Embryo Biopsy

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PGD- Embryo Biopsy

1. Biopsy procedures

- Opening the zona pellucida
- Removal of the cellular material

2. Developmental stage to perform the biopsy

Zona opening

1- MECHANICAL OPENING

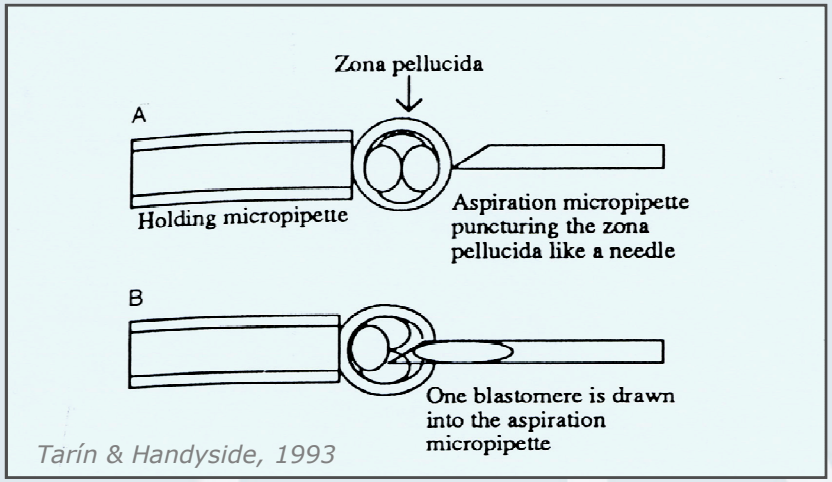
- Direct Puncture
- Partial Zona Dissection

2- CHEMICAL OPENING (Ac. Tyrode's ph=2.3)

3- PHOTOTHERMOLYSIS (Laser)

Zona opening: Mechanical opening

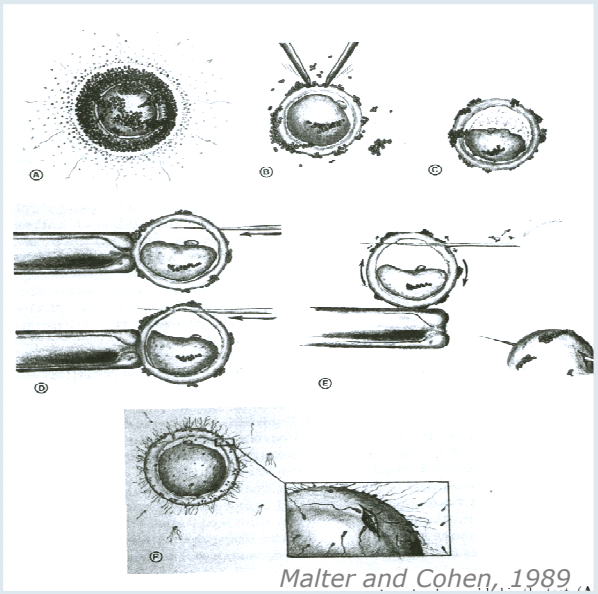
Direct Puncture: Performed with the use of a bevelled pipette. Not clinically applied in the human for blastomere biopsy but used for polar body biopsy Verlinsky and Cieslak, 1993



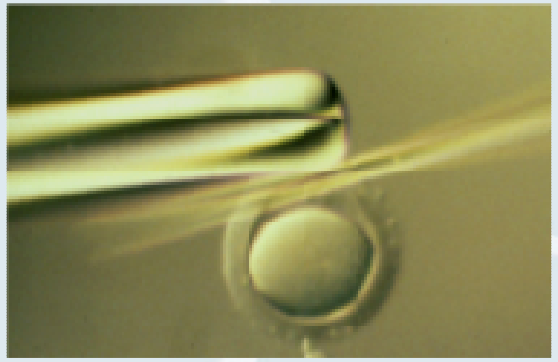
Tarín & Handyside, 1993

Partial Zona Dissection

Described for human oocytes to facilitate sperm penetration
Also useful for Assisted Hatching


Malter and Cohen, 1989

PZD: Involves making a slit in the ZP by a sharp closed microneedle



3-D PZD: Cross shaped slit *(Cieslak,1999)*

Zona opening: Chemical opening

Acid Tyrode's solution (pH 2.3) *Gordon and Talansky, 1983*

- **The most widely used approach** (cheap option)
- Human ZP is more resistant to AT than mouse ZP
- Larger, rounder hole than with PZD. Size of the hole not always easy to control
- **Two** separate **pipettes** are usually used (double holder). Drilling pipette with an inner ø of 5-7 µm plus the aspiration pipette.
- Limiting the extent and duration of AT exposure is necessary to avoid acidification of medium and cell lysis
- Target site: between two blastomeres
- Embryo wash after AT exposure are recommended
- Useful for early cleavage stages but **inappropriate for oocytes**

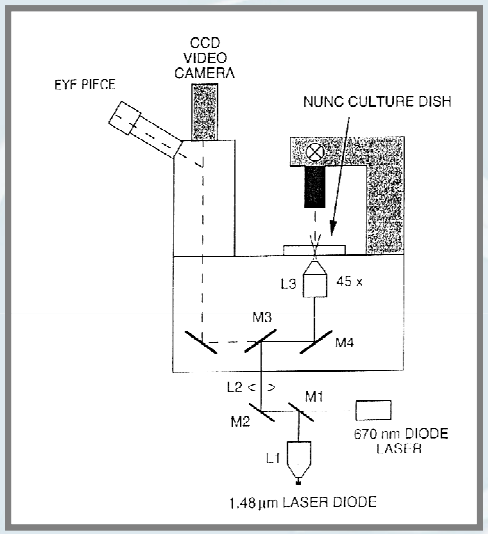
Chen et al, 1998 described **a simplified technique** using only a single, larger pipette to perform zp drilling and blastomere aspiration

Zona opening: Phototermolysis

"Microdissection of mouse and human zona pellucida using a 1.48 µm diode laser beam: efficacy and safety of the procedure."

GERMOND ET AL, 1995. Fertil. Steril 64:604-611

Laser system



- Non contact laser: easily adapted to the microscope. Laser is transmitted trough a 45X objective
- Laser technique reduces time of biopsy procedure
- Quick, simple, safe and efficient procedure with no need for micropipette changes
- A direct relationship between the hole diameter (µm) and the exposure time (ms)
- Effective and focalised without dispersion. Minimal absorption by the culture dish and the medium
- Safe, with no mechanical, thermal or mutagenic effects

Germond et al, 1996: Drilled mouse embryos give rise to normal, fertile offspring and a healthy F2 generation was obtained

Zona opening: Ac. Tyrode's versus Laser

EMBRYO DEVELOPMENT AFTER ZP OPENING (mouse embryos)

Chatzimeletiou et al., RBM Online, 2:178-187, 2001

	24-30 h CAVITATION	48h BLASTOCYST
CONTROL	67.6%	100%
LASER	54.3%	87.5%
ACID TYRODE'S	43.3%	62.5%

Zona-drilled embryos cavitated consistently later than non-drilled controls

	TROPHECTODERM CELLS	ICM CELLS	TOTAL REDUCTION
CONTROL	25.2	10.8	------
LASER	23.2	9.4	9.4%
ACID TYRODE'S	23.3	8.0	13%

There were significantly fewer cells in the zona-drilled embryos compared with non-drilled controls

The AT's drilled embryos had significantly smaller ICM

Zona opening: Ac. Tyrode's versus Laser

Blastocyst development rates in sibling embryos: a prospective randomized trial

Jones et al, 2006

TABLE 2

Day 3 and 5 embryo quality in terms of cell stage and blastocyst development.

Embryos by characteristics eligible for biopsy	ATH	LAH
Embryos with >5 cells on day 3 (n)	59	62
Average cell stage on day 3	7.37	7.09
Blastocyst development, grades A and B combined (%)	47.5%	46.8%

Note: All *P* values comparing the two types of zona drilling were statistically nonsignificant.

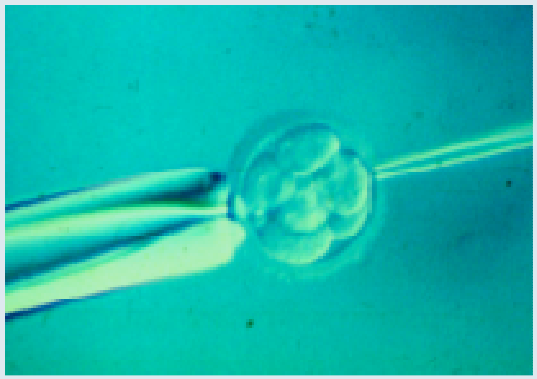
Jones. Hatching methods and blastocyst development. Fertil Steril 2006.

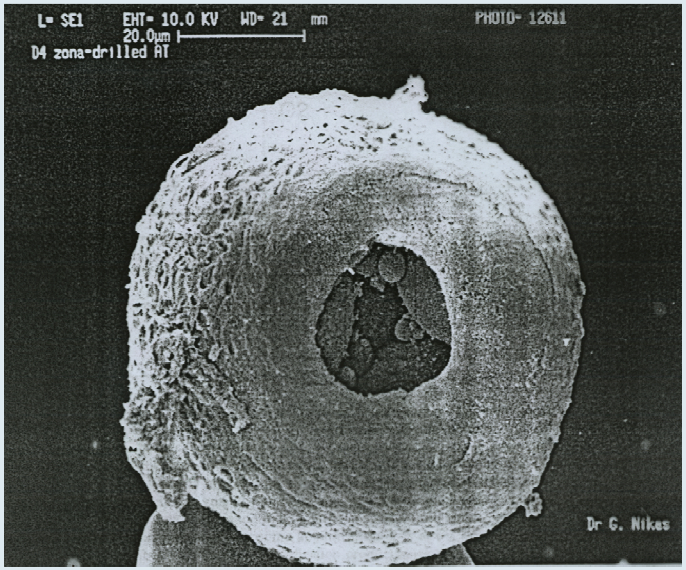
Laser hatching did not impair embryonic development to the blastocyst stage

It did not produce additional risks for embryonic development beyond the blastocyst stage.

Zona opening: Ac. Tyrode's versus Laser

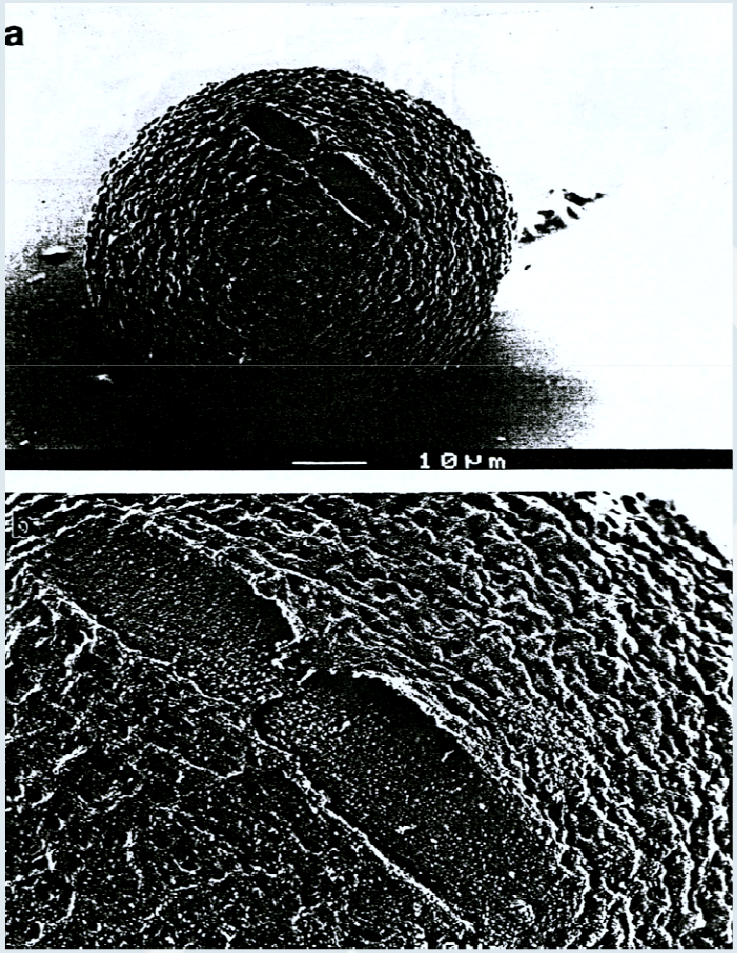
CHEMICAL ZONA OPENING





(G. Nikas)

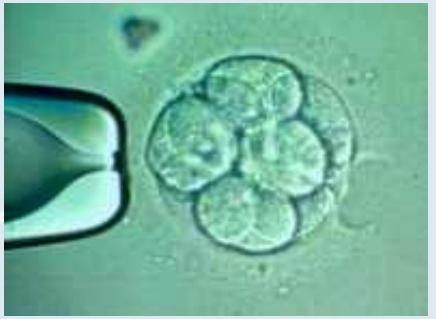
LASER ZONA PELLUCIDA DRILLING



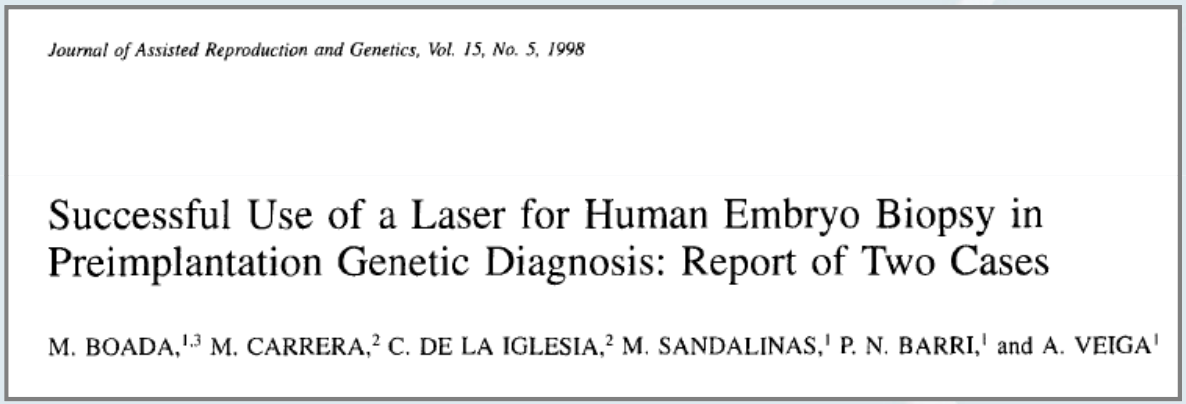
(Germond et al, 1995)

Zona opening: Phototermolysis

LASER ASSISTED BIOPSIES



- **EARLY CLEAVAGE EMBRYO BIOPSY**
  Boada M. et al, 1998
  (J.Assist Reprod Genet 15:302-307)



Journal of Assisted Reproduction and Genetics, Vol. 15, No. 5, 1998

Successful Use of a Laser for Human Embryo Biopsy in Preimplantation Genetic Diagnosis: Report of Two Cases

M. BOADA,[1,3] M. CARRERA,[2] C. DE LA IGLESIA,[2] M. SANDALINAS,[1] P. N. BARRI,[1] and A. VEIGA[1]

- **BLASTOCYST BIOPSY**
  Veiga A. et al, 1997 (Zygote 5:351-354)

- **POLAR BODY BIOPSY**
  Montag M. et al, 1998 (Fertil Steril 69:539-542)

Blastomere Removal

1- ASPIRATION

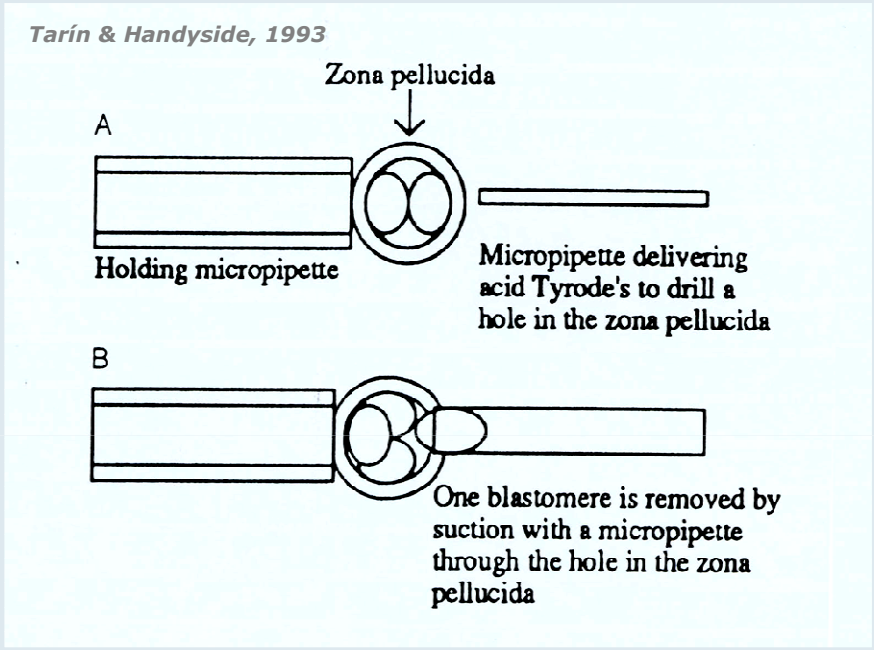
2- EXTRUSION

3- DISPLACEMENT

Blastomere Removal: Aspiration

Ac. Tyrode's & Aspiration

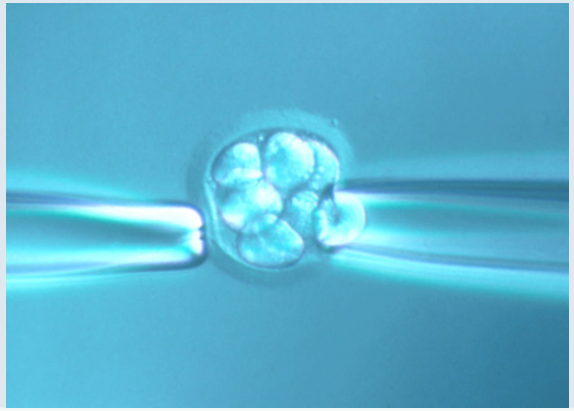
Tarín & Handyside, 1993

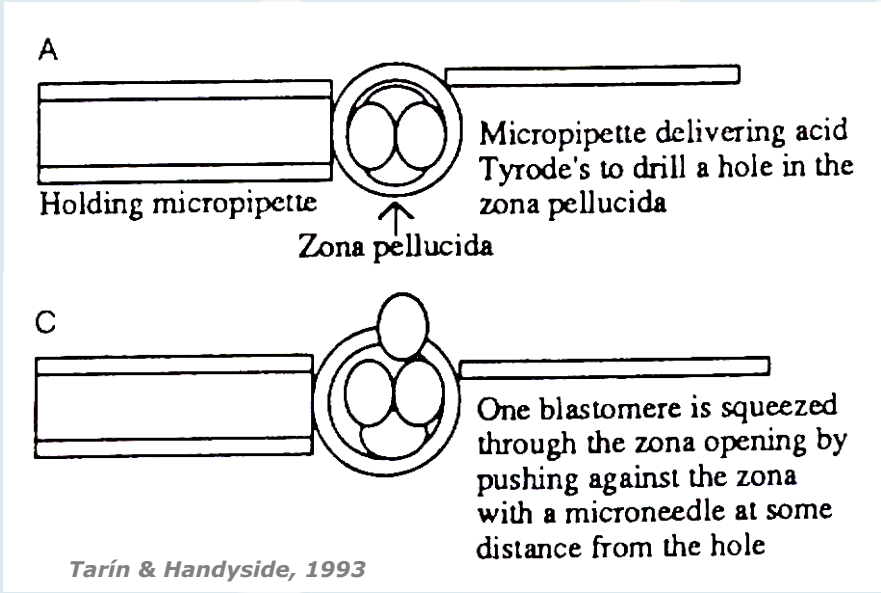


Extrusion:

1.- Dril the zona pellucida

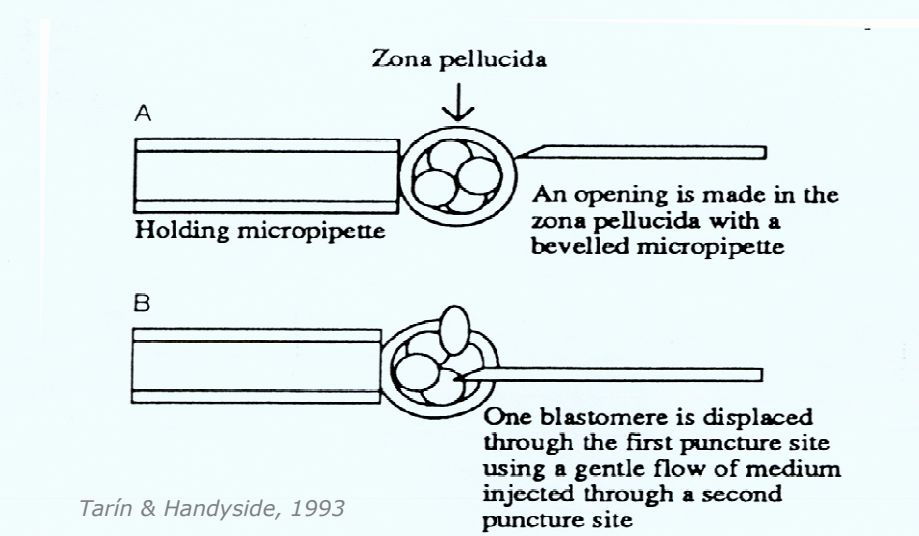
2.- Extrud the blastomere through the hole by pushing against the zp with a microneedle at some distance of the hole





Tarín & Handyside, 1993

Blastomere Removal: Displacement



Tarín & Handyside, 1993

TABLE 1

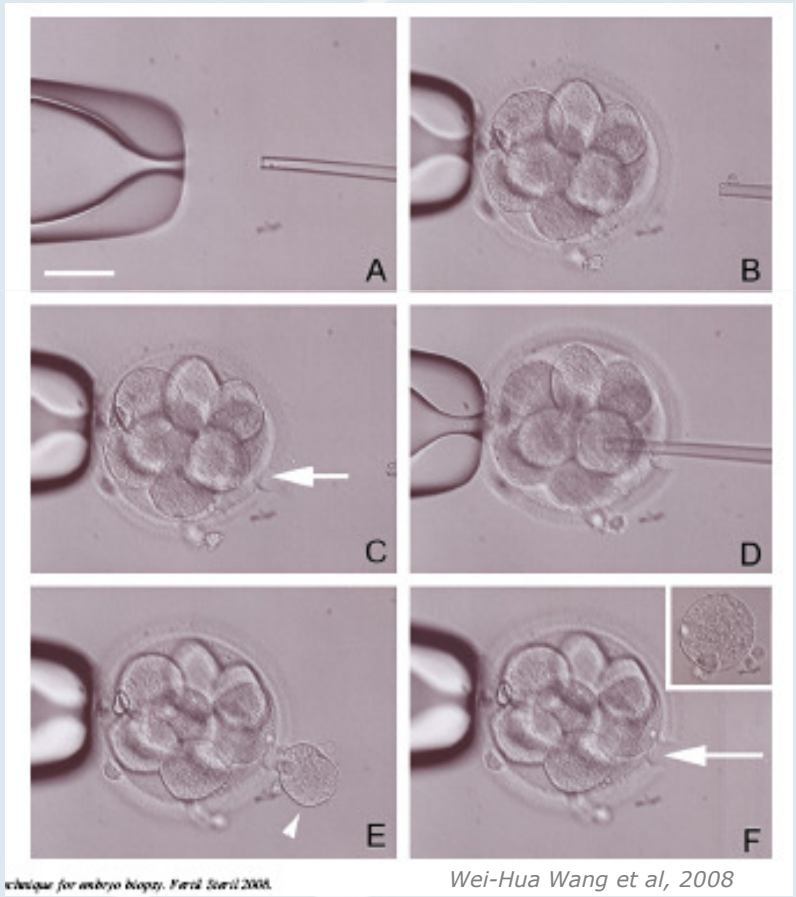
Comparison of embryo development and implantation of human embryos biopsied with two different methods.

Biopsy methods	Aspiration	Displacement
No. of cases	5	14
Age of patients	31.6	37.8
Total No. of eggs retrieved	69	197
Mean No. of eggs	13.8	14.1
No. (%) of M-II	59 (85.5%)	171 (86.8%)
No. (%) of 2PN	51 (86.4%)	156 (91.2%)
No. (%) of embryos biopsied	51 (100%)	151 (96.8%)
No. (%) of blastocysts	29 (56.8%)	84 (55.6%)
No. of patients with transfer	4[a]	10[a]
No. of live birth/ongoing pregnant	2 (50%)	5 (50%)
Total No. of embryo transferred	8	17
Mean No. of embryos for transfer	2	1.7
Total of No. embryo implanted	2	11
Implantation rate (%)	25[b]	64.7[c]

[a] Patients without normal embryos or blastocysts for transfer were not included.
[b,c] $P < .05$.

Wang. Biopsy of human embryos for PGD. Fertil Steril 2008.

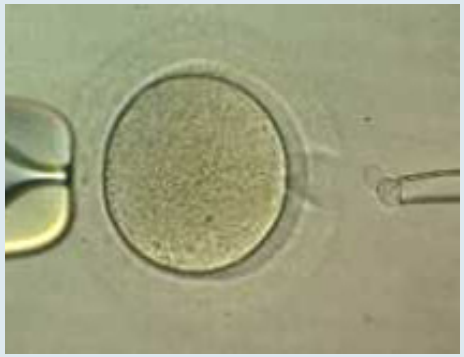
Wei-Hua Wang et al, 2008



chnique for embryo biopsy. Fertil Steril 2008.

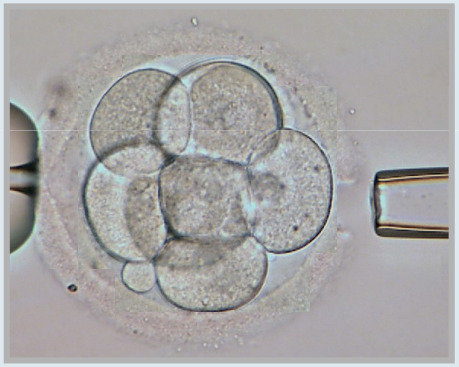
Wei-Hua Wang et al, 2008

2. Developmental stage to perform the biopsy

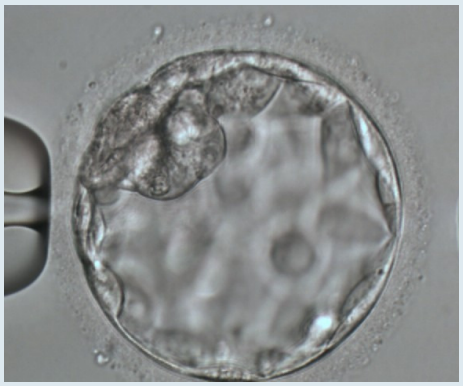


Polar bodies from oocytes

(Day 0/ Day 1)

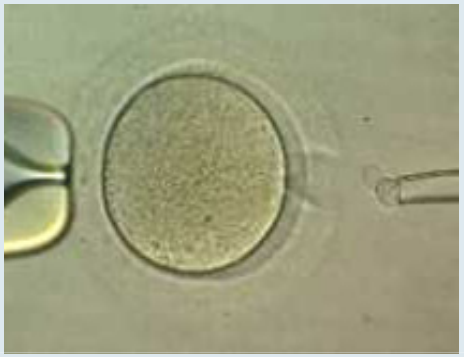


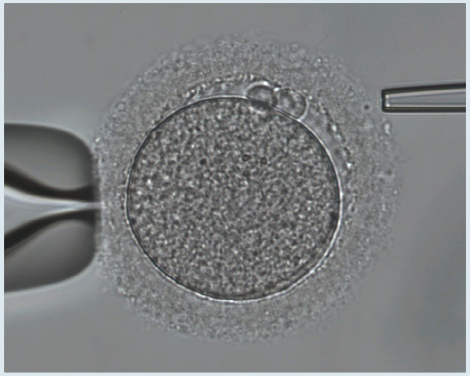
Blastomeres from early cleavage stage embryos (Day 3)



Trophectoderm cells from blastocysts (Day 5)

Polar body biopsy





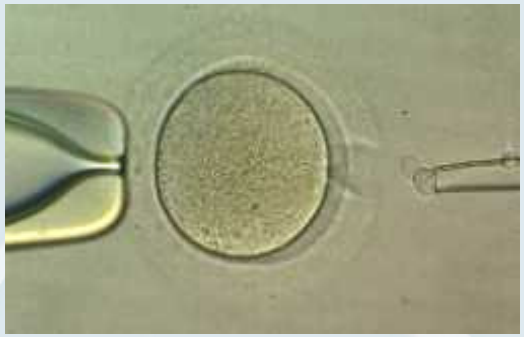
- **Verlinsky et al. Hum Reprod, 1990**
- **1st polar body:** 2-3 hours after oocyte pick up (<6h). Degeneration or fragmentation of the 1stpb
- **Small hole** of 18-25 µm. (not less than 15 µm)
- **Ac. Tyrode's:** is not recommended. It could be harmful and compromise the viability of the oocyte. **Mechanical zona opening** and **laser technology** (Montag et al, 1997) are the best options.
- **Pipettes for pb biopsy**: bevelled or not. Inner ø of 12-15 µm.
- **Sperm Microinjection** (ICSI). Not IVF.
- **Analysis of 1st and 2nd polar bodies:**

  Simultaneous (6-14 h after fertilization; pb_s at the 12 o'clock position)

  Sequential with ICSI between. (Cytoplasmic bridges)

Polar body biopsy

- **Preconceptional manipulation in first pb bipsy** Advantage when ethical objections or legal restrictions to embryo manipulation exist



- **Removal of extra-embryonic material**

  Polar bodies often degenerate. They are expected to have no biological role in the embryo development. No embryonic cells are removed. No reduction of the cellular mass. No effects on the embryo development

- **Indirect Method**

  The chromosomal constitution of the oocyte will be complementary to what is observed in the 1stpb. Each chromosomal set should have two paired chromatids

Polar body biopsy

- **Only genetic maternal contribution can be evaluated** Limitation for detecting the paternal influence and the errors that occurred post-fertilisation

- **Useful for maternal structural and numerical chromosome aberrations and in certain monogenetic diseases**

- **Recombination of homologous chromosomes**
  If it occurs, the 1st pb will be heterozygous and the complement of the oocyte cannot be derived. The additional information from the 2nd pb will be required.

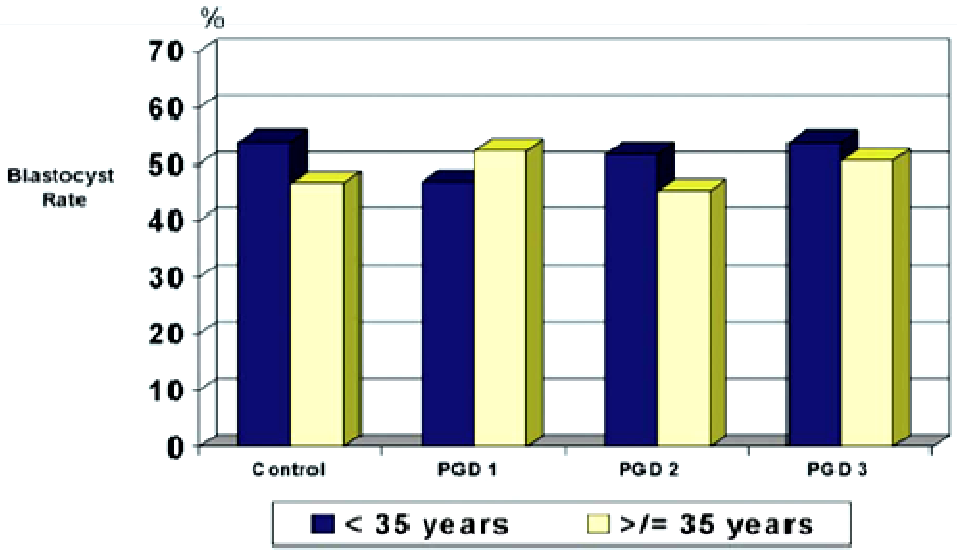
- **Premature division of chromatids** (Angell et al, 1991) could also lead to a difficult interpretation of the results

Polar body biopsy

PGD1- 1st pb (day 0)
PGD2- 1st and 2nd pb_s (day 1)
PGD3- 1st pb + 2nd pb + blastomere biopsy (D+3)

FIGURE 1

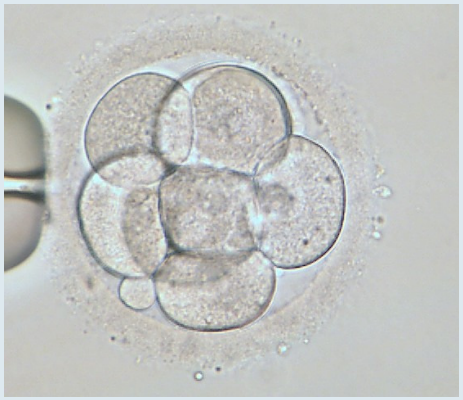
Effect of one to three micromanipulations for PGD on oocytes and embryos on blastocyst development by female age. Bars indicate the percentage of blastocysts in each PGD group and in the control group (*blue bars*, patient ages <35 y; *yellow bars*, patient ages ≥ 35 y). No significant differences were found between groups.



Cieslak-Janzen. Blastocyst development after PGD. Fertil Steril 2006.

Cieslak-Janzen et al, 2006

Blastomere biopsy



Early cleavage stage embryos (Day 3)

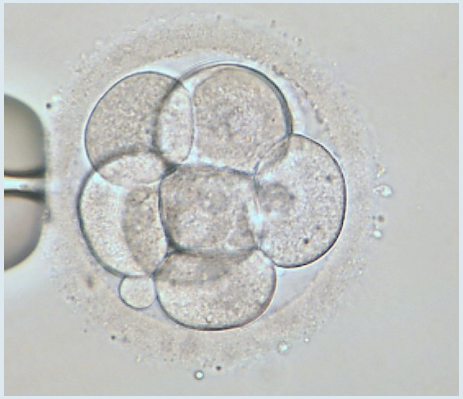
- Handyside et al. Nature, 1990
- The most widely used biopsy procedure

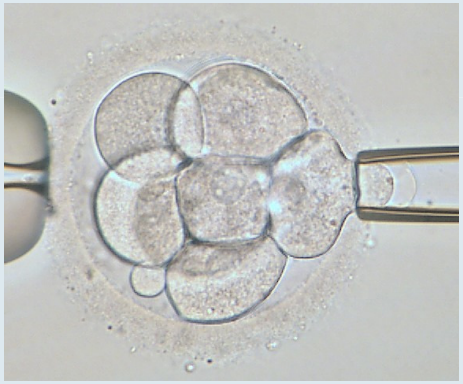
ESHRE PGD Consortium data Collection IX
Goossens et al, 2009

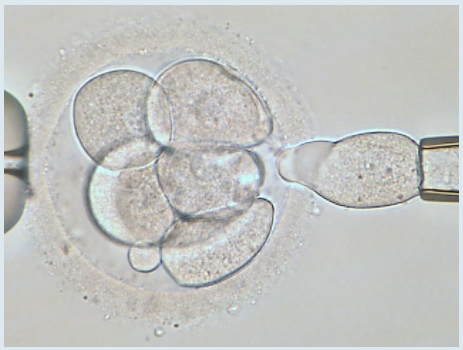
	Dec. 2006-Oct. 2007	
Polar body biopsy	1089	
Blastomere biopsy:		
Cleavage aspiration	12836	92%
Cleavage extrusion	1077	
Cleavage flow displacement	38	
Blastocyst biopsy	57	
Polar body + Cleavage	5	
Unknown	68	

- It allows the detection of maternal, paternal and early post-fertilisation defects
- It gives enough time for the genetic diagnosis if it is performed on day 3 and transfer on day 5

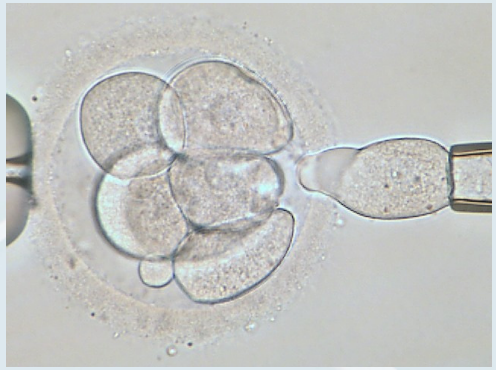
Blastomere biopsy

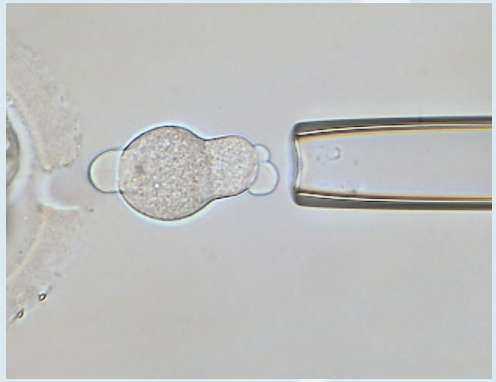


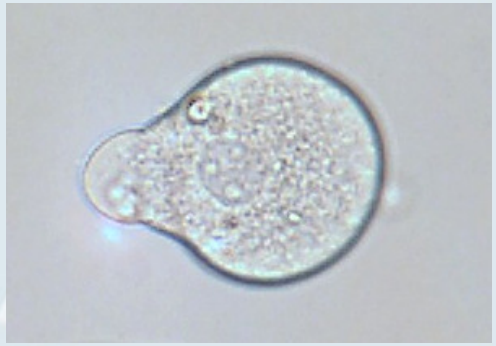




- Place the embryo with the chosen cell to biopsy at the 3 o'clock position. The cell should contain a single, clearly visible nucleus
- Zona opening: mechanical, Acid Tyrode's, Laser. Small hole of approx. 40 µm.
- Cell removal: Aspiration is the most widely used
- Special pipettes for blastomere aspiration. Inner ø of 35-40 µm
- The cell should be partially aspirated and pulled out rather than completely aspirated
- Place the biopsied cell far from the embryo
- Keep the embryo immediately in the incubator

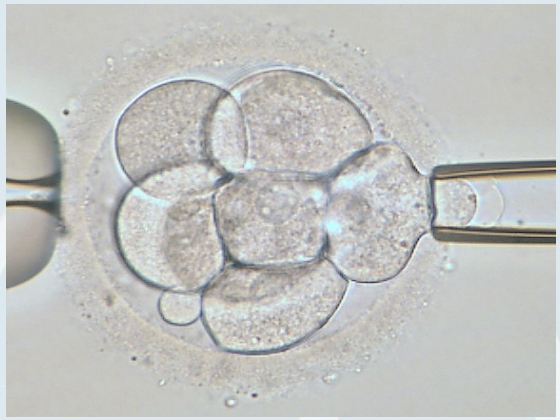






Blastomere biopsy

Compaction: On day 3, blastomeres show a strong tendency to adhere to each other but cells are not yet compacting.



Gap junctions are first detected at the 4 cell stage and the blastomeres adhere to each other (Hardy et al, 1996).

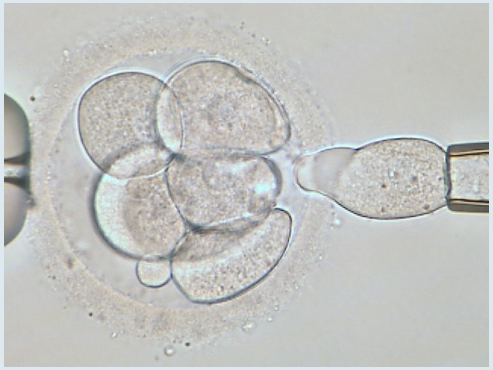
Full compaction does not occur before the 16-32 cell stage

- **Ca^{2+} Mg^{2+} free culture medium** facilitates embryo biopsy with no detrimental effect on embryo development and pregnancy rates

  (Veiga et al 1994; Santaló et al, 1996; Dumoulin et al, 1998)

Limit exposure time: maximum 10 min. After biopsy, gently flush the embryo repeatedly

Blastomere biopsy



Day3

Biopsy should be performed on the morning of Day 3 (68-72 h. after insemination)
Three cleavage cycles: approx. 60 h. Eight-cell stage. (6-10 cells)

- **Cells are still totipotent**
  It is thought that at this stage, blastomeres are allocated to, but not committed to, specific pathways (ICM, Trophectoderm)
- **1 or 2 blastomeres**
  Preferable to remove only one cell. If it is necessary to remove two, the same hole has to be used.
  Controversy: Hardy et al, 1990; Van de Velde et al, 2000; Thornhill et al, 2005; Cohen et al 2007; Goossens et al, 2008; De Vos et al, 2009)
- **The removal of too many blastomeres can be detrimental**
  Risks of formation of a small ICM (Hardy and Handyside, 1993)

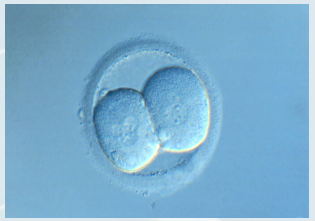
REDUCTION CELLULAR MASS ⟷ **ACCURACY OF DIAGNOSIS**

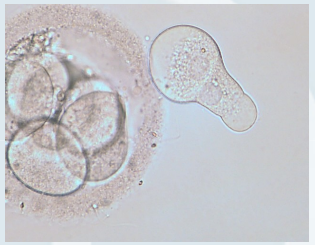
Blastomere biopsy

- **Fragmented embryos** (>35%) or **embryos with a low development rate** should not be biopsied.

- **Multinucleated embryos** should be considered for embryo biopsy?

- Births after transfer of Mn embryos have been reported.
- Decision could vary depending on:
  - The number of available embryos
  - The proportion of Mn blastomeres within the embryo
  - The multinucleation pattern
  - The day of its appearance





Representativeness of a Mn blastomere of the sibling blastomeres

		Cell 2			
		Normal	**Abnormal**	**Inconclusive**	**Total**
Cell 1	**Normal**	**8**	**0**	**2**	**10**
	Abnormal	**7**	**82**	**9**	**98**
	Inconclusive	**1**	**4**	**3**	**8**
	Total	**16**	**86**	**14**	**116**

87% abnormals
6% discordance

Parriego et al 2007
PGDIS- Melbourne

Blastomere biopsy

- **Mosaicism**

  Up to 70% of mosaicism have been reported in preimplantation embryos (Munné, 1995, 1997; Voullaire, 2000; Wells and Delhanty, 2000; Bielanska, 2002)

  Coulam et al, 2007

J Assist Reprod Genet (2007) 24:37–41

Table 2 Concordant and discordant rates for each of the chromosomes analyzed by FISH on 2 blastomeres from 102 embryos biopsied on day 3 of development

Chromosome	X	Y	13	15	16	18	21	22	Total
Concordant	80	89	76	72	72	74	73	81	617
Discordant	22	13	26	30	30	28	29	21	199
% Discordant	21.6	12.8	25.5	29.4	29.4	27.5	28.4	20.6	24.4

- 74.5% rate of discordance when 2 blastomeres biopsied from the same embryo on D+3 were analyzed for 8 chromosomes

- Both technical and biological contributions

J Assist Reprod Genet (2007) 24:37–41

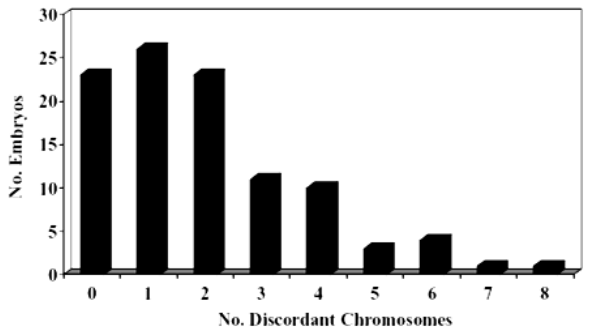


Fig. 1 Number of discordant chromosomes within each embryo undergoing biopsy of two blastomeres on day 3 of development

Blastomere biopsy

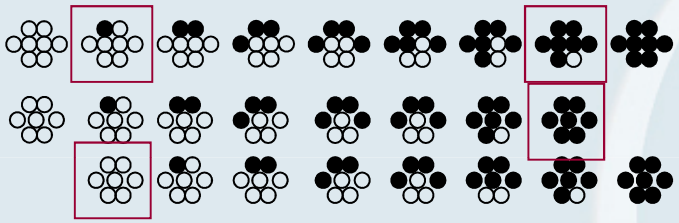
Mosaicism

Probabilities of normal and abnormal results of 1 or 2-cell biopsies taken from 8-cell embryos with different levels of mosaicism

1 cell biopsy

8 cell-stage embryos

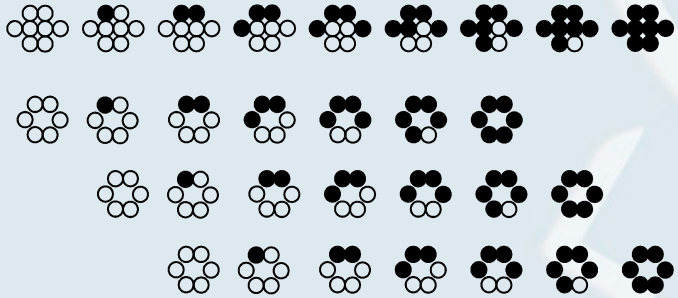
7 cells after biopsy



2 cells biopsy

8 cell-stage embryos

6 cells after biopsy



Los et al, 2004 Hum Reprod Update 10: 79-94

Blastomere biopsy

Mosaicism

Self repair mechanisms

Hypothesis:

- Apoptosis of abnormal cells during early development

- Confination of certain anomalies to extra-embryonic tissues such as placenta, chorion, or amnios, later during pregnancy

Verjaal et al, 1987 found karyotypic differences between cells from placenta and other fetal issues

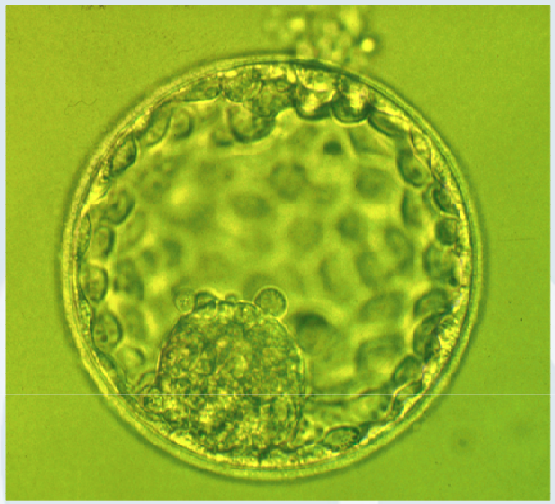
Evsikov and Verlinsky, 1998; Magli, 2000

There is no evidence to support the fact of a preferential allocation of euploid cells to the ICM and aneuploid cells to the trophectoderm

Blastocysts derived from aneuploid embryos revealed a high incidence of mosaicism of ICM cell lineages

Trophectoderm biopsy

Is the trophectoderm representative of the embryo itself?

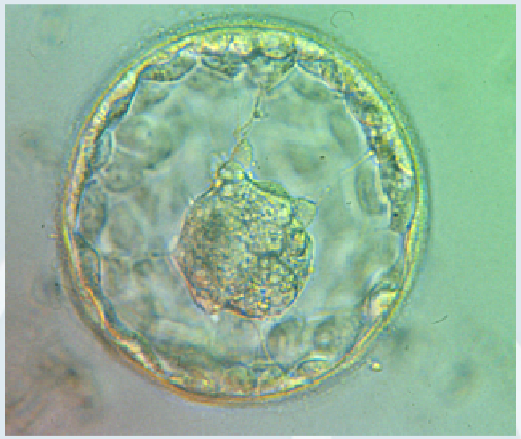


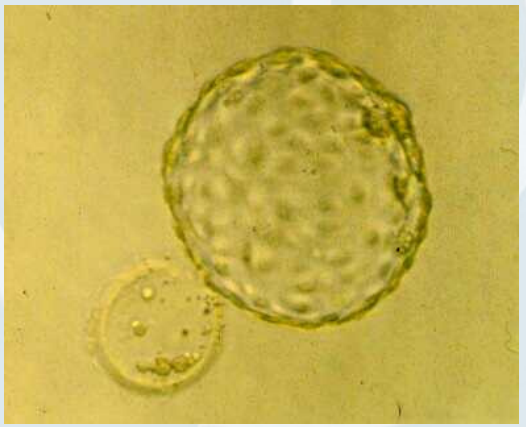
ICM	embryo (ectoderm, mesoderm and endoderm) viteline vesicle amnion

Trophectoderm → non-embryonic tissues (chorion, placenta, umbilical cord)

Trophectoderm biopsy

- Blastocyst biopsy is an emerging technique
- Provides more cells to analyse
- Interesting in monogenic diseases (more DNA available)
- Lower degree of mosaicism
- ICM remains fully intact
- Requires a high blastocyst rate, an optimized culture system and specific laboratory expertise
- Genetic results should be obtained in <24 hours in order to avoid cryopreservation





Double selection by genetic diagnosis and culture to blastocyst stage may lead to high pregnancy and implantation rates

Trophectoderm biopsy

Zygote 5 (November), pp 351–354. © 1997 Cambridge University Press Printed in the United Kingdom

Laser blastocyst biopsy for preimplantation diagnosis in the human

A. Veiga[1], *M. Sandalinas*[1], *M. Benkhalifa*[2], *M. Boada*[1], *M. Carrera*[3], *J. Santaló*[4], *P.N. Barri*[1] *and Y. Ménézo*[2]

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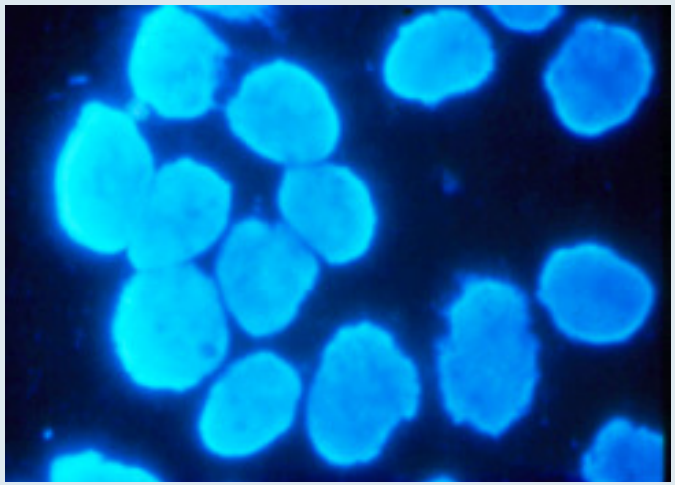
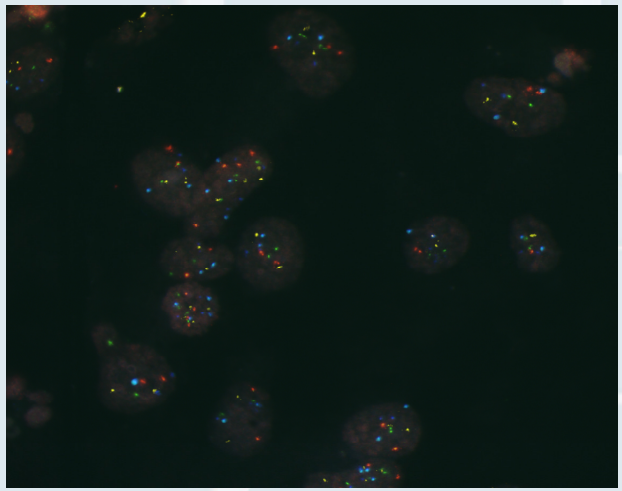


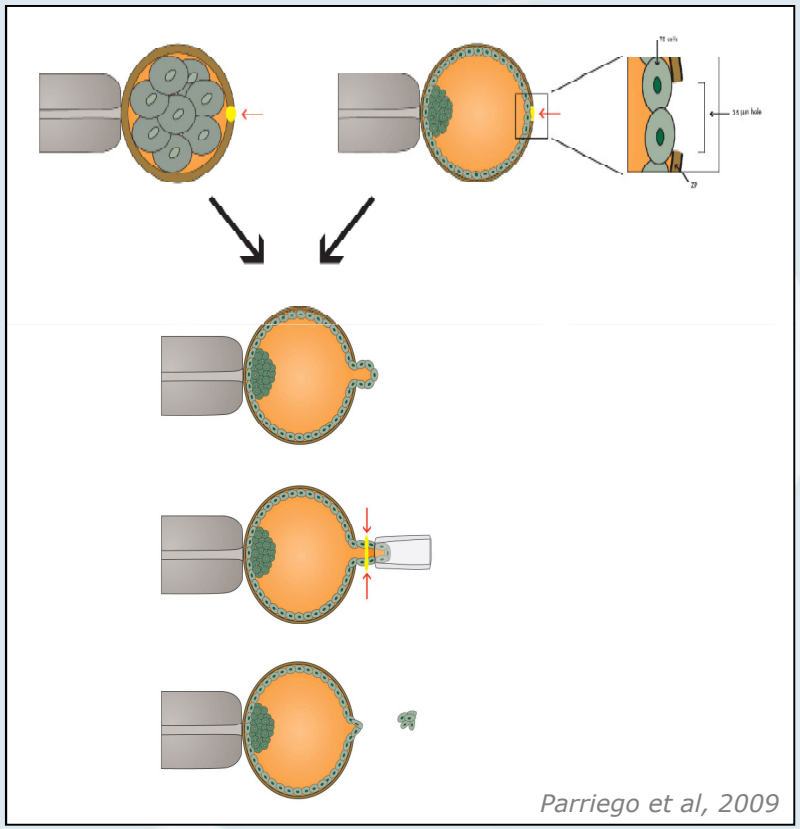


More cells available to analyze

Trophectoderm biopsy

- Zona Pellucida drilling. A small gap 25-30 µm directly opposite the ICM (morning of day 5/ day 3-4)
- Incubation 4 h to allow blastocoele expansion and spontaneous herniation of trophectoderm cells
- Dissection of 3-10 trophectoderm cells using laser pulses
- Blastocyst incubation and transfer on late day 5 or morning day 6 (hatched blastocysts).

First cases reported needed cryopreservation



Parriego et al, 2009

Trophectoderm biopsy

- **Blastocyst biopsy and cryostorage and later transfer of biopsied blastocysts**

De Boer et al, 2004

Mc Arthur et al, 2005

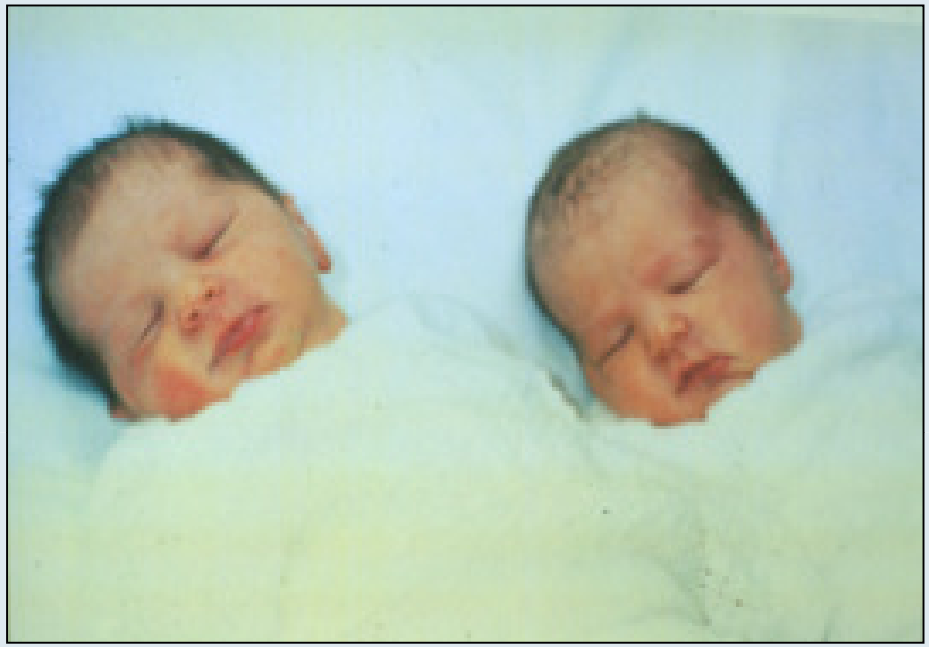
- **Blastocyst biopsy on day 5 and transfer on day 6**

Kokkali et al, 2007

Table I. Overall cycle data for Groups A and B for the diagnosis of β-thalassaemia syndromes

Indication	Group A	Group B
Total cycles	10	10
Female age (years)	36.8 ± 2.82	35 ± 2.94
Day of biopsy	D3	D5
Day of embryo transfer	D5	D6
Biopsy procedure		
Zona breaching	Laser	Laser
Biopsy method	Blastomere	Trophectoderm
Embryology		
Fertilized	131	128
Biopsied	101	53
Diagnosed	76	50
Unaffected	47	26
Transferable at blastocyst	35	26
Transferred	30	21
Average number transferred	3 ± 1.05	2.1 ± 0.99
Frozen	5	5 (+7[a])
Total blastocyst developed	66	60
Blastocysts affected	19	14
Blastocyst not diagnosed	12	3
Clinical outcome		
Cycles to embryo transfer	10	10
HCG positive	6	6
Ectopic	2	
Miscarriage		1
Number of fetal sacs	6 (+2)	10
Implantation rate (%)	26.7%[b]	47.6%

PGD: A celebration of 20 years



Thank you for
your attention!

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Silvia Mateo
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