Accuracy of assessing embryo ploidy of human embryos with PGS in association with IVF

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Conflict Statement

Dr. Gleicher is listed as co-inventor on a number of pending patent applications claiming diagnostic and therapeutic benefits from determination of CGG repeat numbers and ovarian *FMR1* genotypes and sub-genotypes.

Dr. Gleicher is co-inventor of awarded U.S. patents, claiming therapeutic benefits for supplementation of DHEA in women with diminished ovarian reserve, a topic discussed in this talk. Other patent applications in regards to DHEA and other fertility-related claims, with no relationship to this talk, are pending. Dr. Gleicher receives royalties from, and owns shares in Fertility Neutraceuticals, LLC, a distributor of a DHEA product.

Dr. Gleicher is co-inventor of three pending patent applications claiming potential therapeutic benefit for anti-Müllerian hormone (AMH) in infertile women. Dr. Gleicher owns shares in OvaNova Laboratories, LLC.

Outline

- The PGS hypothesis
- Assumptions underlying PGS
- Mathematical evidence that PGS cannot work
- Experimental evidence that PGS cannot work
- Clinical evidence that PGS does not work

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The PGS Hypothesis

- Aneuploidy is a major cause of IVF failure and miscarriages after IVF
- Elimination of aneuploid embryo before embryo transfer, therefore, will improve IVF outcomes and reduce miscarriages

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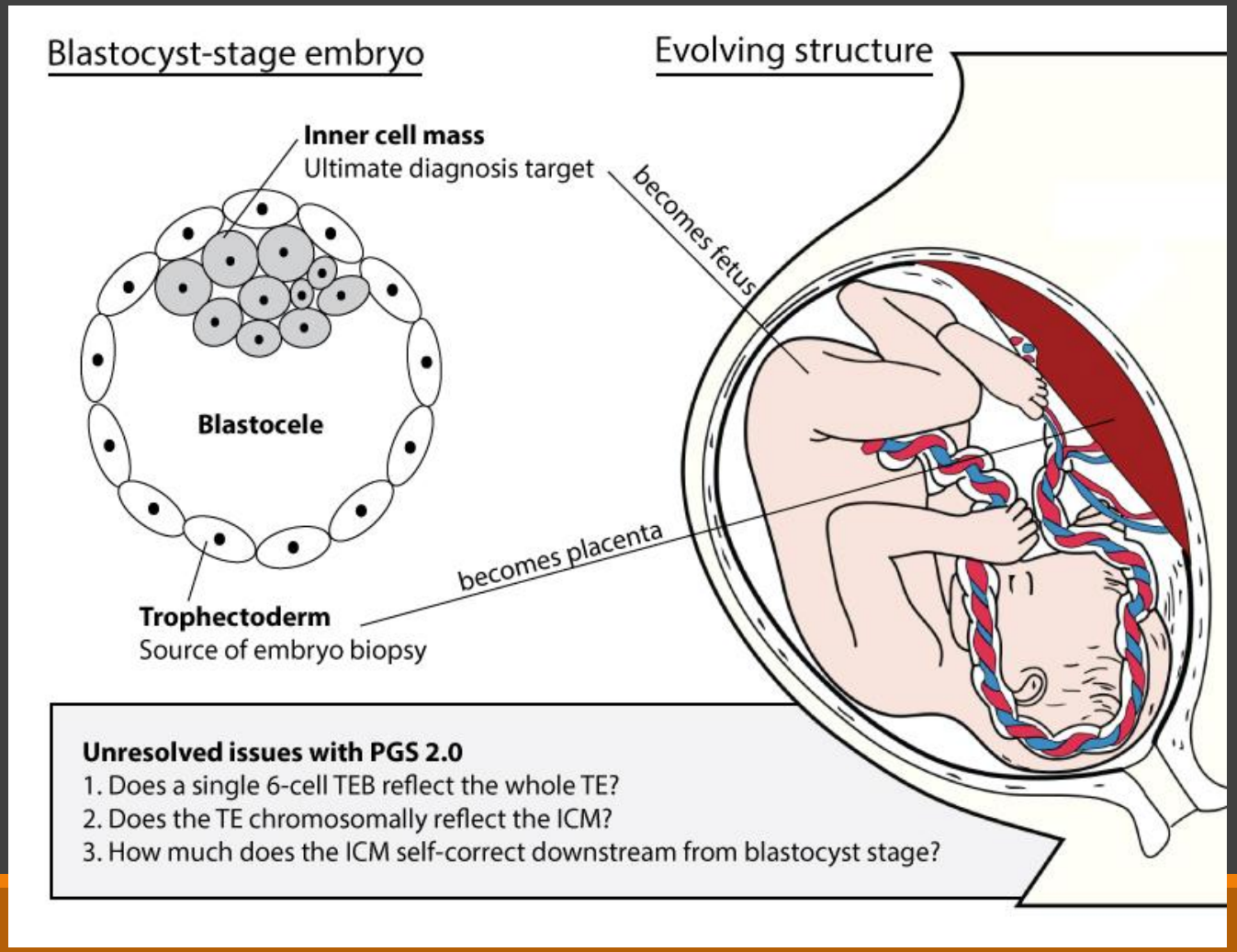
PGS Assumptions

- A TEB can reliably determine embryo ploidy at blastocyst stage
- Ploidy at blastocyst stage reflects ultimate fetal ploidy
- Diagnostic platforms are accurate

The Value of PGS



Outline

- The PGS hypothesis
- Assumptions underlying PGS
- **Mathematical evidence that PGS cannot work**
- Experimental evidence that PGS cannot work
- Clinical evidence that PGS does not work

Can a single TEB reliably reflect the whole TE?

SPECIAL ARTICLE

Can Trophectoderm Biopsies Resolve Whether to Transfer or Discard Embryos?

Norbert Gleicher, M.D., Jakob Metzger, PH.D., Gist Croft, PH.D., Vitaly A. Kushnir, M.D., David H Barad, M.D., M.S.

ABSTRACT

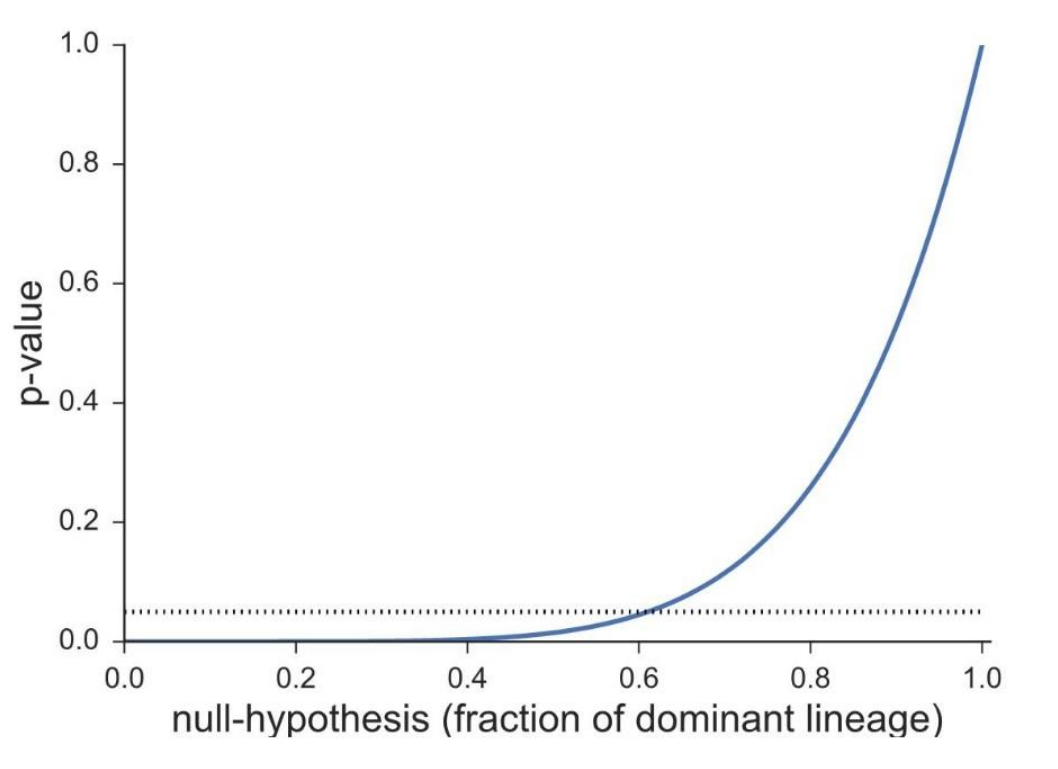
BACKGROUND: Utilization of preimplantation genetic screening (PGS) with in vitro fertilization (IVF) is increasing. Current PGS (PGS 2.0) employs a single trophectoderm (TE) biopsy (TEB) at blastocyst stage to eliminate aneuploid embryos prior to embryo transfer in attempts to increase live births and reduce miscarriages. Because TE mosaicism appears more pronounced than previously appreciated, whether a single TEB can reliably establish embryo ploidy has recently been questioned on biological grounds.

METHODS: We here present models for probabilities of false-negative and false-positive single 6 cell TEBs in a ~300-cell TE.

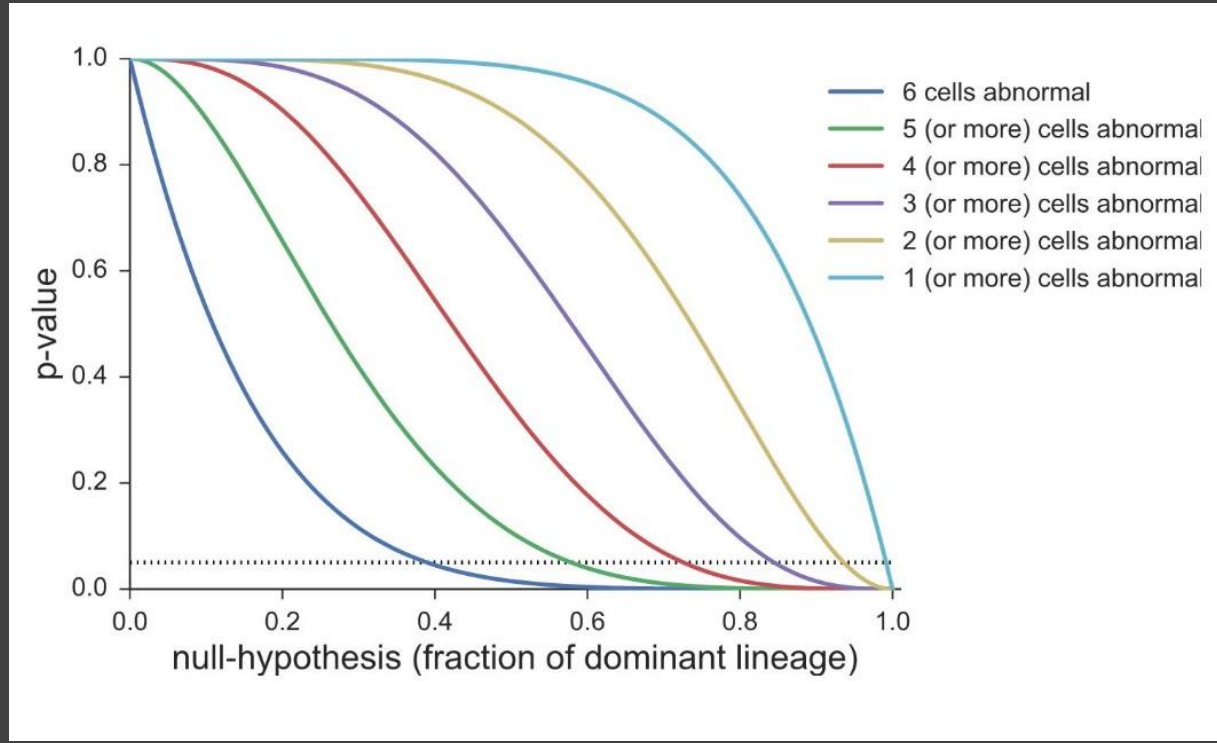
RESULTS: Both models demonstrate that a single TEB is not predictive enough in determining whether an embryo is euploid, mosaic or aneuploid to permit a reliable decision on whether this embryo can be transferred or should be discarded. This conclusion is reached under best scenario assumptions, including even distribution of mosaicism throughout the TE, and does not even consider discrepancies between chromosomal status of TE and inner cell mass (ICM) and the now well established ability of embryos to self-correct downstream from blastocyst stage.

CONCLUSIONS: Since because of too much chromosomal heterogeneity of the TE a single 6-cell TEB in blastocyst-stage embryos does not permit reliable differentiation between euploid, mosaic-normal or mosaic-abnormal (aneuploid) TE, the concept of PGS has to be reconsidered, adding to recently expressed concerns about the clinical efficacy of the procedure but also offering a possible explanation why clinical studies of PGS have so far failed to demonstrate outcome improvements in IVF.

P-values for observing no mosaicism, given different hypotheses r and a threshold of 0.05 (dotted line)



P-values for observed mosaicism, given different hypotheses r, and varying numbers of abnormal-aneuploidy cells in biopsy



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A single TEB, therefore, cannot reliably represent the complete TE

Does the TE reflect the inner cell mass (ICM)?

Not very well!

Orvieto et al 2016 Gynecol Endocrinol
Bolton et al 2016 NATURE Communications

Can the embryo self-correct after blastocyst stage?

Yes!

Bolton et al 2016 NATURE Communications

ARTICLE

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Mouse model of chromosome mosaicism reveals lineage-specific depletion of aneuploid cells and normal developmental potential

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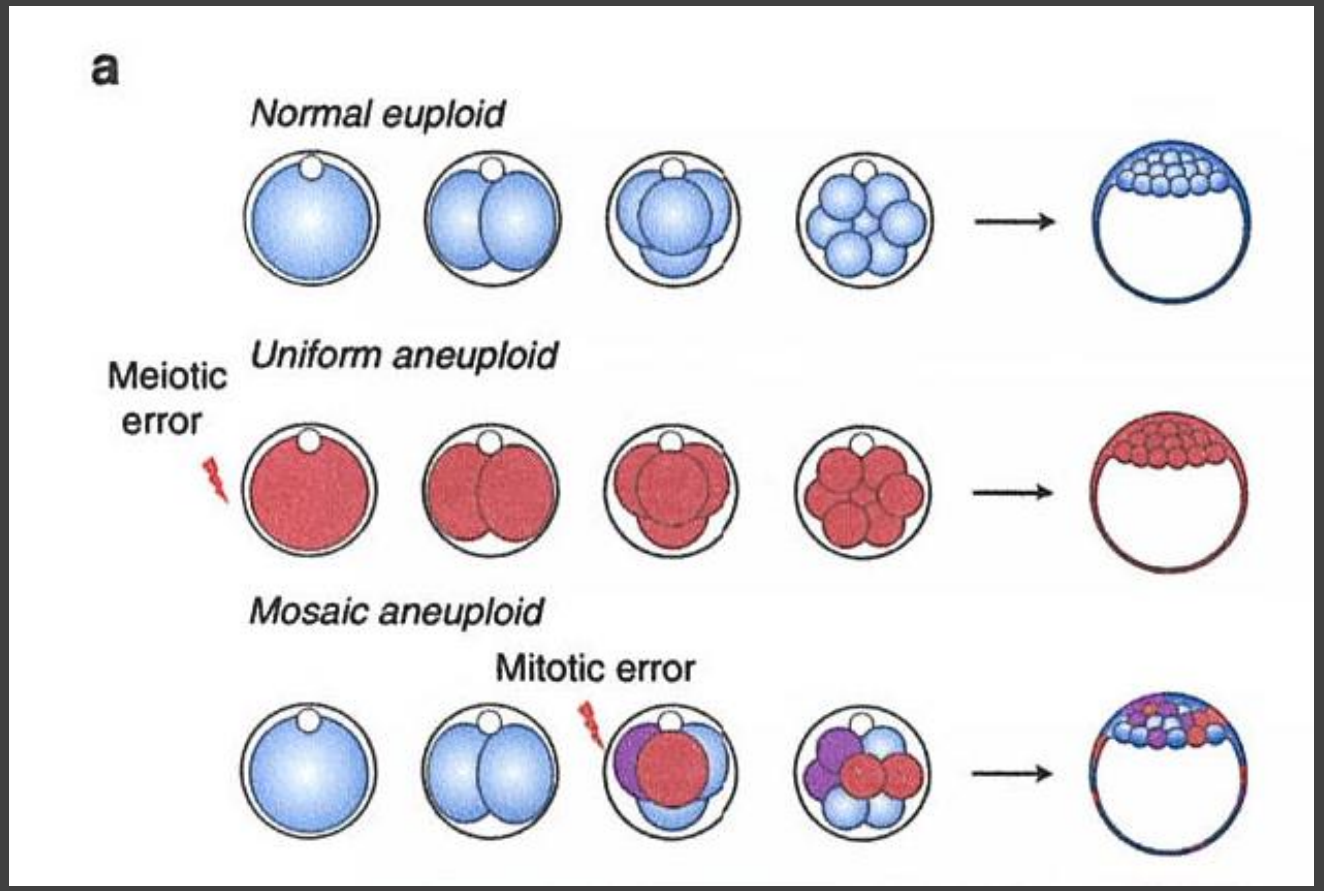
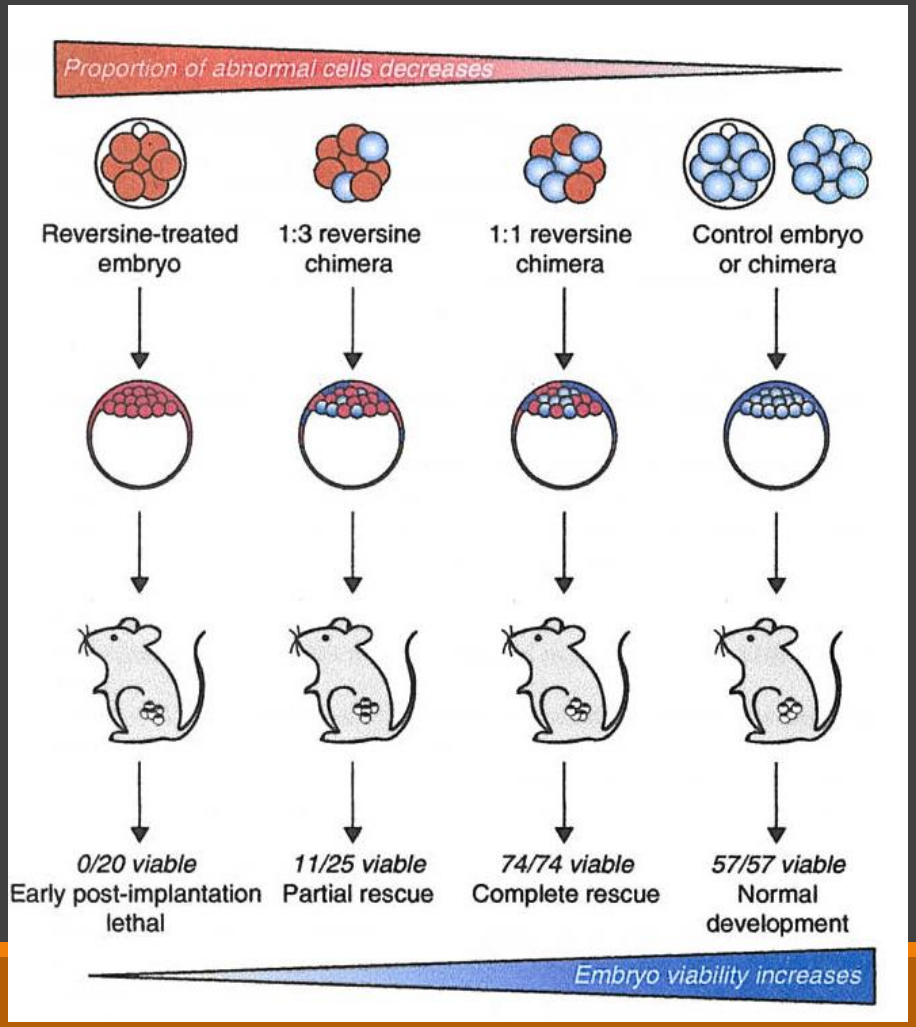
Most human pre-implantation embryos are mosaics of euploid and aneuploid cells. To determine the fate of aneuploid cells and the developmental potential of mosaic embryos, here we generate a mouse model of chromosome mosaicism. By treating embryos with a spindle assembly checkpoint inhibitor during the four- to eight-cell division, we efficiently generate aneuploid cells, resulting in embryo death during peri-implantation development. Live-embryo imaging and single-cell tracking in chimeric embryos, containing aneuploid and euploid cells, reveal that the fate of aneuploid cells depends on lineage: aneuploid cells in the fetal lineage are eliminated by apoptosis, whereas those in the placental lineage show severe proliferative defects. Overall, the proportion of aneuploid cells is progressively depleted from the blastocyst stage onwards. Finally, we show that mosaic embryos have full developmental potential, provided they contain sufficient euploid cells, a finding of significance for the assessment of embryo vitality in the clinic.

Reversine Treatment Induces Aneuploidy in Mouse Embryos



Effects of Pre-Implantation Chromosome Mosaicism on Embryo Development and Survival



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Gleicher *et al. Reproductive Biology and Endocrinology* (2016) 14:54
DOI 10.1186/s12958-016-0193-6

Reproductive Biology and Endocrinology

RESEARCH Open Access

Accuracy of preimplantation genetic screening (PGS) is compromised by degree of mosaicism of human embryos

Norbert Gleicher[1,2,3*], Andrea Vidali[1,4], Jeffrey Braverman[5], Vitaly A. Kushnir[1,6], David H. Barad[1,2,7], Cynthia Hudson[1], Yang-Guan Wu[1], Qi Wang[1], Lin Zhang[1], David F. Albertini[1,8] and the International PGS Consortium Study Group

Abstract

Background: To preclude transfer of aneuploid embryos, current preimplantation genetic screening (PGS) usually involves one trophectoderm biopsy at blastocyst stage, assumed to represent embryo ploidy. Whether one such biopsy can correctly assess embryo ploidy has recently, however, been questioned.

Methods: This descriptive study investigated accuracy of PGS in two ways. Part I: Two infertile couples donated 11 embryos, previously diagnosed as aneuploid and, therefore, destined to be discarded. They were dissected into 37 anonymized specimens, and sent to another national laboratory for repeat analyses to assess (i) inter-laboratory congruity and (ii) intra-embryo congruity of multiple embryo biopsies in a single laboratory. Part II: Reports on human IVF cycle outcomes after transfer of allegedly aneuploid embryos into 8 infertile patients.

Results: Only 2/11 (18.2 %) embryos were identically assessed at two PGS laboratories; 4/11 (36.4 %), on repeat analysis were chromosomally normal, 2 mosaic normal/abnormal, and 5/11 (45.5 %) completely differed in reported aneuploidies. In intra-embryo analyses, 5/10 (50 %) differed between biopsy sites. Eight transfers of previously reported aneuploid embryos resulted in 5 chromosomally normal pregnancies, 4 delivered and 1 ongoing. Three patients did not conceive, though 1 among them experienced a chemical pregnancy.

Conclusions: Though populations of both study parts are too small to draw statistically adequately powered conclusions on specific degrees of inaccuracy of PGS, here presented results do raise concerns especially about false-positive diagnoses. While inter-laboratory variations may at least partially be explained by different diagnostic platforms utilized, they cannot explain observed intra-embryo variations, suggesting more frequent trophectoderm mosiaicsm than previously reported. Together with recentl published mouse studies of lineages-specific degrees of survival of aneuploid cells in early stage embryos, these results call into question the biological basis of PGS, based on the assumption that a single trophectoderm biopsy can reliably determine embryo ploidy.

Keywords: Preimplantation genetic screening (PGS), In vitro fertilization (IVF), Embryos, Embryo mosaicism, Trophectoderm biopsy, Blastocyst

Abbreviations: aCGH, Array comparative genomic hybridization; FOR, Functional ovarian reserve; IVF, In vitro fertilization; LFOR, Low functional ovarian reserve; PGS, Preimplantation genetic screening

Table 1. Comparison of embryo ploidy between two PGS 2.0 assessments

Embryo ID	Biopsy #	Original PGS analysis (all embryos reported as abnormal)	Repeat PGS analysis (multiple biopsies)
A1	1	45,XY, -18	Normal 46,XX
A2	1	Complex aneuploid	XY, +10, -18q
A3	2		XY, +11, +16, -21
A4	3		XX, -3q
A5	1	46,XY, +3, -11, +15, -14	XX, -2
A6	2		Normal 46XX
A7	3		45,XY, -18
A8	4		Normal 46,XX
B1	1	46,XY, +3, -11	45,XY, -14
B2	2		45,XY, -14
B3	3		45,XY, -14
B4	4		45,XY, -14
B5	1	47,XY, +19	47,XY, +3
B6	2		47,XY, +3
B7	3		47,XY, +3
B8	4		Normal 46,XY
C1	1	45,XX, -1	Normal 46,XX
C2	2		Normal 46,XX
C3	3		Normal 46,XX
C4	1	47,XY, +19	Normal 46,XY
C5	2		Normal 46,XY
C6	3		Normal 46,XY
C7	1	47,XY, +19	Normal 46,XY
C8	2		Normal 46,XY
C9	3		Normal 46,XY
C10	4		Normal 46,XY
D1	1	Complex aneuploid	Normal 46,XY
D2	2		47, +18
D3	1	Complex aneuploid	47XY, +8q, -15, +16
D4	2		46,XY, -15, +16
D5	3		46,XY, -15, +16
D6	4		46,XY, -15, +16
D7	5		46,XY, -15, +16
D8	1	46,XX, +14, -15	46,XX, +14, -15
D9	2		46,XX, +14, -15
D10	3		46,XX, +14, -15
D11	4		46,XX, +14, -15

White and shaded areas represent individual embryos.

Table 2. Characteristics of aneuploid embryos transferred that led to implantation

Patient	n Embryos transferred	Embryos transferred	Outcome
1	1	43, XY, -13, -15, -18	Normal birth, 46, XY
2	1	45, XY, -21	Normal birth, 46, XY
3	2*	45, XY, -21 46, XX	Normal birth, 46, XY
4	2**	Partial 47,XX,17p11.2-pter 45, XY, -22	Normal ongoing 46, XX
5	2***	47, XY, +22 Partial 45,XY,-1plar-p36,12	Normal ongoing 46, XY
6	1****	45, XY, -21	Chemical pregnancy

* This patient, who had undergone PGS for sex selection (desired sex male), had a 45, XY, -21 and a normal 46, XX female transferred. Since she delivered a healthy male, the pregnancy had to be the result of the 45, XY, -21 embryo.

** Two embryos were transferred; normal 46, XX per CVS. Pregnancy, therefore, had to arise from partial trisomic embryo transferred. Currently 20 weeks.

*** Two embryos transferred; normal 46, XY per amniocentesis. Embryo leading to pregnancy unknown; Currently 19 weeks;

**** Chemical pregnancy indicates implantation but not considered a clinical pregnancy; Ploidy unknown;

Table 1. Clinical Outcomes of Single Mosaic Blastocysts Transferred.*

Patient No.	Chromosomal Constitution	Mosaicism†	Karyotype‡	Clinical Outcome
		percent		
1	arr(4)x1,(10)x1	40	46,XX	Baby healthy at birth
2	arr(6)x1,(15)x1	50	46,XX	Baby healthy at birth
3	arr(2)x1	40	46,XX	Baby healthy at birth
4	arr(2)x1	35	46,XY	Baby healthy at birth
5	arr(5)x1	50	46,XX	Baby healthy at birth
6	arr(5)x1,(7)x1	40	46,XX	Baby healthy at birth
7	arr(11)x1,(20)x3,(21)x3	30	NA	No pregnancy
8	arr(1)x1,(6)x3,(10)x3,(12)x3,(13)x3,(14)x3,(21)x3	50	NA	No pregnancy
9	arr(3)x1,(10)x3,(21)x3	35	NA	No pregnancy
10	arr(1)x3	50	NA	Biochemical pregnancy§
11	arr 9p21.2q34.3(26,609,645-140,499,771)x3	45	NA	Biochemical pregnancy§
12	arr(15)x3	30	NA	No pregnancy
13	arr(18)x1	50	NA	No pregnancy
14	arr(18)x1	50	NA	No pregnancy
15	arr(18)x1	40	NA	No pregnancy
16	arr(4)x1	50	NA	No pregnancy
17	arr(5)x3	40	NA	No pregnancy
18	arr 10q21.3q26.3(67,216,644-134,326,648)x3	50	NA	No pregnancy

* NA denotes not available.
† The approximate percentage of aneuploid cells in the transferred blastocyst is listed (see the Supplementary Appendix).
‡ The karyotype was determined by means of chorionic-villus sampling.
§ Biochemical pregnancy was defined by the presence of a low peak in levels of the beta subunit of human chorionic gonadotropin (β-hCG) (<100 mIU per milliliter), a rapid decrease in the urinary or serum β-hCG concentration, and no substantial delay in the onset of the next menstrual period, but with no detection of an identifiable pregnancy by means of ultrasonographic examination.

Greco and et al, N Engl J Med 2015;373:2089-90.

To believe that a single TEB can offer information on whether an embryo can be transferred or should be discarded is, therefore, obviously mistaken!

2016 PGDIS Guidelines

Table 1. *PGDIS* Recommendations for PGS laboratories [15]

1. For reliable detection of mosaicism, ideally 5 cells should be biopsied, with as little cell damage as possible. If the biopsy is facilitated using a laser, the identified contact points should be minimal and preferably at cell junctions. Overly aggressive use of the laser may result in cell damage and partial destruction of cellular DNA.
2. Only a validated Next Generation Sequencing (NGS) platform that can quantitatively measure copy numbers should be used for measurement of mosaicism in the biopsy sample. Ideally, a NGS methodology that can accurately and reproducibly measure 20% mosaicism in a known sample.
3. For reporting embryo results, the suggested cut-off point for definition of mosaicism is >20%, so lower levels should be treated as normal (euploid), > 80% abnormal (aneuploid), and the remaining ones between 20-80% mosaic (euploid-aneuploid mosaics).

2016 PGDIS Guidelines

Table 2. *PGDIS* recommendations for the clinician [15]

1. Patients should continue to be advised that any genetic test based on sampling one or small number of cells biopsied from preimplantation embryos cannot be 100% accurate for a combination of technical and biological factors, including chromosome mosaicism.
2. The patient information and consent forms for aneuploidy testing (if used) should be modified to include the possibility of mosaic aneuploid results and any potential risks in the event of transfer and implantation. This needs to be explained to patients by the clinician recommending the aneuploidy testing.
3. Transfer of blastocysts with a normal euploid result should always be prioritized over those with mosaic aneuploid results.
4. In the event of considering the transfer of a blastocyst with only mosaic aneuploidies, the following options should be discussed with the patient:
   a. A further cycle of IVF with aneuploidy testing to increase the chance of identifying a normal euploid blastocyst for transfer
   b. Transfer of a blastocyst with mosaic aneuploidies for low risk chromosomes only, after appropriate genetic counseling if available
   c. Appropriate monitoring and prenatal diagnosis of any resulting pregnancy, preferably by early amniocentesis (> 14 weeks gestation).

Effectiveness of in vitro fertilization with preimplantation genetic screening: a reanalysis of United States assisted reproductive technology data 2011–2012

Vitaly A. Kushnir, M.D.,[a,c] Sarah K. Darmon, Ph.D.,[a] David F. Albertini, Ph.D.,[a,d] David H. Barad, M.D.,[a,b] and Norbert Gleicher, M.D.[a,b,e]

[a] The Center for Human Reproduction, New York, New York; [b] Foundation for Reproductive Medicine, New York, New York; [c] Wake Forest School of Medicine, Winston-Salem, North Carolina; [d] University of Kansas Medical Center, Kansas City, Kansas; and [e] The Rockefeller University, New York, New York

Objective: To assess effectiveness of preimplantation genetic screening (PGS) in fresh IVF cycles.
Design: Reanalysis of retrospective US national data.
Setting: Not applicable.
Patient(s): A total of 5,471 fresh autologous IVF cycles with PGS and 97,069 cycles without PGS, reported in 2011–2012 to the Centers for Disease Control and Prevention.
Intervention(s): Not applicable.
Main Outcome Measure(s): Cycles that reached ET, miscarriage rates, live birth rates per cycle and per transfer.
Result(s): More PGS than non-PGS cycles reached ET (64.2% vs. 62.3%), suggesting favorable patient selection bias for patients using PGS. Nevertheless, live births rates per cycle start (25.2% vs. 28.8%) and per ET (39.3% vs. 46.2%) were significantly better in non-PGS cycles, whereas miscarriage rates were similar (13.7% vs. 13.9%). With a maternal age >37 years significantly more cycles in the PGS group reached ET (53.1% vs. 41.9%), suggesting a significant selection bias for more favorable patients in the PGS population. This bias rather than the PGS procedure may partially explain the observed improved live birth rate per cycle (17.7% vs. 12.7%) and lower miscarriage rate (16.8% vs. 26.0%) in the older PGS group.
Conclusion(s): Overall, PGS decreased chances of live birth in association with IVF. National improvements in live birth and miscarriage rates reported with PGS in older women are likely the consequence of favorable patient selection biases. (Fertil Steril® 2016;106: 75–9. ©2016 by American Society for Reproductive Medicine.)
Key Words: In vitro fertilization, preimplantation genetic diagnosis, preimplantation genetic screening, aneuploidy, embryo selection

RESEARCH ARTICLE

Preimplantation Genetic Screening Effects on Donor Egg-Recipient Cycles

David H. Barad, M.D., M.S., Sarah. K. Darmon, Ph.D., M.S., Vitaly. A. Kushnir, M.D., David. F. Albertini, Ph.D., and Norbert Gleicher, M.D.

ABSTRACT

BACKGROUND: In 2015, at least 20% of U.S. in vitro fertilization (IVF) cycles utilized preimplantation genetic screening (PGS), even though its effectiveness in improving IVF outcomes has recently been questioned.

METHODS: Utilizing data between 2005-2013 from the national Assisted Reproductive Technology Database of the Society for Assisted Reproductive Technology (SART), we report on the utility of PGS based on birth outcomes in first fresh oocyte donor-recipient cycles (ODRCs) and their subsequent frozen-thawed embryo cycles. Statistical models adjusted for patient and donor ages, number of embryos transferred, race, and cycle year were created to compare outcomes in PGS and non-PGS cycles.

RESULTS: 33,756 patients initiated a first ODRC, among which 468 (1.39%) underwent PGS for assessment of aneuploidy alone. Live birth rates were significantly lower for PGS than non-PGS cycles (51.1 vs. 55.7%, P=0.04). Adjusted for patient and donor ages, oocytes retrieved, embryos transferred, race and reporting year, the odds of live birth in cycles with PGS were reduced by 25% (OR 0. 75 95% CI 0. 62 to 0.91; P = 0.003) in comparison to non-PGS cycles. IVF outcomes in PGS cycles improved over the study period, while non-PGS cycles remained stable. Even in the last two years PGS patients, however, still, demonstrated lower live births (49.8% vs. 56.5%, P < 0.05).

CONCLUSIONS: Even in best prognosis ODRCs, PGS did not offer promised IVF outcome improvements but, actually, significantly reduced live birth rates. These observations raise serious doubts about the increasing utilization of PGS in routine IVF cycles.

	PGS	Non-PGS	
Cycles (n)	69	3,246	
eSET usage 2005-2013 (%)	18.0	13.0	*
eSET usage 2012-2013 (%)	26.0	26.0	
Clinical pregnancies (%)	62.3	69.7	

Hypothesis

RESEARCH ARTICLE SUMMARY

CANCER

Tumor aneuploidy corre
markers of immune evas
reduced response to imm

Teresa Davoli,

"The interplay between chromosomal abnormalities and immune surveillance is...an important new frontier...in cancer research..."

Cancer cell aneuploidy, immune surveillance, and immunotherapy

When tumors have low-level aneuploidy and high mutation (neoantigen) burden, exhausted T cells that recognize neoantigens can be reactivated by immunotherapy. High-level aneuploidy with either low or high mutation burden depletes infiltrating immune cells (T cells) and favors M2 cells, suppressing local immunity.

	LOW ANEUPLOIDY LEVEL	HIGH ANEUPLOIDY LEVEL
Cancer cell	High mutation burden High neoantigens	High/low mutation burden
Tumor microenvironment	Increased immune cell infiltration M1 macrophages (immune stimulatory)	Decreased immune cell infiltration M2 macrophages (immune suppressive)
Therapy	Immunotherapy (immune checkpoint blockade)	Alternative forms of immunotherapy combined with reversal of suppressive microenvironment

Is there anybody in the room who believes we should continue with PGS?

CHR Staff

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