Role of Embryo Biopsy in preimplantation genetic testing

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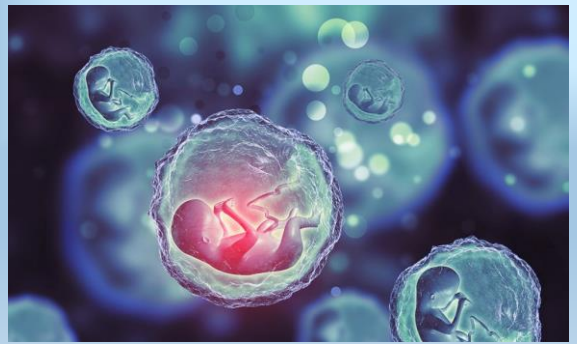
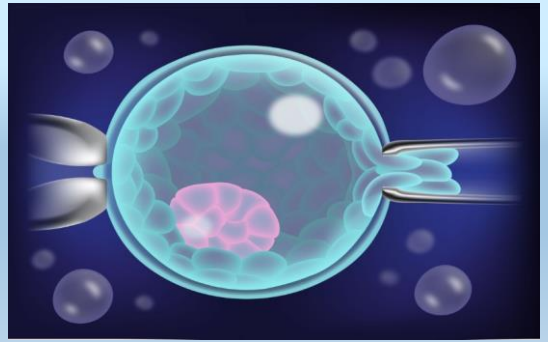
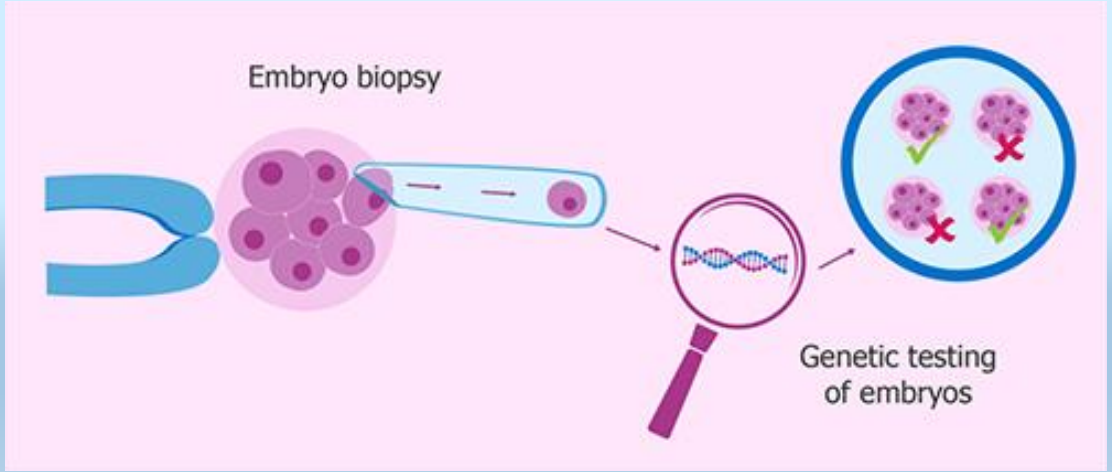
Clinical Embryologist
Associate Professor of Clinical Biochemistry
Nanobiotechnology Research Center,
Avicenna Research Institute





Preimplantation genetic screening or testing-aneuploidy (PGS/PGT-A) has been introduced in clinical practice as a tool for selecting ‘healthy’ embryos before their transfer in utero.



Who is suggested for embryo biopsy?

1. Sex selection

2. Some cases of infertility:

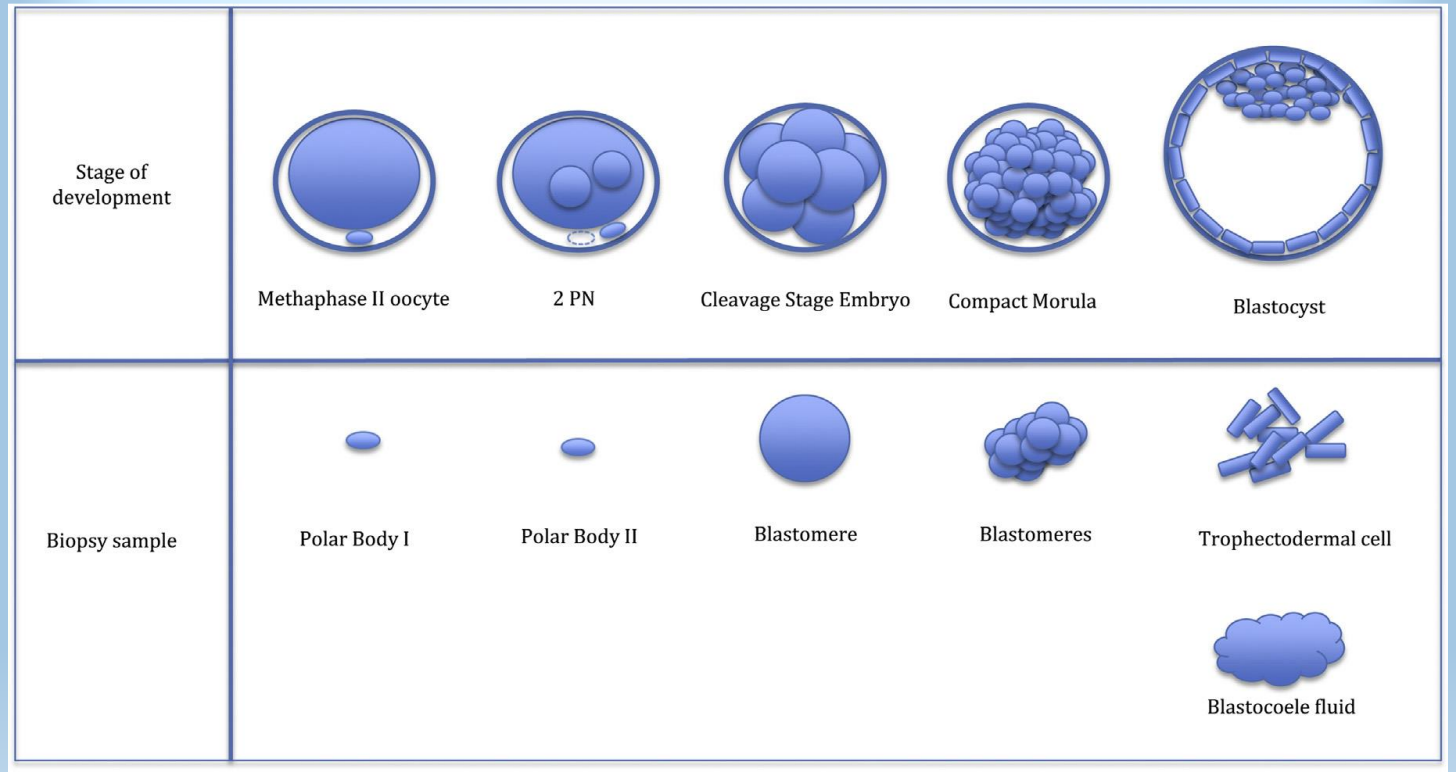
- Patients with recurrent abortions
- Recurrent implantation failures
- Unexplained infertility

3. Advanced maternal age

4. Genetic abnormality:

- Patients with specific genetic disorders/ inherited genetic disorders
- History of chromosomally abnormal child or pregnancy.
- Single gene disorder

Different types of biopsy samples obtained at various stages of early development



Polar body biopsy

- Advantage:
- Less invasive
- is useful in certain cases, to detect abnormal patterns of maternal chromosome segregation

- Disadvantage:
- the effect of the paternal genome is ignored
- False results
- Time consuming

Herrera / Journal of Equine Veterinary Science 41 (2016)

Polar body biopsied-embryo

Developed Embryo quality

- higher fragmentation rate,
- a lower embryo quality,
- a higher cleavage arrest rate
- a lower mean number of blastomeres in day 3

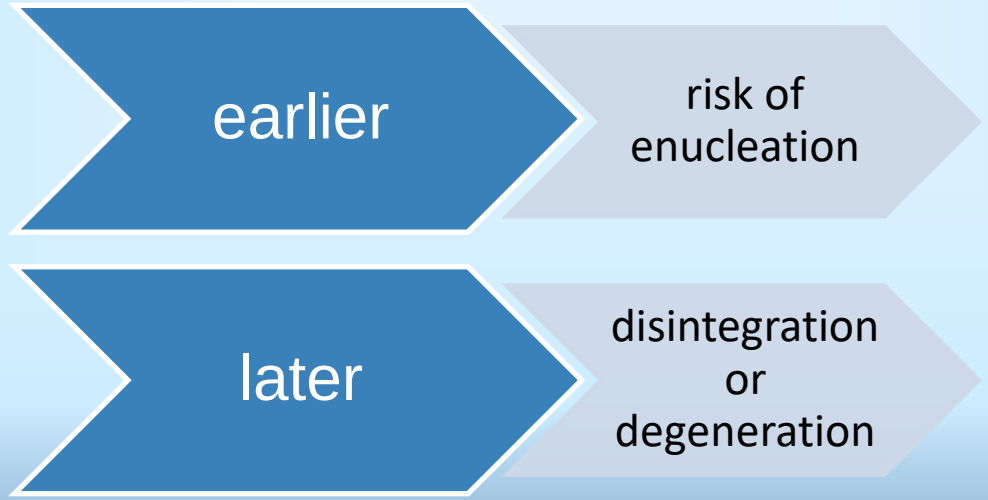
Neonatal outcome

- comparable to those obtained with cleavage stage-based approach

Cimadomo et al. Biomed research international. 2016

Key technical points in polar body biopsy

- ✓ Proper timing of biopsy: 8-14 hours after fertilization



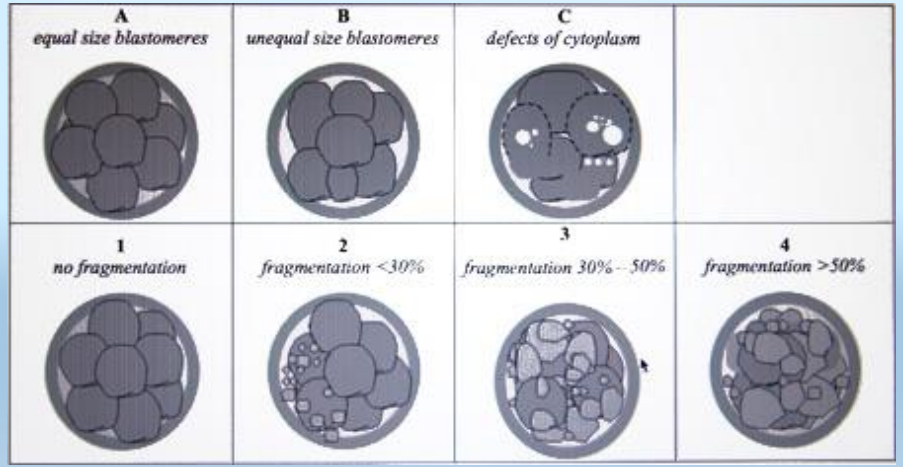
- ✓ Simultaneous biopsy is preferred

Cimadomo et al. Biomed research international. 2016

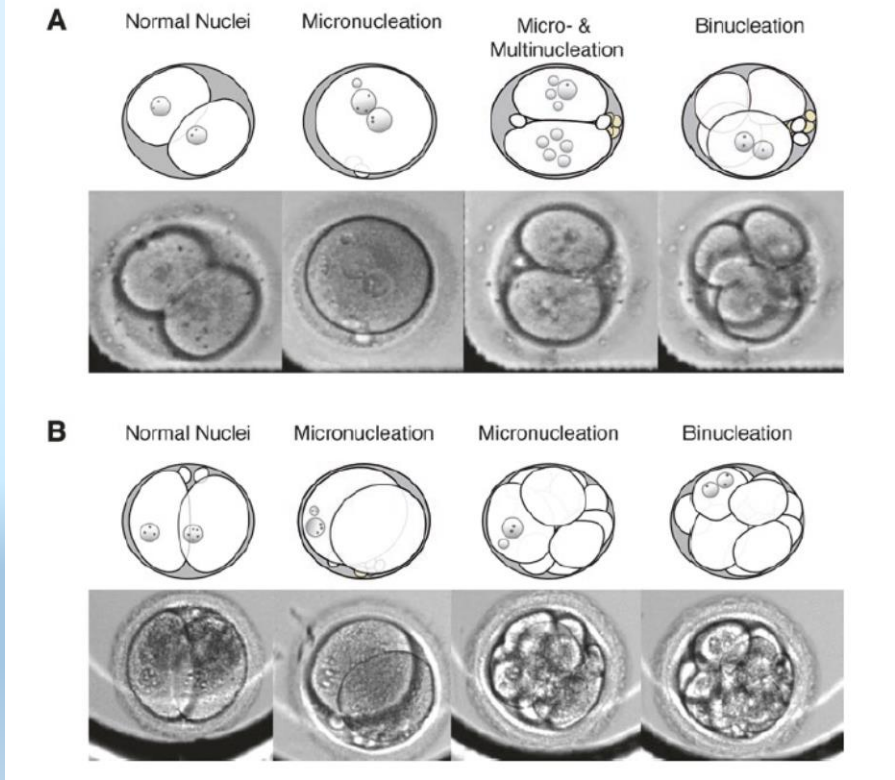
Cleavage stage Biopsy

Evaluation of embryo quality

- Cells number
- fragmentation
- nucleation

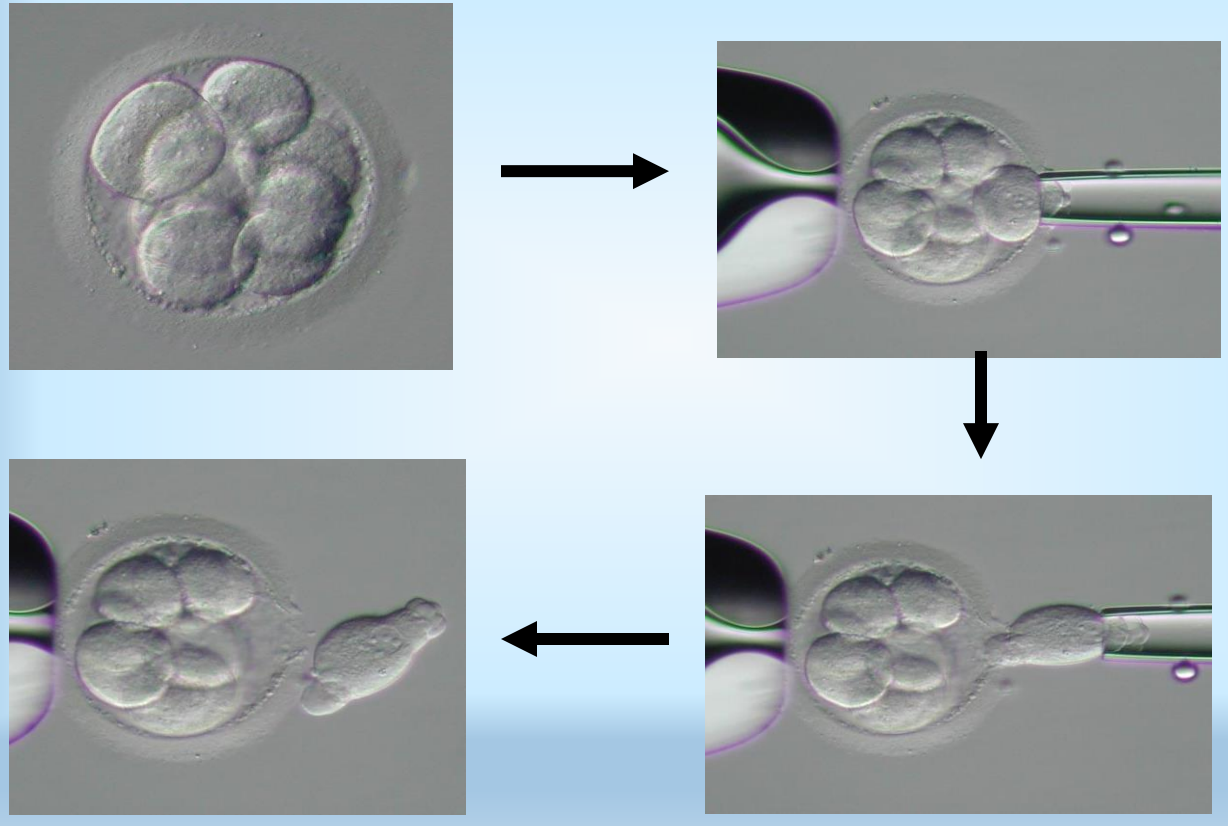


Nuclear morphologies indicative of chromosomal instability



Franck Pellestor (ed.), *Chromothripsis: Methods and Protocols*, Methods in Molecular Biology, 2018

Cleavage stage Biopsy



Day 3 embryo biopsy

Optimal method:

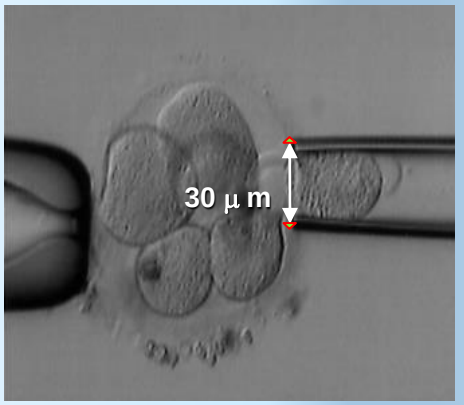
- Hole ideal size is 30 µm
- Ca/Mg free media with amino acids
- Only ONE cell biopsied

Advantages:

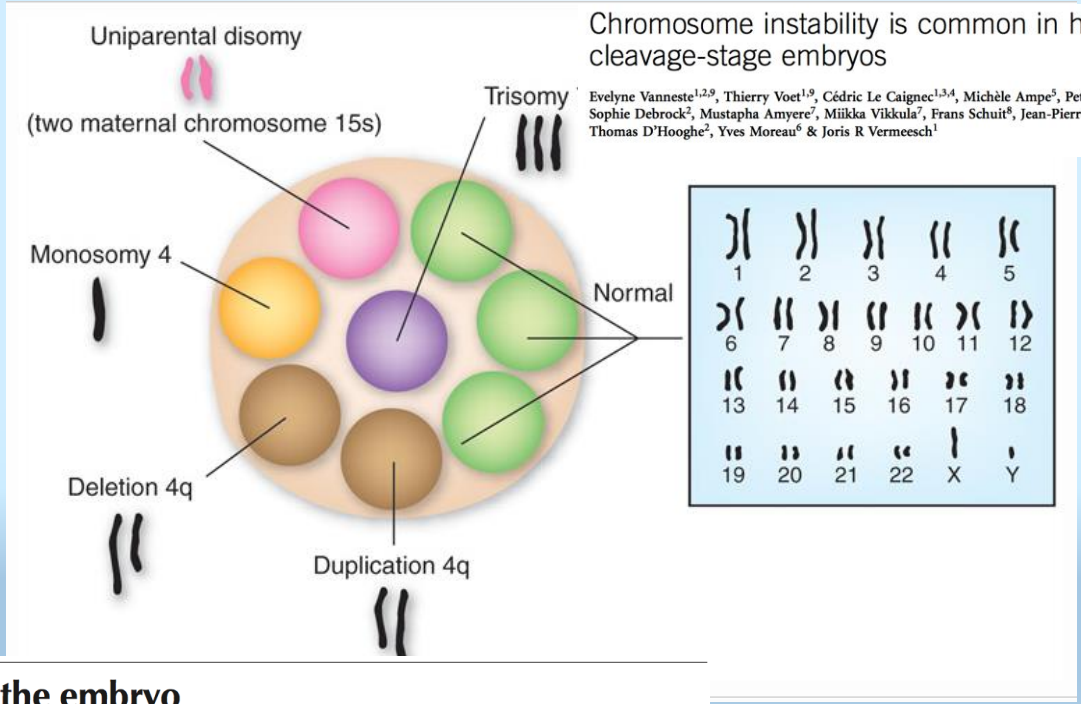
- 30% more abnormalities detected than PB biopsy

Disadvantages:

- 1/8 cells = 12.5% body mass
- Cell polarization?
- Inexperience = damage



Cleavage stage mosaicism



Chromosome instability is common in human cleavage-stage embryos

Evelyne Vanneste[1,2,9], Thierry Voet[1,9], Cédric Le Caignec[1,3,4], Michèle Ampe[5], Peter Konings[6], Cindy Melotte[1], Sophie Debrock[2], Mustapha Amyere[7], Miikka Vikkula[7], Frans Schuit[8], Jean-Pierre Fryns[1], Geert Verbeke[5], Thomas D'Hooghe[2], Yves Moreau[6] & Joris R Vermeesch[1]

Chaos in the embryo

David H Ledbetter

The chromosomes of human embryos seem to be more unstable than previously thought. An analysis of embryos derived from *in vitro* fertilization reveals high rates of structural abnormalities (pages 577–583).

nature medicine

15, 490 - 491 (2009)

The NEW ENGLAND JOURNAL of MEDICINE

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CORRESPONDENCE

Healthy Babies after Intrauterine Transfer of Mosaic Aneuploid Blastocysts

N Engl J Med 2015; 373:2089-2090 | November 19, 2015 | DOI: 10.1056/NEJMc1500421

Ermanno Greco, M.D.
Maria Giulia Minasi, M.Sc.
Francesco Fiorentino, PhD.
European Hospital & Genoma Molecular Genetics Laboratory
Rome, Italy

The Mosaicism of a Preimplantation Embryo Presents Formidable Challenges to The Accuracy of PGT-A Methods

Even More so if an Embryo Has The Ability to Self-correct

Bolton et al., 2016

Impaired developmental dynamic in :

- Timing of blastulation
- Timing of expansion/contraction
- Movements and hatching

Though the majority of studies did not show an increased incidence of pregnancy complications following embryo biopsy, some reported that Blastomere Biopsy is associated with reduced implantation rates, increased risks of low birth weight or preterm birth (Banerjee et al. 2008, Thomaidis et al. 2012, Cimadomo et al. 2016) and, in early childhood, with impaired cognitive and/or motor development (Banerjee et al. 2008, Middelburg et al. 2011, Thomaidis et al. 2012, Cimadomo et al. 2016).

There is no consensus on correlation between embryo biopsy and pregnancy complications.

Reproductive fitness in mice conceived following blastomere biopsy

In the aged female mice, born following BB, had reduced pregnancy rates, numbers of developing follicles and ovarian weights, in addition to altered steroid levels.

Ovarian tissues from both BB-pubertal and BB-aged females were characterized by impaired expression of three proteins (HSPA4, PSMB8 and ALDH1A1) associated with ovarian development, follicle formation and could indicate a higher risk of ovarian cancer (Yu *et al.* 2013, Zacchini et al, 2017).

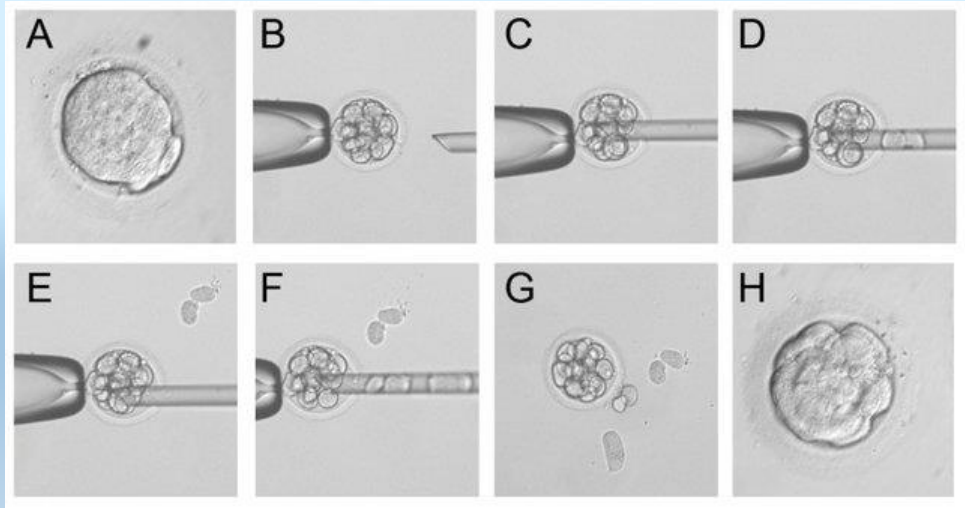
Morula Stage Biopsy

Disadvantage

the need for Ca++/Mg++-free buffer to loosen compaction

Advantage

the number of cells retrieved.



Zakharova et al. PLOS ONE 2014

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

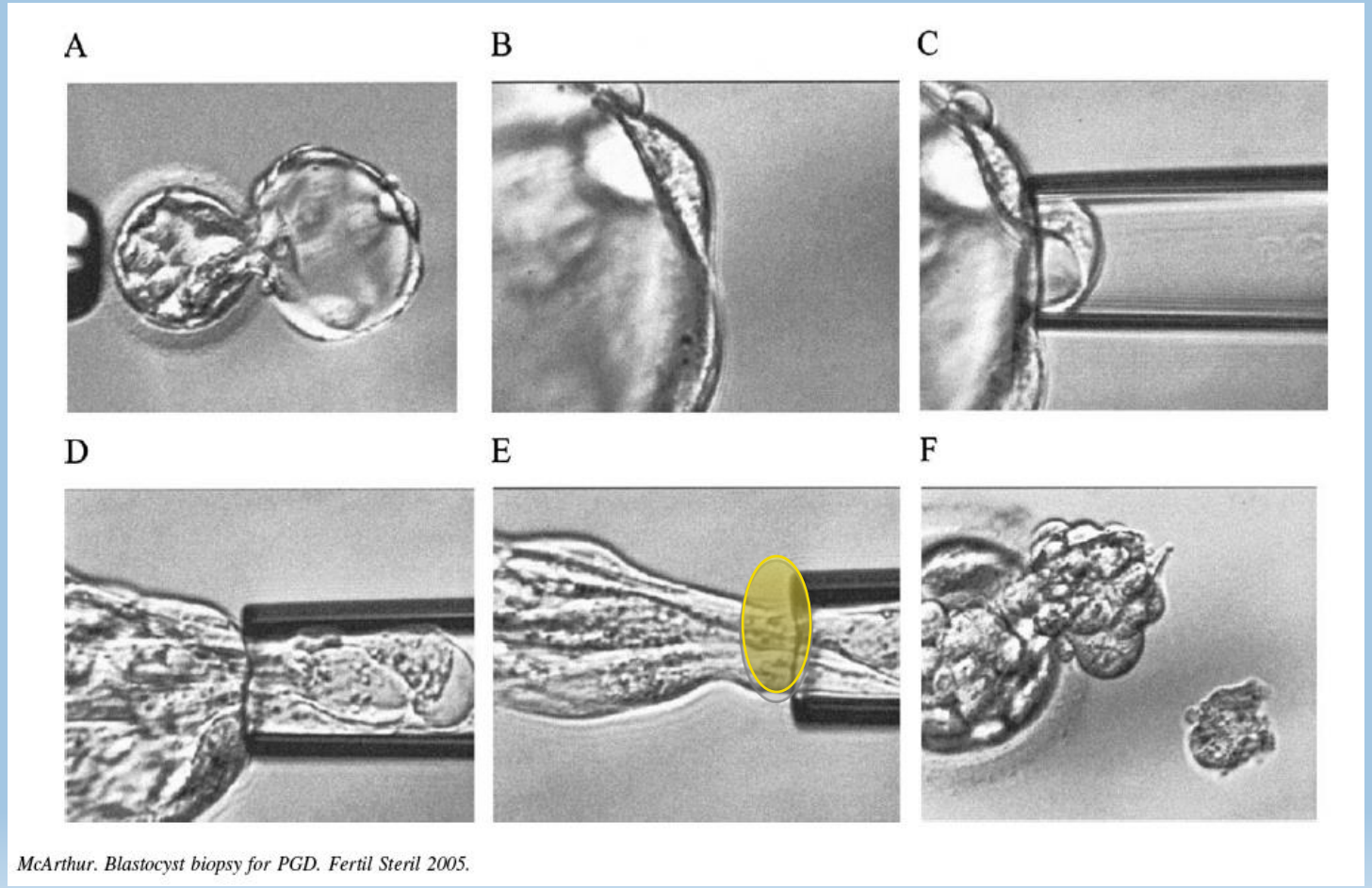
Center for Reproductive Medicine MAMA, Moscow, Russian Federation

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.

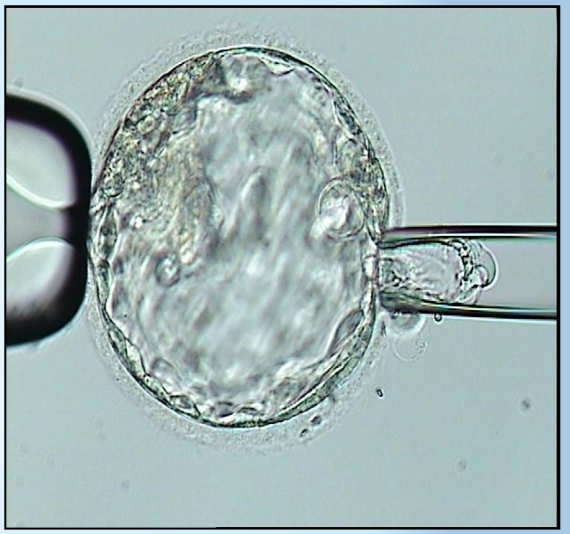
Citation: Zakharova EE, Zaletova VV, Krivokharchenko AS (2014) Biopsy of Human Morula-Stage Embryos: Outcome of 215 IVF/ICSI Cycles with PGS. PLoS ONE 9(9): e106433. doi:10.1371/journal.pone.0106433

Blastocyst Biopsy



McArthur. Blastocyst biopsy for PGD. Fertil Steril 2005.

Blastocyst biopsy: Advantages



1) **More DNA: More robust diagnosis**

2) Lower aneuploidy rate:
Blastocysts: 38.8% , embryos at earlier stages:51%

3) Blastocysts have less mosaicism:
day 3 embryo: from 55% to 73%, Blastocyst: 3.9%

4) Higher reliability of blastocyst stage analysis compared to cleavage stage biopsy (48.2% versus 29.2%)

Scott Jr, et al. Fertility and Sterility. 2012

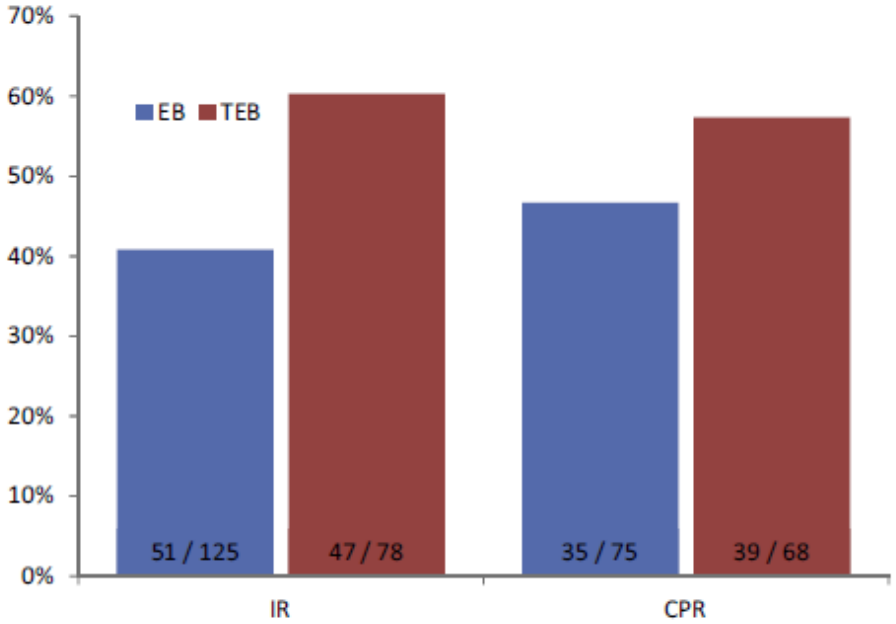
Reproductive BioMedicine Online (2014) 28, 485–491

www.sciencedirect.com
www.rbmonline.com

ARTICLE

Blastocyst culture selects for euploid embryos: comparison of blastomere and trophectoderm biopsies

Alexis Adler *, Hsaio-Ling Lee, David H McCulloh, Esmeralda Ampeloquio, Melicia Clarke-Williams, Brooke Hodes Wertz, James Grifo

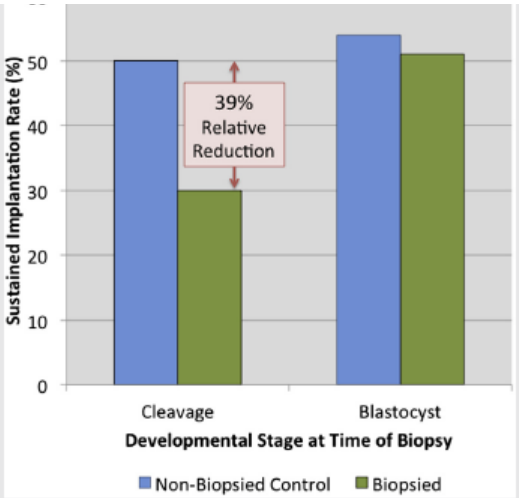


Selecting the optimal time to perform biopsy for preimplantation genetic testing

Katherine L. Scott, M.S.,[a] Kathleen H. Hong, M.D.,[b] and Richard T. Scott Jr., M.D.[b,c]

[a] Atlantic Reproductive Medicine, Raleigh, North Carolina; [b] Department of Obstetrics, Gynecology, and Reproductive Science, Robert Wood Johnson Medical School, Rutgers University, New Brunswick, New Jersey; and [c] Reproductive Medicine Associates of New Jersey, Basking Ridge, New Jersey

Fertility and Sterility® Vol. 100, No. 3, September 2013



Biopsy at the cleavage stage adversely impacts the reproductive potential of the embryo, resulting in an absolute reduction in implantation rates of 19% (relative reductions of 39%). Biopsy at the blastocyst stage does not adversely impact the probability that an embryo will implant and progress to delivery.

Scott. Embryo biopsy for PGD. Fertil Steril 2013.

Journal of Clinical Medicine

MDPI

Review

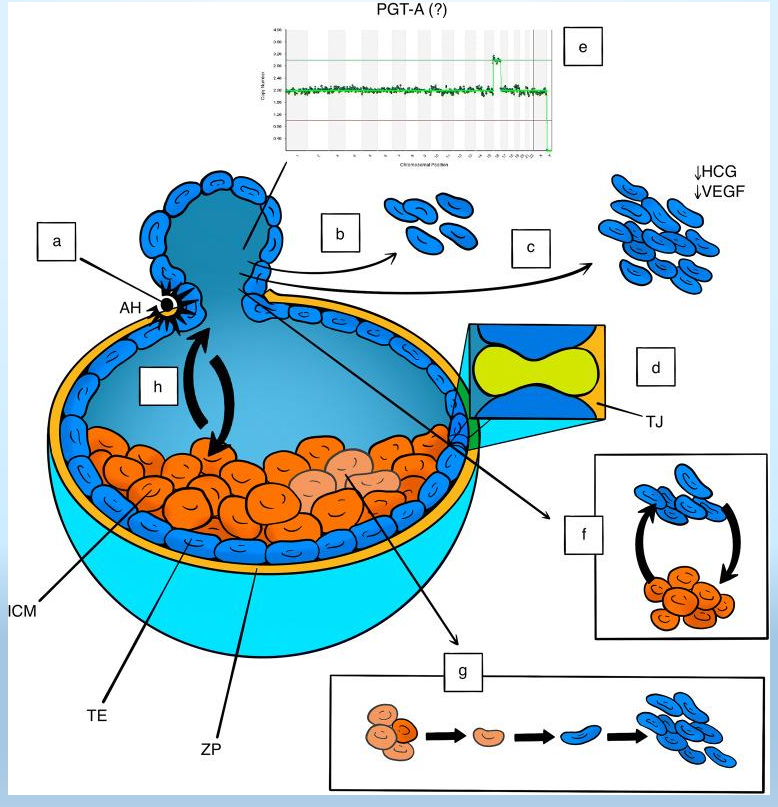
Different Strategies of Preimplantation Genetic Testing for Aneuploidies in Women of Advanced Maternal Age: A Systematic Review and Meta-Analysis

Wei-Hui Shi [1,2,†], Zi-Ru Jiang [3,†], Zhi-Yang Zhou [1,2], Mu-Jin Ye [1,2], Ning-Xin Qin [4], He-Feng Huang [1,2,3], Song-Chang Chen [2,3,*] and Chen-Ming Xu [1,2,3,*]

J. Clin. Med. **2021**, *10*, 3895.

Abstract: Background: Preimplantation genetic testing for aneuploidies (PGT-A) is widely used in women of advanced maternal age (AMA). However, the effectiveness remains controversial. Method: We conducted a comprehensive literature review comparing outcomes of IVF with or without PGT-A in women of AMA in PubMed, Embase, and the Cochrane Central Register of Controlled Trials in January 2021. All included trials met the criteria that constituted a randomized controlled trial for PGT-A involving women of AMA (≥35 years). Reviews, conference abstracts, and observational studies were excluded. The primary outcome was the live birth rate in included random control trials (RCTs). Results: Nine randomized controlled trials met our inclusion criteria. For techniques of genetic analysis, three trials (270 events) performed with comprehensive chromosomal screening showed that the live birth rate was significantly higher in the women randomized to IVF/ICSI with PGT-A (RR = 1.30, 95% CI 1.03–1.65), which was not observed in six trials used with FISH as well as all nine trials. For different stages of embryo biopsy, only the subgroup of blastocyst biopsy showed a higher live birth rate in women with PGT-A (RR = 1.36, 95% CI 1.04–1.79). Conclusion: The application of comprehensive chromosome screening showed a beneficial effect of PGT-A in women of AMA compared with FISH. Moreover, blastocyst biopsy seemed to be associated with a better outcome than polar body biopsy and cleavage-stage biopsy.

key points for blastocyst biopsy



Tocci. J Assist Reprod Genet. 2020

Is the trophectoderm representative of the ICM?

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

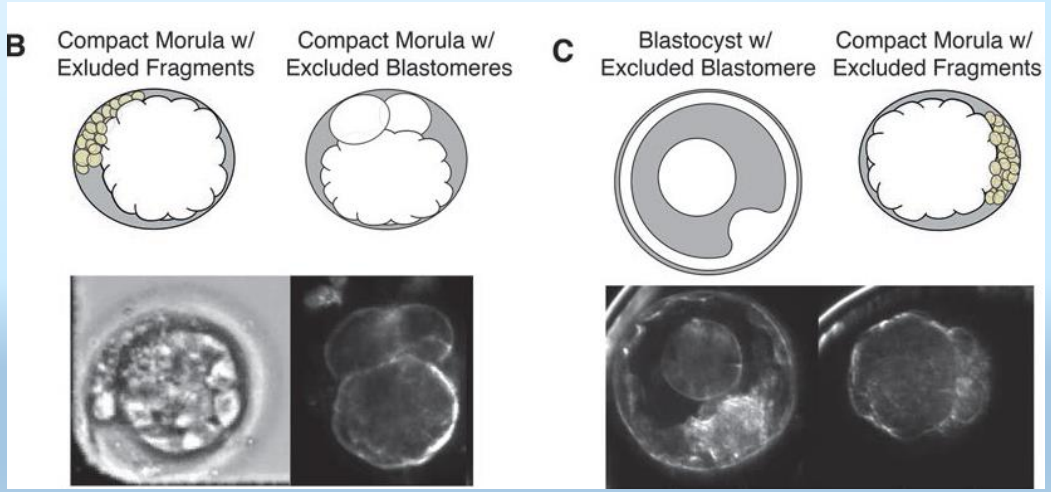
Trophectoderm: non-embryonic tissues (chorion, placenta, umbilical cord)

- ICM and TE were concordant in 97% embryos.

Capalbo, Wright, Themaat, Elliott, Rienzi, Nagy (2011) ASRM meeting

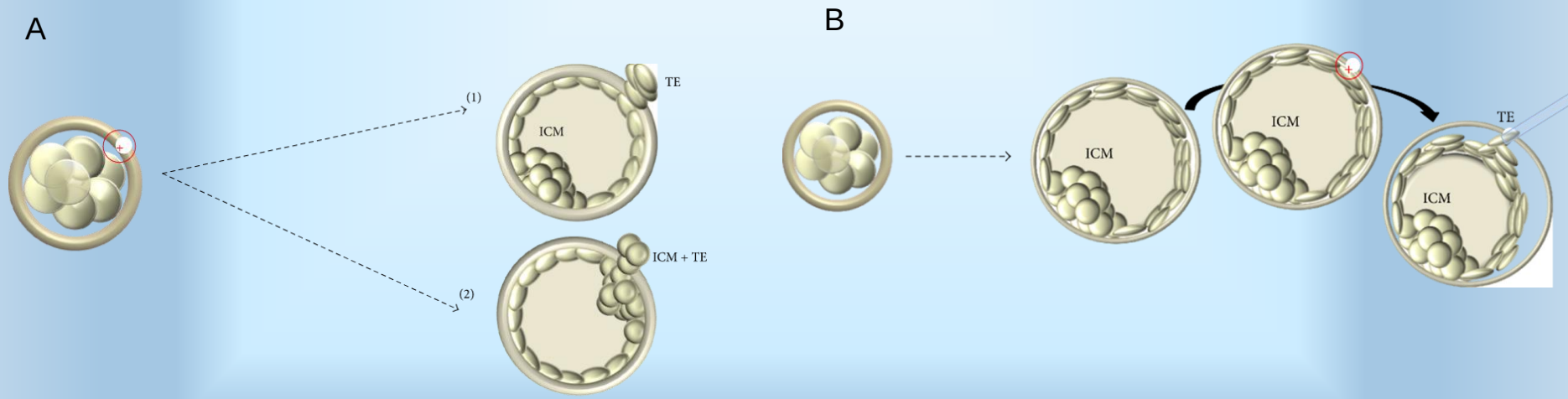
key points for blastocyst biopsy

- No pick up excluded cells in morola and blastocysts



L. Daughtry. *Chromothripsis: Methods and Protocols*, Methods in Molecular Biology, 2018.

Impact of blastocyst biopsy procedure in technical and clinical results



A. Capalbo et al. Human Reproduction. 2015

No significant differences across 7 different operators from 3 IVF centers in terms of both technical and clinical results were reported. In particular, amplification rate, qPCR data concurrence, and estimated number of cells retrieved, as well as ongoing implantation, biochemical, and miscarriage rates, were comparable.

A. Capalbo et al. Human Reproduction. 2015

Archives of Gynecology and Obstetrics
https://doi.org/10.1007/s00404-019-05084-1

GYNECOLOGIC ENDOCRINOLOGY AND REPRODUCTIVE MEDICINE

Comparison of two protocols of blastocyst biopsy submitted to preimplantation genetic testing for aneuploidies: a randomized controlled trial

Haibin Zhao[1] · Wenrong Tao[1] · Mei Li[1] · Hui Liu[1] · Keliang Wu[1] · Shuiying Ma[1]

Received: 22 August 2018 / Accepted: 2 February 2019

Abstract

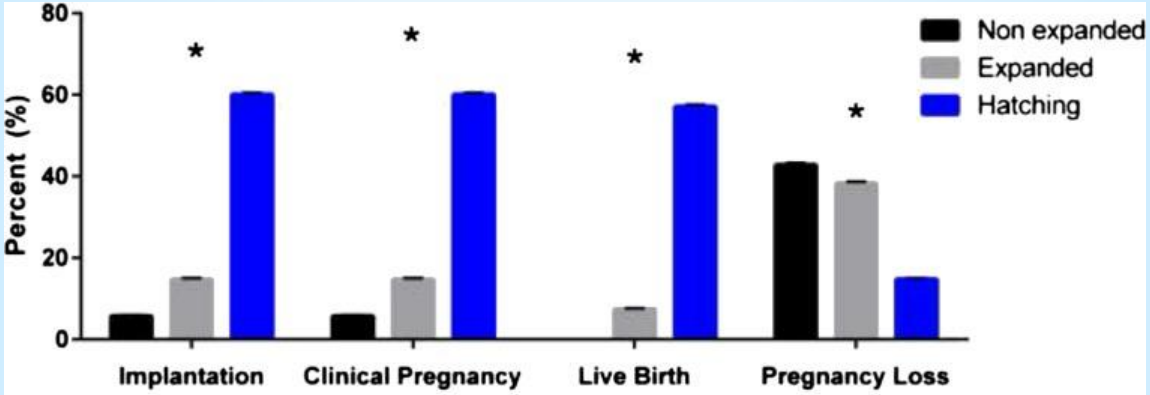
Purpose To compare the effectiveness of two protocols of blastocyst biopsy submitted to preimplantation genetic testing for aneuploidies (PGT-A).

Methods This is a randomized controlled trial of a cohort of 221 patients undergoing PGT-A. 106 female patients aged ≤ 40 years with no less than 8 mature oocytes retrieved and ≥ 3 good-quality embryos on day 3 were randomly assigned to the day-3 hatching-based TE biopsy. The remaining 115 females aged ≤ 40 years with ≥ 8 MII oocytes obtained and no less than 3 high-quality embryos on day 3 were assigned to the TE biopsy without hatching group (also called the new biopsy group). The primary outcome was measured by a live birth after the first embryo transfer.

Results The live birth rate did not differ significantly between the two groups (50.00% vs. 59.26%, $P > 0.05$, OR 1.46; 95% CI 0.78–2.70). There was no significant between-group difference in the rates of implantation, clinical pregnancy, and miscarriage. However, the frozen blastocyst rate was significantly lower in the day-3 hatching-based TE biopsy compared with the new biopsy group (47.54% vs. 53.96%, $P < 0.05$, OR 1.29; 95% CI 1.08–1.56).

Conclusions Our study provides strong evidence that the new blastocyst biopsy method exhibits advantages over day-3 hatching-based TE biopsy method. Using this method, we were able to obtain more blastocysts to perform trophectoderm biopsy in patients subjected to PGT-A.

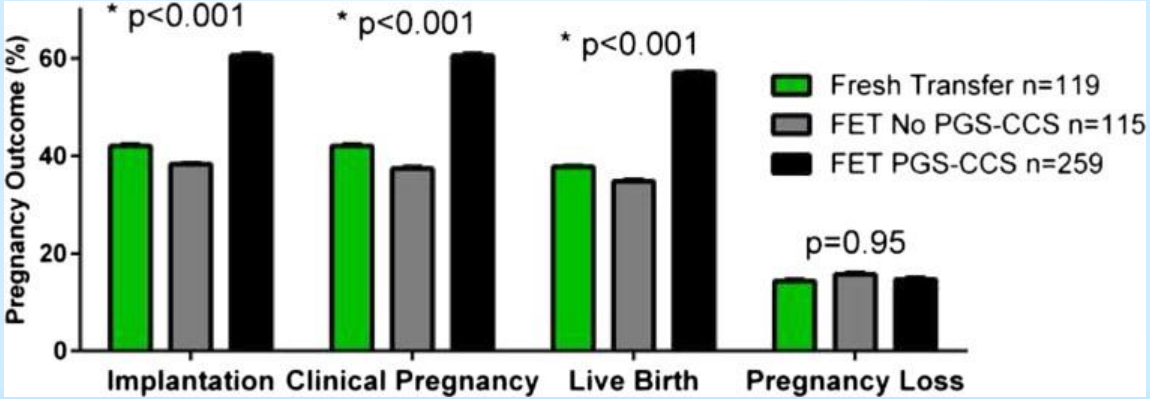
Pregnancy outcomes with PGS-CCS by embryo expansion at time of embryo biopsy: impact of blastocyte expansion



* Denotes statistical significance. Includes all age groups combined.Overall comparisons made by Kruskal Wallis and post hoc DunnTest. All p values <0.001 for comparisons between non-expanded and hatching as well as between expanded and hatching. No significant differences between non expanded versus expanded embryos for any pregnancy outcomes.

Singh et al. Journal of Assisted Reproduction and Genetics (2019)

Higher Pregnancy outcomes for PGS-CCS of hatching embryos compared to fresh and frozen non-PGS-CCS cycles



Singh et al. Journal of Assisted Reproduction and Genetics (2019)

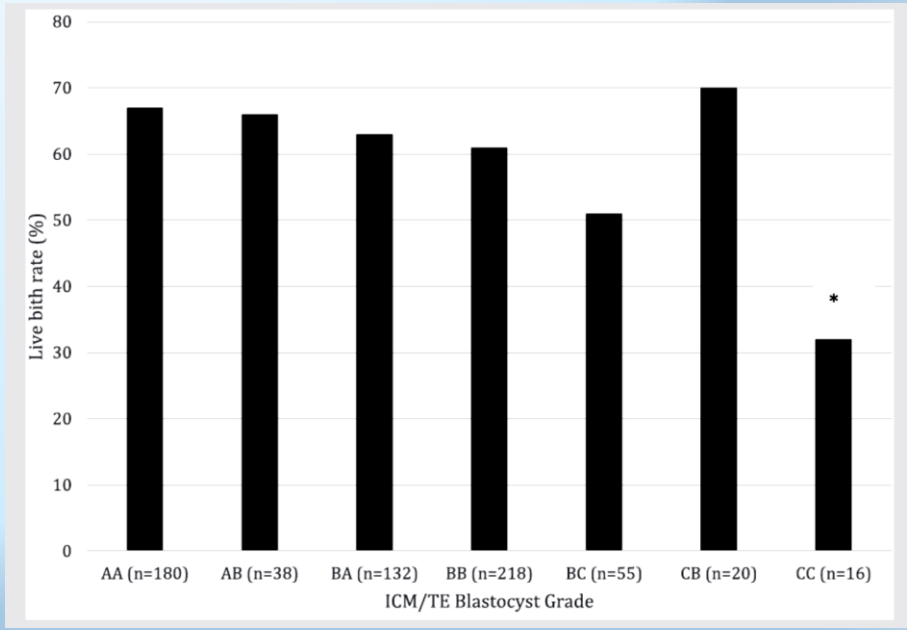
Prognostic value of blastocyst grade after frozen euploid embryo transfer in patients with recurrent pregnancy loss

Gayathree Murugappan, M.D.,[a] Julia G. Kim, M.D.,[b,c] Jonathan D. Kort, M.D.,[d] Brent M. Hanson, M.D.,[b,c] Shelby A. Neal, M.D.,[b,c] Ashley W. Tiegs, M.D.,[b,c] Emily K. Osman, M.D.,[b,c] Richard T. Scott, M.D.,[b,c] and Ruth B. Lathi, M.D.[a]

Fertil Steril Rep® Vol. 1, No. 2, September 2020

Objective: To determine whether trophectoderm (TE) grade or inner cell mass (ICM) grade have predictive value after euploid frozen embryo transfer (euFET) among recurrent pregnancy loss (RPL) patients.
Design: Retrospective cohort study.
Setting: Single fertility center.
Patient(s): Women with ≥2 prior pregnancy losses with ≥1 euploid embryo for transfer undergoing preimplantation genetic testing for aneuploidy.
Intervention(s): Intracytoplasmic sperm injection, TE biopsy, blastocyst grading and vitrification, and single euFET, with first transfer outcome recorded.
Main Outcome Measure(s): Live birth and clinical miscarriage rates.
Result(s): The study included 660 euFET cycles. In a binomial logistic regression analysis accounting for age, body mass index, antimüllerian hormone level, and day of blastocyst biopsy, or ICM grade C was not significantly associated with odds of live birth, miscarriage, or biochemical pregnancy loss. TE grade C was significantly associated with odds of live birth and was not associated with odds of miscarriage or biochemical pregnancy loss. Blastocyst grade CC had significantly lower live birth rate compared with all other blastocyst grades.
Conclusion(s): Embryo grade CC and TE grade C are associated with decreased odds of live birth after euFET in RPL patients. Embryo grade is not associated with odds of clinical miscarriage in this cohort of RPL patients, suggesting that additional embryonic or uterine factors may influence the risk of pregnancy loss. (Fertil Steril Rep® 2020;1:113–8. ©2020 by American Society for Reproductive Medicine.)

embryo grade was not predictive of clinical miscarriage rate in this cohort of RPL patients, suggesting that additional embryonic or uterine factors may influence their risk of pregnancy loss.



Impact of PGT-A in results of RIF patients

- Two RCTs (Rubio, C. *et al, 2013*, Blockeel, C. *et al, 2008)* and three observational studies investigated the potential role of PGT-A in improving IVF outcomes in women with RIF.
- *Primary outcomes* Meta-analysis of RCTs failed to show an improvement in both clinical pregnancy and live birth chances in women who underwent PGT-A.
- Pooling of results of observational studies did not show a beneficial effect of PGT-A on both pregnancy and live birth chances.
- *Secondary outcomes* Rubio et al. did not observe an impact of PGT-A on chances of embryo implantation and miscarriage in women who underwent PGT-A.

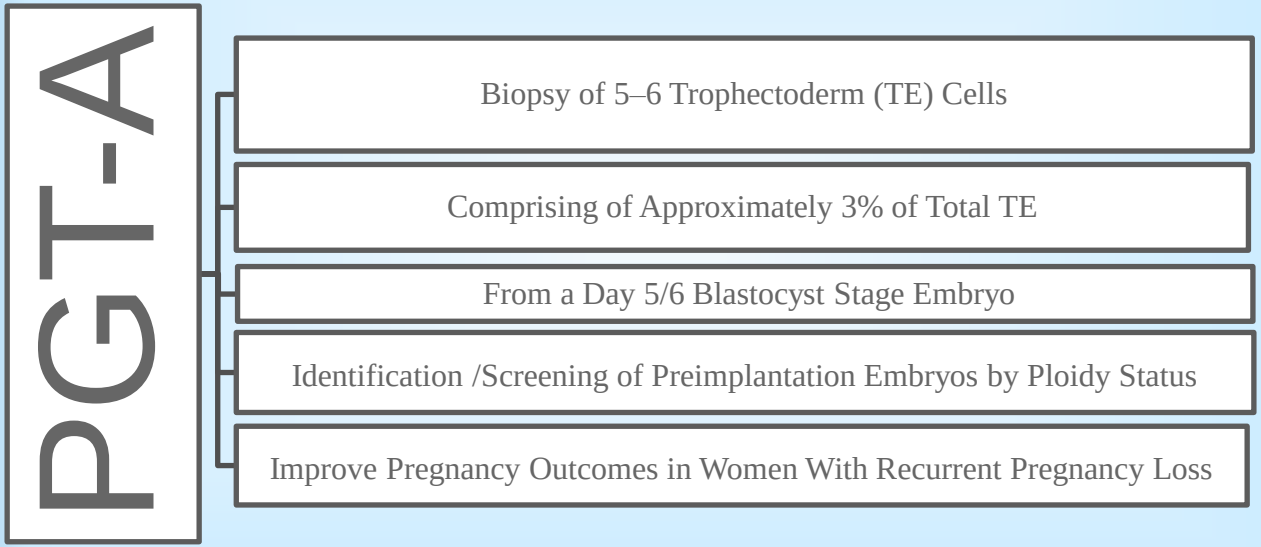
Potential role of NGS-based PGT-A for RIF patients with advanced maternal age

Variable	Group A	Group B	*P* value
No. of patients obtained euploidy embryo	91	19	
No. of transfer cycles	102	23	
Pregnancy rate per transfer % (n)	64.7 (66)	43.5 (10)	NS
Clinical pregnancy rate per transfer % (n)	48.0 (49)	39.1 (9)	NS
Implantation rate per transfer % (n)	51.0 (52)	39.1 (9)	NS
Miscarriage rate per transfer % (n)	7.8 (8)	4.3 (1)	NS

NS not statistically significantly different

Tong et al. Reproductive Science.2021

Effect of blastocyst biopsy in recurrent pregnancy loss



Bhatt et al., 2019; Kim et al., 2019

Higher chromosomal abnormality rate in blastocysts from young patients with idiopathic recurrent pregnancy loss

Xin-Yan Liu, Ph.D., Qi Fan, M.D., Jing Wang, M.D., Rong Li, M.D., Yan Xu, Ph.D., Jing Guo, Ph.D., Yi-Zi Wang, Ph.D., Yan-Hong Zeng, M.D., Chen-Hui Ding, Ph.D., Bing Cai, Ph.D., Can-Quan Zhou, Ph.D., and Yan-Wen Xu, Ph.D.

Guangdong Provincial Key Laboratory of Reproductive Medicine, First Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong, People's Republic of China

- A total of 62 patients with idiopatic RPL underwent 101 PGT-A cycles (iRPL group), and 212 patients underwent 311 PGT-M cycles (control group).

- significantly higher rate of chromosomal abnormalities in blastocysts of young women with RPL.

- euploid embryos were transferred after PGT-A, young patients with iRPL had a higher miscarriage rate

- chromosomal abnormalities might not be the only causal factor for iRPL.

Fertility and Sterility® Vol. 113, No. 4, April 2020

A Randomized Clinical Trial in 2019 Showed That:

✓ improve the ongoing pregnancy rate in woman aged *35–40*

but

✓ PGT-A did not improve the ongoing pregnancy rate in women aged *25–40*

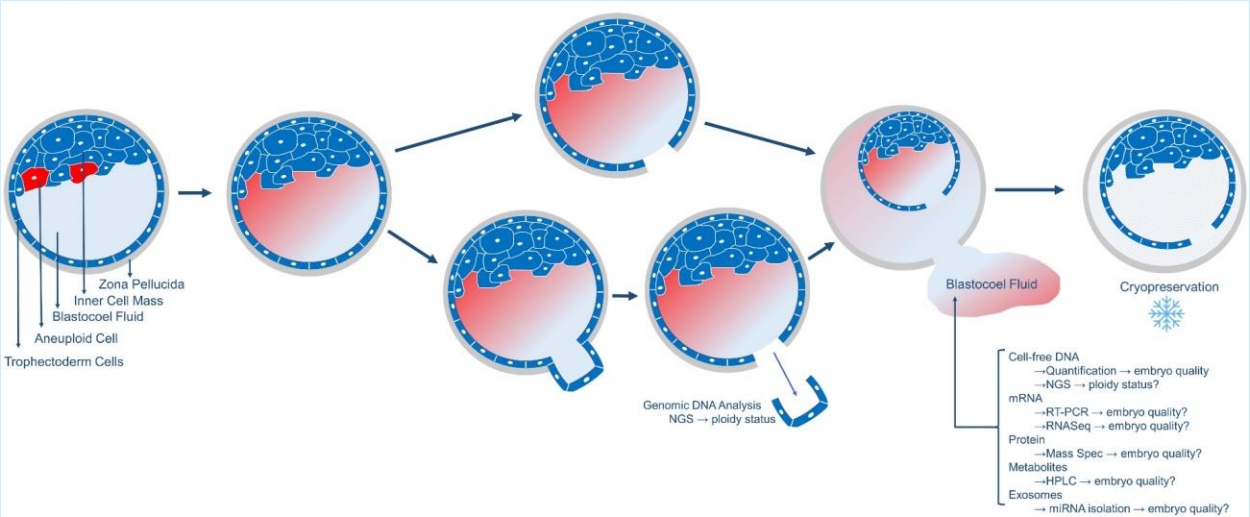
Why?

✓ actual mosaic preimplantation embryos that are identified as euploid by PGT-A
✓ there are likely other factors beyond ploidy status that allow a preimplantation embryo to successfully implant and lead to an ongoing pregnancy

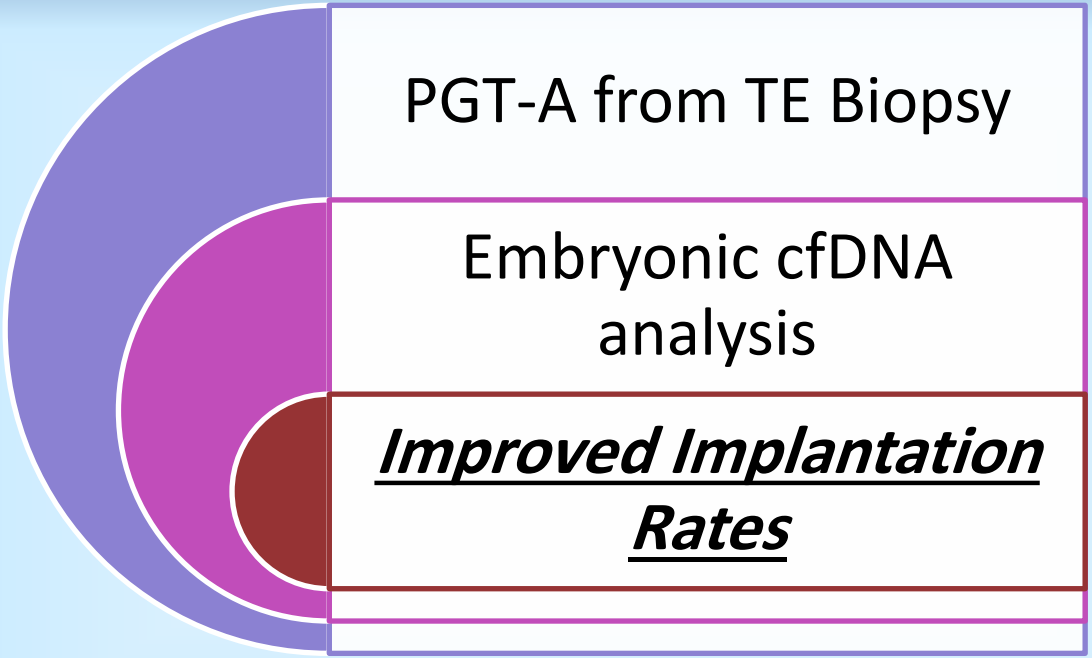
Therefore:

✓ The Most Viable Preimplantation Embryo May Be Selected by observing a Euploid Ploidy Status Via PGT-A Analysis and Detection of Specific Proteins, mRNA or Metabolites Within Its Blastocoel Fluid

Blastocoel Fluid?



Ng, Chang, and Wu. 1999



Rubio et al., 2019

To sum up:

-Embryo biopsy and consequent PGT-A are useful especially for patients with advanced maternal age.

-Hatched blastocyst are best developmental stage for biopsy.

-Technical procedure of blastocyte biopsy does not interfere in the clinical results.

-Due to mosaicism even in blastocyst stage, transfer of euploid embryo in not assured.

-Combination of PGT-A and cell-free DNA is suggested to assure healthy euploid embryo.

THANKS FOR YOUR ATTENTION

I wonder," he said, "whether the stars are set alight in heaven so that one day each one of us may find his own again..."

— Antoine de Saint-Exupéry, The Little Princ