

Leuven Institute for  
Fertility and Embryology



## Oocyte and Embryo Biopsy

S. Gordts, L. Gianaroli\*, C. Maggi\*, B. Vandamme, R. Campo

# 1. Indications

These techniques are indicated for the following groups of patients:

1. Couples carrying genetic disorders which can be transmitted to the offspring. A list of such diseases (not complete and on continuous updating) is reported in Table I.
2. Patients affected by infertility or sub-fertility undergoing assisted reproduction treatments (IVF or ICSI) with poor prognosis, having already experienced failures both in the natural and in the assisted conception. This technique is particularly indicated for couples with female partner aged more than 38 years, or who failed 3 IVF or ICSI treatment cycles, even if embryos with pregnancy-achieving potential had been transferred.
3. Patients carrying translocations in their chromosomal inheritance. Translocations may be described as abnormalities in the position of more or less long "chromosome pieces", which may lead to the birth of children with more severe chromosomal abnormalities than the parental ones. However such aberrations are very often a hindrance to conception, both natural and assisted. From the medical point of view, translocations are divided in: Robertsonian and reciprocal translocations (see Table 2)
4. Patients having in their reproductive case-history two or more miscarriages not caused by "mechanical" alterations, such as uterine pathologies (adhesions, fibromas, congenital malformations, etc.).

Other pathologies could benefit from oocyte and embryo biopsy but, in the absence of proved scientific data, they are not described here, since studies about them are still ongoing.

## 2. Some notice on the background

At the middle of the Eighties, Australian scientist Alan Trounson assumed the possibility to retrieve one cell from an 8-cell embryo, without damaging it. (1).

At the beginning of the Nineties, Alan Handyside, researcher in London, published the first pregnancy achieved after embryo biopsy performed to exclude the transfer of embryos with gene disorders (2).

In 1995, during an international congress, a meeting between Santiago Munnè and Luca Gianaroli opened the opportunity of a co-operation which proved - in the course of time - to be unique for its potential to produce scientific information aiming at supporting some categories of infertile couples. At that time, Munnè was working out a technique capable of "counting" the chromosomes of a single cell. These studies suggested the hypothesis of the existence of categories of patients producing a high percentage of embryos with chromosome abnormalities and, as a consequence, with no implantation power if transferred in the patient's uterus (3).

At the same time, by a different approach Yuri Verlinski (Chicago) began producing similar data by removing the by-product of the egg-cell before and after insemination (4).

This technique, very promising and less invasive for the embryo, suffers from the fact that obviously it is not able to study the male part of the possible gene or chromosome disorder. In 1997 the first papers with full acknowledgement and appreciation of the international scientific community were published (5-6): since then the specialized scientific journals of greatest world repute (like Fertility and Sterility, Human Reproduction, Molecular Human Reproduction, Journal of Assisted Reproduction and Genetics, Prenatal Diagnosis, Molecular and Cellular Endocrinology) regularly publish the studies of the team of Dr> Gianaroli and of other groups that in the meantime started to use these techniques (7-18).

To date two scientific organizations dealing specifically with this topic have been instituted: the International Working Group On Preimplantation Genetic Diagnosis (IWG on PGD) and the Preimplantation Genetic Diagnosis Consortium of the European Society of Human Reproduction and Embryology (ESHRE PGD Consortium). Both groups regularly meet at least once in the year, in order to exchange ideas, projects and opinions, aiming at having these techniques more and more advanced as rapidly as possible.

### **3. How biopsy techniques are performed**

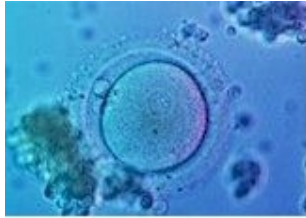
#### ***3.1. Oocyte biopsy***

Oocyte biopsy is performed by removal of the polar body/bodies. The first polar body, containing a set of 46 chromosomes, is extruded from the oocyte prior to its fertilization in order to "make room" to the 23 chromosomes of the spermatozoon, whereas the second polar body is extruded from the oocyte after fertilization. It is precisely in this lapse of time that - using a chemical or mechanical technique or resorting to a source of coherent light (laser), the zona pellucida is pierced and a thin microneedle is inserted, then the microneedle aspirates the 2 polar bodies which will then be prepared for screening and diagnosis.

#### ***3.2. Embryo biopsy***

Embryo biopsy is performed 3 days after a successful insemination. The procedure is very similar to the removal of polar bodies, but to retrieve one cell requires a more gentle manipulation, owing to the risk of damaging the surrounding cells. If the procedure has been performed correctly, there is no risk for the embryo, as it has been proved by several studies on animals and humans. As it occurs with polar body biopsy, the removed cell is then prepared for slide fixing or for screening by means of sophisticated biochemistry techniques (for inst. PCR: Polymerase Chain Reaction).

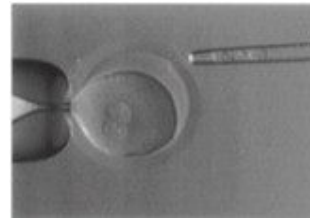
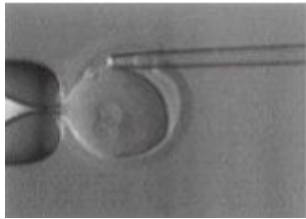
#### ***Oocyte biopsy***



**Fig. 1:** A fertilised oocyte - as shown by the presence of the 2 central pronuclei - is subjected to polar bodies biopsy..

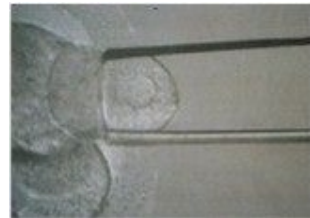
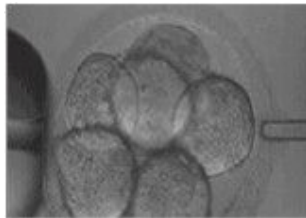


**Fig. 2:** The oocyte is kept in position by a round-edged holding pipette (left side of the picture).



**Fig. 3 en 4:** A thin microneedle is introduced into the layer surrounding the oocyte (left); the polar bodies are removed through a very slight suction of the microneedle (right).

### *Embryo biopsy*



**Fig. 5:** Embryo biopsy is performed by removing one cell from a 6-8 cells embryo. While the round-edged holding pipette keeps the embryo in position (left side in the pictures), a slit is opened in the membrane containing the embryo by using a proper solution which is expelled by a micropipette placed close to the cell to be retrieved

**Fig. 6:** Once the membrane is pierced, a new micropipette, with a larger diameter than the previous one, is inserted in the right position; this will allow to remove, by suction, one cell which will then be released by negative pressure



**Fig. 7:** This photo shows the cell that has been removed.

## 4. How screening and diagnosis techniques are performed

Two are the main cell screening (and consequently diagnosis ) techniques: FISH (Fluorescent In-Situ Hybridization) and PCR (Polymerase Chain Reaction).

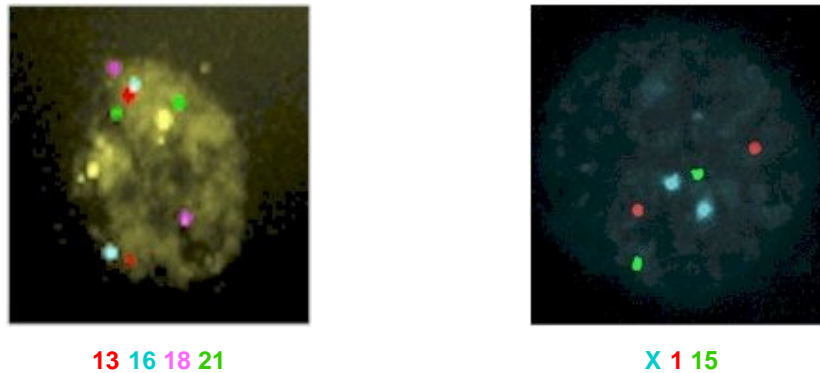
## 4.1. FISH

The first one is used to detect chromosomes and consists of various steps: to expose the small quantity of DNA contained in the nucleus of the removed cell, to open its double helix and to bring it in contact, for competition, with a large quantity of external DNA properly "stained" by fluorochromes of different colours depending on the chromosome under examination. The cell nucleus, slide-fixed and properly arranged, is then examined through a special fluorescence microscope capable of perceiving differences among the single chromosomes. The same procedure may be repeated similarly, in order to enlarge the quantity of the analyzed chromosomes.

## 4.2. PCR

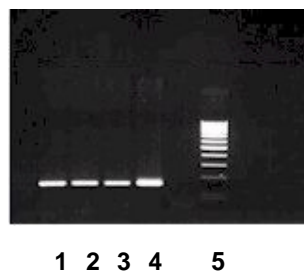
PCR (which, incidentally, won a Nobel award to its inventor) is used to detect very small pieces of chromosomes (like gene-sequences, single genes or even pieces of a single gene) through a sophisticated system of amplification of the minimum quantity of DNA retrieved from the nucleus of the single cell (Fig. 10). Once the amplification has been performed, it is possible to detect even single-base mutations, responsible for the pathology at issue (Fig. 11)

### FISH



**Fig. 8 en 9:** The fluorochromes detected by microscope screening show the presence of two copies for each one of chromosomes 13 (red), 16 (blue), 18 (deep pink), 21 (green) and 22 (yellow) after the first hybridization cycle (left); two copies of chromosomes X (blue), 1 (red) and 15 (green) result from the second hybridization cycle performed on the same cell (right). Therefore this cell is classified as normal for the 8 chromosomes analysed.

### PCR



**Fig. 10:** By using the PCR technique, a region has been enlarged, corresponding to the gene responsible for cystic fibrosis, in case of mutation. The bright band which can be seen in the three

columns on the left shows the amplification. The same experiment includes control systems: the fourth band represents the positive control where - if the system works correctly - the amplification band will be seen, whereas the same will be absent from the fifth band, which is the negative control. The last band on the right represents the molecular weight markers, which are useful to identify the amplification product.

## 5. Effectiveness and efficiency of these techniques

To the best of our current knowledge, it may be stated that these techniques can be effective in 90-93% of cases and that 100% may be reached depending on the possibility to develop new technologies: that is the reason why the above techniques cannot be considered as an alternative solution to the routine prenatal diagnosis (chorionic villus sampling or amniocentesis) , but rather as an additional procedure, though it is to be recalled that their use reduces by 90% at least the transfer and possible implantation in the uterus of embryos affected by transmittable pathologies, or - in the presence of infertile couples - they may avoid the transfer to uterus of embryos not capable to plant. However it is important to remind that the international literature reports some discrepancies - even if not many - between the results obtained after embryo biopsy and those found in the prenatal diagnosis or at the birth<sup>1</sup>. This confirms on the one hand the effectiveness of the above techniques and on the other hand the importance to undergo prenatal diagnosis - in case of pregnancy - in order to validate and to complete the lab results.

## 6. Outcome of the techniques

The results of the techniques performed at SISMER (Bologna) and in our center are shown in the following tables. It is to be recalled that:

- aneuploidy stands for an alteration of chromosome count, namely a quantity difference (higher or lower) in the normal set of 46 chromosomes;
- clinical pregnancy stands for on-term pregnancy or a current pregnancy beyond the 3rd month, with BCF (fetal heart beat) ultrasound evidence;
- implantation rate stands for the comparison between fetuses with BCF ultrasound evidence during the pregnancy and the number of embryos transferred.

---

### <sup>1</sup> References:

Hardy K. and Handyside A.H (1992) Biopsy of cleavage stage human embryos and diagnosis of single gene defects by DNA amplification. *Arch. Patol. Lab. Med.* 116, 388-392.

Harper K. and Handyside A.H. (1994) The current status of preimplantation diagnosis. *Curr. Obstet. Gynecol.* 4, 143-149.

Sermon K., Goossens V., Seneca S. et al. (1998) Preimplantation diagnosis of Huntington's disease (HD): clinical application and analysis of the HD expansions in affected embryos. *Prenat Diagn* 18, 1427-1436.

ESHRE PGD Consortium (2000) ESHRE Preimplantation genetic diagnosis (PGD) Consortium: data collection II (May 2000) *Hum. Reprod.* 15, 2673-2683.

**Table 1. Gene disorders transmittable to the offspring, which can be analysed by genetic diagnosis after oocyte and embryo biopsy..**

Achondroplasia	Central core disease
Agammaglobulinemia	Gaucer's disease
Sickle-cell anemia	Huntington's disease
Fanconi's anemia	Alport's disease
Spinal/bulbar muscular atrophy	Tay-Sachs' disease
Alpha1- antitrypsin deficiency	MELAS
Long chain hydroxyacyl CoA dehydrogenase deficiency	X-linked myotubular myopathy
Ornithine transcarbamilase deficiency	Neurofibromatosis I and II
Deficiency of the mitochondrial trifunctional protein	Multiple endocrine neoplasia type II
Multiple epiphyseal dysplasia	Osteogenesis imperfecta I and IV
<b>Myotonic dystrophy</b>	<b>Familial adenomatous polyposis coli</b>
Becker's muscular dystrophy	Rhetinitis pigmentosa
Duchenne's muscular dystrophy	Rhesus (Rh D)
Haemofilia A and B	Tuberous sclerosis
Epidermolysis bullosa	Crouzon's syndrome
<b>Exclusion HD</b>	Di George's syndrome
FAP-Gardner	Hunter's syndrome MPS II
Phenylketonuria	Lesch-Nyhan's syndrome
Cistic fibrosis	Marfan's syndrome
X-linked hydrocephalus	<b>Digital oro-facial-syndrome type 1</b>
Incontinentia pigmenti	Stickler's syndrome
Hyperinsulinemic hypoglycemia PHH1	Fragile X syndrome
Early onset Alzheimer's disease	Wiskott-Aldrich syndrome
Charcot-Marie-Tooth's disease 1 and 2A	Thalassemia

**Table 2. Translocations: structure rearrangements between 2 non-homologous chromosomes. Types of translocations treated and under examination for probes' setting.**

<b>Robertsonian translocations:</b>	<b>Reciprocal translocations:</b>
occurring between chromosomes 13, 14, 15, 21, 22	occurring between all other chromosomes
13:14	1:3
13:21	1:11
14:21	1:22
	2:8
	3:12
	4:15
	4:20
	11:22
	12:13

**Table 3. Outcome for patients with maternal age  $\geq 38$  years (September 1996 - December 2000).**

N° cycles	174
Total N° embryos	1078
N° embryos analysed	920
N° normal embryos (%)	257 (28)
N° aneuploid embryos (%)	655 (71)
N° embryos with no result(%)	8 (1)

N° transfers (%)	107 (61)
N° clinical pregnancies (%)	36 (34)
N° abortions(%)	5 (14)
Implantation rate (%)	20.0

**Table 4. Outcome in patients with >=3 IVF failures (September 1996 - December 2000).**

N° cycles	105
Total N° embryos	675
N° embryos analysed	588
N° normal embryos (%)	214 (36)
N° aneuploid embryos (%)	361 (61)
N° embryos with no result (%)	13 (2)
N° transfers (%)	76 (72)
N° clinical pregnancies (%)	24 (32)
N° abortions (%)	1 (4)*
Implantation rate (%)	22.4

\*Extra uterine pregnancy

**Table 5. Outcome in patients with altered karyotype owing to mosaics of the sexual chromosomes (September 1996 - december 2000).**

N° cycles	38
Total N° embryos	232
N° embryos analysed	192
N° normal embryos (%)	69 (36)
N° aneuploid embryos (%)	118 (61)
N° embryos with no result(%)	5 (3)
N° transfers (%)	27 (71)
N° clinical pregnancies (%)	13 (48)
N° abortions (%)	0
Implantation rate (%)	36.2

**Table 6. Outcome in patients carrying robertsonian and reciprocal translocations (September 1996 - December 2000).**

	<b>Robertsonian translocations</b>	<b>Reciprocal translocations</b>
N° cycles	22	15
Total N° embryos	125	86
N° embryos analysed	107	73
N° normal embryos (%)	26 (24)	19 (26)
N° aneuploid embryos (%)	81 (76)	53 (73)
N° embryos with no result (%)	0	1 (1)
N° transfers (%)	13 (59)	9 (60)
N° clinical pregnancies (%)	8 (62)	2 (22)
N° abortions (%)	2 (25)*	1 (50)
Implantation rate (%)	43.5	10.5

\*1 miscarriage after amniocentesis (normal fetal karyotype)

**Table 7. Outcome in patients with recurrent abortion (September 1996 - December 2000).**

N° cycles	49
Total N° embryos	331
N° embryos analysed	276

N° normal embryos (%)	76 (27)
N° aneuploid embryos (%)	198 (72)
N° embryos with no result(%)	2 (1)
N° transfers (%)	32 (65)
N° clinical pregnancies(%)	13 (41)
N° abortions (%)	1 (8)
Implantation rate (%)	25.8

**Table 8. Outcome in patients carrying single gene disorders (September 1996 - July 2001).**

N° cycles	15
Total N° embryos	95
N° embryos analysed	87
N° normal embryos (%)	28 (32)
N° healthy carrier embryos (%)	42 (48)
N° embryos affected for the studied pathology (%)	14 (16)
N° embryos zonder resultaat (%)	3 (4)
N° transfers (%)	12 (80)
N° klinische zwangerschappen (%)	4 (33)*
N° miskramen (%)	0
Implantatie rate (%)	23.5

*\*All diagnoses performed after embryo biopsy were confirmed by the following prenatal diagnosis.*

**Table 9. Outcome of cycles with oocyte and embryo biopsy.**

N° clinical pregnancies	98
N° abortions	15
N° tubal pregnancies	1
N° deliveries	82
- singleton	60
- twin	20
- triplet	2
Total N° infants	106
- newborns with minor or severe malformations	2*

*\*one polymalformed (not chromosome-linked pathology); one carrier of Down syndrome.*

## 7. How to enter the programme

The indications for the embryo biopsy program, which are explained to the couple during the counselling with the physician team of our Centre, are:

- patients at risk of transmitting genetic diseases to the offspring;
- infertile couples at high risk of aneuploidies, who are recommended to undergo aneuploidy screening in order to increase their on term pregnancy rate;
- patients carrying robertsonian or reciprocal translocations.

Whereas infertile couples or carriers of robertsonian translocations may enter the program according to procedures not different from those of a normal cycle of medically assisted reproduction, for other cases a particular preliminary stage is required. In this phase it is necessary to take a blood sampling which allows to arrange for the specific probes used in the analysis of reciprocal translocations, or for the diagnosis of the genetic disease at issue. . Once the preliminary phase is concluded, the patients may enter the treatment cycle. Owing to the complexity of

this organization (which even requires the co-operation of a laboratory specialised in molecular genetics in the case of embryo biopsy for genetic diseases) these treatment cycles may only be performed in fixed times. The patients undergoing a cycle of oocyte and embryo biopsy will be required to undersign, in addition to the usual informed consent forms for assisted reproduction cycles (which are reported in the page "Assisted Reproduction Technologies") a specific informed consent by which they authorise the execution of a biopsy on the oocytes or embryos produced in the course of the treatment cycle.

# INFORMED CONSENT FOR PREIMPLANTATION GENETIC DIAGNOSIS FOR ANEUPLOIDIES

Granted that at present no existing technology can really assure that the pre-embryo transferred or to be transferred is not affected by chromosomal abnormalities, gene disorders or by malformations, embryo biopsy, by retrieving one or more cells from the embryo, or the polar bodies from the oocyte, allows to analyse some of its chromosomes in order to identify embryos at a very low risk of aneuploidies (alteration of chromosome number).

Aneuploidies are searched by means of technologies which can give an answer within a few hours.

We are aware that the above mentioned technique:

- 1) is presently used in order to increase the pregnancy rate in selected groups of patients (maternal age >36 years, repeated failures of assisted reproduction cycles, recurrent abortions, presence of chromosomal abnormalities in the couple);
- 2) can lead to cancellation of embryo transfer, if embryos have chromosomal alterations;
- 3) does not exclude that the embryo/embryos which will implant after transfer to the uterus may be carrier of chromosomal or genetic diseases;
- 4) does not involve any further discomfort, besides those connected with a normal cycle of assisted reproduction;
- 5) may not be applied to our embryos, in spite of our agreement, due to unforeseen technical problems in the genetic laboratory.
- 6) Though many experimental studies on man and animals demonstrated that embryo microsurgery - which is necessary to retrieve cells - does not affect the normal embryo development, there is no absolute certainty that the procedure

is 100% safe, since these analyses have been carried out only for a limited number of clinical cases.

- 7) The above mentioned technique could not be effective in spite of all the efforts aimed at obtaining the best result. It may also occur that a normal embryo is erroneously identified as abnormal, thus being not transferred, or that an abnormal embryo is identified as normal. and therefore transferred into the uterus. Other risks include the possibility of a genetic and development damage which could accidentally occur during the analysis.

In view of all the above considerations, and in particular with reference to n° 3, 6 and 7, the pregnancy must be monitored.

In particular, between the 10<sup>th</sup> and the 16<sup>th</sup> week gestation it is recommended to undergo chorionic villus sampling or amniocentesis. These tests will provide a complete chromosomal analysis of the fetus whose growth and development must then be monitored by aid of ultrasound analysis.

Furthermore we have been informed about the possibility that the embryos which cannot be transferred because arrested or due to the detection of aneuploidies, may be further analyzed later on, in order to verify the results.

We hereby confirm that we have read this 'informed consent' form, that Dr. .... has carefully explained it to us and that we have clearly understood its content.

<b>Name of female partner</b>	<b>Signature</b>
<b>Name of male partner</b>	<b>Signature</b>
<b>Name of witness</b>	<b>Signature</b>
<b>Date</b>	