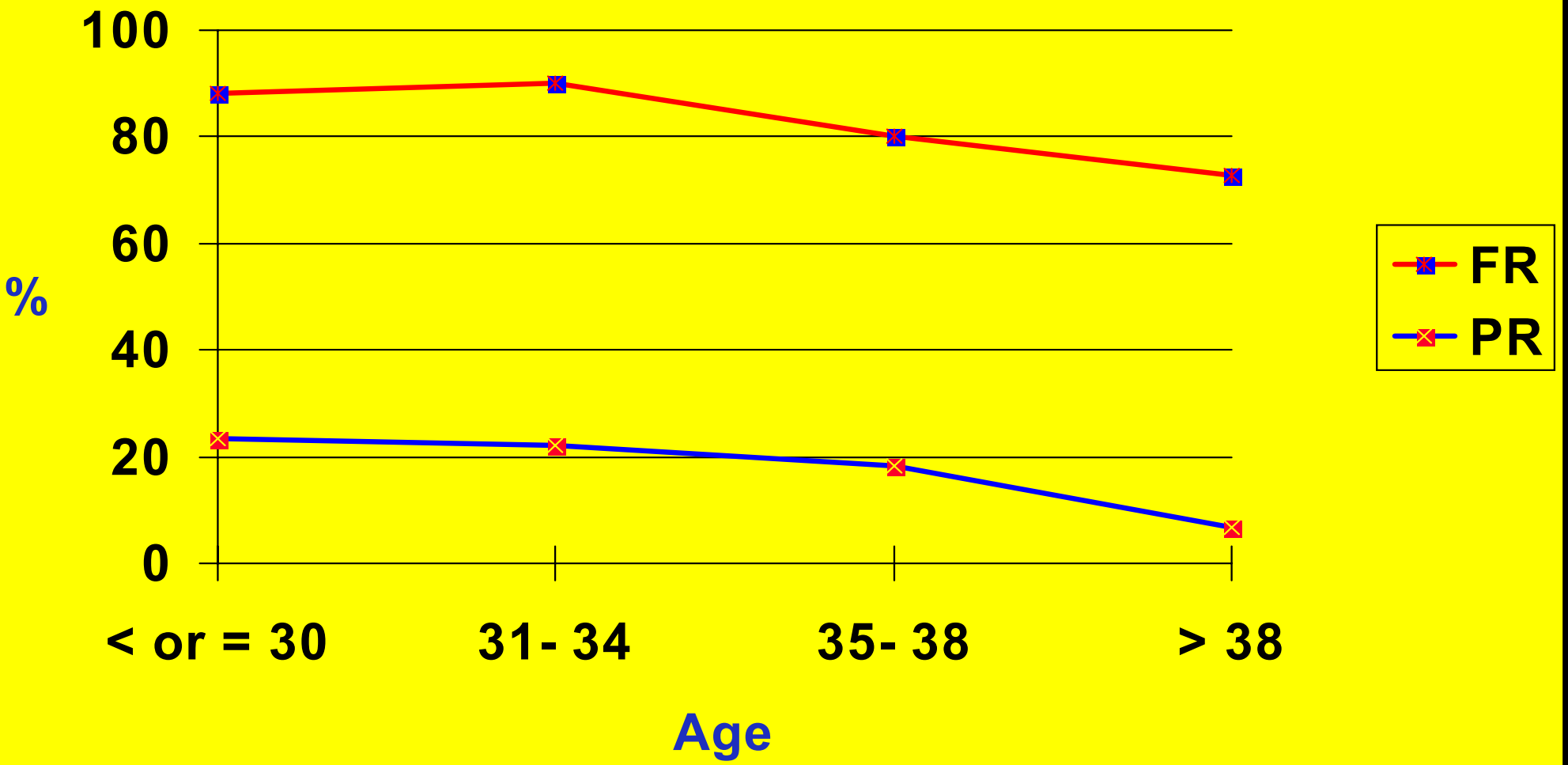
Impact of Age on IVF Outcome

Age (years)	≤ 30	31-34	35-38	> 38
No. of retrieved oocytes (mean)	10.5	11.9	7.5	5.8
Fertilization rate (%)	88.2	90	80	72.7
Pregnancy rate (%)	23.5	22	18.2	6.8

Correlation Between Number of Retrieved Oocytes and Pregnancy Rate

No. of retrieved oocytes	Pregnancy rate per transfer (%)
< 6	8.5
7-15	21.6
16-20	15.6
> 20	0

Impact of Age on IVF Outcome



Evaluation of embryo viability

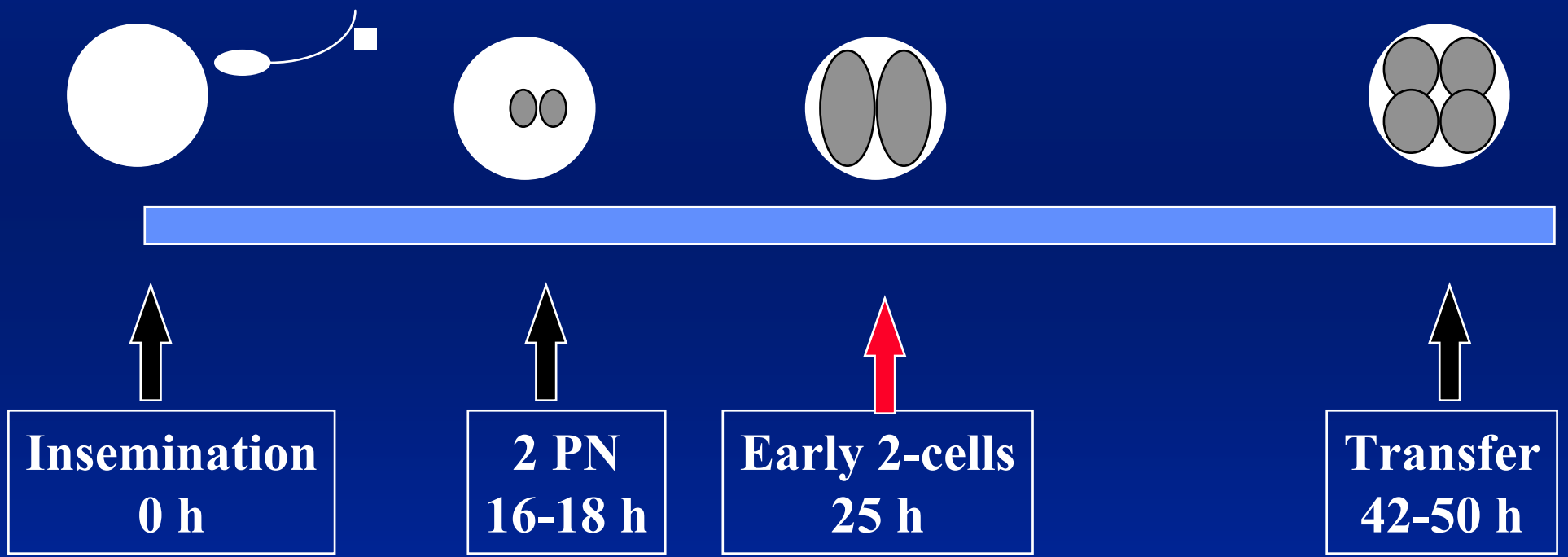
Measurement of metabolic parameters:

- O_2 consumption
- Pyruvate uptake
- Glucose uptake and lactate production
- Secretion of platelet activating factor

Morphological assessment of embryos according to:

- Morphology
- Cleavage stage
- Both

Assessment of early cleaving 2-cell embryos



Clinical pregnancy rates according to the number of embryos transferred in patients who had early and no early cleaving embryos.

N° of embryos transferred	No early cleavage	Early cleavage
1	0/8 (0)	0/3 (0)
2	2/21 (9.5)	0/3 (0)
3	14/76 (18.4)	9/21 (42.9)*
4	1/11 (9.1)	-

* significantly different P<0.05 compared to no early cleavage

Parameters of patients according to whether embryos had or had not undergone early cleavage to the 2-cell stage by 25 h post insemination.

Parameter	No Early Cleavage	Early Cleavage
N° of cycles	116	27
N° of oocytes (mean ± SD)	999 (8.7 ± 6.5)	229 (8.5 ± 4.8)
N° of 2 PN (mean ± SD)	607 (5.23 ± 3.5)	160 (6.07 ± 3.9)
Early 2 cells (mean ± SD)	0	75 (2.78 ± 2.4)
N° of embryos on day 2 (mean ± SD)	511 (4.41 ± 3.2)	156 (5.78 ± 3.9)
N° of embryos transferred (mean ± SD)	322 (2.78 ± 0.7)	72 (2.67 ± 0.6)
Implantation rate (%)	**24/322 (7.5)**	**17/72 (23.6)***
N° of clinical pregnancies (%)	**17 (14.7)**	**9 (33.3)***

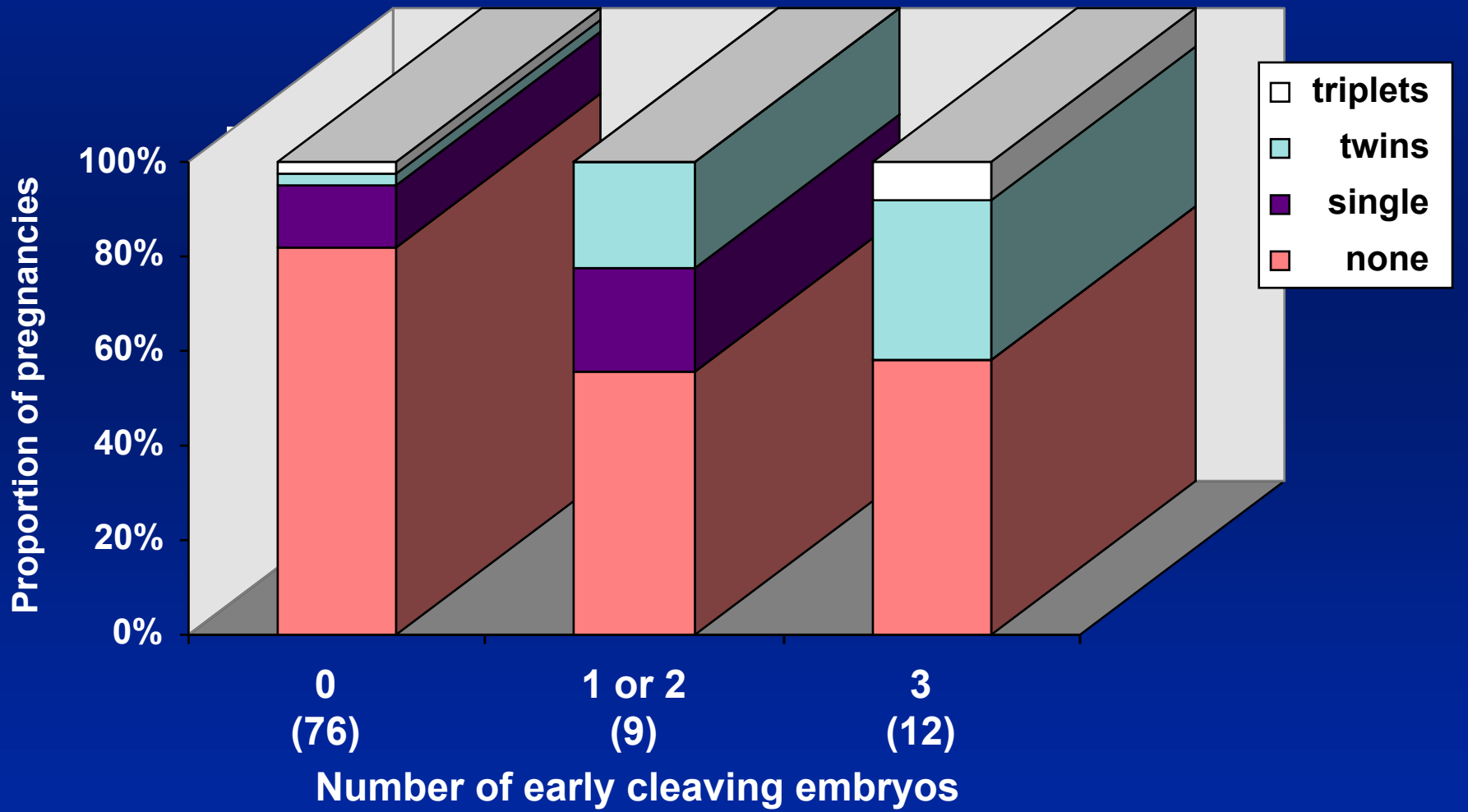
*** significantly different P<0.05 compared to no early cleavage**

Parameters of patients according to whether embryos had or had not undergone early cleavage to the 2-cell stage by 27 h post ICSI.

Parameter	No Early Cleavage	Early Cleavage
N° of cycles	34	54
N° of oocytes (mean ± SD)	296 (8.7 ± 5.0)	489 (9.0 ± 5.3)
N° of oocytes injected (mean ± SD)	238 (7.0 ± 4.2)	440 (8.2 ± 4.8)
N° of 2 PN (mean ± SD)	137 (4.0 ± 2.2)	263 (4.9 ± 2.8)
Fertilization rate (%)	(57.6)	(59.8)
Early 2 cells (mean ± SD)	0	122 (2.2 ± 1.6)
N° of embryos on day 2 (mean ± SD)	96 (2.8 ± 1.9)	231 (4.3 ± 2.4)*
N° of embryos transferred (mean ± SD)	93 (2.7 ± 0.8)	150 (2.8 ± 0.7)
Implantation rate (%)	**3/93 (3.2)**	**21/150 (14.0)***
N° of clinical pregnancies (%)	**2 (5.9)**	**14 (25.9)***

*** significantly different P<0.05 compared to no early cleavage**

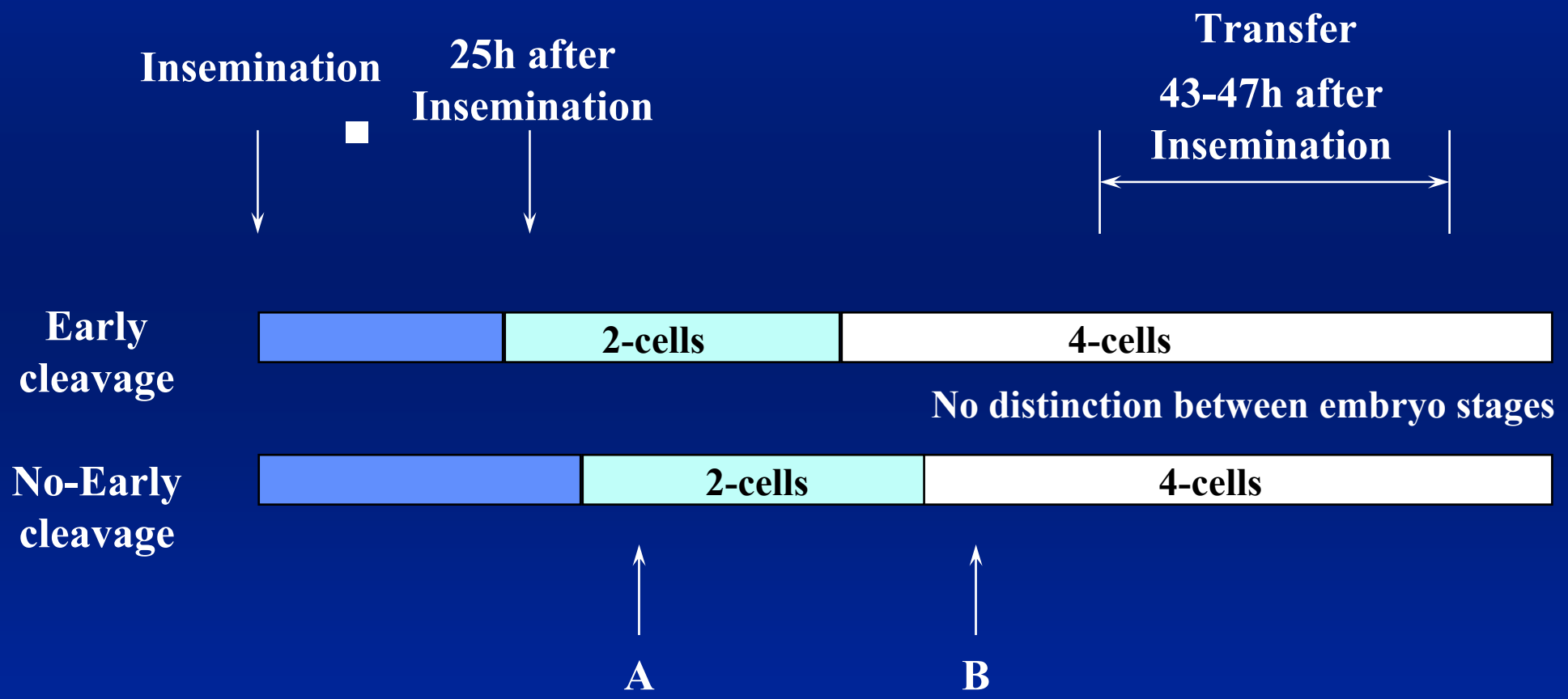
Pregnancy in relation to the number of early and no early cleavage embryos transferred in patients receiving three embryos at transfer.



Main Results

- The pregnancy rate in the early cleavage group was double the rate of the no-early cleavage group.
- Early cleaving embryos implanted at a rate 3-fold higher than no early cleaving embryos.

The importance of specific time for distinguishing embryo cleavage during screening.



Conclusions for Early cleavage embryos

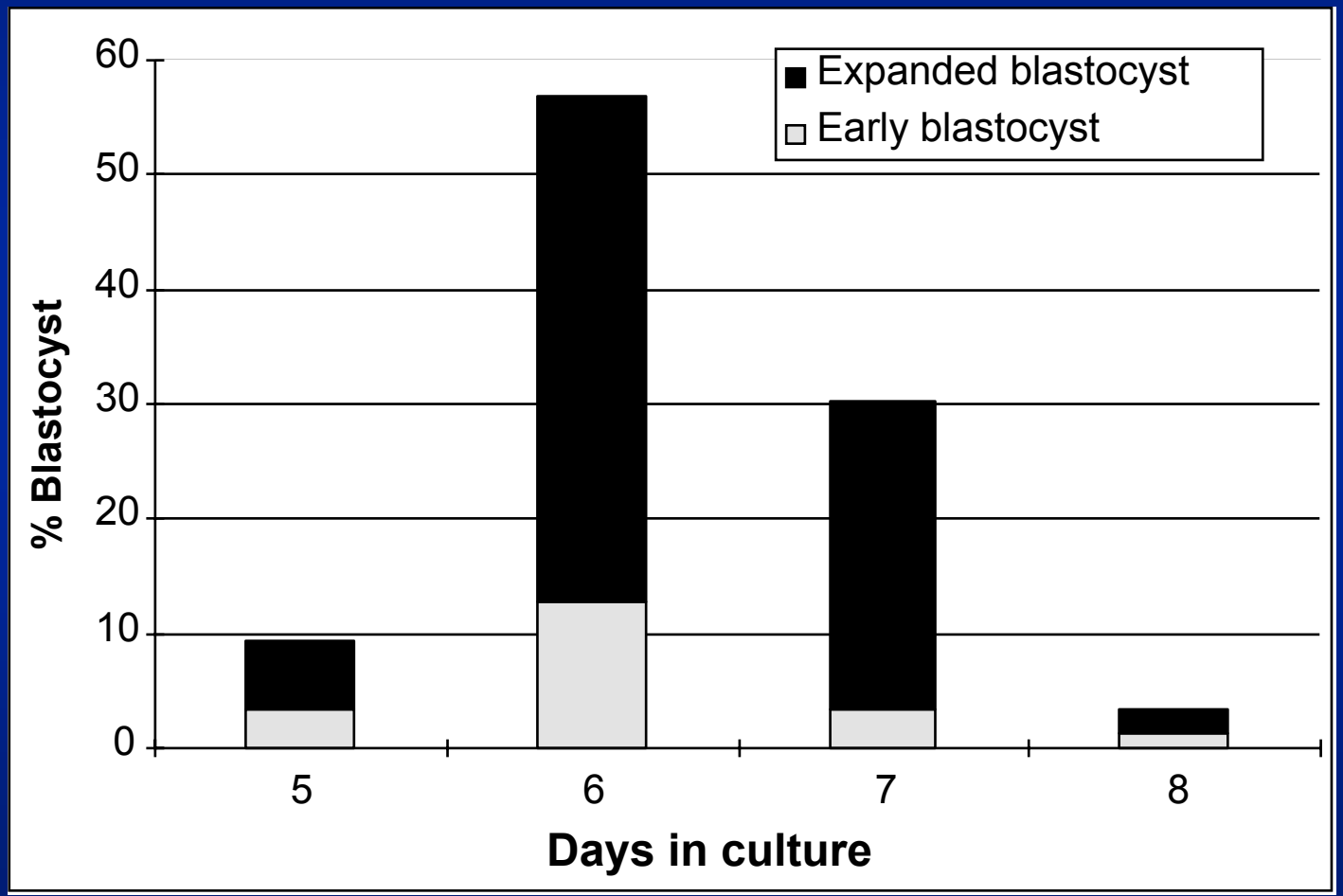
- Assessment of early cleavage to the 2-cell stage can be used as an indicator of embryo viability and is subsequently a strong prognostic factor of the likelihood of pregnancy.
- Selecting early cleaving 2-cell embryos alleviates the problem of guessing which are the more advanced embryos at the time of transfer.
- ICSI results have shown that early cleavage is not influenced by the timing of fertilization.
- Early cleavage is likely due to intrinsic factors within the oocyte or embryos that promote embryo cleavage after fertilization.

SELECTION OF THE BEST SPARE EMBRYOS BY CULTURE TO THE BLASTOCYST STAGE

DEVELOPMENT OF SPARE EMBRYOS TO THE BLASTOCYST STAGE

N° of cycles	90
N° of cycles with blastocysts (%)	70 (77.7)
N° of spare embryos	423
N° of blastocysts (%)	200 (47.3)

DEVELOPMENT OF SPARE EMBRYOS TO THE BLASTOCYST STAGE



The influence of the day of freezing and the day of transfer (after LH peak) on pregnancy rate

Day of transfer from LH peak ■	Day of freezing		Total (%)
	Day-5 and 6 blastocysts	Day-7 and 8 blastocysts	
4	0/2	0/3	0/5 (0)
5	1/4	1/4	2/8 (25)
6	4/10	0/7	4/17 (23.5)
7	1/1	0/2	1/3 (33.3)
9	1/1	-	1/1 (100)
Total (%)	7/18 (38.9)	1/16 (6.2)*	8/34 (23.5)

* P=0.04 comparing Day 7-8 blastocysts to Day 5-6 blastocysts

Conclusions for Blastocysts

- Culture of spare embryos to the blastocyst stage allows a selection of better quality embryos for freezing
- Blastocysts frozen on the 5th or 6th day of culture have a significantly higher viability than blastocysts that formed after the 6th day
- The rate of blastocyst development is more important than the timing of the transfer

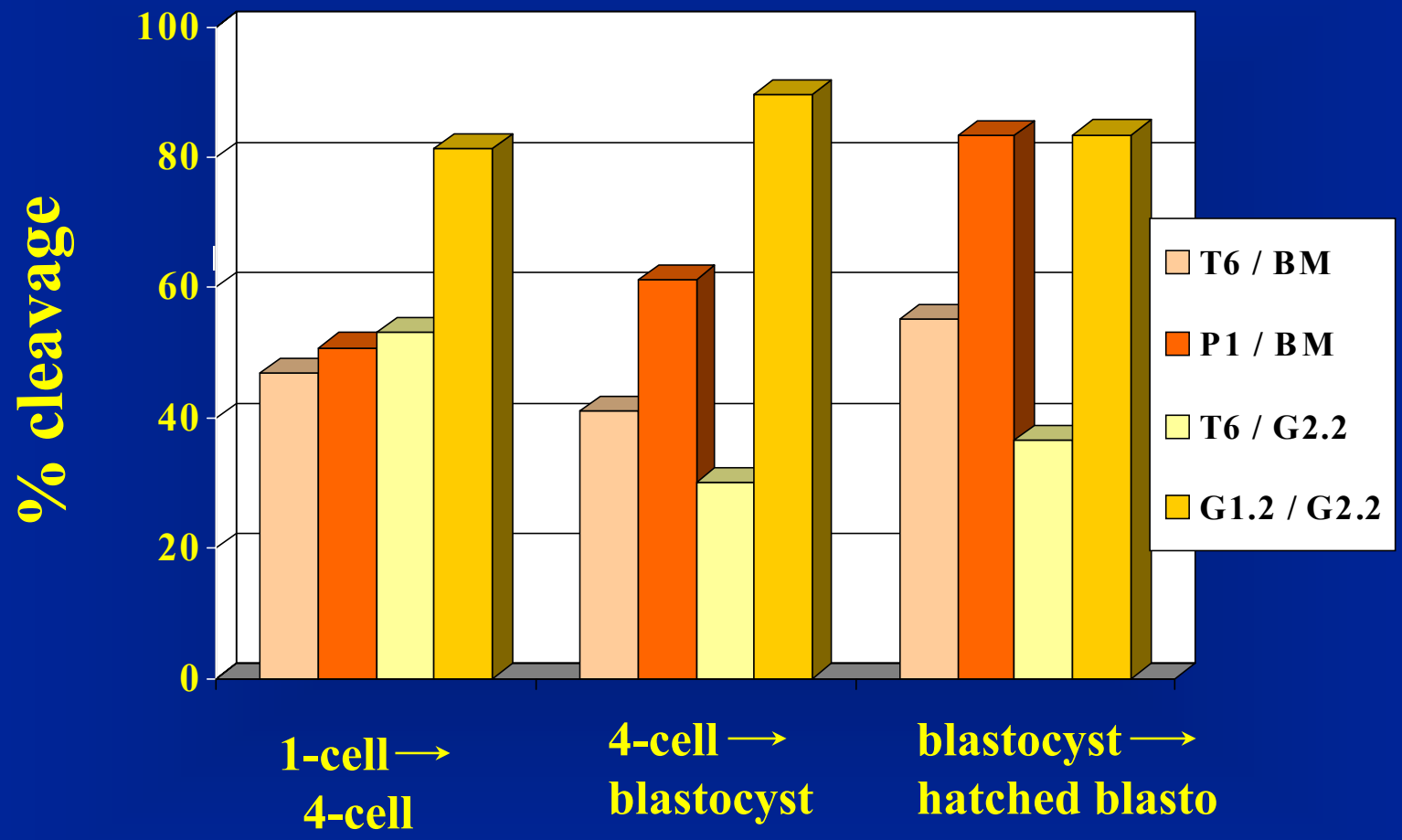
Glucose requirement in culture media during the different steps of fertilization and embryo development *in vitro*

	Fertilization	Zygote ⇨ 8¢	8¢ ⇨ Blastocyst
Glucose	+	-	+

The use of sequential media is necessary for optimum embryo development in human

Stage	Medium	Glucose levels
Sperm capacitation	IVF-50 ™	High
Fertilization	IVF-50 ™	High
Zygote to 8-cell	G1.2 ™	Low
8-cell to balstocyst	G2.2 ™	High

Effect of different culture media on mouse embryo development



BM ™ = Blastocyst Medium G1.2 ™ & G2.2 ™ = Gardner's media

Final hatching results according to the source of embryos treated by the different methods

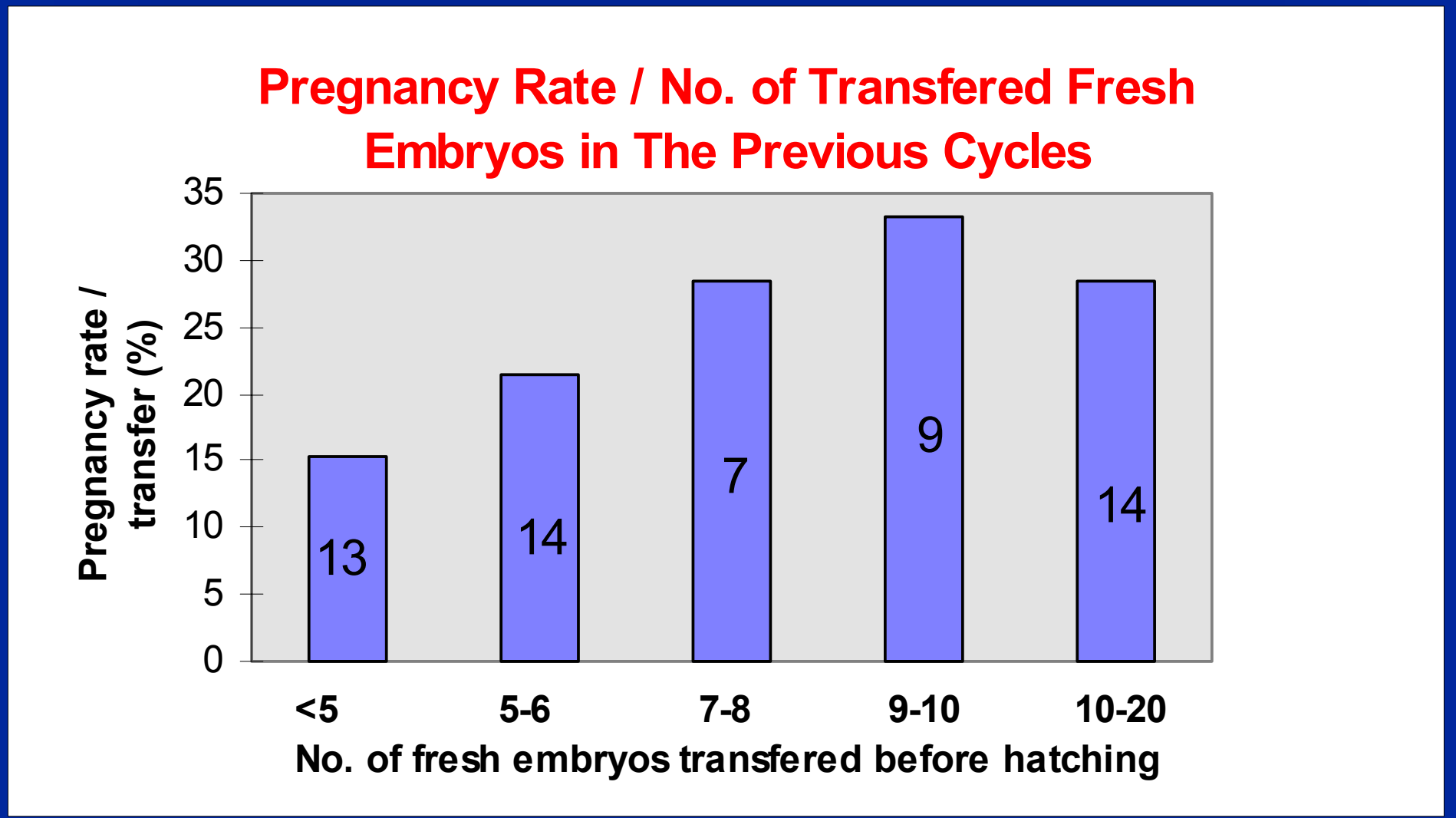
Source of embryos ➔	IVF	ICSI	IVF or ICSI thawed
No. of Patients	30	26	22
Mean Age (±SD)	36,5 (±3,7)	34,12 (±4,6)	35,22 (±5,1)
Range of Cycles	3,54 (±1,6)	3,83 (±2,3)	2,88 (±1,6)
Fertilisation rate (mean) / M II	73.85	66.5	75.7
Cleavage rate (mean) / M II	72.19	66.5	76.2
No. of transfered hatched embryos	31	29	24
Total no. of embryos hatched	80	69	54
Mean of hatched embryos/cycle	2,58 (±0,8)	2,37 (±0,7)	2,25 (±0,7)
Pregnancy rate/Transfer(No. of pregnancies)	22,58% (7)	24,13% (7)	8,30% (2)
Pregnancy rate/Patient	23.33%	26.92%	9.10%
Implantation rate(No. sacs)	11,25% (9)	11,59% (8)	3,70% (2)

Conclusion : No significant benefit to treat thawed embryos by assisted hatching.

Success rate after assisted hatching in patients with primary and secondary infertility

Final results of hatched embryos	IVF + ICSI Fresh embryos hatched		
	Primary Infertility	**Secondary Infertility**	**Total**
No. of transfer with hatching	**39**	**21**	**60**
Mean number of fresh embryo transferred in the previous cycles	**6,54** (±3,5)	**8,38** (±4,5)	**7,18** (±4,0)
Total number of hatched embryos	**92**	**57**	**149**
Mean number of hatched embryos	**2,36** (±0,7)	**2,71** (±0,8)	**2,48** (±0,8)
Pregnancy rate/Transfer. (No.)	**17,95%** (7)	**24,14%** (7)	**23,33%** (14)
Implantation rate (No. of sacs)	**9,78%** (9)	**14,04%** (8)	**11,41%** (17)

Correlation Between the Number of Embryos Transferred in the Previous Cycles and the Pregnancy Rate After the Assisted Hatching



Assisted hatching was indicated in :

- Failed Embryo Transfer (more than 3 transfers of 2 good quality embryos).

and/or

- Thick zona pellucida (> 15 μm).

Conclusions:

- Patients with secondary infertility seem to get benefit from assisted hatching. This may be due to change in the quality of the ZP corresponding to the increase in age.
- The practice of assisted hatching is still controversial. ESHRE metanalysis is currently runnining to have a conclusion.