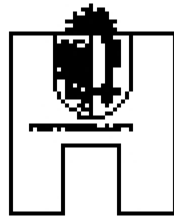


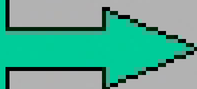
WHO Collaborating Centre
for Research in Human Reproduction
Clinic for Infertility and Gynecological Endocrinology
University Hospital, Geneva, Switzerland

ASSISTED REPRODUCTIVE TECHNOLOGIES

DR HERVE LUCAS, MD PHD,

September 28, 2000

Assisted Reproductive Technologies (ART)



★ Artificial Insemination (AI)

Origin of sperm :

- AIC : AI with conjoined sperm
- AIC : AI with donor sperm

Site of insemination :

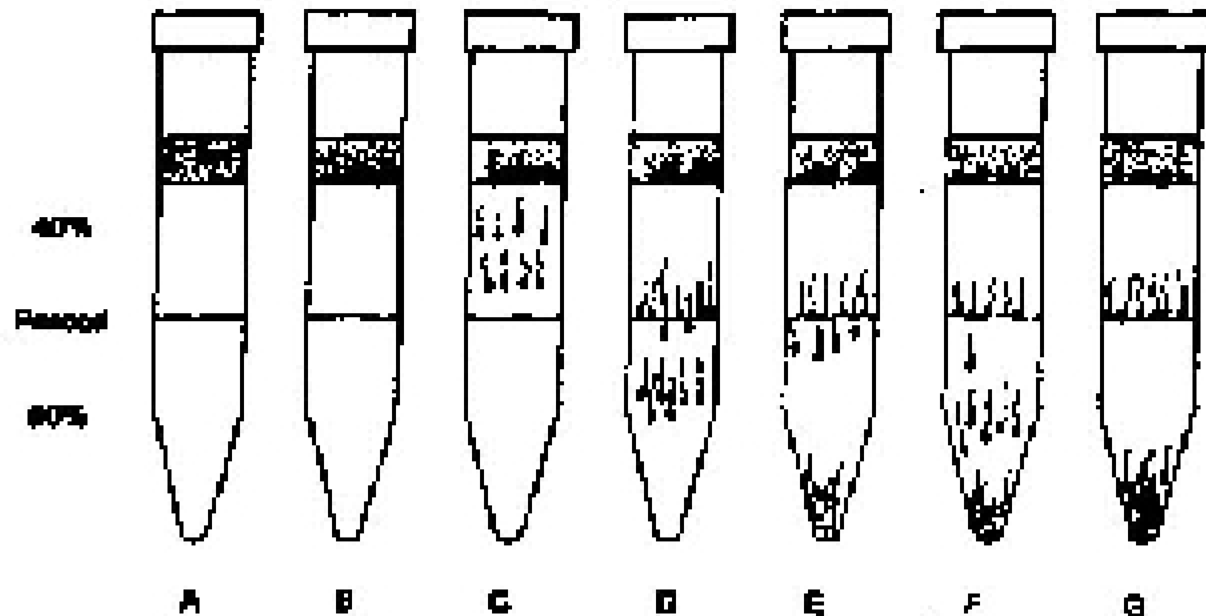
- ICI : intra-cervicale insémination
- IUI : intra-utérine insémination

★ In vitro fertilization

- IVF : classical IVF
- ICSI : assisted IVF

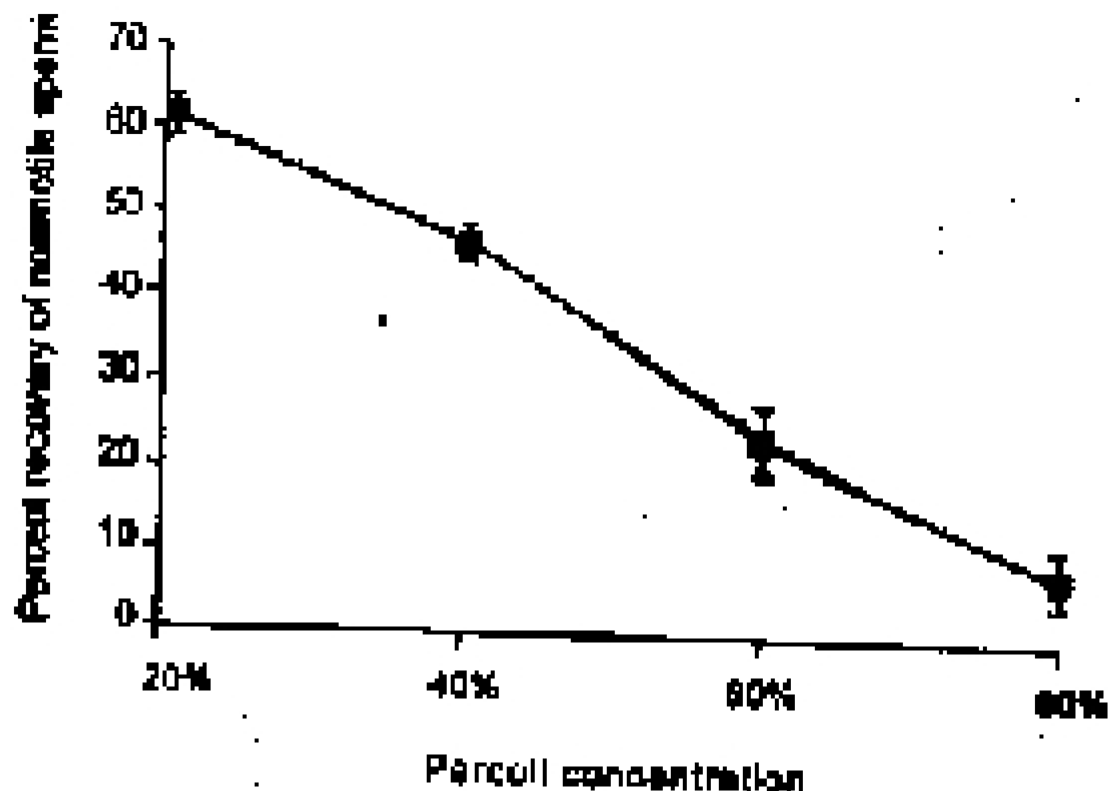
Sperm Preparation (1)

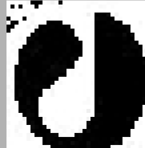
Sequence of events during sperm separation with the use of two Percoll layers. Randomly oriented sperm, spread on the top layer (A), turn their heads downward as soon as centrifugation starts (B). A gap is formed between sperm swimming down by their own tail movements and nonmotile sperm precipitating just by the gravitational force (C). Part of the nonmotile and abnormal forms are trapped at the isopycnic zone of the interface (D). In 5-10 minutes, fast-moving sperm reach the first, while nonmotile sperm, decelerated by the high-density 80% Percoll, form a gap (E). Between 10 and 20 minutes, nonmotile sperm approach the motile sperm (F), gradually reversing the separation.



Sperm Preparation (2)

Recovery of nonmotile sperm from the bottom of the test tube after aliquots of 0.5 mL from the same specimen were spread on a 30-mm-high, single layer of 20%-80% Percoll and centrifuged at 300 x g for 10 minutes (n = 8). Each point indicates mean ± SEM. P<.01 for the difference between every two adjacent time points.





Pharmacia Biotech

January 13, 1997

Assistance Hôpitaux
Publique De Paris
123, Boulevard de Port-Royal
75679 Paris Cedex 14
France

Formal Notification

Percoll® is NOT to be Used for the Isolation of Spermatozoa used in Assisted Reproduction in Humans

Dear

We are writing to you on an extremely important matter concerning the use of our product, Percoll, for the isolation of human spermatozoa which are subsequently reintroduced into humans through assisted reproduction technologies (ART). Percoll is a density gradient centrifugation reagent specifically designed for the separation of cells, viruses and subcellular particles from tissues, cell cultures and blood samples. Percoll is marketed and sold for RESEARCH PURPOSES ONLY.

Human Reproduction vol.13 no.11 pp.3139–3143, 1998

Evaluation of three substitutes for Percoll in sperm isolation by density gradient centrifugation

O.E.Claassens[1,3], R.Menkveld[1] and K.L.Harrison[1]

Table II. Comparison between semen parameters and [illegible] values after Isolate, OptiPrep, Percoll and PureSperm gradient centrifugation for the oligozoospermic and normal donor groups (values are mean ± SD)

Sperm parameter	Unwashed	Isolate	OptiPrep	Percoll	PureSperm
Oligozoospermic (n = 10)					
Concentration ($\times 10^6$/ml)	10.6 ± 6.3	3.8 ± 1.4	[illegible]	2.7 ± [illegible]	[illegible]
Motility (%)	35.8 ± 10.5[b]	65.2 ± 16.1	[illegible]	[illegible]	62.0 ± 13.4
Progressive motility (%)	43.0 ± 9.1[b]	85.3 ± 16.8	[illegible]	87.4 ± 9.1	[illegible]
Morphology normal (%)	3.2 ± 1.9	4.6 ± 3.5	4.2 ± 2.5	[illegible]	4.3 ± 2.5
Teratozoospermia index	1.7 ± 0.1[d]	1.49 ± 0.2	[illegible]	1.6 ± 0.3	1.5 ± 0.2
Acrosome index (%)	4.3 ± 2.1	5.5 ± 3.2	5.1 ± 3.7	4.0 ± 1.8	4.4 ± 2.7
Normal chromatin condensation (%)	64.0 ± 13.2[a]	90.3 ± [illegible]	[illegible] ± 10.0	95.5 ± 5.3	88.2 ± 10.0
Normal sperm donors (n = 10)					
Concentration ($\times 10^6$/ml)	77.8 ± 34	[illegible]	82 ± 5.8	20.5 ± 13.6	23.3 ± [illegible]
Motility (%)	54.6 ± 16.2[a]	84.8 ± 3.7	87.9 ± 9.6	85.2 ± 6.9	82.6 ± 8.5
Progressive motility (%)	[illegible] ± 18.1[b]	45.4 ± [illegible]	[illegible] ± 11.5	94.7 ± 6.4	87.4 ± 10.5
Morphology normal (%)	6.9 ± 4.3	7.1 ± 4.1	5.9 ± 3.9	5.4 ± 3.6	[illegible] ± 4.3
Teratozoospermia index	1.5 ± 0.2[c]	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.2 ± 0.1
Acrosome index (%)	8.1 ± 1.9	[illegible] ± 1.6	7.2 ± 4.4	[illegible] ± 5.2	9.7 ± 4.5
Normal chromatin condensation (%)	80.1 ± 12.5	90.0 ± 14.5	88.4 ± 11.5[e]	[illegible] ± 22.1	93.5 ± [illegible]

[a]Statistical differences not determined.
[b]Significantly lower than other values in same row (P < 0.005).
[c]No significant differences between groups.
[d]Significantly higher than other values in same row (P < 0.0005).
[e]Significantly higher than unwashed sample (P < 0.005).

Influence of centrifugation

Swim-up with elimination of decapacitant factors

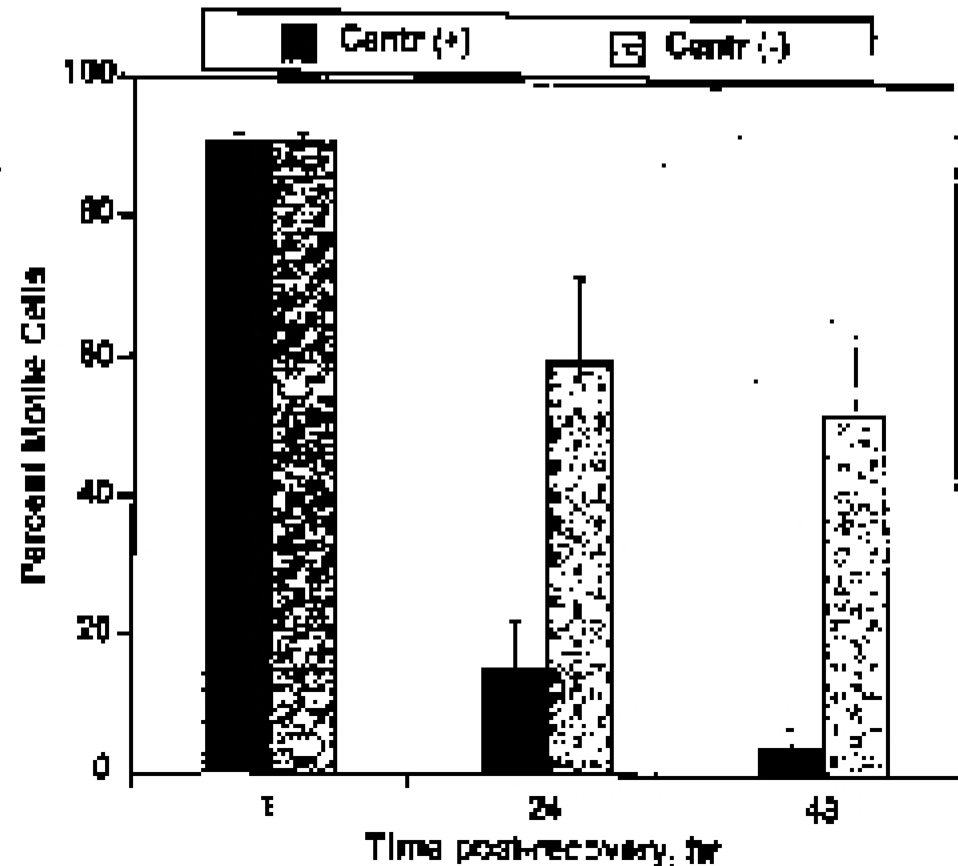


Fig. 2. Effect of centrifugation after swim-up on percentage of motile cells assessed over 48 h incubation at 24°C in samples obtained by the dextran swim-up procedure. The sample obtained by the swim-up procedure was split; one aliquot was centrifuged at 600 g for 8 min, followed by resuspension of the cells to the original volume [Centr(+)], while the other aliquot remained uncentrifuged [Centr(−)]. Values were the means for 10 samples, each from a separate donor; error bars indicate SD. After 1 h incubation, there was no significant difference between treatments ($P = 0.28$). At 24 and 48 h, the difference was highly significant ($P < 10^{-7}$).

Conclusion :

The quality of the sperm preparation is very important

Swim-up : decapacitant factors, influence of centrifugation

Association of a gradient preparation technique with swim-up

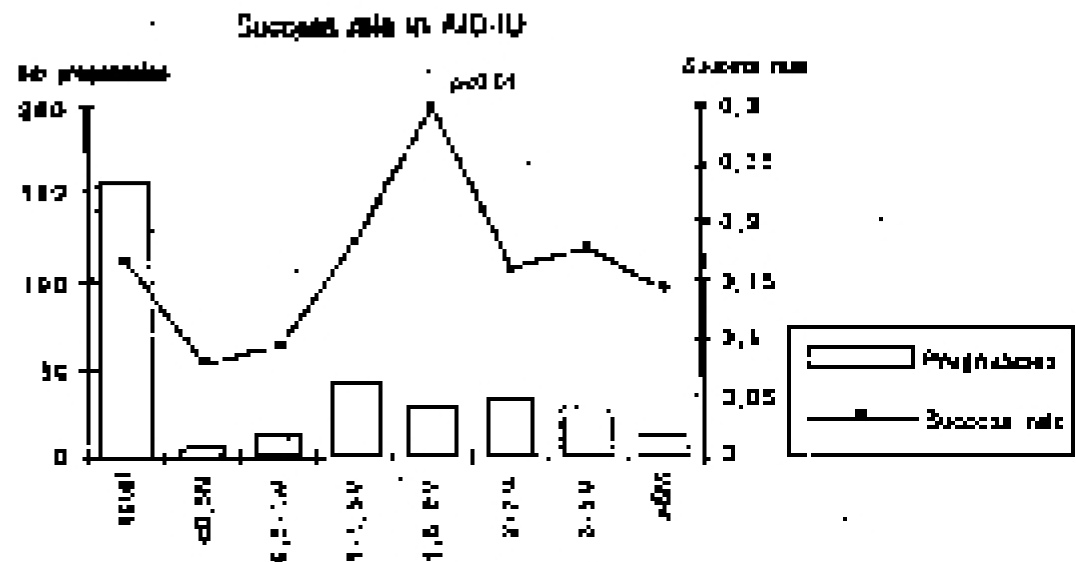
For FIV, ICSI or IIU (<15 M/spz inseminated)

Modify the number of phases in the gradient

Modify the density of the phases (frozen sperm, testicular sperm)

AID

AIC



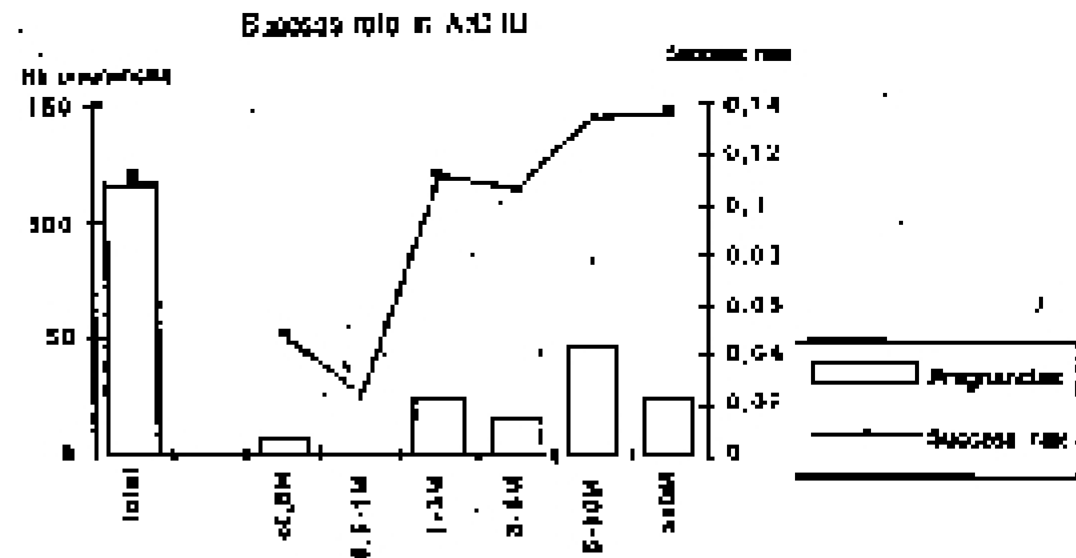
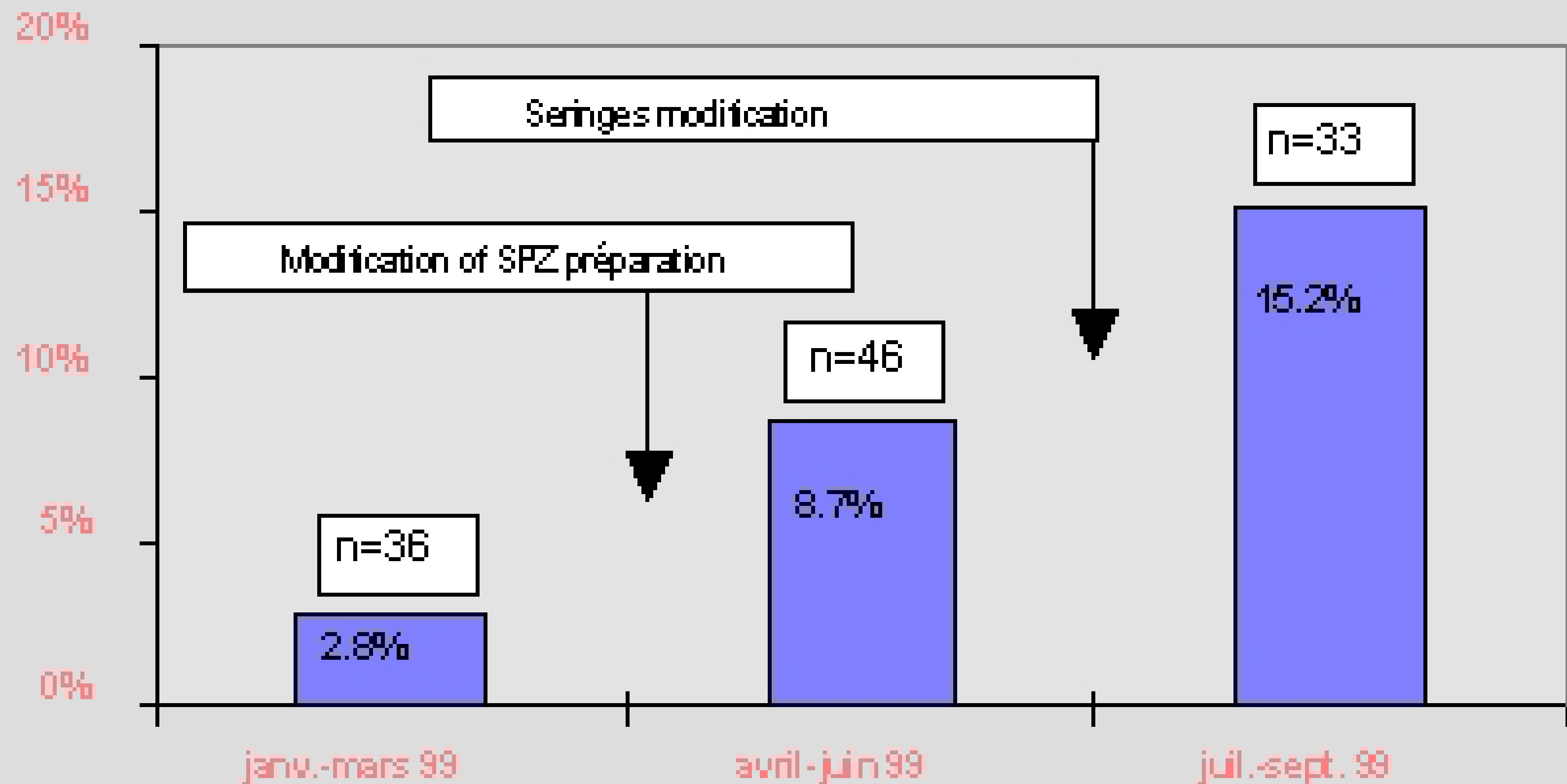


Figure 1 : Taux de succès en insémination intrautérine avec sperme frais de conjoint ou avec sperme congelé de donneur, en fonction du nombre de spermatozoïdes mobiles inséminés.

Variation of PR in IIU in 1999



ORIENTATION THERAPEUTIQUE EN A.M.P.

*Critères

- mobilité
- morphologie

*Nombre total de spermatozoïdes mobiles dans la préparation

$\geq 1 \times 10^6$ ---------- IAC - IAD
IU

$\geq 0,5 \times 10^6$ -------- FIV classique

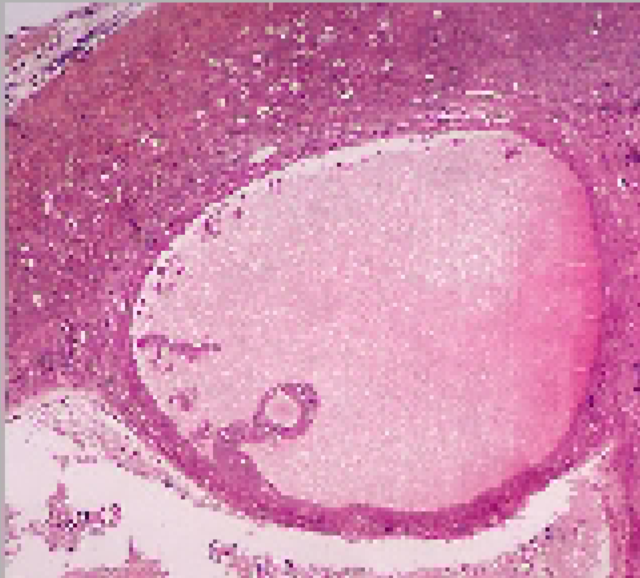
$\geq 0,2 \times 10^6$ -------- FIV microgouttes
(10 000 spermatozoïdes/ 20 µl)

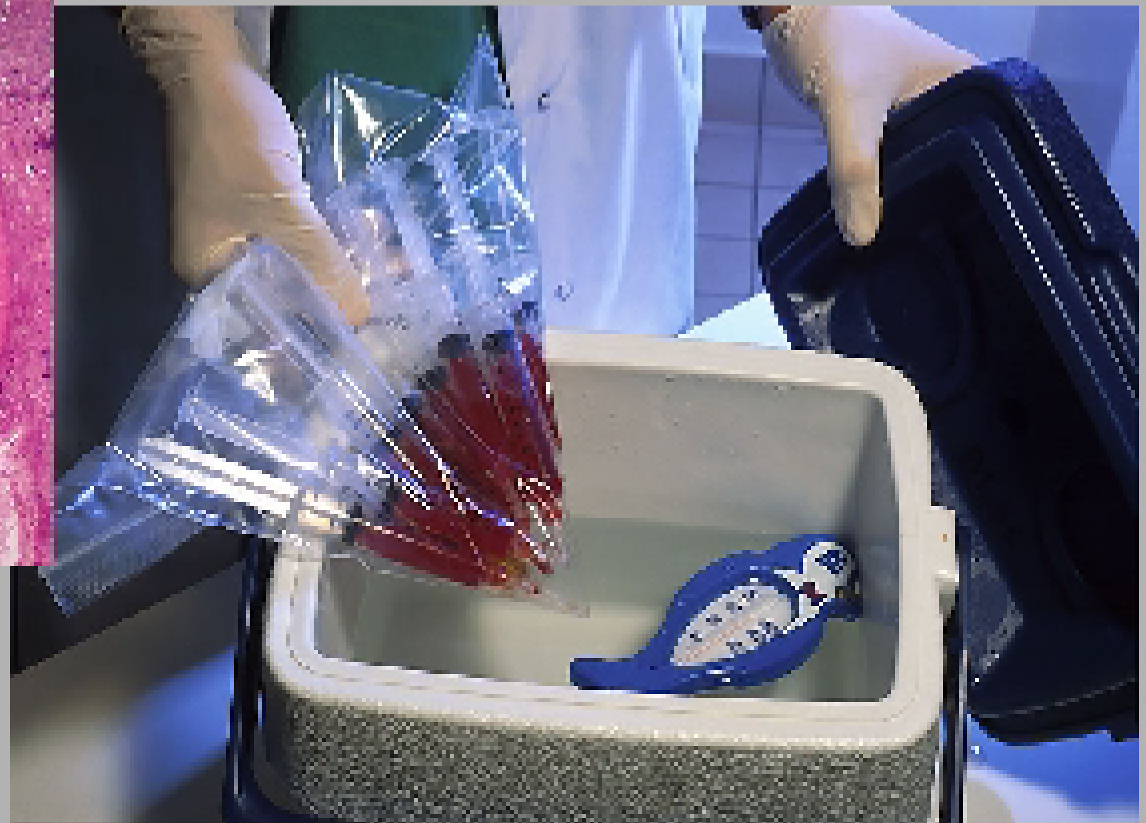
$< 0,2 \times 10^6$ ---------- I C S I

Different steps of IVF

Section of a mature follicle

J0

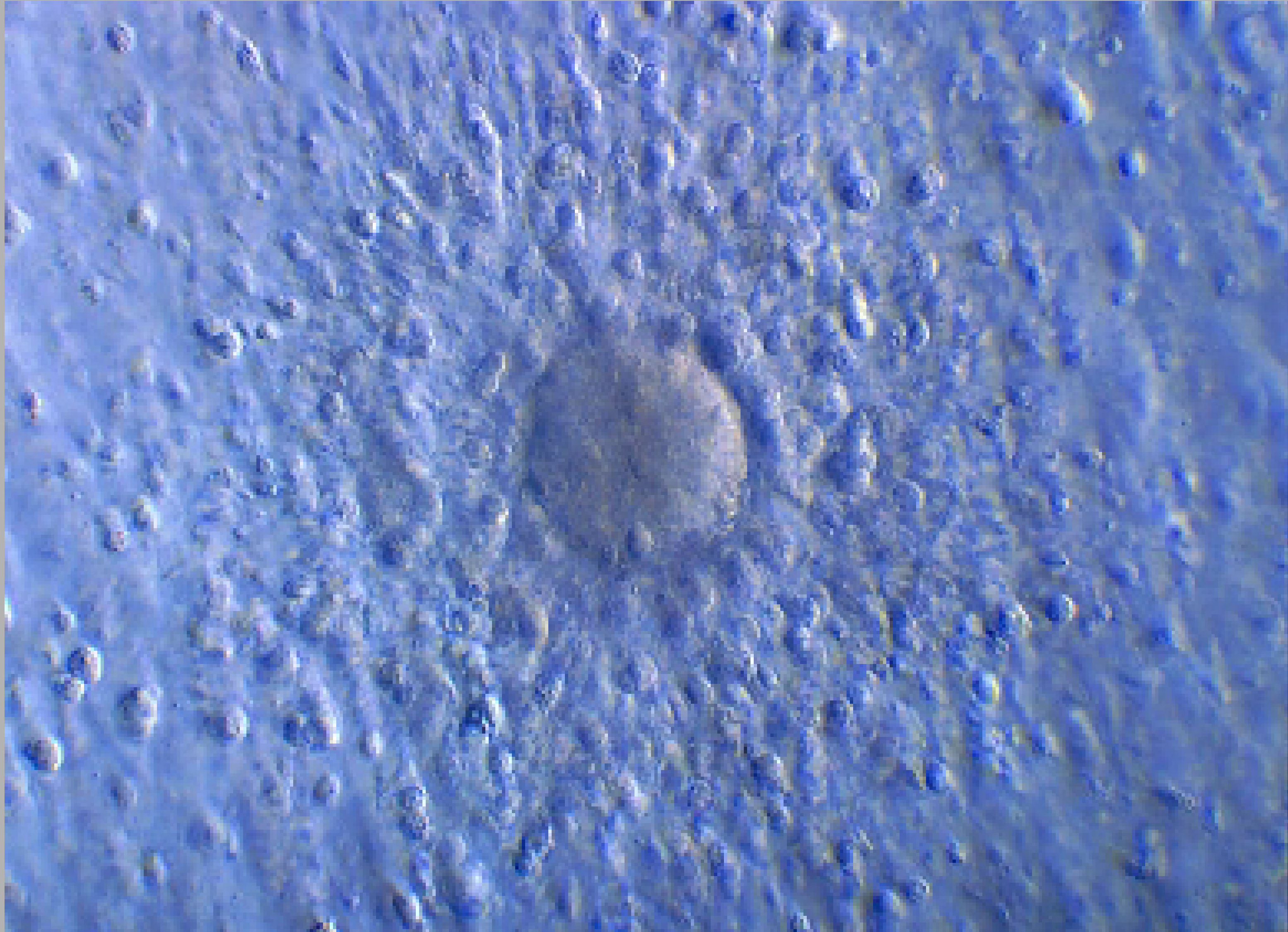




Seringes with cumulus-oocyte complexes in follicular fluid

JO

Ovocyte surrounded by the cumulus-oocyte complex

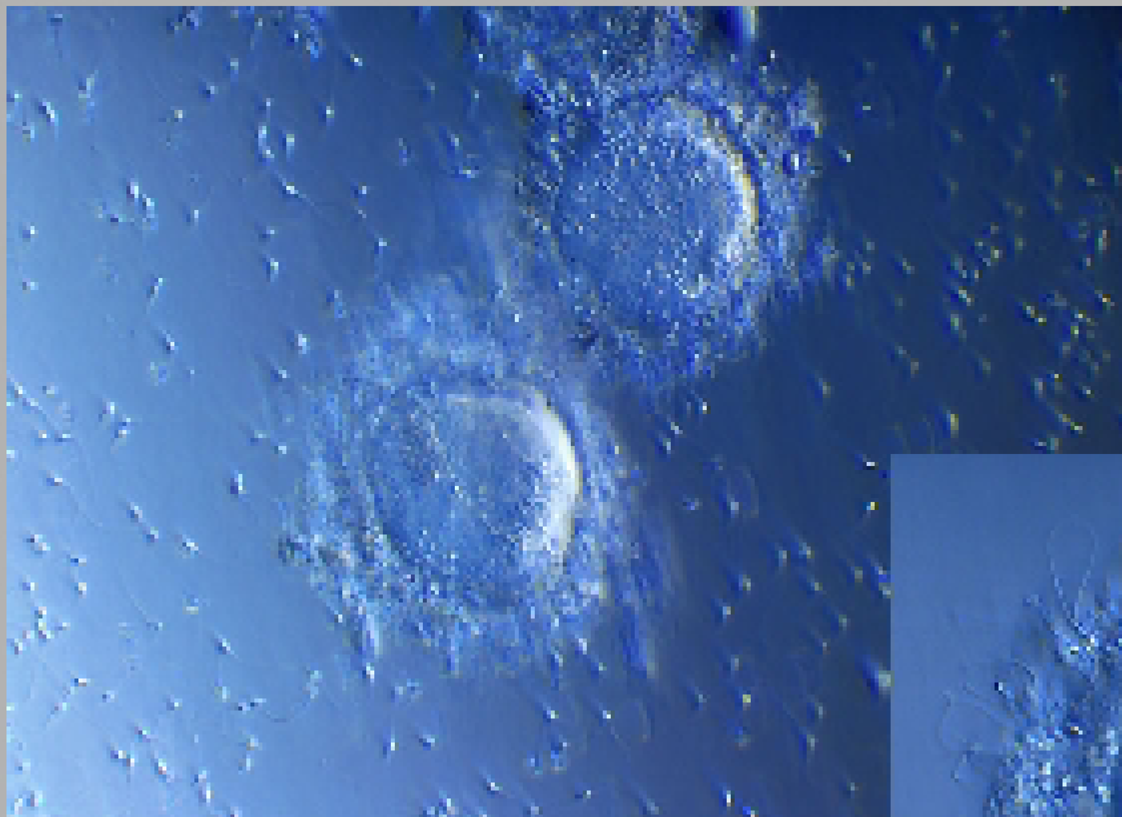


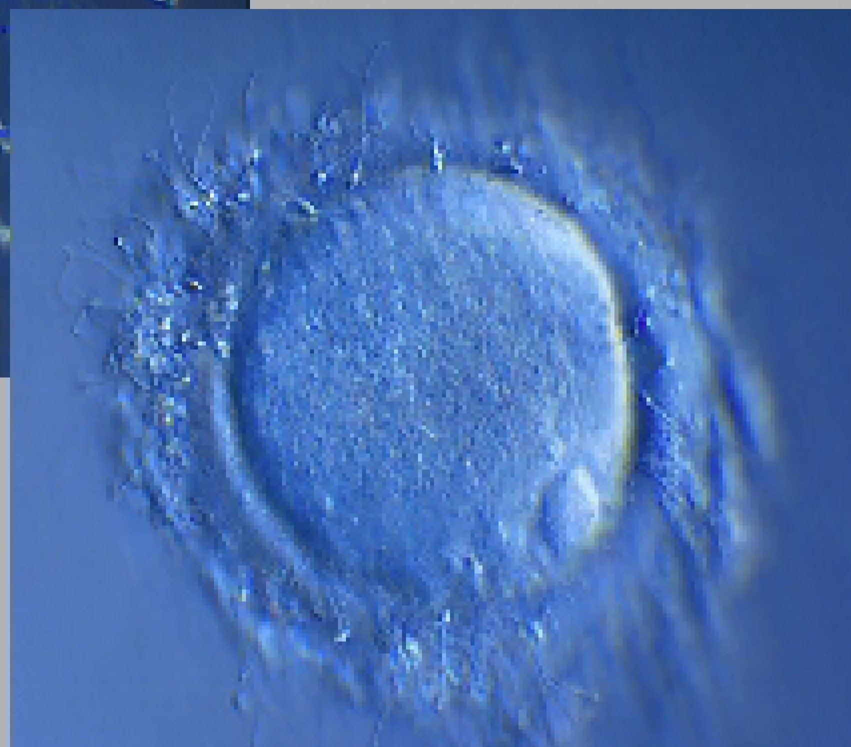
JO

Technician with the sperm preparation

J1

Decoronisation after classical IVF

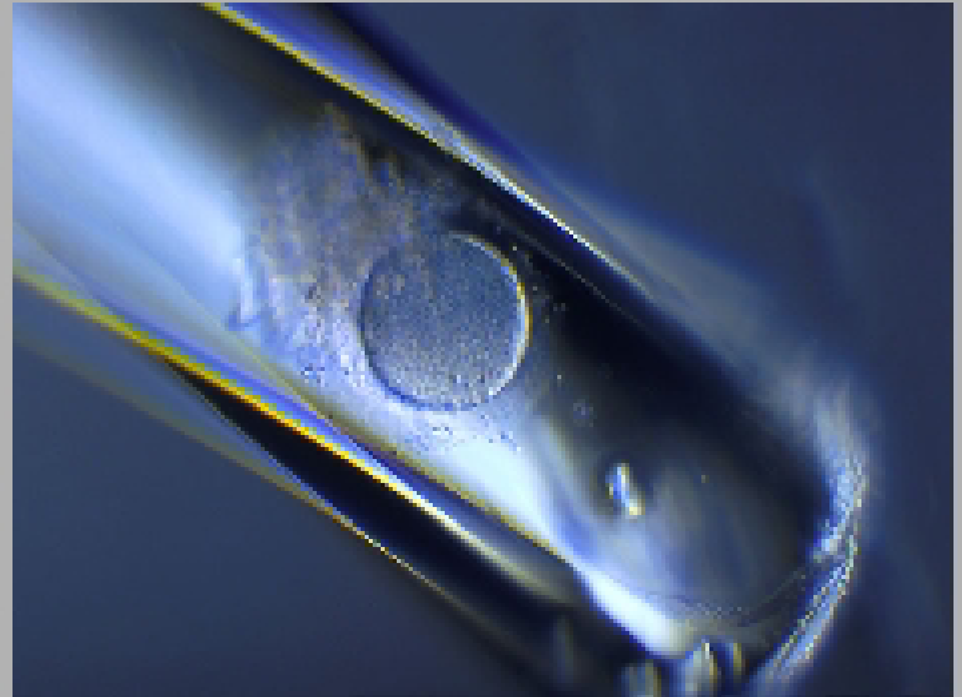




Unfertilized oocyte

J1

Décoronisation

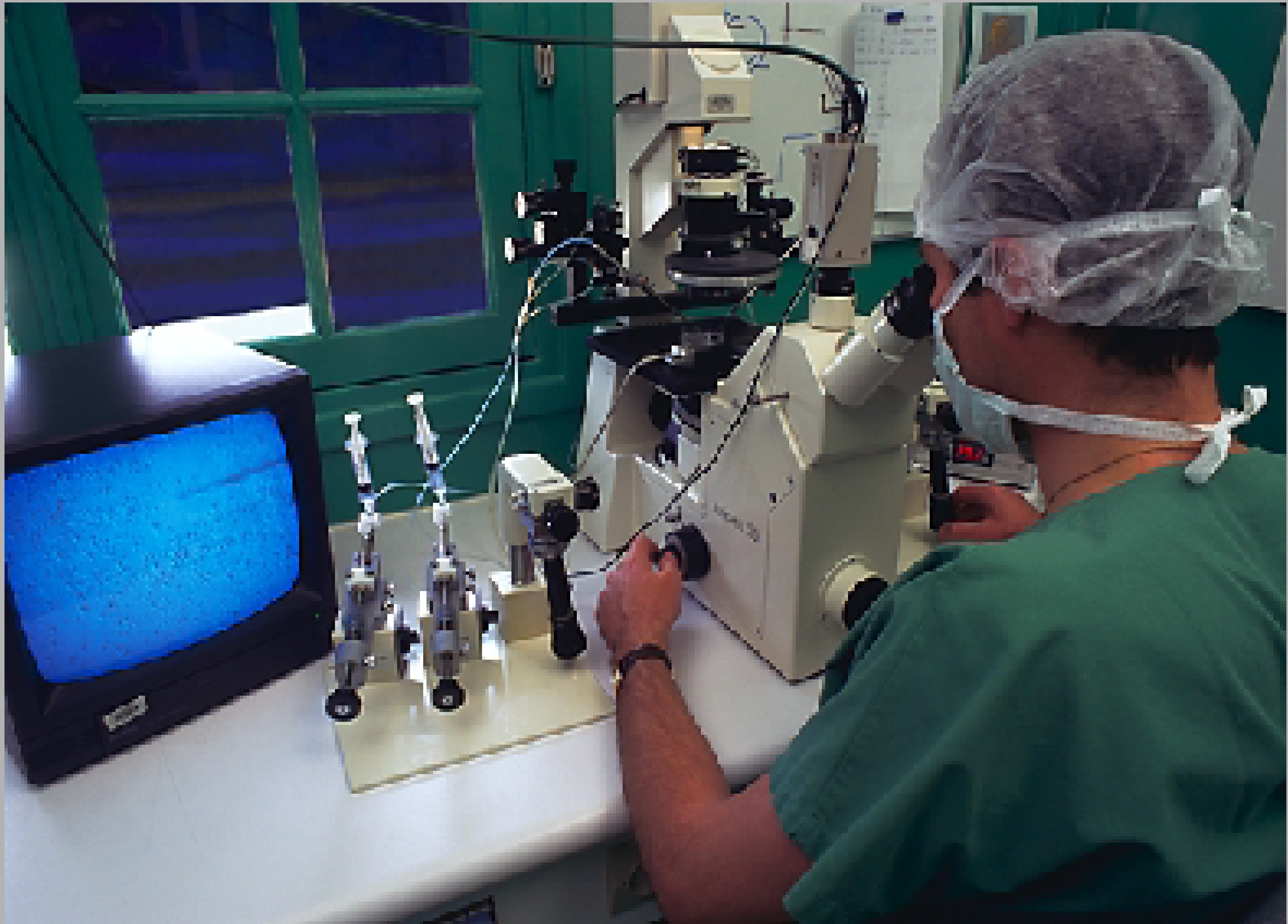


Different steps of ICSI

Decoronisation <u>at J0</u> before injection of the mature oocytes (MII)

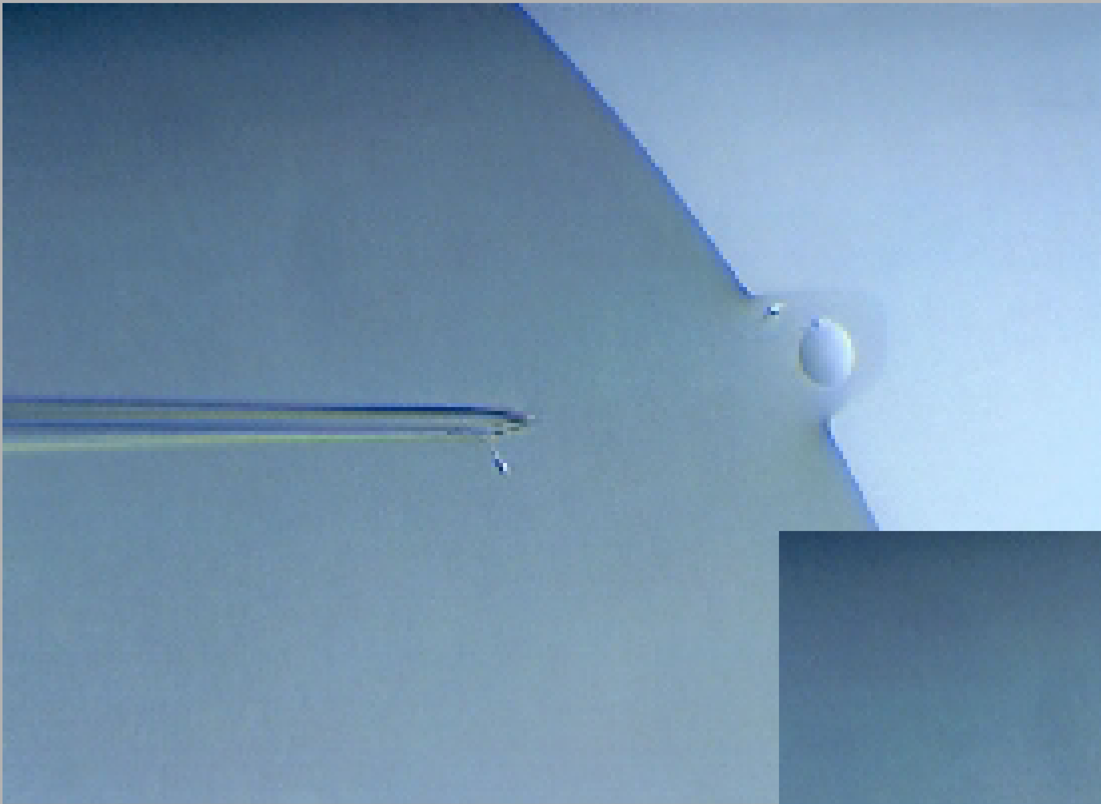
J0

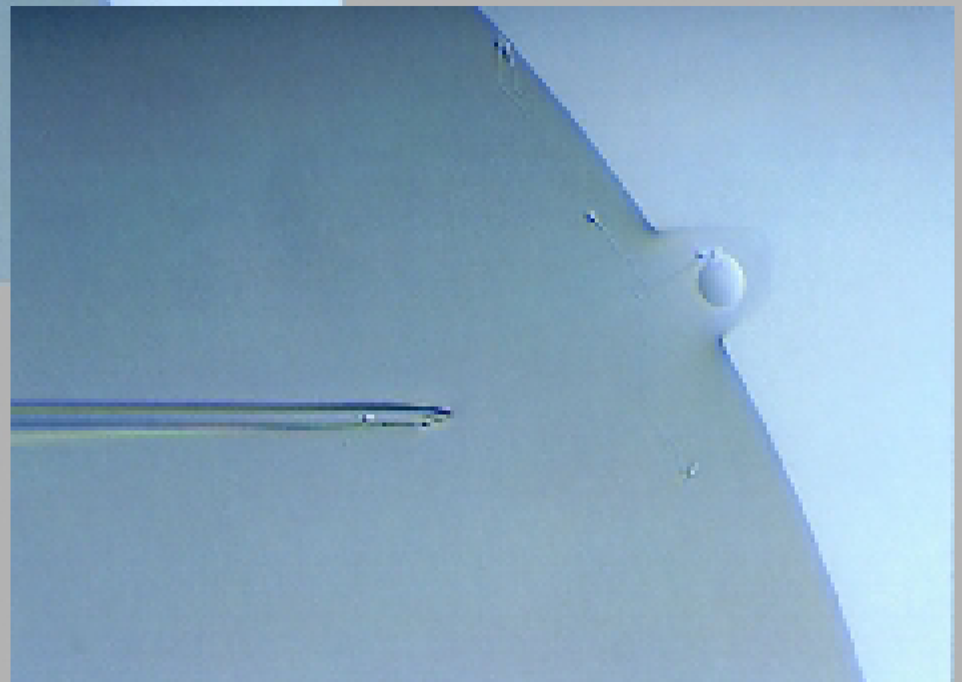
ICSI 1



J0

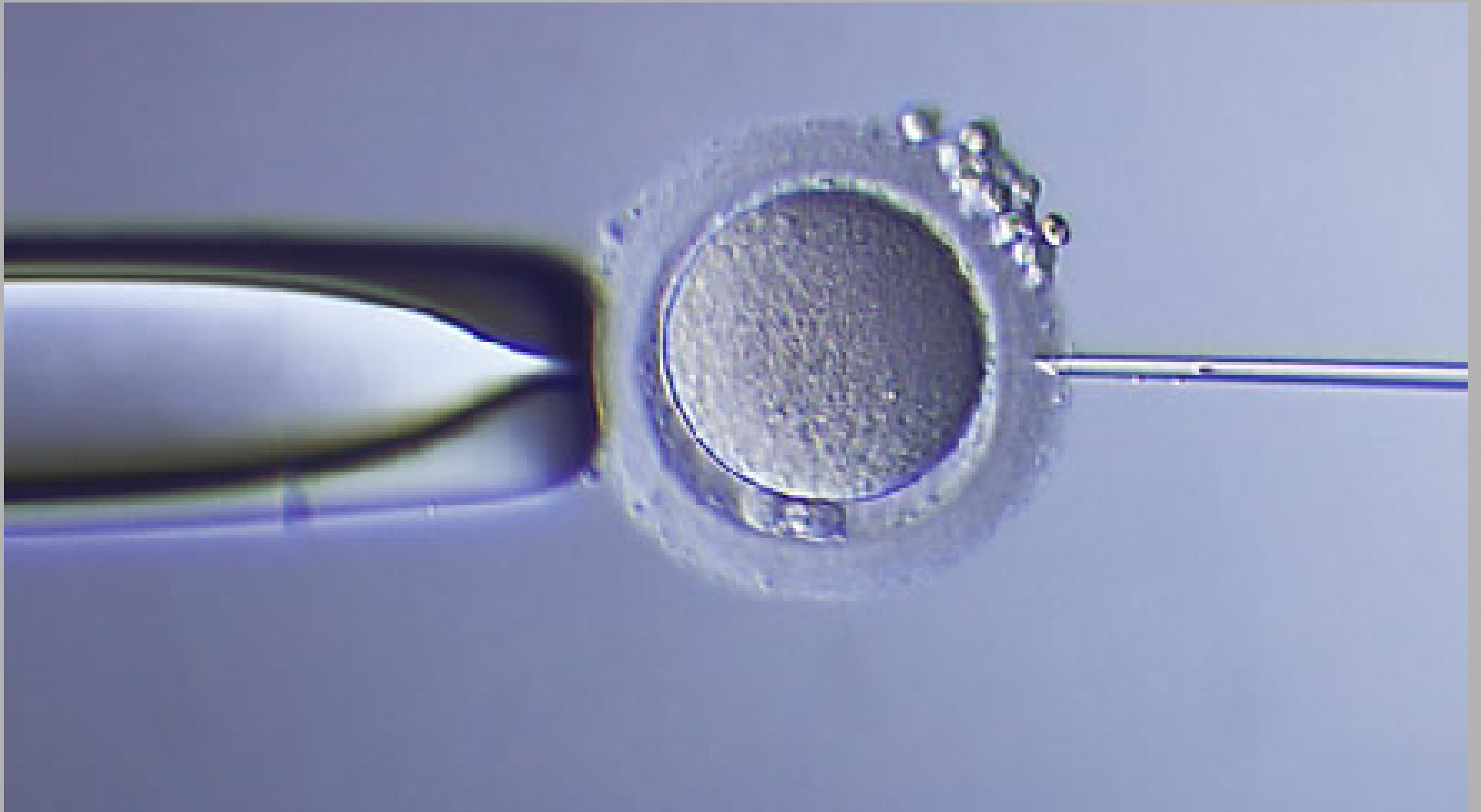
ICSI 2





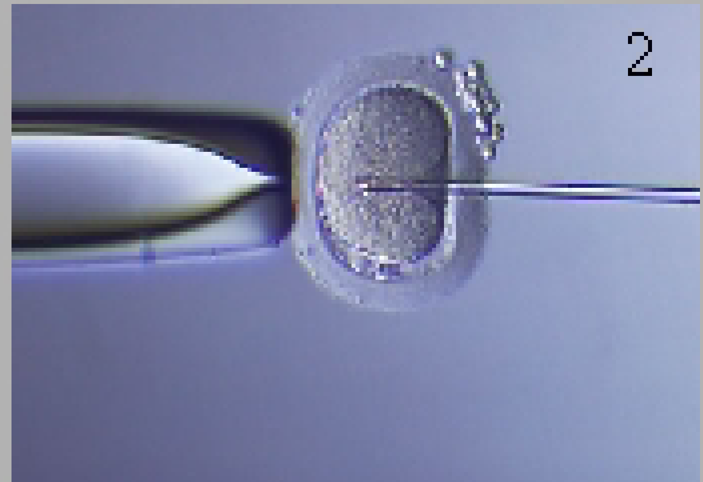
J0

ICSI 3

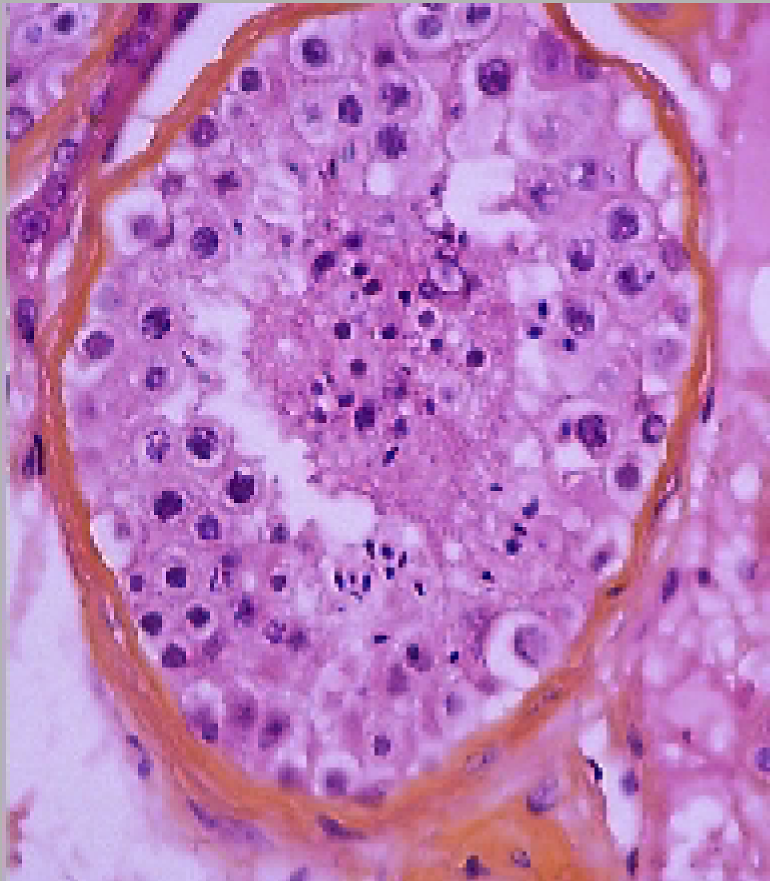


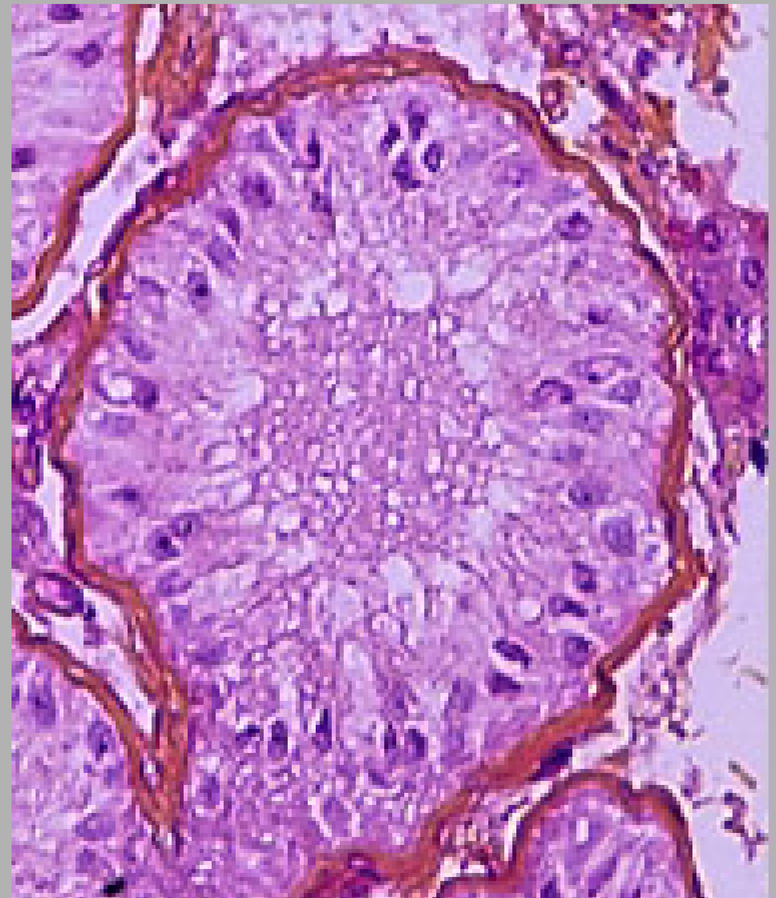
J0

ICSI 4



Normal testicular section



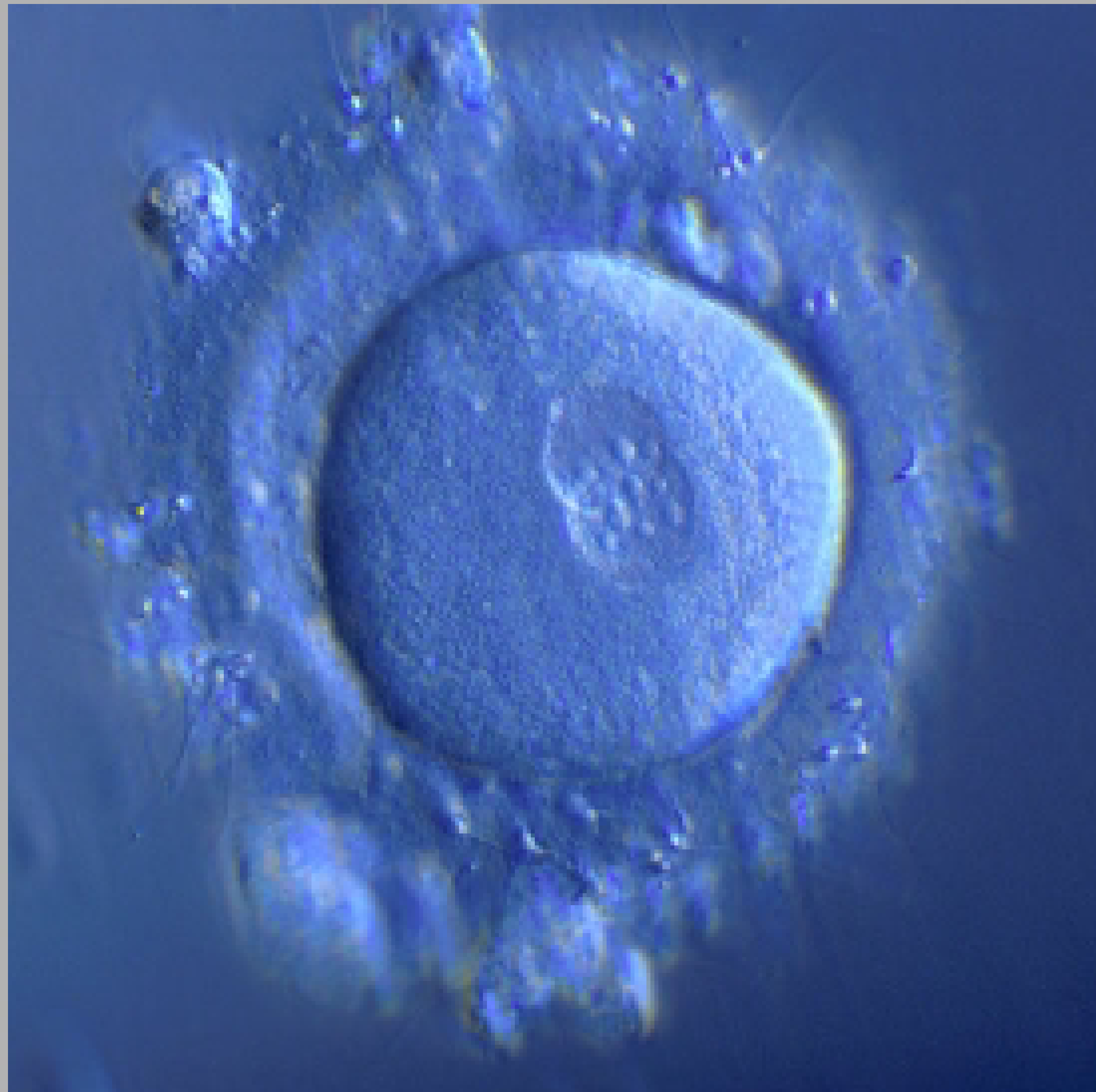


Sertoli cell only syndrome

IVF and ICSI

Observation of zygotes 1

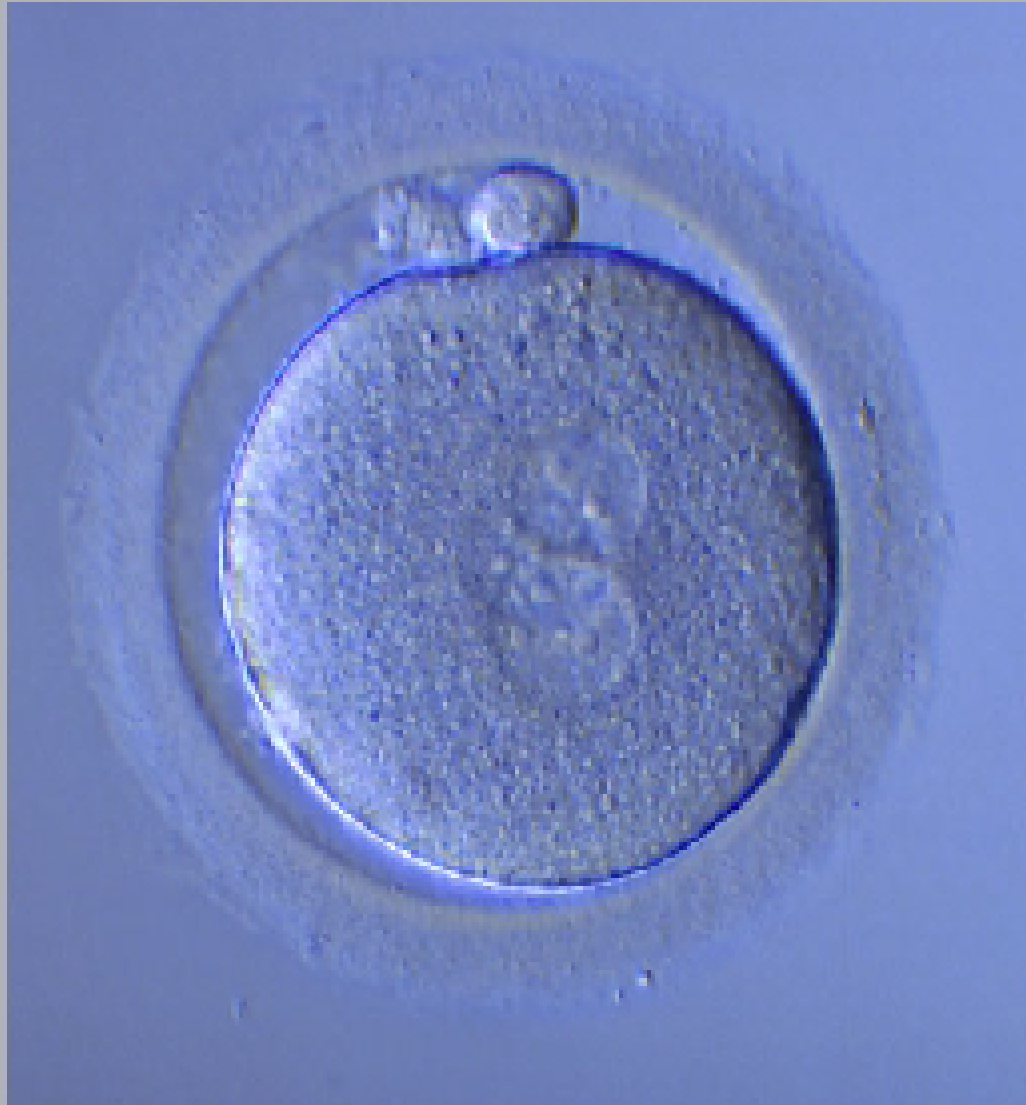
J1



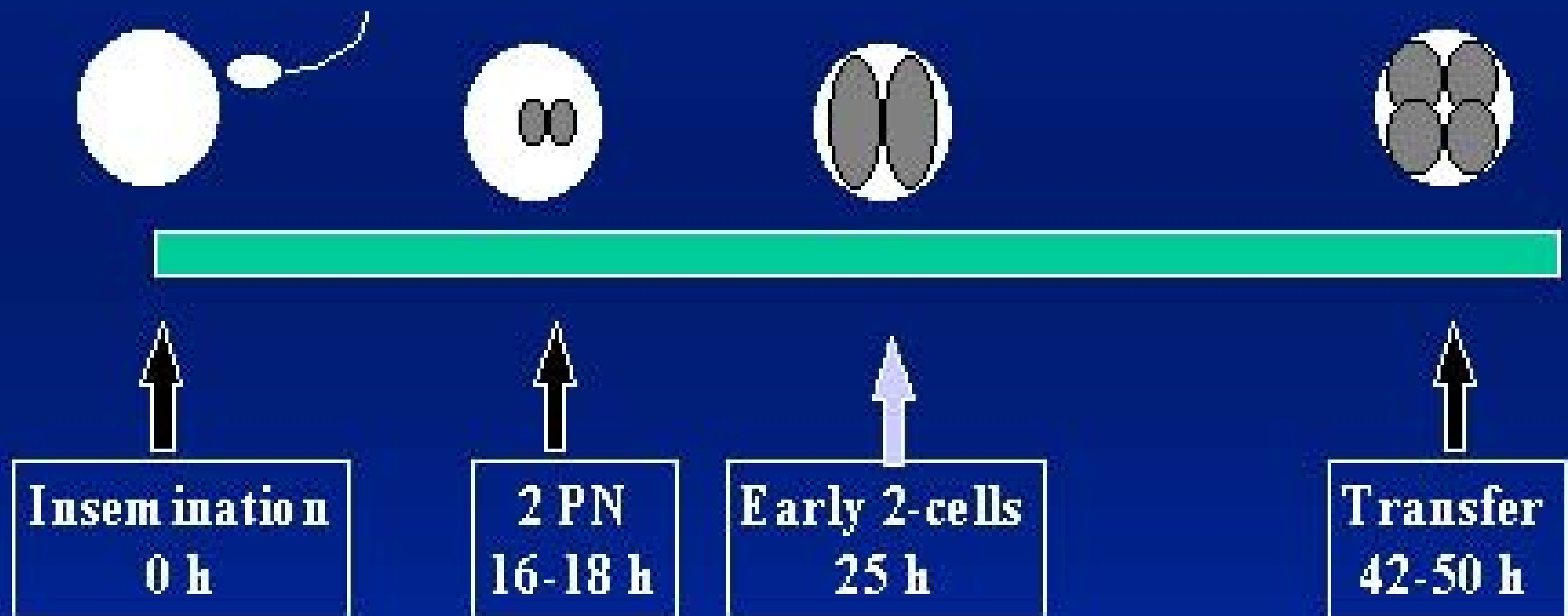
IVF and ICSI

Observation of zygotes 2

J1

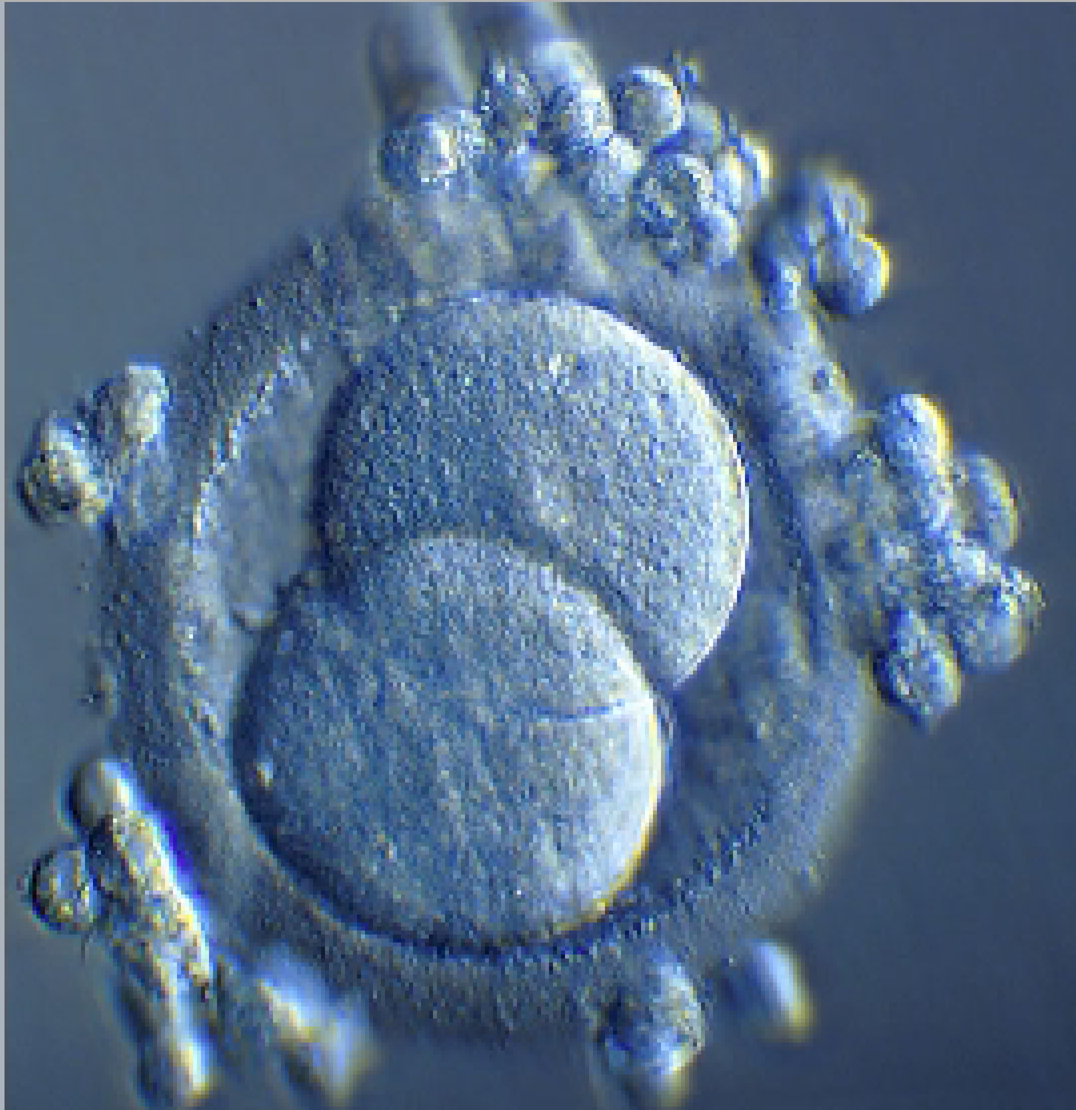


Assessment of early cleaving 2-cell embryos



Two-cell embryo

J1



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
Nº of cycles	116	27
Nº of oocytes (mean ± SD)	999 (8.7 ± 6.5)	229 (8.5 ± 4.8)
Nº 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)
Nº of embryos on day 2 (mean ± SD)	511 (4.41 ± 3.2)	156 (5.78 ± 3.9)
Nº 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)*
Nº of clinical pregnancies (%)	17 (14.7)	9 (33.3)*

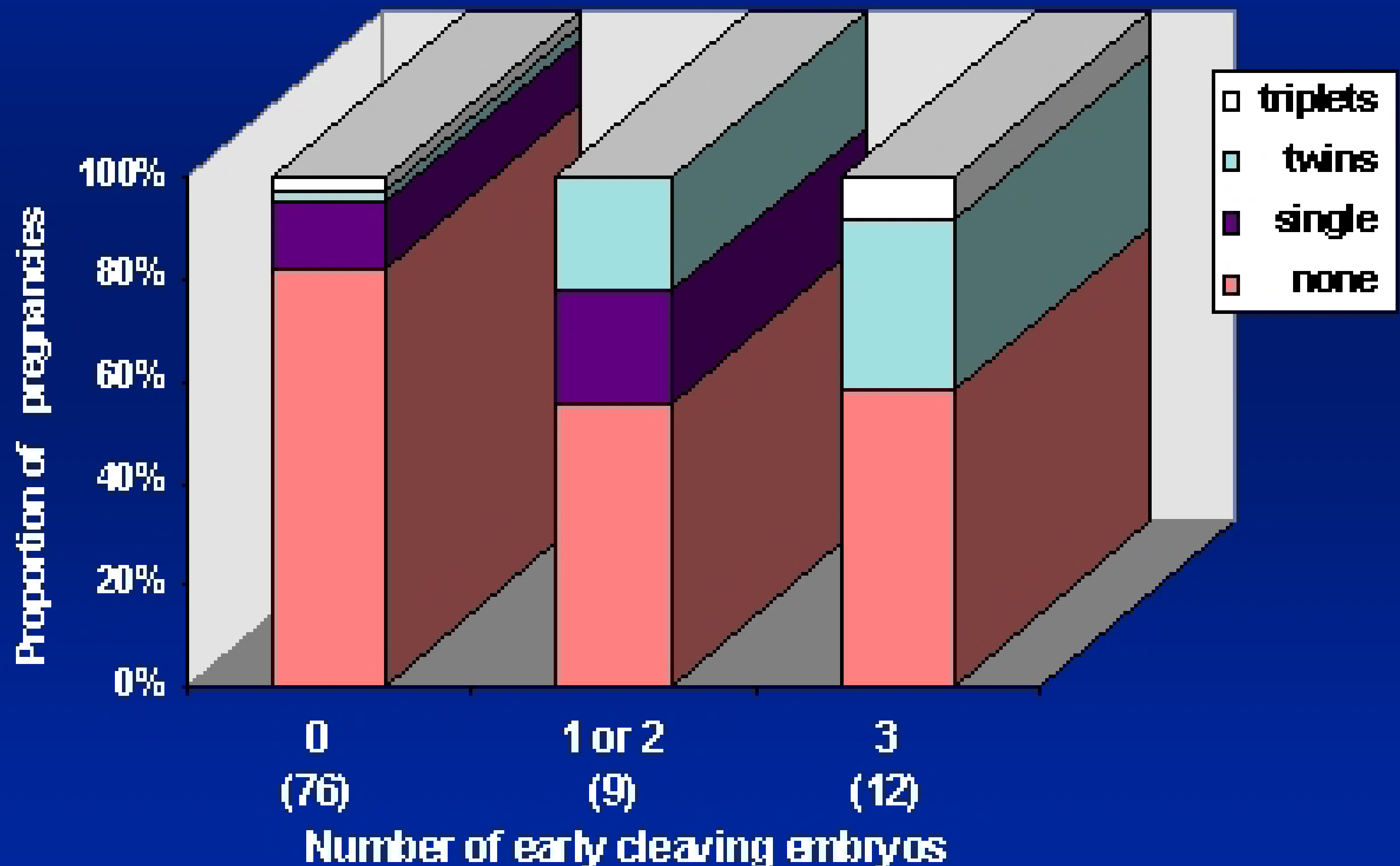
* significantly different P<0.05 compared to no early cleavage

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

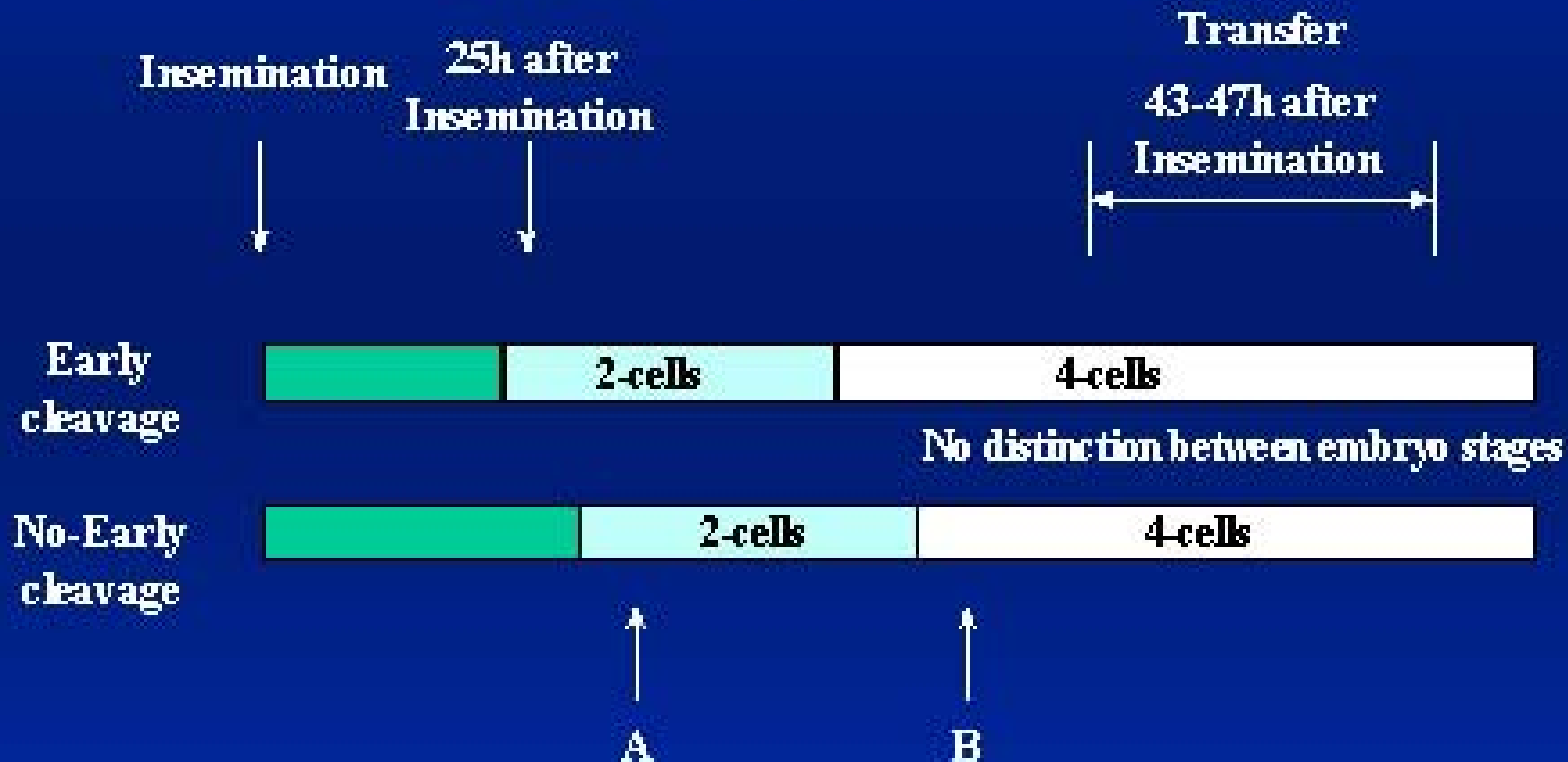
Pregnancy in relation to the number of early and no early cleavage embryos transferred in patients receiving three embryos at transfer.



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.

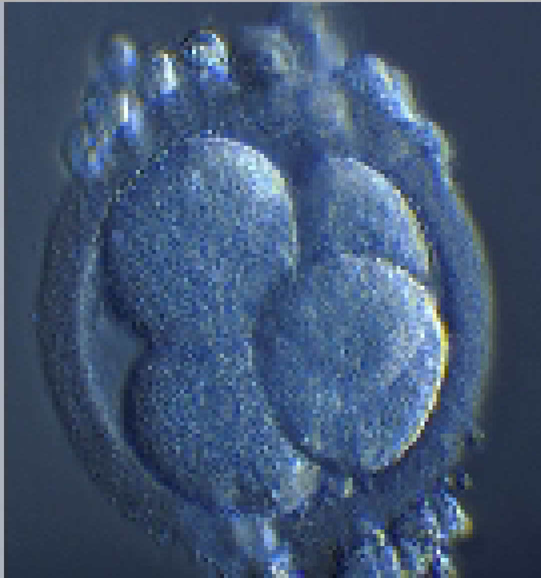
The importance of specific time for distinguishing embryo cleavage during screening.



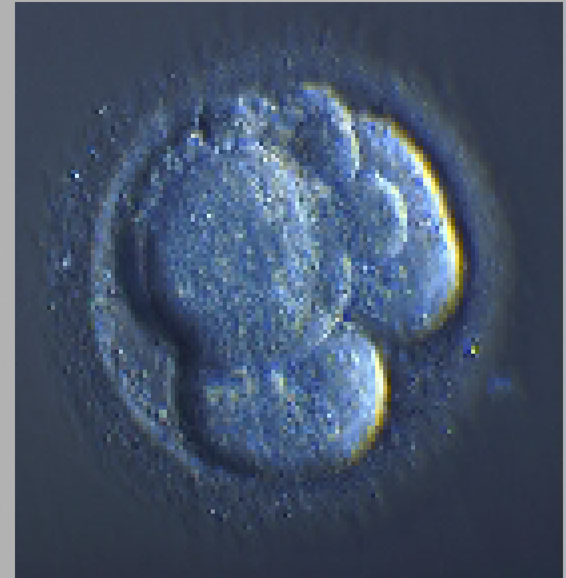
Conclusions for Early cleavage embryos

- 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.

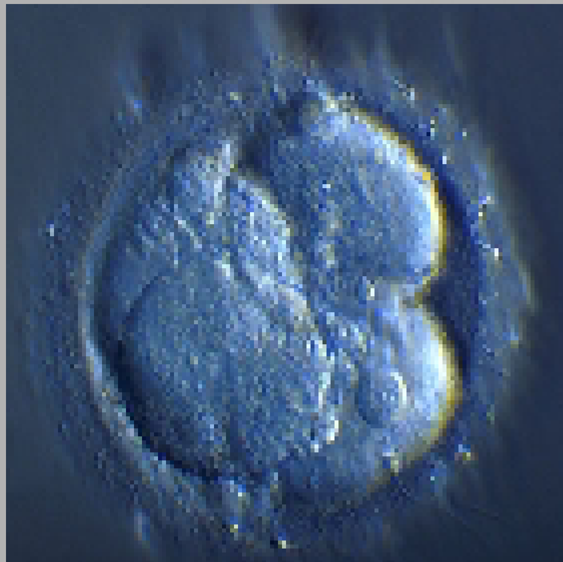
Embryo quality at J2



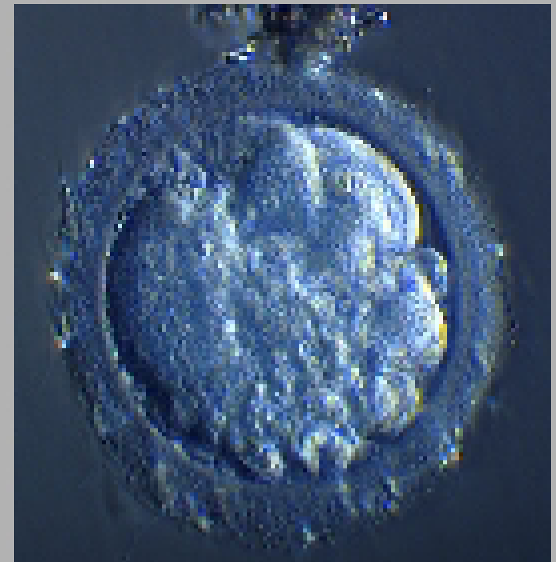
A



B

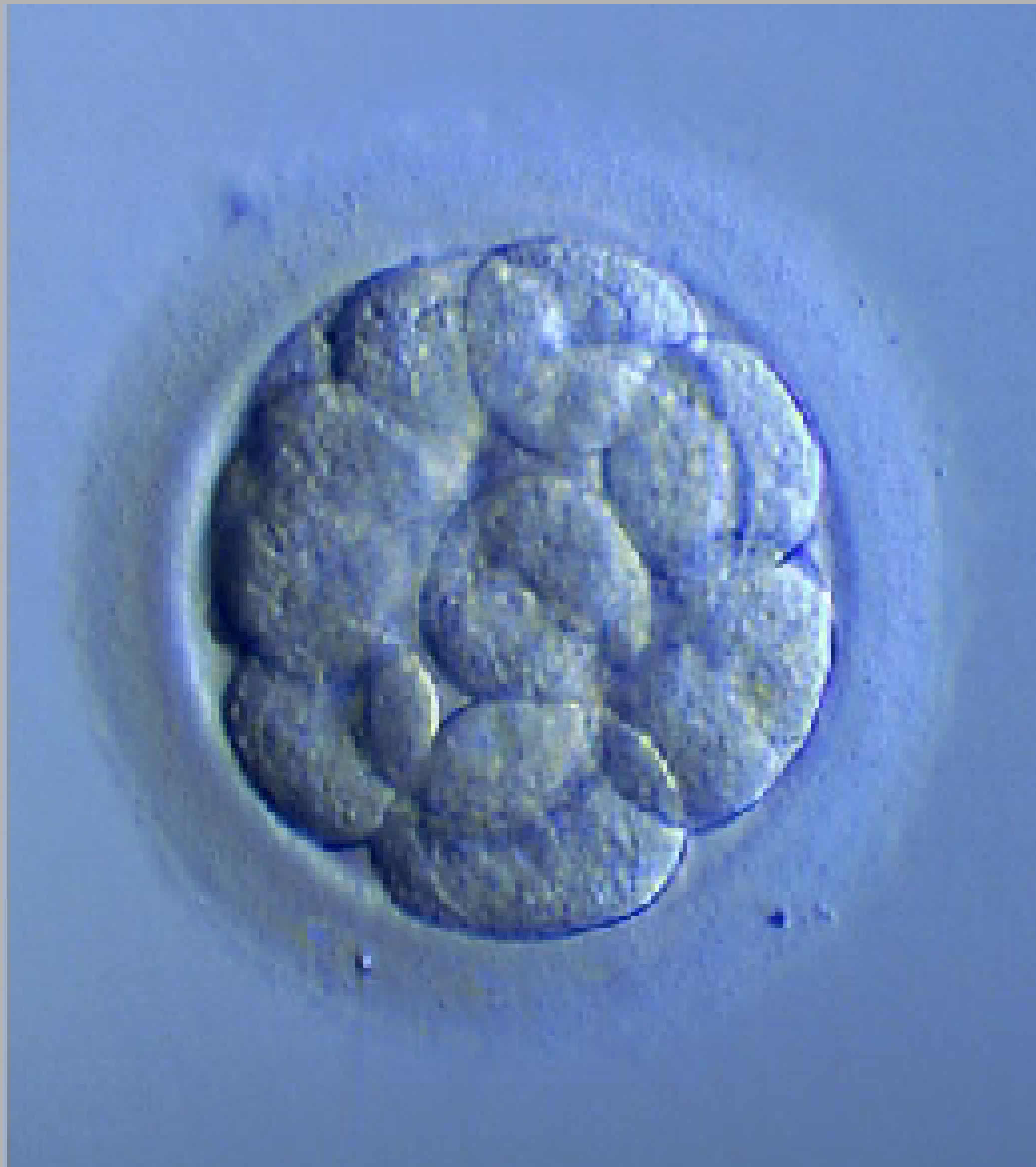


C

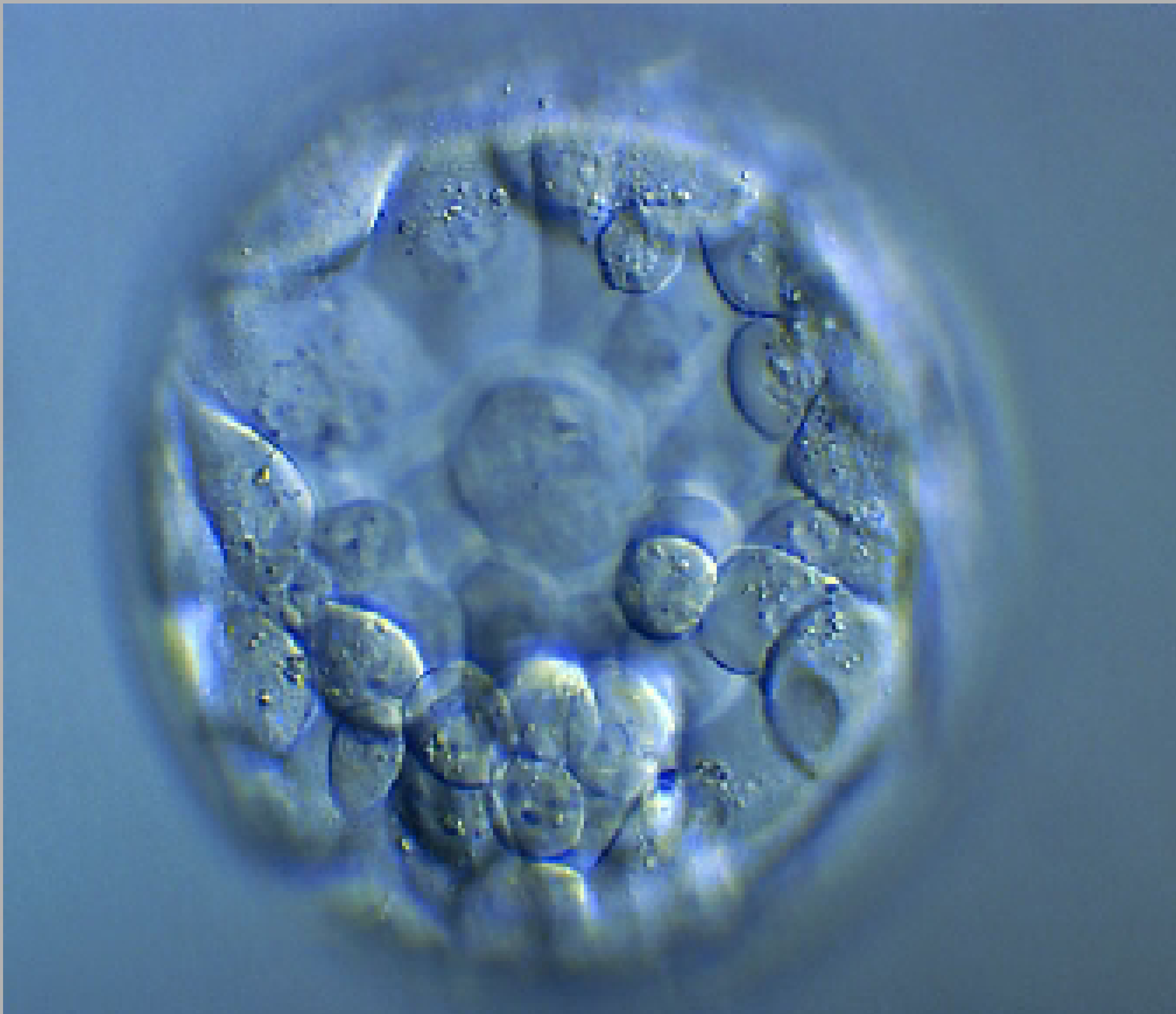


D

Morula stage J4



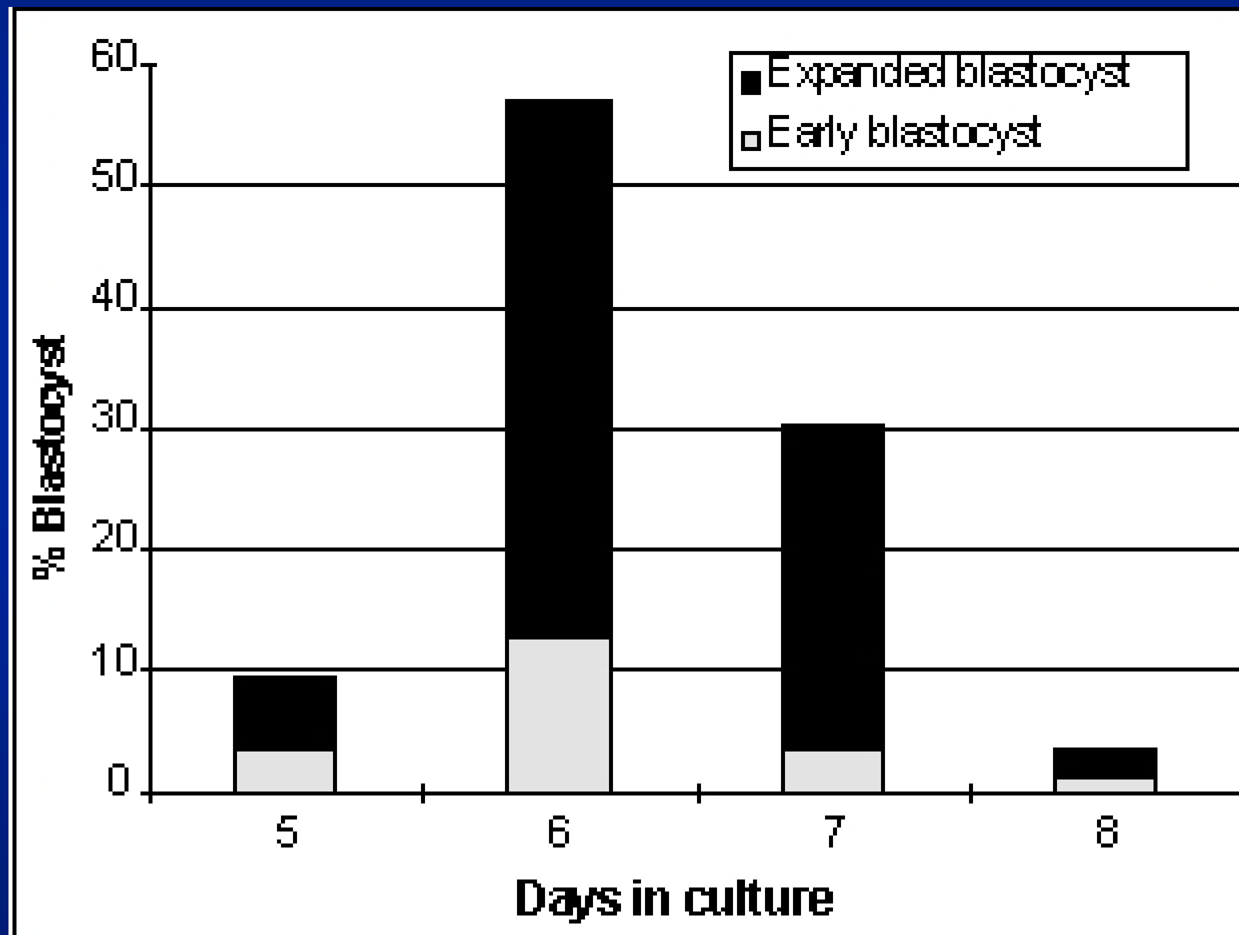
Blastocyst stage



SELECTION OF THE BEST EMBRYOS BY CULTURE 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

Choose a culture media

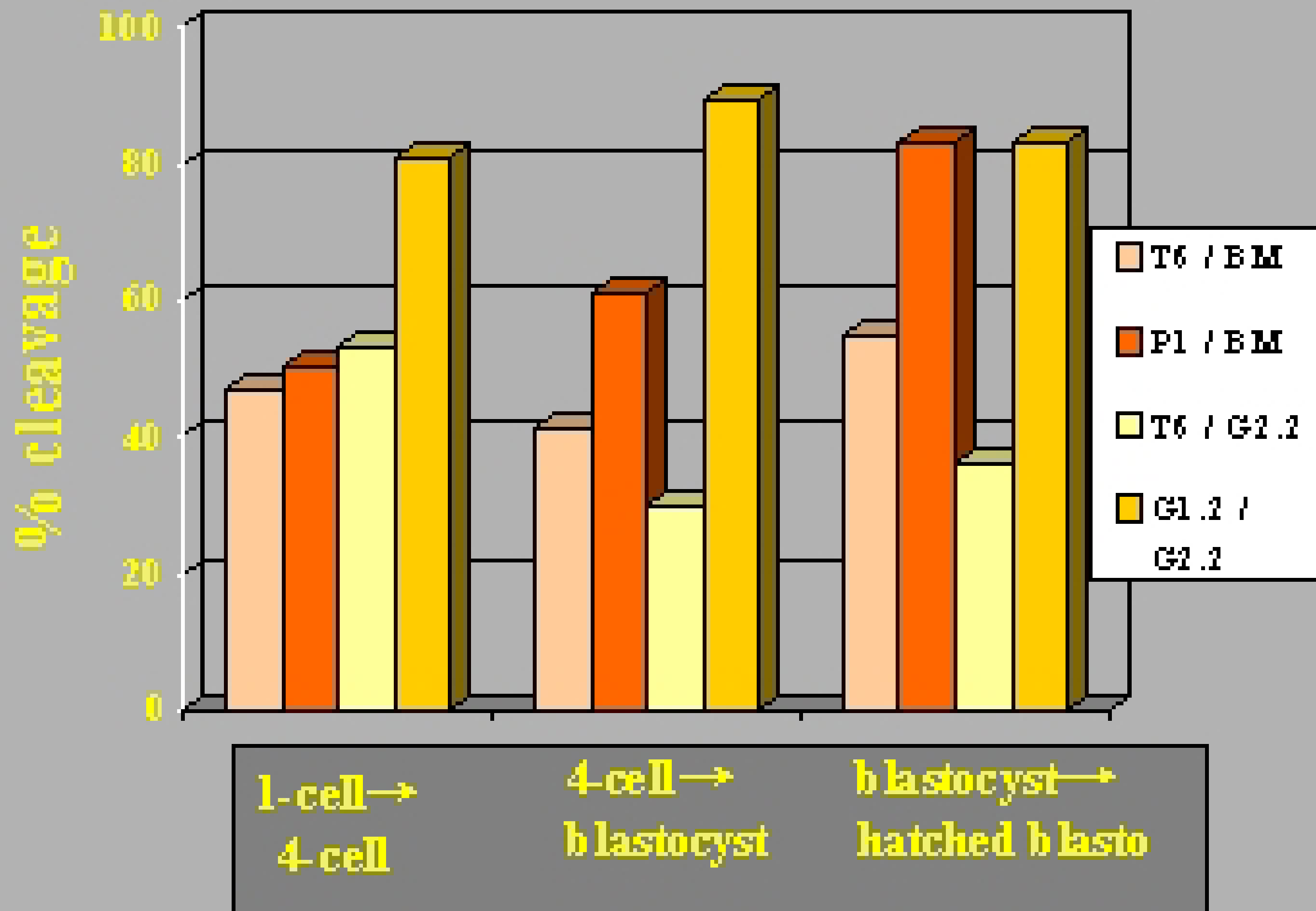
Glucose requirement in culture media during the different steps of fertilization and embryo development *in vitro*

	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

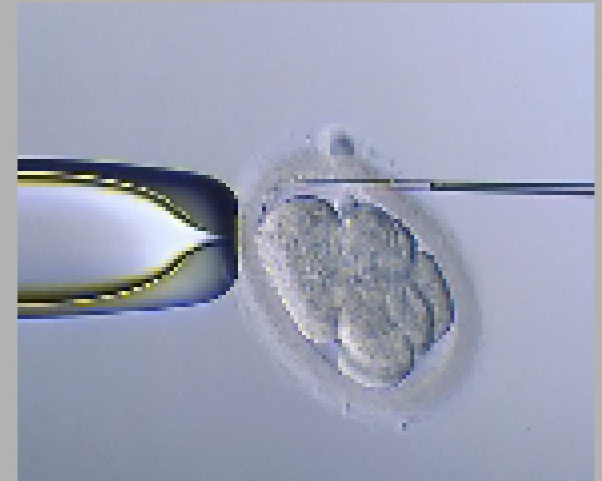
Effect of different culture media on mouse embryo development



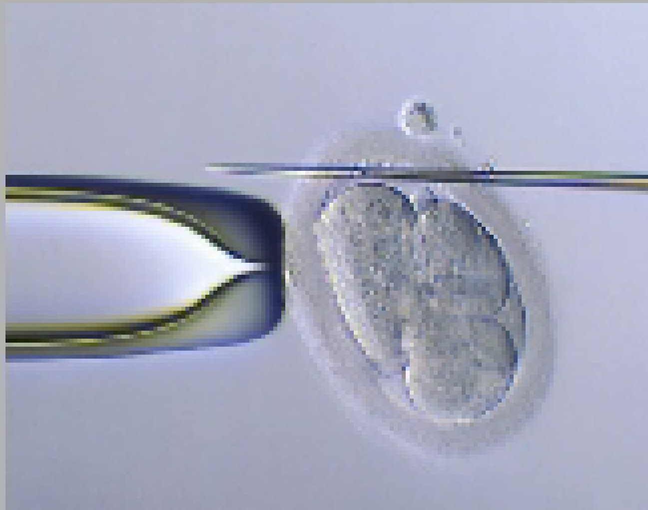
BM ™ = Blastocyst Medium G1.2 ™ & G2.2 ™ = Gardner's media

Mechanical Assisted Hatching

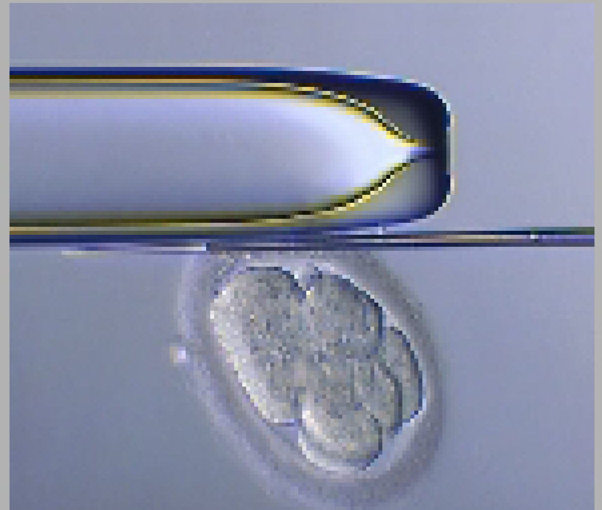
1



2



3



4

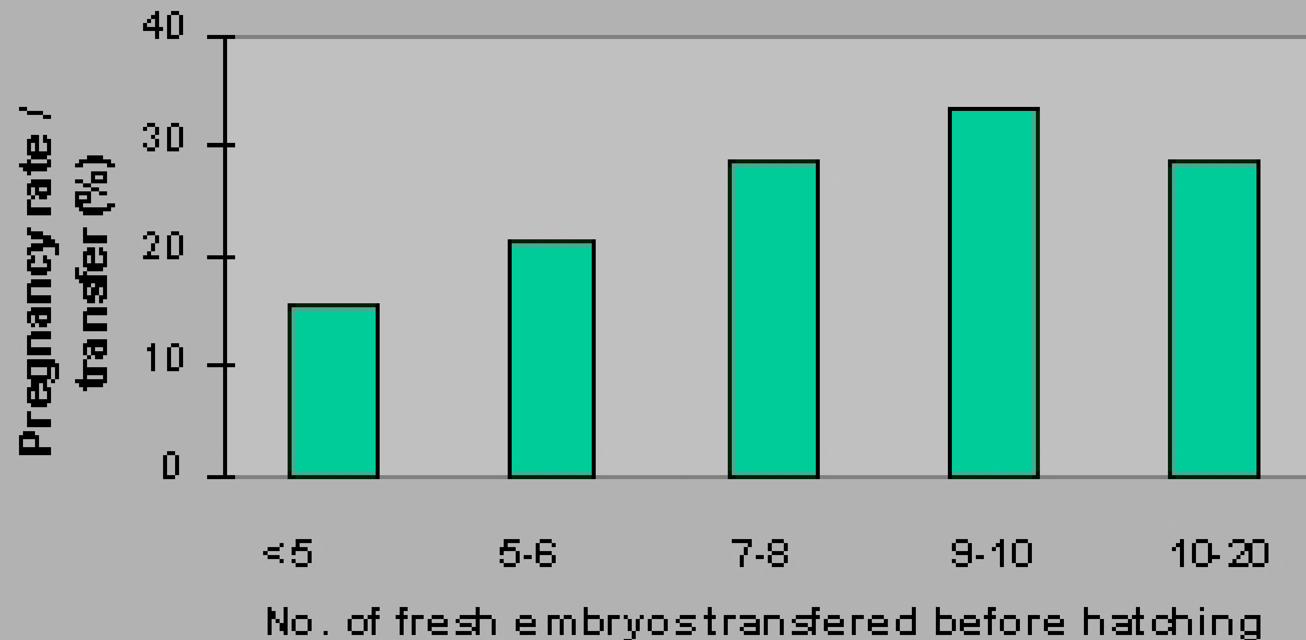
Final hatching results according to the source of embryos treated by the different methods

Source of embryos →	IVF	ICSI	IVF or ICSI thawed
No. of Patients	90	28	22
Mean Age (±SD)	98.5 (±9.7)	94.12 (±4.8)	95.22 (±5.1)
Range of Cycles	9.54 (±1.8)	9.39 (±2.9)	2.33 (±1.8)
Fertilization rate (mean) / M II	79.35	88.5	75.7
Cleavage rate (mean) / M II	72.19	88.5	78.2
No. of transferred hatched embryos	91	29	24
Total no. of embryos hatched	80	89	54
Mean of hatched embryos/cycle	2.53 (±0.8)	2.97 (±0.7)	2.25 (±0.7)
Pregnancy rate/Transfer (No. of pregnancies)	22.58% (7)	24.19% (7)	8.90% (2)
Pregnancy rate/Patient	29.99%	28.92%	9.10%
Implantation rate (No. sacs)	11.25% (9)	11.59% (8)	9.70% (2)

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 runmining to have a conclusion.

Preimplantation genetic diagnosis