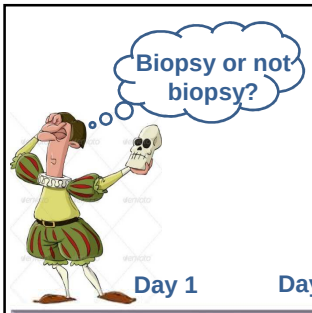
Poor embryo development: biopsy or not biopsy?

A. Pomante, MC Magli, L. Gianaroli, AP Ferraretti

S.I.S.Me.R. Reproductive Medicine Unit, Bologna, Italy

Bologna, May 8, 2016

Embryo biopsy



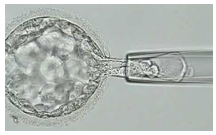
Day 1 Day 2 Day 3 Day 4 Day 5 Day 6

Polar Body Biopsy

Cleavage stage biopsy

Morula stage biopsy

Blastocyst biopsy
Trophectoderm Blastocoelic fluid



1. Polar Body Biopsy

- Polar bodies are by-products of meiosis which have no influence on further embryo development
- Generally more accepted from a legal point of view
- Minimally invasive
- More time to carry out tests
- Not under division
- No mosaicism

- No information about paternal genome

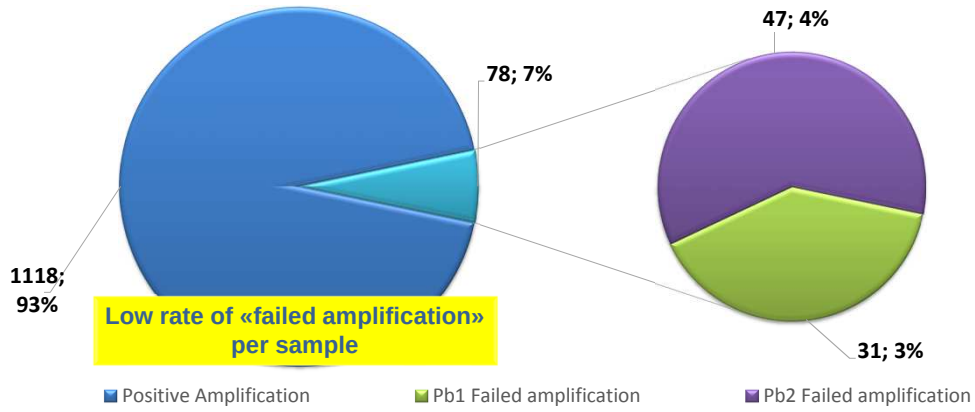
1. Polar Body Biopsy

DIAGNOSTIC EFFICIENCY

2013-2015

122 cycles-115 couples →PGS for advanced maternal age

598 biopsied zygotes-> 1196 polar bodies analyzed



Low rate of «failed amplification» per sample

S.I.S.Me.R. data

1. Polar Body Biopsy

DIAGNOSTIC EFFICIENCY

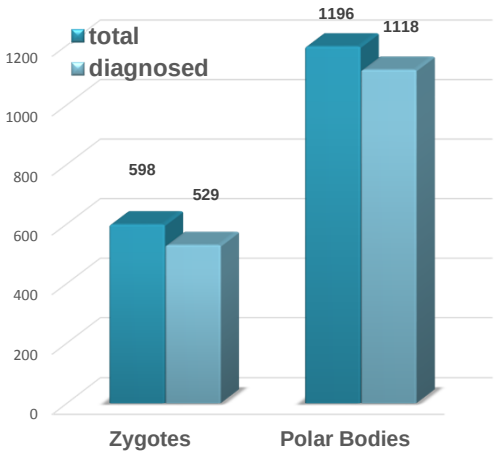
529 zygotes diagnosed on 598 (88.5%)

69 undiagnosed zygotes

24 embryos (34.8%) day 3 rebiopsy

41 embryos arrested on day 2/3 (65.2%)

100% diagnosed



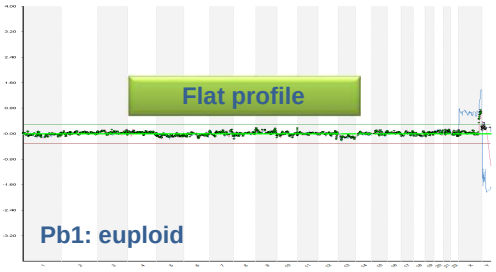
Zygotes/PBs' morphology not related to positive results

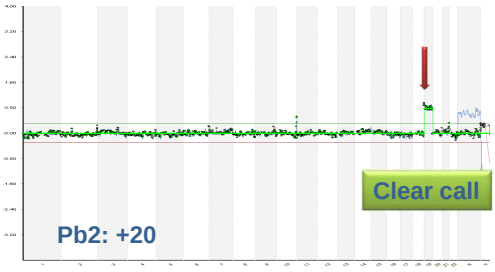
Negative results mostly related to poor embryo quality

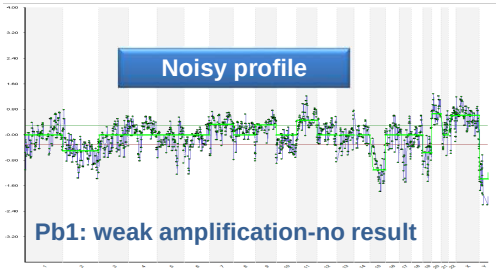
PB Storage and delivery can be an issue

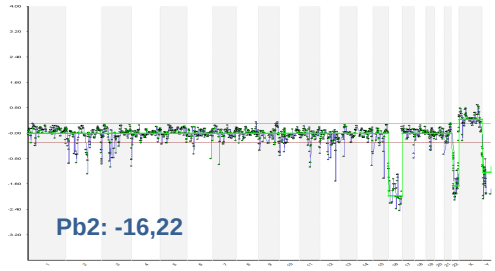
S.I.S.Me.R. data

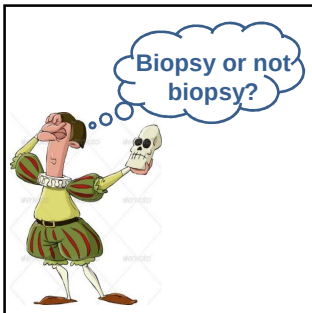
1. Polar Body Biopsy









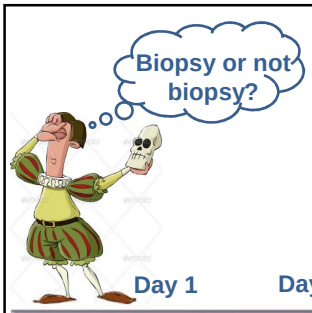


1. Polar Bodies Biopsy

From the genetic lab point of view :

DO IT!

11.5% of zygotes do not have a complete diagnosis→2/3 arrest
only 4% of developing zygotes undiagnosed



Embryo biopsy

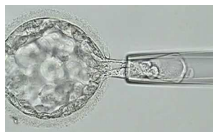
Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6

Polar Body Biopsy

Cleavage stage biopsy

Morula stage biopsy

Blastocyst biopsy
Trophectoderm **Blastocoelic fluid**



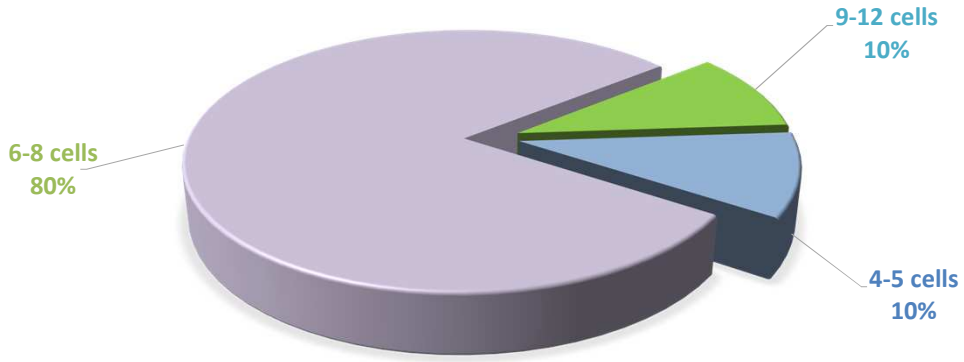
2. Cleavage stage biopsy

- Performed on day 3 embryos (ESHRE 2010 guidelines: ≥6 cells with less than 30% of fragmentation)
- Historically most widely used method
- Information about paternal and maternal genomes
- Mitotic contribution to aneuploidy
- Multiple embryos available
- Fresh transfer

- Mosaicism and genetic instability
- Only 1 cell

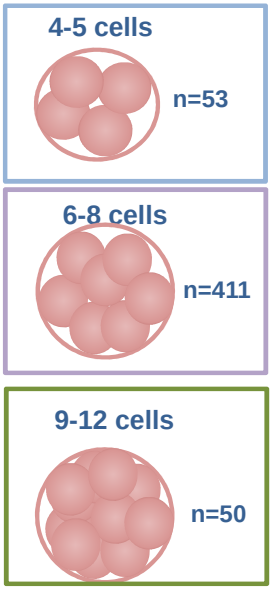
2. Cleavage stage biopsy

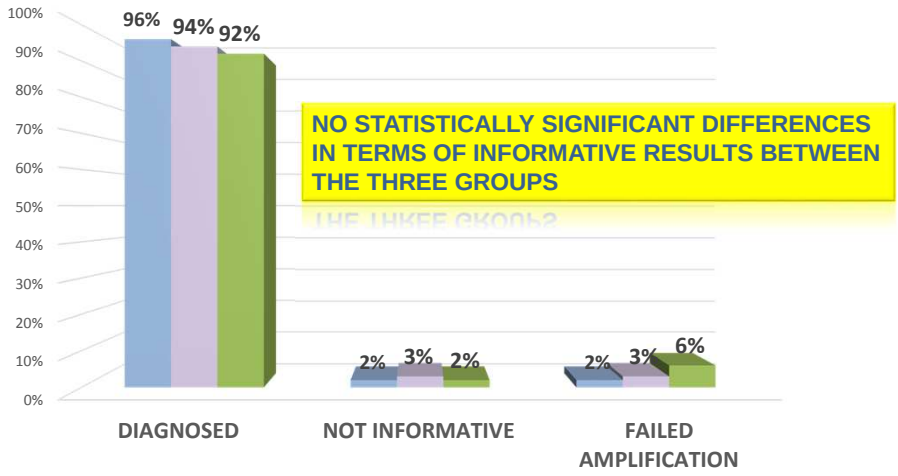
514 embryos



S.I.S.Me.R. data

2. Cleavage stage biopsy



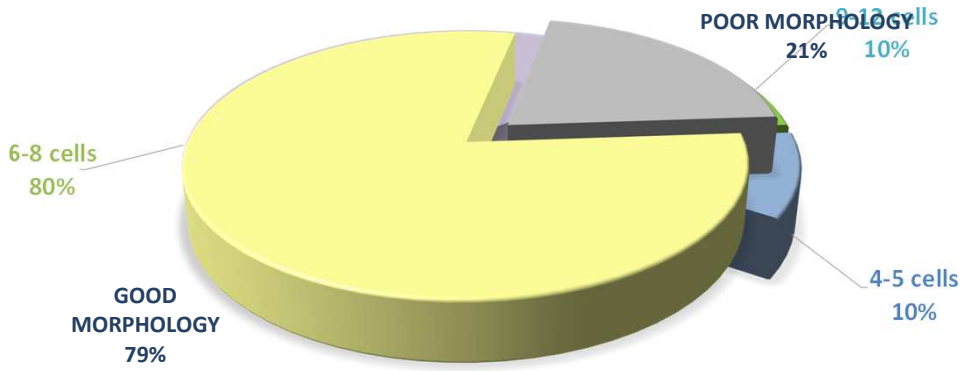


NO STATISTICALLY SIGNIFICANT DIFFERENCES IN TERMS OF INFORMATIVE RESULTS BETWEEN THE THREE GROUPS

iiarg

2. Cleavage stage biopsy

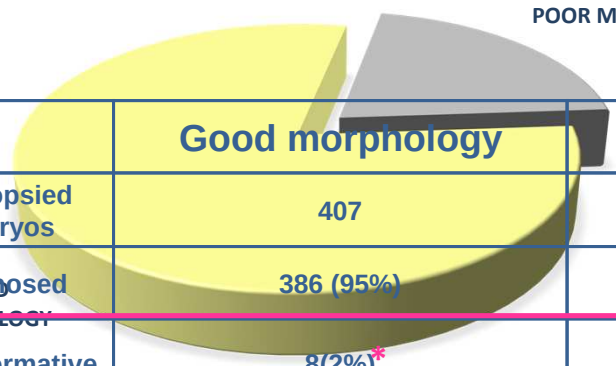
514 embryos



iiarg

2. Cleavage stage biopsy

514 embryos

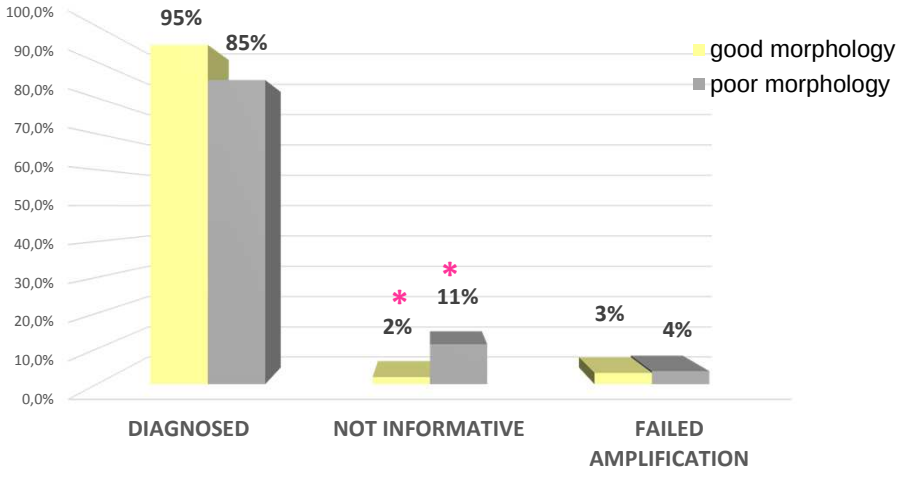


- ≥30% fragmentation
- Vacuoles
- Granularity
- multinucleation

	Good morphology	Poor morphology
N. Biopsied embryos	407	107
Diagnosed	386 (95%)	91 (85%)
Not informative	8(2%)*	12 (11%) *
Failed amplification	13(3%)	4 (4%)

p=0.001

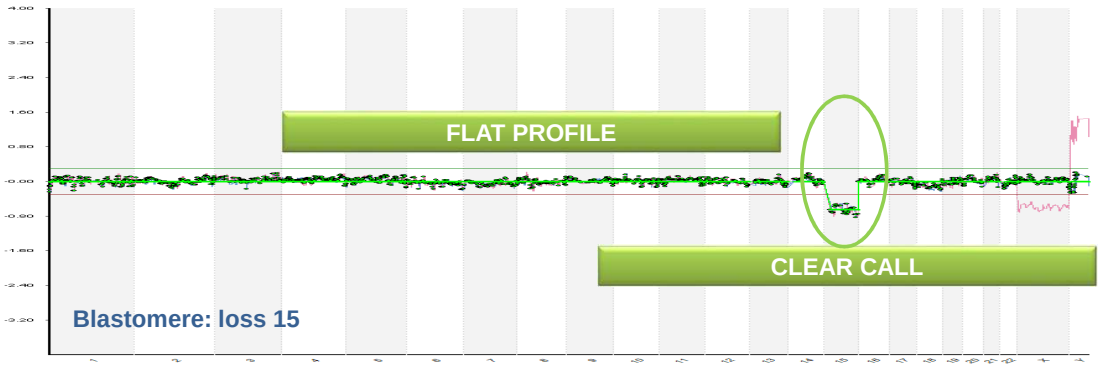
2. Cleavage stage biopsy



p=0.001

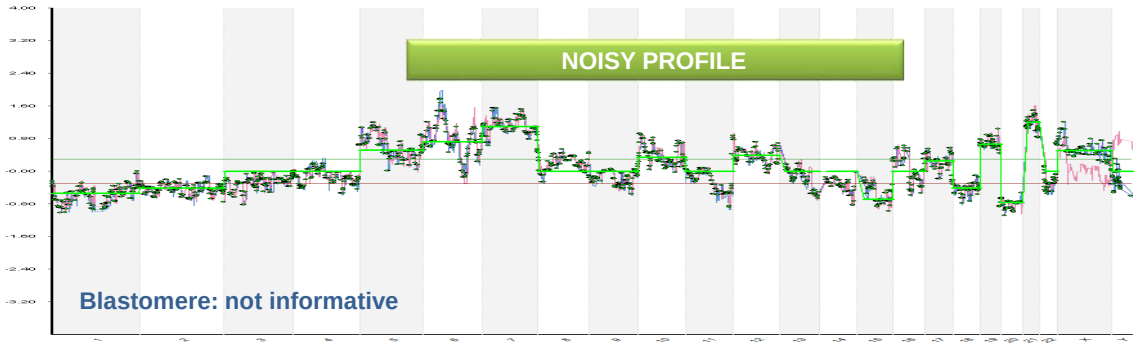
2. Cleavage stage biopsy

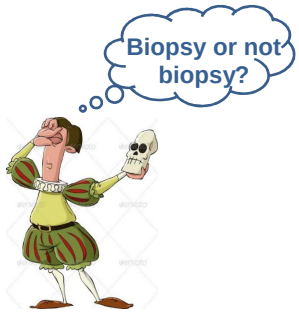
Good morphology embryo



2. Cleavage stage biopsy

Poor morphology embryo





2. Cleavage stage biopsy

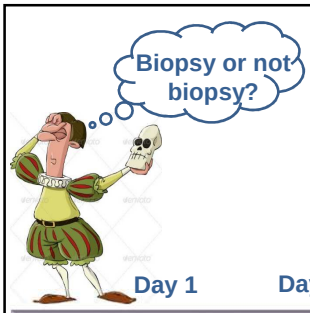
From the genetic lab point of view :

Good morphology embryos: DO IT!

Poor morphology embryos:
11.2% of undiagnosed embryos Biopsy?

iiarg

Embryo biopsy



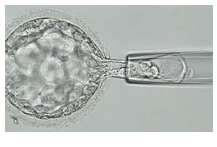
Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6

Polar Body Biopsy

Cleavage stage biopsy

Morula stage biopsy

Blastocyst biopsy
Trophectoderm **Blastocoelic fluid**



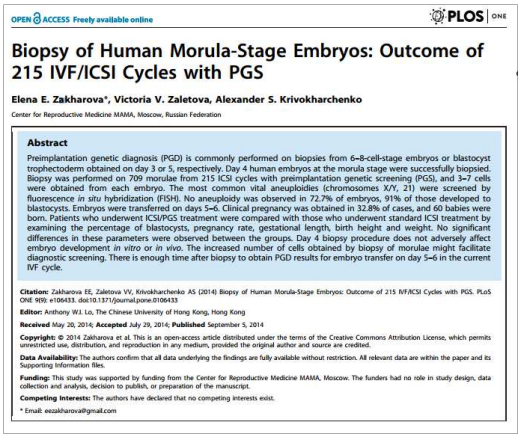
iiarg

3. Morula stage biopsy

- Performed on day 4 embryos
- Information about paternal and maternal genomes
- Multiple cells availables for diagnosis
- Fresh transfer

- More data needed to evaluate its feasibility

3.Morula stage biopsy



OPEN ACCESS Freely available online PLOS ONE

Biopsy of Human Morula-Stage Embryos: Outcome of 215 IVF/ICSI Cycles with PGS

Elena E. Zakharova*, Victoria V. Zaletova, Alexander S. Krivokharchenko

Center for Reproductive Medicine MAMA, Moscow, Russian Federation

Abstract

Preimplantation genetic diagnosis (PGD) is commonly performed on biopsies from 6–8-cell-stage embryos or blastocyst trophectoderm obtained on day 3 or 5, respectively. Day 4 human embryos at the morula stage were successfully biopsied. Biopsy was performed on 709 morulae from 215 ICSI cycles with preimplantation genetic screening (PGS), and 3–7 cells were obtained from each embryo. The most common vital aneuploidies (chromosomes X/Y, 21) were screened by fluorescence *in situ* hybridization (FISH). No aneuploidy was observed in 72.7% of embryos, 91% of those developed to blastocysts. Embryos were transferred on days 5–6. Clinical pregnancy was obtained in 32.8% of cases, and 60 babies were born. Patients who underwent ICSI/PGS treatment were compared with those who underwent standard ICSI treatment by examining the percentage of blastocysts, pregnancy rate, gestational length, birth height and weight. No significant differences in these parameters were observed between the groups. Day 4 biopsy procedure does not adversely affect embryo development *in vitro* or *in vivo*. The increased number of cells obtained by biopsy of morulae might facilitate diagnostic screening. There is enough time after biopsy to obtain PGD results for embryo transfer on day 5–6 in the current IVF cycle.

Citation: Zakharova EE, Zaletova VV, Krivokharchenko AS (2014) Biopsy of Human Morula-Stage Embryos: Outcome of 215 IVF/ICSI Cycles with PGS. PLoS ONE 9(9): e106433. doi:10.1371/journal.pone.0106433

Editor: Anthony W.I. Lo, The Chinese University of Hong Kong, Hong Kong

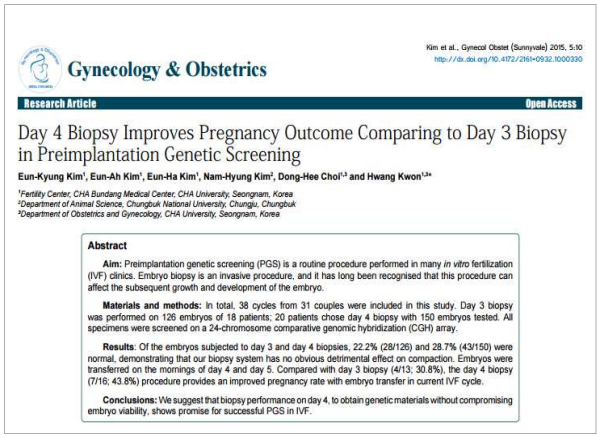
Received May 20, 2014; **Accepted** July 29, 2014; **Published** September 5, 2014

Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper and its Supporting Information files.

Funding: This study was supported by funding from the Center for Reproductive Medicine MAMA, Moscow. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* Email: eezakharova@gmail.com



Kim et al., Gynecol Obstet (Sunnyvale) 2015, 5:10
http://dx.doi.org/10.4172/2161-0932.1000330

Gynecology & Obstetrics

Research Article Open Access

Day 4 Biopsy Improves Pregnancy Outcome Comparing to Day 3 Biopsy in Preimplantation Genetic Screening

Eun-Kyung Kim[1], Eun-Ah Kim[1], Eun-Ha Kim[1], Nam-Hyung Kim[2], Dong-Hee Choi[1,3] and Hwang Kwon[1,3]*

[1]Fertility Center, CHA Bundang Medical Center, CHA University, Seongnam, Korea
[2]Department of Animal Science, Chungbuk National University, Chungju, Chungbuk
[3]Department of Obstetrics and Gynecology, CHA University, Seongnam, Korea

Abstract

Aim: Preimplantation genetic screening (PGS) is a routine procedure performed in many *in vitro* fertilization (IVF) clinics. Embryo biopsy is an invasive procedure, and it has long been recognised that this procedure can affect the subsequent growth and development of the embryo.

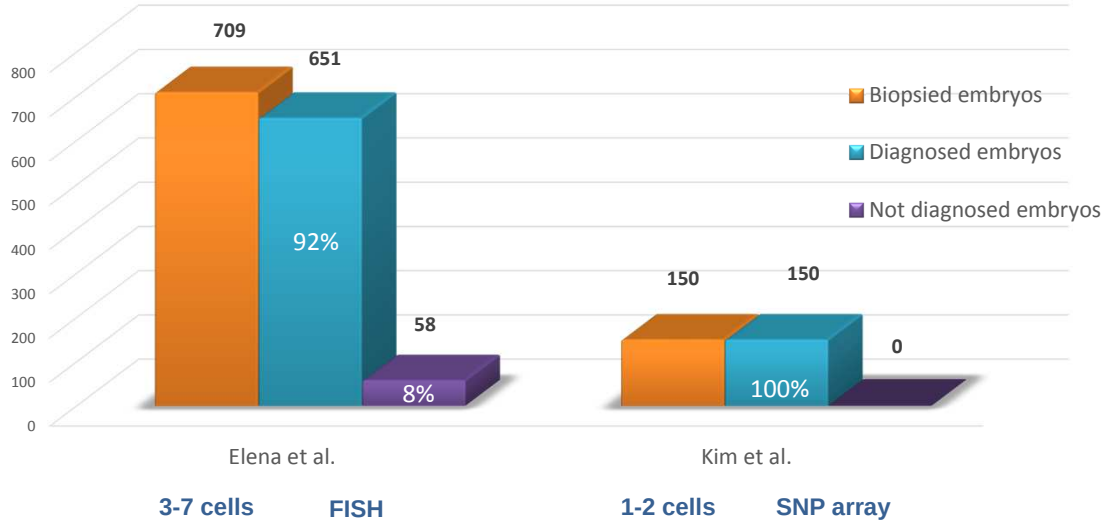
Materials and methods: In total, 38 cycles from 31 couples were included in this study. Day 3 biopsy was performed on 126 embryos of 18 patients; 20 patients chose day 4 biopsy with 150 embryos tested. All specimens were screened on a 24-chromosome comparative genomic hybridization (CGH) array.

Results: Of the embryos subjected to day 3 and day 4 biopsies, 22.2% (28/126) and 28.7% (43/150) were normal, demonstrating that our biopsy system has no obvious detrimental effect on compaction. Embryos were transferred on the mornings of day 4 and day 5. Compared with day 3 biopsy (4/13; 30.8%), the day 4 biopsy (7/16; 43.8%) procedure provides an improved pregnancy rate with embryo transfer in current IVF cycle.

Conclusions: We suggest that biopsy performance on day 4, to obtain genetic materials without compromising embryo viability, shows promise for successful PGS in IVF.

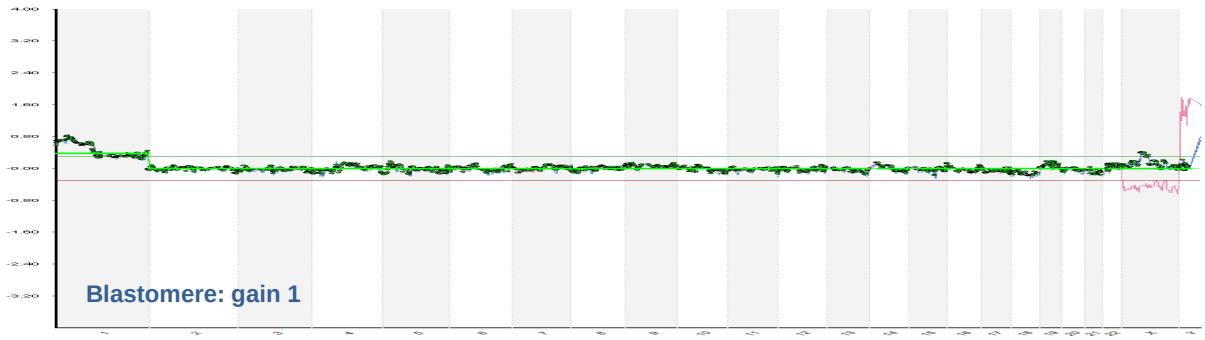
3. Morula stage biopsy

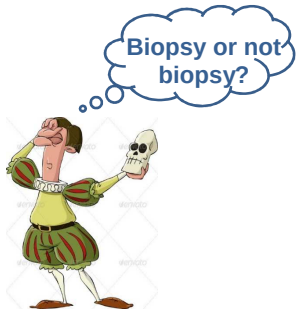
Biospy performed on embryos without fragmentation



3-7 cells **FISH** **1-2 cells** **SNP array**

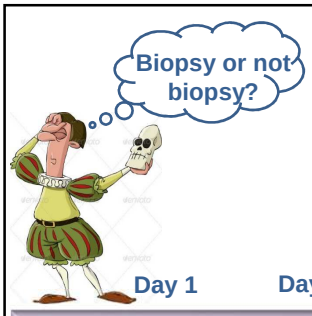
3. Morula stage biopsy





3. Morula stage biopsy

Wait for more data!



Embryo biopsy

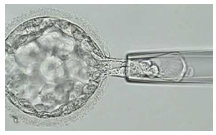
Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6

Polar Body Biopsy

Cleavage stage biopsy

Morula stage biopsy

Blastocyst biopsy
Trophectoderm
Blastocoelic fluid



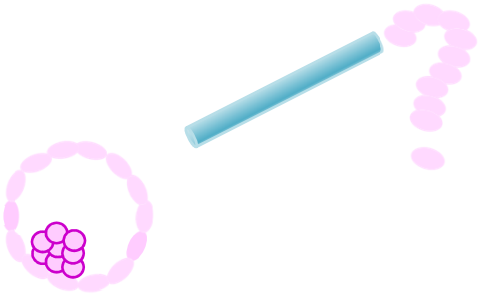
4.Trophectoderm biopsy

- Performed on day 5/6 embryos
- Increasingly the preferred method
- Multiple cells availables for diagnosis-removal of a low proportion of total blastocyst's cell number
- Fewer embryos available
- Less mosaicism

- Training!
- Over extended embryo culture-frozen transfer

4. Trophectoderm biopsy

- Widely demostrated that there are no detrimental effects on the blastocyst after TE biopsy
- Diagnostic efficiency: 96-98% of diagnosed embryos (depending on the lab)



How many cells can be removed?

Number of biopsied trophectoderm cells is likely to affect the implantation potential of blastocysts with poor trophectoderm quality

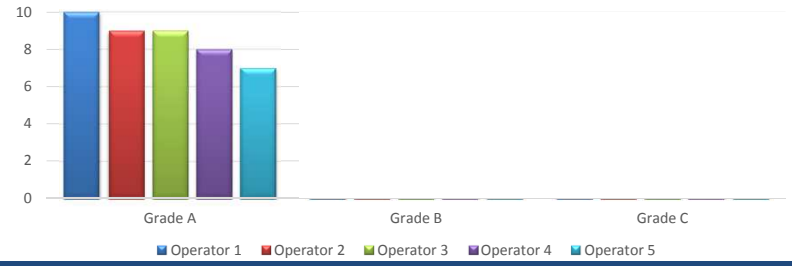
Shuoping Zhang, M.Sc.,[a,b] Keli Luo, M.D., Ph.D.,[a,b,c] Dehua Cheng, M.Sc.,[a,b] Yueqiu Tan, Ph.D.,[a,b,c] Changfu Lu, Ph.D.,[a,b,c] Hui He, M.Sc.,[a,c] Yifan Gu, Ph.D.,[a,b,c] Guangxiu Lu, M.D.,[a,b,c,d] Fei Gong, M.D., Ph.D.,[a,b,c] and Ge Lin, M.D., Ph.D.[a,b,c,d]

[a] Institute of Reproduction and Stem Cell Engineering, Central South University, [b] Reproductive and Genetic Hospital of Citic-Xiangya, [c] Key Laboratory of Stem Cells and Reproductive Engineering, Ministry of Health, and [d] National Engineering and Research Center of Human Stem Cell, Changsha, People's Republic of China

4. Trophectoderm biopsy

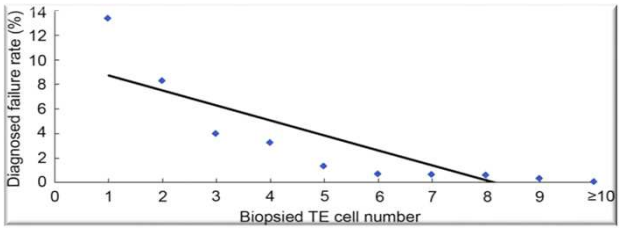
Significant difference in the median number of cells in the biopsies obtained by different embryologists

More biopsied cells in embryos with a higher TE score



Zhang et al., *Fert Ster* 2016

4. Trophectoderm biopsy
DIAGNOSTIC EFFICIENCY

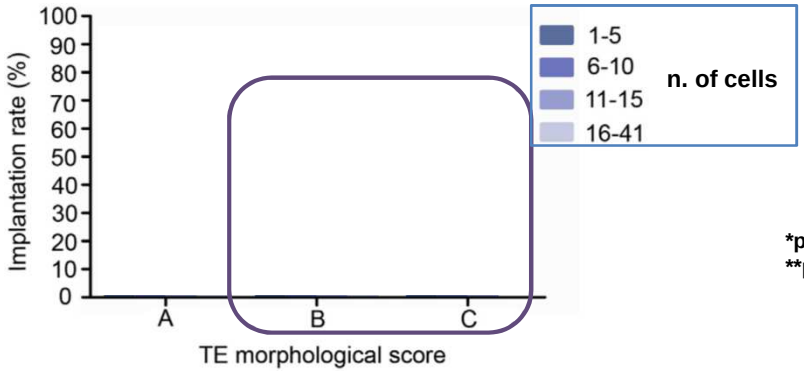


Diagnostic efficiency decreases with the number of biopsied cells

Zhang et al., *Fert Ster* 2016

Trophectoderm biopsy

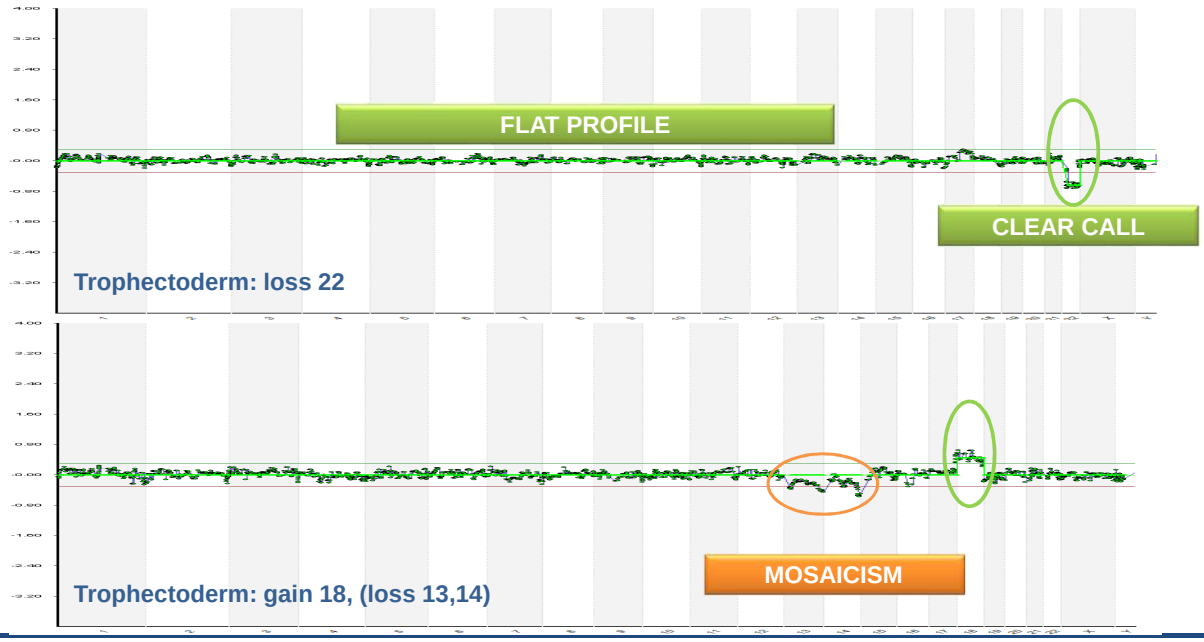
IMPACT ON IMPLANTATION RATE

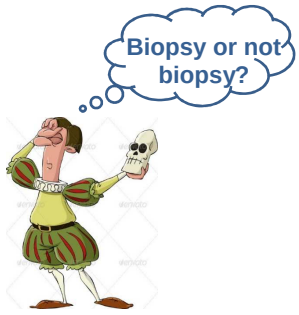


*p<0.05
**p<0.01

In embryos with a low TE score the number of biopsied cells negatively influences the IR

Zhang et al., *Fert Ster* 2016





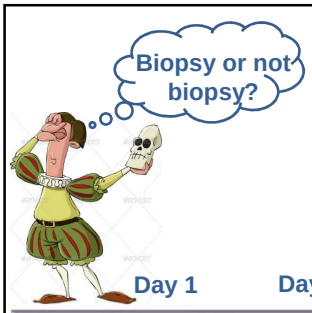
4. Trophectoderm biopsy

From the genetic lab point of view :

DO IT!

CONSIDER:
- **TE quality**
- **number of biopsied cells**

Embryo biopsy



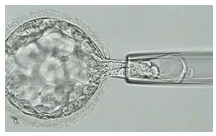
Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6

Polar Body Biopsy

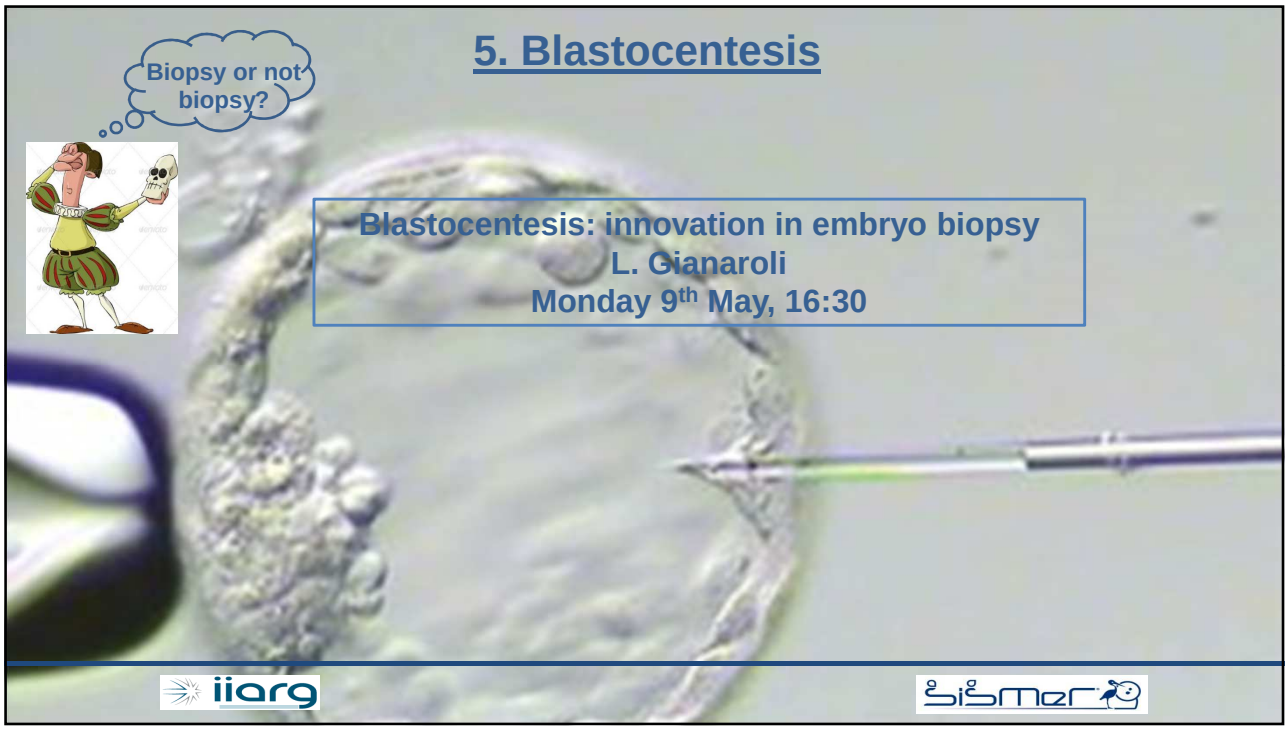
Cleavage stage biopsy

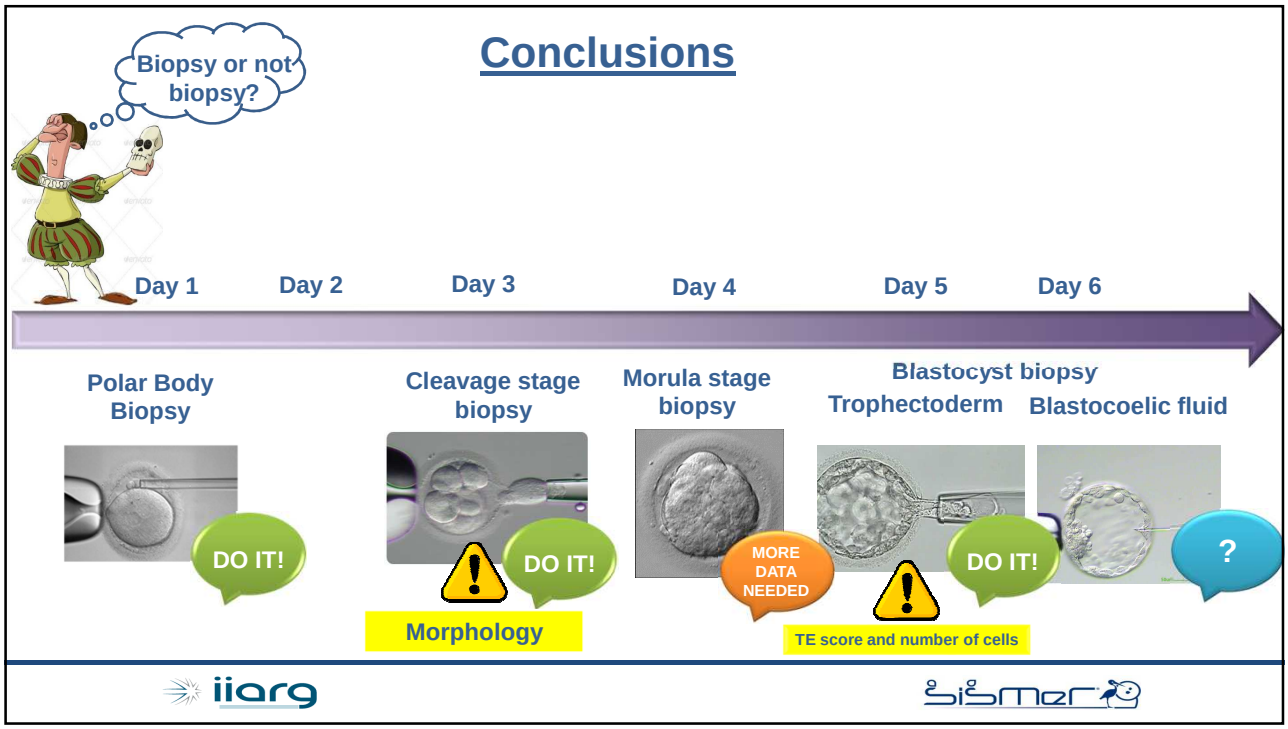
Morula stage biopsy

Blastocyst biopsy
Trophectoderm



Blastocoelic fluid


Biopsy or not biopsy?
5. Blastocentesis
Blastocentesis: innovation in embryo biopsy
L. Gianaroli
Monday 9th May, 16:30
iiarg
SISMER


Biopsy or not biopsy?
Conclusions
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Polar Body Biopsy
DO IT!
Cleavage stage biopsy
DO IT!
Morphology
Morula stage biopsy
MORE DATA NEEDED
Blastocyst biopsy
Trophectoderm
Blastocoelic fluid
DO IT!
TE score and number of cells
?
iiarg
SISMER

Poor embryo development: biopsy or not biopsy?

A. Pomante, MC Magli, L. Gianaroli, AP Ferraretti

S.I.S.Me.R. Reproductive Medicine Unit, Bologna, Italy

Bologna, May 8, 2016