Non-Invasive PGT

Mandy Katz-Jaffe, PhD

Disclosures and Learning Objectives

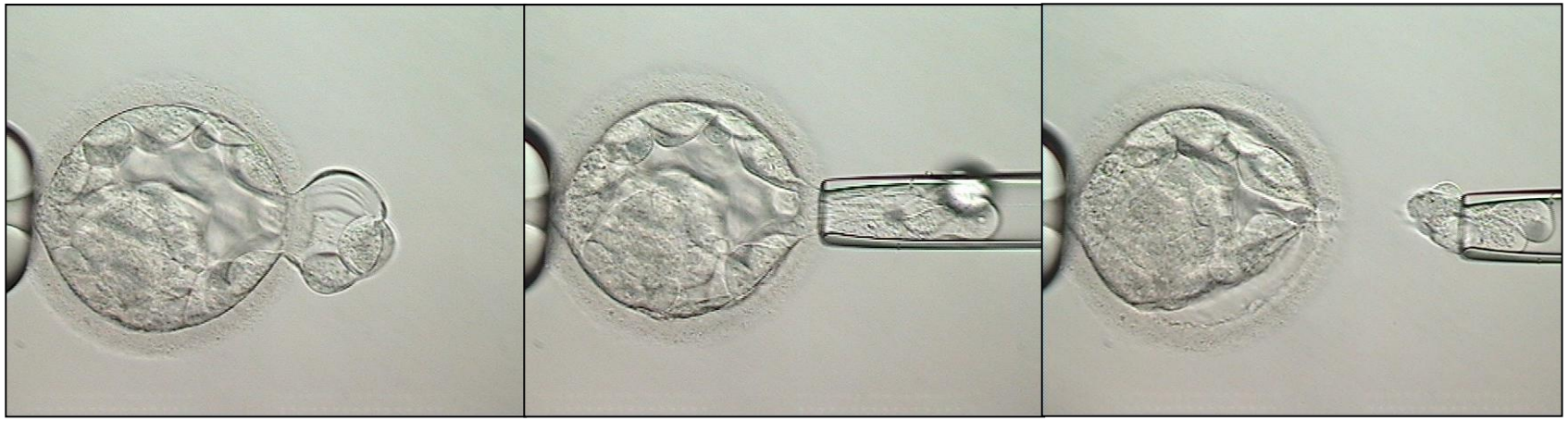
- No Disclosures

Participants should be able to differentiate the advantages and disadvantages among the different methods of PGT-A:

- Invasive
- Minimally invasive
- Non-invasive

Invasive PGT-Aneuploidy

- Biopsy of intact trophectoderm cells allows for PGT-A testing and the selection of euploid blastocysts for embryo transfer.
- Specialized micromanipulation equipment.
- Highly skilled embryologists to perform biopsy.
- Dedicated time across 2-3 days per patient cycle.



Invasive PGT-A Outcomes

Non-selection studies have shown the significantly greater reproductive potential of euploid blastocysts after invasive PGT-A

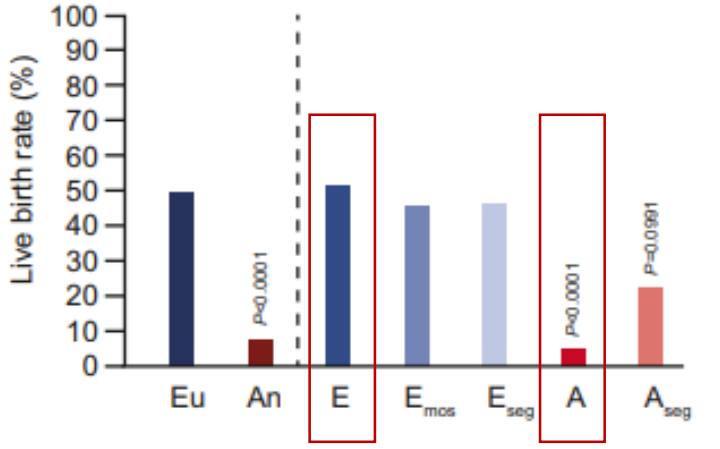


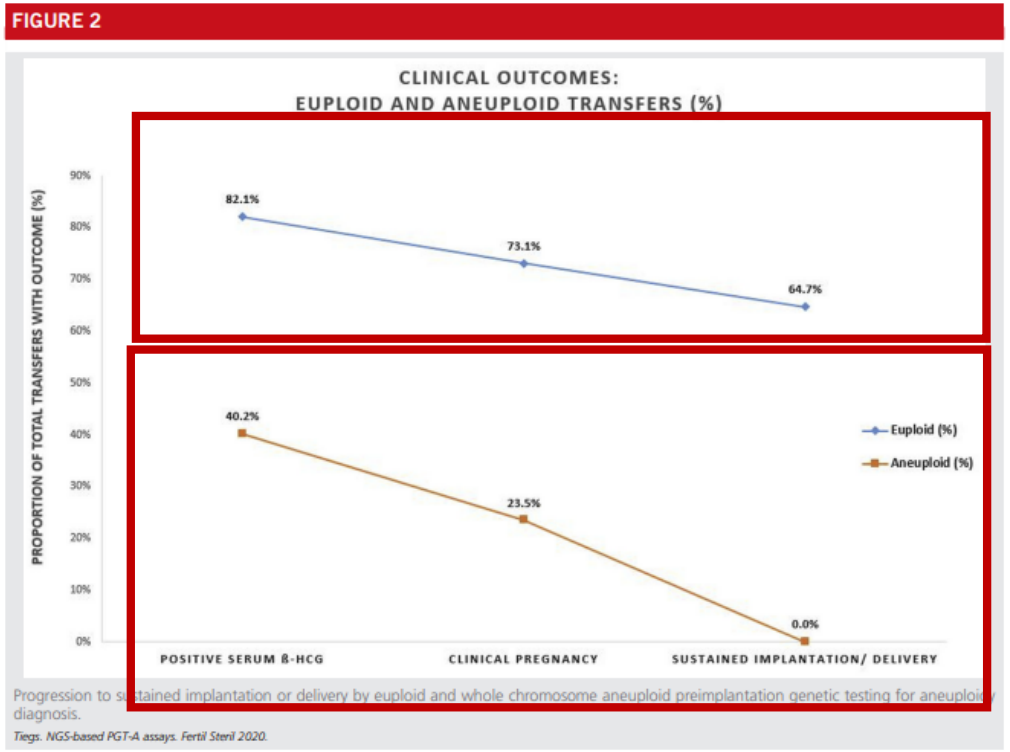
FIGURE 2 **Live birth rates versus embryo chromosome status.** Left panel shows live birth outcomes euploid embryos compared to aneuploid embryos. Right panel shows E embryos compared to E_{mos}, E_{seg}, A and A_{seg} embryos. Differences in outcomes are indicated by *p* values, with values less <0.05 being statistically significant and values between 0.05 and 0.2 being clinically significant [Colour figure can be viewed at wileyonlinelibrary.com]

Wang et al, 2021

- Whole chromosome aneuploids showed significantly higher failure rates from implantation to ongoing clinical pregnancy and live birth ($p < 0.0001$).
- Euploid embryo live birth rates were significantly higher at 51% versus 4.5% for whole chromosome aneuploids embryo (n=2).
- Approximately 85% of whole chromosome aneuploid embryos that initiated a pregnancy miscarried within the first trimester, with more than half after 6.5 week ultrasound.
- Nearly 75% of the patients that did have an abnormal embryo transferred could have preferentially transferred a euploid embryo.

Invasive PGT-A Outcomes

Non-selection studies have shown the significantly greater reproductive potential of euploid blastocysts after invasive PGT-A

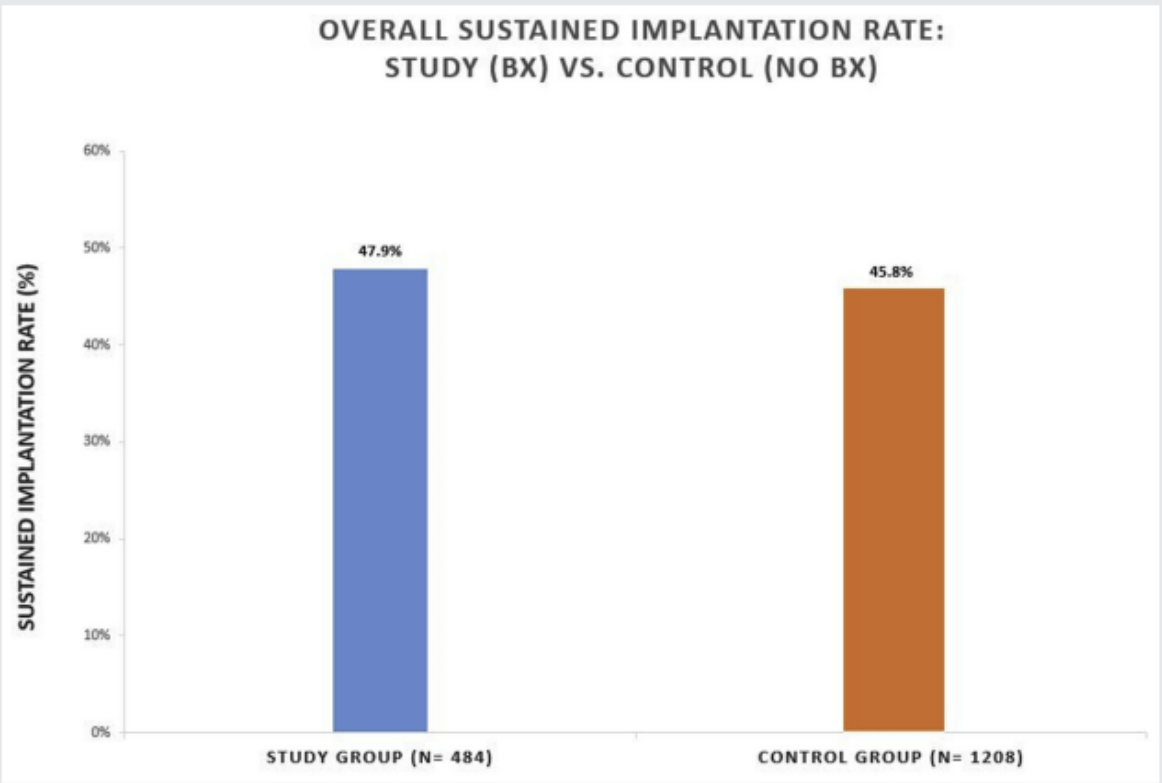


After PGT-A results were unblinded:

- 40.2% of patients who underwent a transfer with an aneuploid embryo had a positive serum bhCG.
- 23.5% of patients who underwent a transfer with an aneuploid embryo were identified with a clinical pregnancy but all of these patients experienced a spontaneous pregnancy loss.
- 100% of aneuploid embryos failed to reach live birth.
- In contrast, embryos labeled as euploid resulted in 73.1% clinical pregnancy and 64.7% live birth rate.

Minimal Developmental Impact

FIGURE 3



Comparison of the overall sustained implantation/live birth rates of the study group and an age-matched control group. No significant difference in sustained implantation/live birth was identified between groups ($P > .05$).

Tiegs. NGS-based PGT-A assays. Fertil Steril 2020.

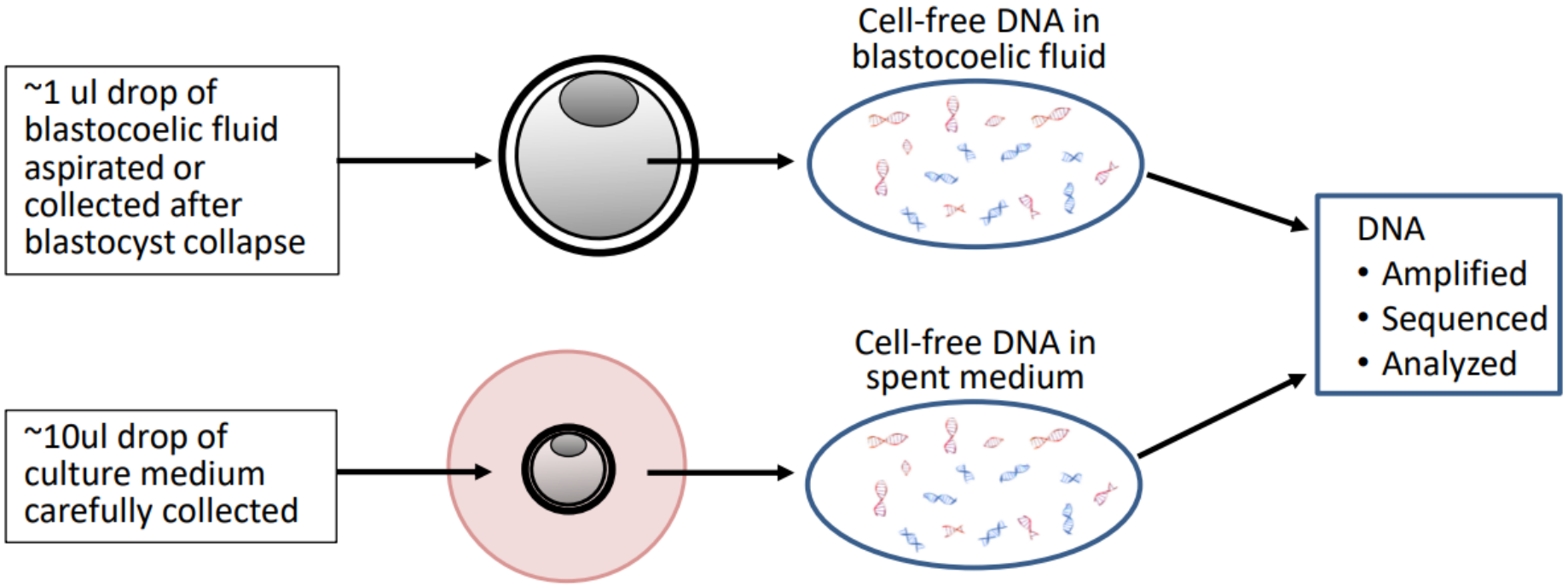
- To evaluate the impact of biopsy, the sustained implantation rate was compared between the study group, which underwent a biopsy but no PGT-A analysis, and an age-matched control group, which similarly underwent IVF/ICSI followed by FET without TE biopsy.
- The overall sustained implantation rates were similar for the study group with biopsy at 47.9% and the control group at 45.8% without biopsy.
- Adjusted for maternal age, BMI, embryo quality and endometrial thickness before FET.
- Highly reassuring data with respect to the safety of TE biopsy in regard to achieving live birth.

Why a Non-Invasive PGT-A Approach?

A less invasive approach to PGT-A is always welcomed:

- Especially with lower quality blastocysts that maybe more susceptible to further micromanipulation.
- Data is still needed beyond the neonatal period to establish long term safety of TE biopsy.
- Possible link between TE biopsy and a significant increase in preeclampsia (Zhang et al, 2019) and hypertensive disorders among mothers (Makhijani et al, 2021).
- Biopsy requires a high degree of technical skills.
- Significant costs associated with both specialized equipment costs and operator training.
- However, there are concerns regarding quality, accuracy, sensitivity and specificity associated with cell free degraded DNA!

Non-Invasive and Minimally Invasive PGT-A Approaches



Minimally Invasive PGT-A Publications

Reproductive BioMedicine Online
Volume 26, Issue 6, June 2013, Pages 603-610

Genomic DNA in human blastocoele fluid

S. Palini [a] [1] ✉, L. Galluzzi [b, 1], S. De Stefani [a], M. Bianchi [b], D. Wells [c], M. Magnani [b], C. Bulletti [a]

Molecular analysis of DNA in blastocoele fluid using next-generation sequencing

Yixin Zhang, Na Li, Li Wang, Huiying Sun, Minyue Ma, Hui Wang, Xiaofei Xu, Wenke Zhang, Yingyu Liu, David S. Cram, Baofa Sun ✉ & Yuanqing Yao ✉

Journal of Assisted Reproduction and Genetics **33**, 637–645 (2016) | Cite this article

Blastocentesis: a source of DNA for preimplantation genetic testing. Results from a pilot study

Luca Gianaroli, M.D., M. Cristina Magli, M.Sc., Alessandra Pomante, Ph.D., Anna M. Crivello, B.Sc., Giulia Cafueri, B.Sc., Marzia Valerio, B.Sc., and Anna P. Ferraretti, M.D.
Reproductive Medicine Unit, Società Italiana Studi di Medicina della Riproduzione, Bologna, Italy

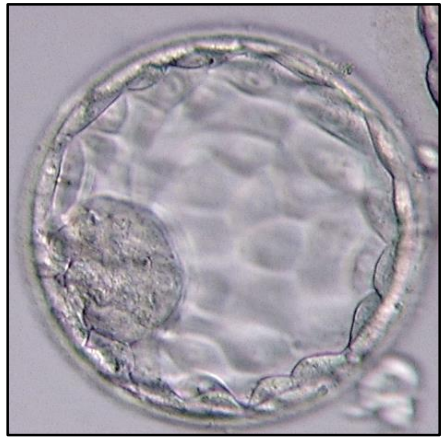
Deoxyribonucleic acid detection in blastocoelic fluid: a new predictor of embryo ploidy and viable pregnancy

M. Cristina Magli, M.Sc., Cristina Albanese, M.Sc., Andor Crippa, Ph.D., Carla Tabanelli, M.D., Anna P. Ferraretti, M.D., and Luca Gianaroli, M.D.
Reproductive Medicine Unit, S.I.S.Me.R., Bologna, Italy

Molecular Karyotyping of Cell-Free DNA from Blastocoele Fluid as a Basis for Noninvasive Preimplantation Genetic Screening of Aneuploidy

N. A. Skryabin[a, b], I. N. Lebedev[a, b], V. G. Artukhova[c], D. I. Zhigalina[b], I. A. Stepanov[d], G. V. Krivoschekova[d], and A. V. Svetlakov[c]

Minimally-Invasive PGT-A: Blastocoel Fluid Aspiration



Magli et al, 2019 published the most comprehensive data on blastocoel fluid PGT-A.

Most extensive experience with TE biopsy and BF aspiration on 256 blastocysts from 91 patients.

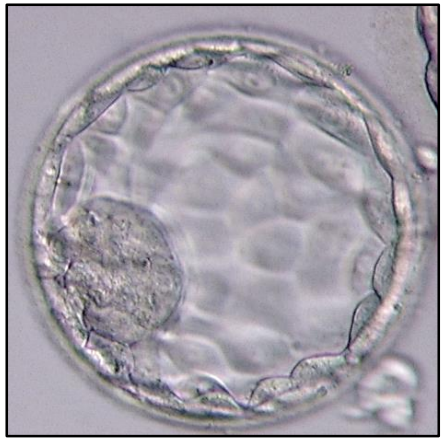
Variable	BF successful amplification	BF failed amplification	*P* value
Maternal age (y), mean ± SD	35.4 ± 4.4	36.9 ± 4	
No. transferred blastocysts	19	34	
No. euploid BFs	17	0	
No. aneuploid BFs	1	0	
No. BFs with no result	1	34	
No. clinical pregnancies	7	26	
No. ongoing	6	23	
No. miscarriages	1	3	
Total clinical pregnancy rate (%)	7/19 (37)	26/34 (77)	.005
Ongoing pregnancy rate (%)	6/19 (31.5)	23/34 (68)	.010

Note: The BF results were related to implantation.

Results:

1. Only 45% of euploid BF's amplified compared to 81% of aneuploid BF's
2. Chromosome concordance with TE result was observed in 93.6% of the amplified BF's
3. Authors suggested selection criteria of euploid embryos with failed BF amplification had improved implantation potential.
4. BF PGT-A results in high amplification failure rates and overall poor ability to positively confirm a euploid chromosome constitution.

Minimally-Invasive PGT-A: Blastocoel Fluid Aspiration



Conclusions

Even though aspiration of the blastocoel fluid at the expanded blastocyst stage is a promising technique there are several limitations:

- With overall low concordance, the DNA in the blastocoel fluid, at least in part, could originate from cells that are necrotic or apoptotic, compromising DNA quantity and integrity.
- Early stage blastocysts would be excluded from PGT-A testing and for women of AMA with slower developing embryos this could represent a significant portion of the embryo cohort.

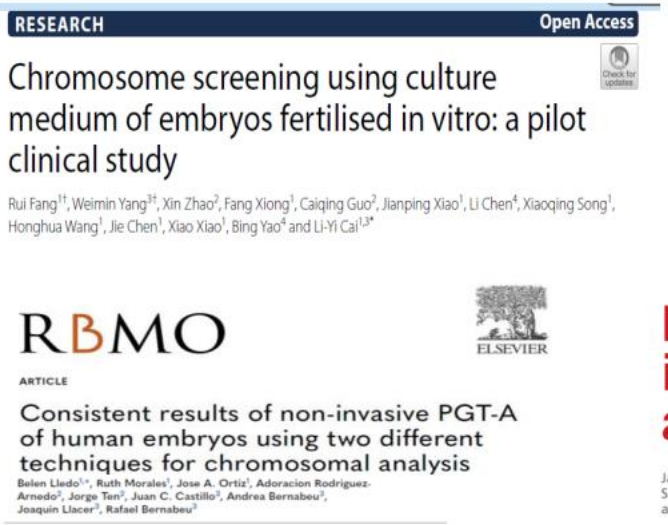
Non-Invasive PGT-A Publications

Human Reproduction, Vol.33, No.4 pp. 745–756, 2018
Advanced Access publication on February 20, 2018 doi:10.1093/humrep/dey028

human reproduction ORIGINAL ARTICLE *Reproductive genetics*

Origin and composition of cell-free DNA in spent medium from human embryo culture during preimplantation development

M. Vera-Rodriguez[1], A. Diez-Juan[1], J. Jimenez-Almazan[1], S. Martinez[1], R. Navarro[1], V. Peinado[1], A. Mercader[2], M. Meseguer[2], D. Blesa[1], I. Moreno[1], D. Valbuena[1], C. Rubio[1], and C. Simon[1,2,3,4,*]



RESEARCH Open Access

Chromosome screening using culture medium of embryos fertilised in vitro: a pilot clinical study

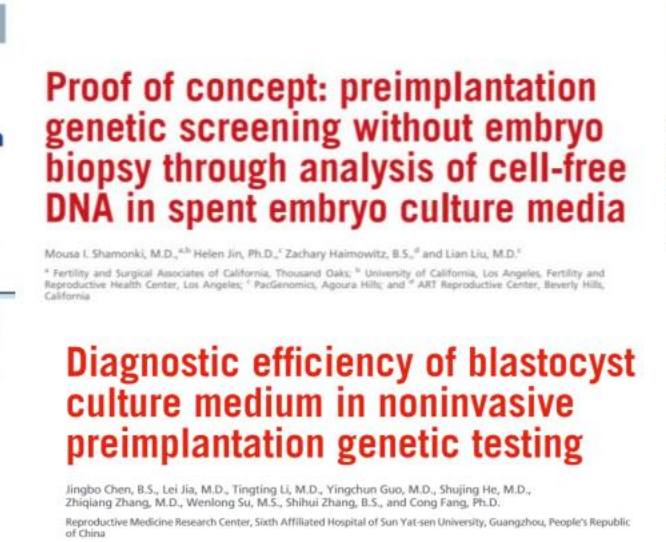
Rui Fang[†], Weimin Yang[3†], Xin Zhao[2], Fang Xiong[1], Caiqing Guo[2], Jianping Xiao[1], Li Chen[4], Xiaoqing Song[1], Honghua Wang[1], Jie Chen[1], Xiao Xiao[1], Bing Yao[4] and Li-Yi Cai[1,3*]

RBMO ELSEVIER

ARTICLE

Consistent results of non-invasive PGT-A of human embryos using two different techniques for chromosomal analysis

Belen Ledo[1,*], Ruth Morales[1], Jose A. Ortiz[1], Adoracion Rodriguez-Arnedo[2], Jorge Ten[2], Juan C. Castillo[3], Andrea Bernabeu[3], Joaquin Llacer[3], Rafael Bernabeu[3]



Proof of concept: preimplantation genetic screening without embryo biopsy through analysis of cell-free DNA in spent embryo culture media

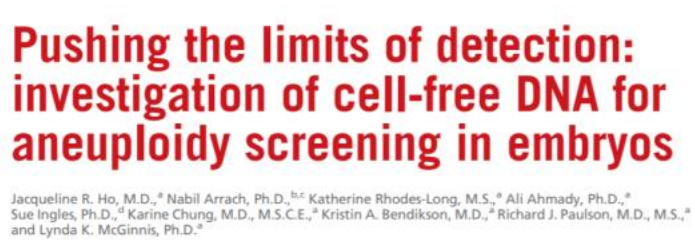
Mousa I. Shamonki, M.D.,[a,b] Helen Jin, Ph.D.,[c] Zachary Haimowitz, B.S.,[d] and Lian Liu, M.D.[c]

[a] Fertility and Surgical Associates of California, Thousand Oaks; [b] University of California, Los Angeles, Fertility and Reproductive Health Center, Los Angeles; [c] PacGenomics, Agoura Hills; and [d] ART Reproductive Center, Beverly Hills, California

Diagnostic efficiency of blastocyst culture medium in noninvasive preimplantation genetic testing

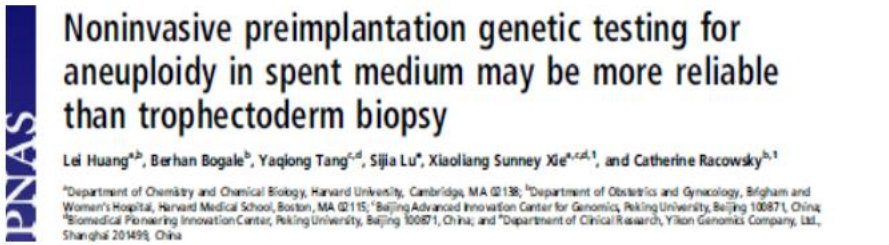
Jingbo Chen, B.S., Lei Jia, M.D., Tingting Li, M.D., Yingchun Guo, M.D., Shujing He, M.D., Zhiqiang Zhang, M.D., Wenlong Su, M.S., Shihui Zhang, B.S., and Cong Fang, Ph.D.

Reproductive Medicine Research Center, Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou, People's Republic of China



Pushing the limits of detection: investigation of cell-free DNA for aneuploidy screening in embryos

Jacqueline R. Ho, M.D.,[a] Nabil Arrach, Ph.D.,[b,c] Katherine Rhodes-Long, M.S.,[a] Ali Ahmady, Ph.D.,[a] Sue Ingles, Ph.D.,[d] Karine Chung, M.D., M.S.C.E.,[a] Kristin A. Bendikson, M.D.,[a] Richard J. Paulson, M.D., M.S.,[a] and Lynda K. McGinnis, Ph.D.[a]



PNAS

Noninvasive preimplantation genetic testing for aneuploidy in spent medium may be more reliable than trophectoderm biopsy

Lei Huang[a,2], Berhan Bogale[b], Yaqiong Tang[c,d], Sijia Lu[a], Xiaoliang Sunney Xie[a,c,d,1], and Catherine Racowsky[b,1]

[a]Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138; [b]Department of Obstetrics and Gynecology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115; [c]Beijing Advanced Innovation Center for Genomics, Peking University, Beijing 100871, China; [d]Biomedical Pioneering Innovation Center, Peking University, Beijing 100871, China; and [e]Department of Clinical Research, Yikon Genomics Company, Ltd., Shanghai 201499, China

PLOS ONE

Evaluation of a novel non-invasive preimplantation genetic screening approach

Valeriy Kuznyetsov[1], Svetlana Madjunkova[1*], Ran Antes[1], Rina Abramov[1], Gelareh Motamedi[1], Zenon Ibarrientos[1], Clifford Librach[1,2,3,4,5*]

biomedicines MDPI

Article

Validation of Non-Invasive Preimplantation Genetic Screening Using a Routine IVF Laboratory Workflow

Ni-Chin Tsai [1,2], Yun-Chiao Chang [3], Yi-Ru Su [4], Yi-Chi Lin [4], Pei-Ling Weng [3], Yin-Hua Cheng [3], Yi-Ling Li [4,5] and Kuo-Chung Lan [1,4,5,*]

PGT-Aneuploidy: Non-Invasive

A non-invasive approach to PGT-A has the potential to be both embryo and lab friendly (no specialist equipment, no biopsy training & expertise, and a more simplified work flow).

Cell-free DNA is readily available in the spent embryo culture media, providing an easier, more economical removal of genetic material for PGT-A.

Considerations:

- Sampling of the media without disrupting embryo development and IVF lab workflow.
- Cell-free DNA is of lower quantity and lesser quality.
- The origin of the cell-free DNA? Percentage of apoptotic events?
- Or are aneuploid cells preferentially excluded from the embryo?
- For clinical use, a niPGT result must reflect the blastocyst chromosome constitution.

Embryonic cell-free DNA versus trophectoderm biopsy for aneuploidy testing: concordance rate and clinical implications

Carmen Rubio, Ph.D.,[a] Laura Rienzi, M.Sc.,[b] Luis Navarro-Sánchez, Ph.D.,[a] Danilo Cimadomo, Ph.D.,[b] Carmen María García-Pascual, Ph.D.,[a] Laura Albricci, Ph.D.,[b] Daria Soscia, M.Sc.,[b] Diana Valbuena, M.D., Ph.D.,[c] Antonio Capalbo, Ph.D.,[d] Filippo Ubaldi, M.D., Ph.D.,[b] and Carlos Simón, M.D., Ph.D.[a,e,f,g,h]

Results Summary:

Prospective blinded study

Concordance = 78.7%

Sensitivity = 94.5%

Specificity = 71.7%

Media drops at day 6/7 showed higher concordance

TABLE 1

Results according to sample type and day of biopsy, n (%).

Result		Day 5	Day 6/7	Total	*P* value	OR (95% CI)
General Information	No. of blastocysts analyzed, n	33	82	115	–	–
	Female age, y, mean ± SD	37.3 ± 2.8	38.4 ± 3.2	38.8 ± 3.3	NS	–
	Hours in culture, mean ± SD	26.0 ± 2.8	50.8 ± 8.8	44.6 ± 13.3	<.0001	24.800 (21.697–27.903)
Informative NGS results	Informative TE biopsies	33 (100%)	81 (98.2%)	114 (99.1%)	NS	–
	Informative SBM	27 (81.8%)	82 (100%)	109 (94.8%)	.0004	0.02564 (0.001398–0.4704)
	Blastocysts with informative TE + SBM	27 (81.8%)	81 (98.8%)	108 (93.9%)	.0022	0.05556 (0.006395–0.4826)
Embryo concordances[a]	Total concordance for ploidy with different sex	18 (66.7%)	72 (88.9%)	90 (83.3%)	.0144	0.7500 (0.5682–0.9900)
	Total concordances for ploidy and sex	17 (63.0%)	68 (84.0%)	85 (78.7%)	.0299	0.3250 (0.1218–0.8669)
	Full concordances for ploidy and sex	11 (40.7%)	58 (71.6%)	69 (63.9%)	.0055	0.2726 (0.1100–0.6754)
	Partial concordances for ploidy and sex	6 (22.3%)	10 (12.4%)	16 (14.8%)	NS	–
Chromosome concordances[b]	Concordant autosomes	399/418 (95.5%)	1,542/1,584 (97.3%)	1,941/2,002 (97.0%)	NS	–
	Concordant sex chromosomes	35/38 (92.1%)	138/144 (95.8%)	173/182 (95.1%)	NS	–

Note: NGS = next-generation sequencing; NS = nonsignificant ($P \geq .5$); SBM = spent blastocyst media; TE = trophectoderm.

[a] For embryo concordance, total concordance includes embryos with full + partial concordances; full concordance is defined as TE and SBM both euploid or aneuploid for the same chromosomes, and partial concordance is defined as aneuploid embryos but with at least one different aneuploid chromosome in TE and SBM. All results were calculated considering the blastocysts with informative TE + SBM.

[b] For chromosome concordance, SBM with chaotic profiles were excluded for calculations.

Rubio. Embryonic cfDNA analysis for niPGT-A. Fertil Steril 2019.

Embryonic cell-free DNA versus trophectoderm biopsy for aneuploidy testing: concordance rate and clinical implications

Carmen Rubio, Ph.D.,[a] Laura Rienzi, M.Sc.,[b] Luis Navarro-Sánchez, Ph.D.,[a] Danilo Cimadomo, Ph.D.,[b] Carmen María García-Pascual, Ph.D.,[a] Laura Albricci, Ph.D.,[b] Daria Soscia, M.Sc.,[b] Diana Valbuena, M.D., Ph.D.,[c] Antonio Capalbo, Ph.D.,[d] Filippo Ubaldi, M.D., Ph.D.,[b] and Carlos Simón, M.D., Ph.D.[a,e,f,g,h]

TABLE 2

Clinical outcome after single embryo transfer of thawed blastocysts diagnosed as euploid after TE biopsy.

Outcome	Euploid TE/euploid SBM	Euploid TE/aneuploid SBM	Total
No. of transfers	17	12	29
Mean female age, y	37.5 ± 2.5	37.4 ± 2.3	37.5 ± 2.4
Positive pregnancy test	11 (64.7%)	4 (33.3%)	15 (51.7%)
Biochemical pregnancy loss	2 (18.2%)	0	2 (13.3%)
Clinical pregnancy rate	9 (52.9%)	4 (33.3%)	13 (44.8%)
Clinical miscarriage	0	2 (50.0%)	2 (15.4%)
Ongoing implantation rate	9 (52.9%)	2 (16.7%)	11 (37.9%)

Note: Abbreviations as in Table 1.

Rubio. Embryonic cfDNA analysis for niPGT-A. Fertil Steril 2019.

FET Outcomes:

- When euploid TE was concordant with euploid SBM the ongoing implantation rate was 52.9%.
- This was significantly higher than the ongoing implantation rate for euploid TE with discordant aneuploid SBM at 16.7%.
- However, this sample size was very small so differences were not significant and indeed a limitation for interpretation.

Journal of Assisted Reproduction and Genetics (2019) 36:1609–1621
https://doi.org/10.1007/s10815-019-01517-7

ASSISTED REPRODUCTION TECHNOLOGIES

A prospective study of non-invasive preimplantation genetic testing for aneuploidies (NiPGT-A) using next-generation sequencing (NGS) on spent culture media (SCM)

Queenie S. Y. Yeung[1] · Ying Xin Zhang[2] · Jacqueline P. W. Chung[1] · Wai Ting Lui[2] · Yvonne K. Y. Kwok[2] · Baoheng Gui[3,4] · Grace W. S. Kong[1] · Ye Cao[2] · Tin Chiu Li[1] · Kwong Wai Choy[2,3]

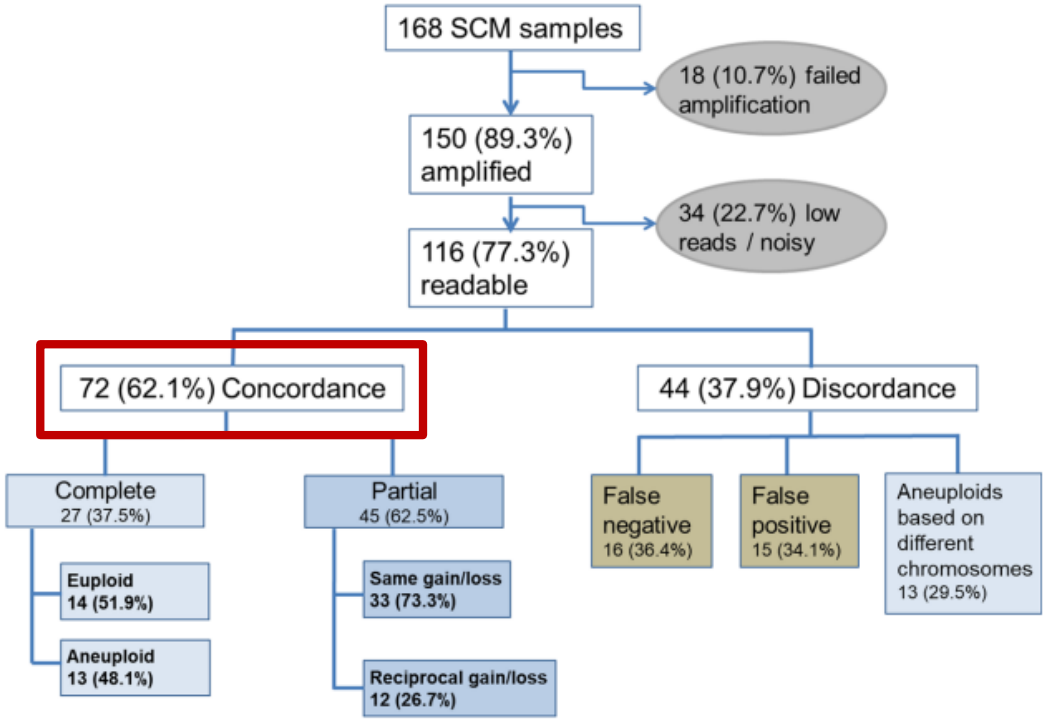


Table 2 Spent culture media sampling as a screening test for aneuploidy and sex determination

Stage of collection	Sensitivity	Specificity	Positive predictive value (PPV)	Negative predictive value (NPV)
Day 5 ($n = 50$)	83.8%	53.8%	83.8%	53.8%
Day 6 ($n = 66$)	80.0%	43.8%	81.6%	41.2%
Blastocysts (day 5 and day 6 combined)	81.6% [71.9–89.1%]	48.3% [29.5–67.5%]	82.6% [76.7–87.1%]	46.7% [32.9–61.0%]

Values in parentheses were test at 95% confidence interval

Performance	All embryos ($n = 116$)	Full maternal contamination excluded ($n = 109$)	Mosaic embryos excluded ($n = 107$)
Sensitivity	81.6% [71.9–89.1%]	88.8% [79.7–94.7%]	91.0% [82.4–96.3%]
Specificity	48.3% [29.5–67.5%]	48.3% [29.5–67.5%]	48.3% [29.5–67.5%]
Positive predictive value	82.6% [76.7–87.1%]	82.6% [76.8–87.2%]	82.6% [76.7–87.1%]
Negative predictive value	46.7% [32.9–61.0%]	60.9% [43.1–76.2%]	66.7% [47.3–81.7%]

Values in square brackets were test at 95% confidence interval

Noninvasive preimplantation genetic testing for aneuploidy in spent medium may be more reliable than trophectoderm biopsy

Lei Huang[a,b], Berhan Bogale[b], Yaqiong Tang[c,d], Sijia Lu[e], Xiaoliang Sunney Xie[a,c,d,1], and Catherine Racowsky[b,1]

[a]Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138; [b]Department of Obstetrics and Gynecology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115; [c]Beijing Advanced Innovation Center for Genomics, Peking University, Beijing 100871, China; [d]Biomedical Pioneering Innovation Center, Peking University, Beijing 100871, China; and [e]Department of Clinical Research, Yikon Genomics Company, Ltd., Shanghai 201499, China

Edited by R. Michael Roberts, University of Missouri, Columbia, MO, and approved May 24, 2019 (received for review January 31, 2019)

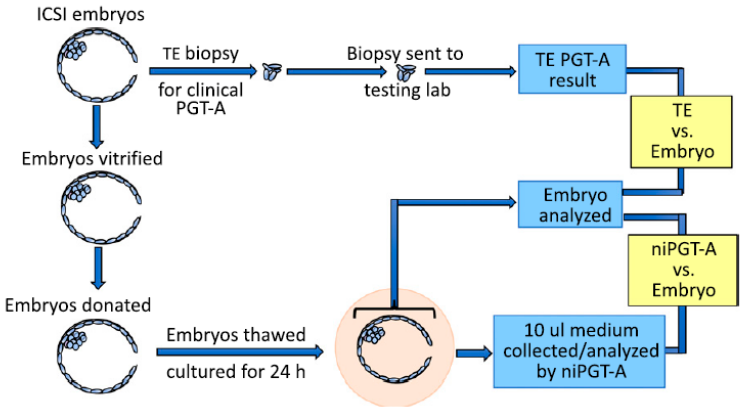


Fig. 2. Workflow of sample processing for PGT-A analysis of TE biopsy, embryo, and spent culture media.

Table 2. Comparison of the performance of niPGT-A versus TE biopsy for PGT-A

Performance characteristic	niPGT-A (n = 48)	TE-biopsy (n = 50)
FPR	20.0% (3/15)	50.0% (9/18)
FNR	0.0% (0/33)	0.0% (0/32)
PPV	91.7% (33/36)	78.0% (32/41)
NPV	100.0% (15/15)	100.0% (18/18)
Sensitivity	100.0% (33/33)	100.0% (32/32)
Specificity	80.0% (12/15)	50.0% (9/18)
% Concordance for embryo ploidy	93.8% (45/48)	82.0% (41/50)
% Concordance for chromosome CNs	83.3% (40/48)	62.0% (31/50)

niPGT-A and TE biopsy results were compared with those of the embryo. Sequencing threshold was set at 60% mosaicism.

Study Limitations:

Blastocysts were warmed and cultured for 24 hours which does not represent routine in vitro embryo culture.

Four different PGT-A labs were utilized for TE biopsy testing from only n=13 patients.

Very small sample size (n=48) to conclude that niPGT-A is possibly more reliable than nucleated TE cells.

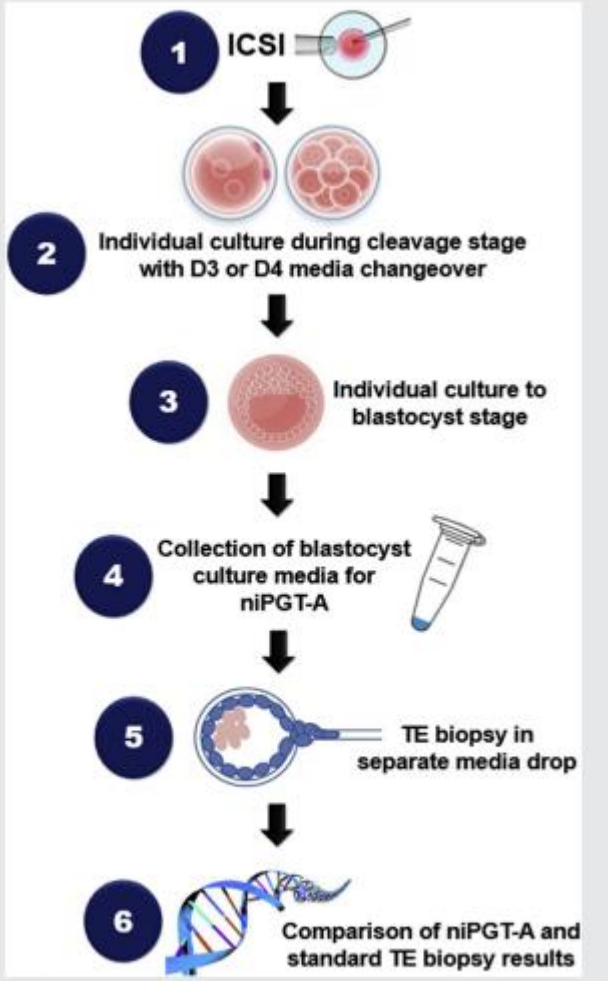
Noninvasive preimplantation genetic testing for aneuploidy exhibits high rates of deoxyribonucleic acid amplification failure and poor correlation with results obtained using trophectoderm biopsy

Brent M. Hanson, M.D.,[a,b] Xin Tao, Ph.D.,[c] Kathleen H. Hong, M.D., H.C.L.D.,[a] Cynthia E. Comito, M.T.,[a] Rosanna Pangasnan, B.A.,[a] Emre Seli, M.D.,[a,d] Chaim Jalas,[c] and Richard T. Scott Jr., M.D., H.C.L.D.[a,b]

[a] Reproductive Medicine Associates of New Jersey, Basking Ridge, New Jersey; [b] Sidney Kimmel Medical College at Thomas Jefferson University, Philadelphia, Pennsylvania; [c] Foundation for Embryonic Competence, Basking Ridge, New Jersey; and [d] Yale School of Medicine, New Haven, Connecticut

- Embryos were cultured in 30ul medium.
- Day of medium changeover was investigated (Day 3 or Day 4).
- Day of blastocyst culture and biopsy (Day 5, 6 or 7) studied.
- Number of days of embryo exposure to culture medium (1, 2, 3 or 4) studied.

FIGURE 1



Flow diagram detailing sample processing for niPGT-A using spent culture media and standard PGT-A analysis of TE biopsy. niPGT-A = noninvasive preimplantation genetic testing for aneuploidy; PGT-A = preimplantation genetic testing for aneuploidy; TE = trophectoderm.

Hanson. Clinical validation of noninvasive PGT-A. Fertil Steril 2021.

Noninvasive preimplantation genetic testing for aneuploidy exhibits high rates of deoxyribonucleic acid amplification failure and poor correlation with results obtained using trophectoderm biopsy

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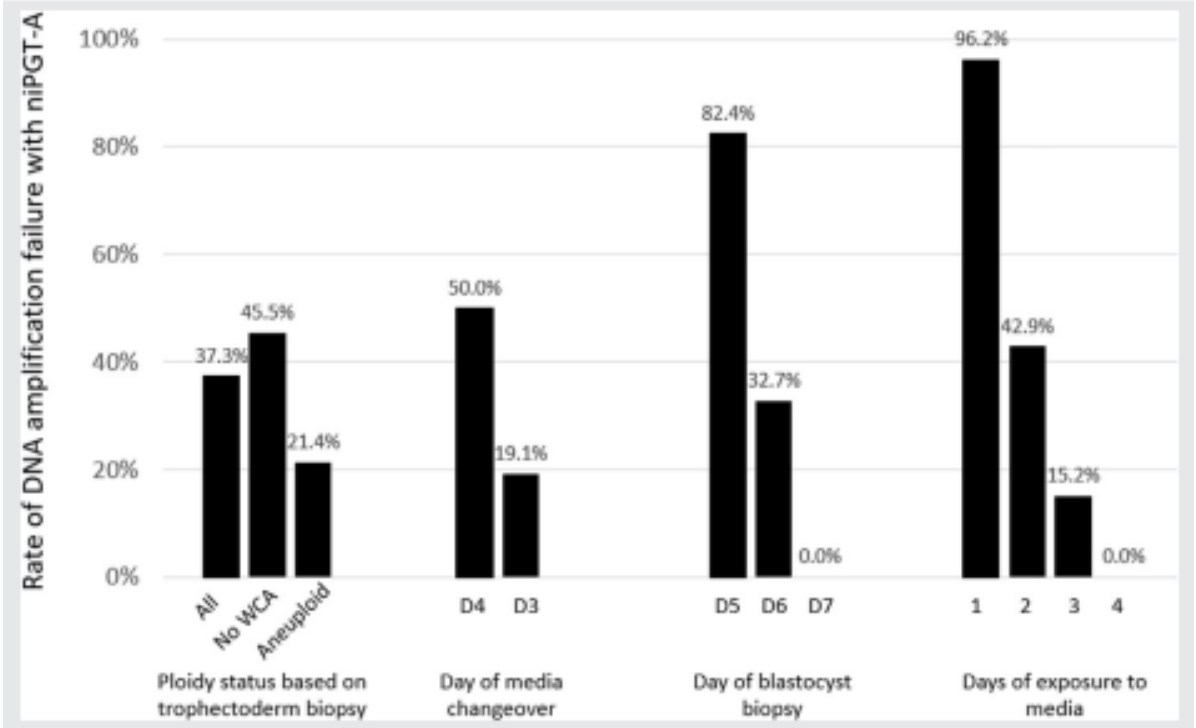

- DNA amplification failure occurred with niPGT-A in 37.3% (62/166) of the spent media samples *versus* 0% in the TE biopsy group.
- Of 104 embryos with both niPGT-A and TE biopsy results available, whole-chromosome discordance was noted in 42 cases (40.4%).
- Conclusion: The rates of DNA amplification failure were high among the niPGT-A samples, precluding the clinical applicability of niPGT-A in its current form.

REVIEW

Non-invasive preimplantation genetic testing for aneuploidies: an update

BIOGRAPHY

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Luis Navarro-Sánchez[1,*], Carmen García-Pascual[1,2], Carmen Rubio[1,2], Carlos Simón[1,2,3,4,5]

TABLE 2 SUMMARY OF STUDIES COMPARING RESULTS BETWEEN SBM AND WB

Authors	No. of SBM	Informative media% (n/N)	Concordance WB–SBM[a] % (n/N)	False positives % (n/N)	False negatives % (n/N)	Drop volume[b] (µL)	Embryo manipulation	Time in culture	WGA method	PGT-A technique
Xu et al. (2016)	42	100 (42/42)	Ploidy: 85.7 (36/42) Full: 66.7 (28/42) Partial: 19.0 (8/42)	9.5 (4/42)	4.8 (2/42)	30 (5 to 20)	Vitrification on D3	D3–D5	MALBAC (Yikon)	NGS (Illumina)
Ho et al. (2018)	41	97.6 (40/41)	Ploidy: 45.5 (15/33)[c]	–	–	25 (5)	AH on D3 versus no AH	D1–D5	PicoPLEX (Rubicon)	NGS (Thermo Fisher)
Huang et al. (2019)	52	92.3 (48/52)	Ploidy: 93.8 (45/48) Full: 85.4 (41/48) Partial: 8.3 (4/48)	6.3 (3/48)	–	15 (3.5)	AH on D3, TE biopsy plus vitrification on D5/6	D5–D6 D6–D7 Cultured for 24 h after warming	MALBAC (Yikon)	NGS (Illumina)
Rubio et al. (2020b)[d]	81	90.1 (73/81)	Ploidy: 84.4 (54/64)[c] Full: 68.8 (44/64) Partial: 15.6 (10/64)	6.3 (4/64)	9.4 (6/64)	10 (10)	None	D4–D6/7	ReproSeq (Thermo Fisher)	NGS (Thermo Fisher)
Yin et al. (2021)	75	78.7 (59/75)	Ploidy: 89.8 (53/59) Full: 32.2 (19/59) Partial: 57.6 (34/59)	10.2 (6/59)	–	25 (25)	Biopsy on D5/6 and vitrification	Cultured for 24 h after warming	MALBAC (Yikon)	NGS (Illumina)
Shitara et al. (2021)	20	95 (19/20)	Ploidy: 93.8 (15/16)[c] Full: 56.3 (9/16) Partial: 37.5 (6/16)	–	6.3 (1/16)	–	Vitrified D5/6 Zona pellucida removed	24 h for D5 3 h for D6 blastocysts	SurePlex (Illumina)	NGS (Illumina)
Chen et al. (2021a)	265	96.6 (256/265)	Ploidy: 78.1 (200/256)	16.8 (43/256)[3]	5.1 (13/256)[e]	– (20–25)	None	D3–D5/6	MALBAC (Yikon)	NGS (Illumina)

Spent Blastocyst Media versus Blastocyst:

- Whole embryo is considered the gold standard for comparison.
- Varied amplification rates ranging from 78.7% to 100%.
- Varied concordance rates ranging from 45.5% to 93.8%.
- Varied drop volume ranging from 10ul to 30ul.
- Varied time in culture including D3-D5, D1-D5, D5-D6, D4-D6, D6-D7 etc..
- The significant heterogeneity of all these procedures limits the comparison and interpretation of these data sets.

TABLE 1 SUMMARY OF STUDIES COMPARING RESULTS BETWEEN SBM AND TE OR PB BIOPSIES

Authors	No. of SBM	Informative media % (n/N)	Concordance TE–SBM[a] % (n/N)	False positives % (n/N)	False negatives % (n/N)	Drop volume (µl)[a]	Embryo manipulation	Time in culture	WGA method	PGT-A technique
Shamonki et al. (2016)	57	96.5 (55/57)	Ploidy (all full): 33.3 (2/6)[c]	–	–	15 (15)	AH on D3	D3–D5/6	Repli-G (Qiagen)	aCGH (Agilent Technologies)
Feichtinger et al. (2017)[d]	22	81.8 (18/22)	Ploidy: 72.2 (13/18) Full: 22.2 (4/18) Partial: 50.0 (9/18)	5.6 (1/18)	22.2 (4/18)	25 (5)	PB biopsy, AH on D3	D0–D5/6	SurePlex (Illumina)	aCGH (Illumina)
Vera-Rodríguez et al. (2018)	56	91.1 (51/56)	Ploidy: 33.3 (17/51) Full: 17.6 (9/51) Partial: 15.7 (8/51)	–	66.7 (34/51)	25 (20)	AH on D3	D3–D5	SurePlex (Illumina) + ReproSeq (Thermo Fisher)	NGS (Thermo Fisher)
Ho et al. (2018)	41	97.6 (40/41)	Ploidy: 65.0 (26/40)	–	–	25 (5)	AH on D3 versus no AH	D1–D5	PicoPLEX (Rubicon)	NGS (Thermo Fisher)
Huang et al. (2019)	52	92.3 (48/52)	Ploidy: 89.1 (41/46)[e] Full: 65.2 (30/46) Partial: 23.9 (11/46)	2.2 (1/46)	8.7 (4/46)	15 (3.5)	AH on D3, TE biopsy plus vitrification on D5/6	D5–D6 D6–D7 24 h culture after thawing	MALBAC (Yikon)	NGS (Illumina)
Yeung et al. (2019)	168	69.0 (116/168) D5: 55.6 (50/90) D6: 84.6 (66/78)	Ploidy: 73.3 (85/116) Full: 23.3 (27/116) Partial: 50.0 (58/116) D5: Ploidy 76 (38/50) D6: Ploidy 71.2 (47/66)	12.9 (15/116) D5: 12 (6/50) D6: 13.6 (9/66)	13.8 (16/116) D5: 12 (6/50) D6: 15.2 (10/66)	30 (3)	AH on D3	D3–D5 D3–D6	SurePlex (Illumina)	NGS (Illumina)
Rubio et al. (2019)[f]	115	93.9 (108/115) D5: 81.8 (27/33) D6/7: 98.8 (81/82)	Ploidy: 78.7 (85/108) Full: 63.9 (69/108) Partial: 14.8 (16/108) D5: Ploidy 63 (17/27)[g] D6/7: Ploidy 84 (68/81)	13.9 (15/108) D5: 29.6 (8/27) D6/7: 8.6 (7/81)	2.8 (3/108) D5: 3.7 (1/27) D6/7: 2.5 (2/81)	10 (10)	None	D4–D5 D4–D6/7	ReproSeq (Thermo Fisher)	NGS (Thermo Fisher)
Rubio et al. (2020b)[f]	1301	85.2 (1108/1301)	Ploidy: 78.2 (866/1108) Full: 67.7 (750/1108) Partial: 10.5 (116/1108)	12.4 (137/1108)	8.3 (92/1108)	10 (10)	None	D4–D6/7	ReproSeq (Thermo Fisher)	NGS (Thermo Fisher)
Lledo et al. (2020)[h]	92	92.4 (85/92)	Ploidy: 74.7 (62/83) or 72.3 (60/83)[e]	12.0 or 15.7 (10/83 or 13/83)	13.3 or 12.0 (11/83 or 10/83)	20 (7.5 each method)	AH on D3	D3–D5/6	MALBAC (Yikon) or SurePlex (Illumina)	NGS (Illumina)
Shitara et al. (2021)	20	95 (19/20)	Ploidy: 88.9 (16/18)[e] Full: 66.7 (12/18) Partial: 22.2 (4/18)	5.6 (1/18)	5.6 (1/18)	–	Vitrified D5/6 embryos Zona pellucida removed	24 h for D5 3 h for D6 blastocysts	SurePlex (Illumina)	NGS (Illumina)
Hanson et al. (2021)[f]	166	62.7 (104/166) D3/4–D5: 17.6 (6/34) D3/4–D6/7: 74.2 (98/132)	Ploidy: 63.5 (66/104) D3/4–D5: Ploidy (and full) 50.0 (3/6) D3/4–D6/7: Ploidy 64.3 (63/98) Full: 30.6 (30/98) Partial: 33.7 (33/98)	26.9 (28/104) D3/4–D5: 33.3 (2/6) D3/4–D6/7: 26.5 (26/98)	8.7 (9/104) D3/4–D5: 16.7 (1/6) D3/4–D6/7: 8.2 (8/98)[4]	30 (–)	AH on D3	D5: 24–48 h D6: 48–72 h D7: 72–96 h	MALBAC (Yikon)	NGS (Illumina)
Chen et al. (2021a)	265	96.6 (256/265)	Ploidy: 74.2 (190/256)[i]	14.5 (37/256)[i]	11.3 (29/256)[i]	– (20–25)	None	D3–D5/6	MALBAC (Yikon)	NGS (Illumina)

REVIEW

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TE Biopsy versus Spent Blastocyst Media:

- Varied amplification rates ranging from 69% to 97.6%.
- Varied concordance rates ranging from 33.3% to 89.1%.
- Varied drop volume ranging from 10ul to 30ul.
- Varied time in culture including D3-D5, D0-D5, D1-D5, D4-D5, D5-D6, D4-D6 etc..
- The significant heterogeneity of these procedures limits the translation of these approaches into the clinic.

TABLE 3 SUMMARY OF STUDIES COMPARING RESULTS BETWEEN SBM PLUS BF AND TE BIOPSIES OR WB

Authors	No. of SBM	Informative media % (n/N)	Concordance TE-SB-M+BF[a] % (n/N)	False positives % (n/N)	False negatives % (n/N)	Concordance WB-SB-M+BF % (n/N)	False positives % (n/N)	False negatives % (n/N)	Drop volume (µl)[b]	Embryo manipulation	Time in culture	WGA method	PGT-A technique
Kuznyetsov et al. (2018)	47	100 (47/47)	Ploidy: 88.4 (38/43)[c] Full: 69.8 (30/43) Partial: 18.6 (8/43)	2.3 (1/43)	9.3 (4/43)	Ploidy: 89.3 (25/28)[c] Full: 78.6 (22/28) Partial: 10.7 (3/28)	7.1 (2/28)	3.6 (1/28)	25 (25)	28 vitrified; 24 with previous TE biopsy; all laser collapse	D4–D5/6	SurePlex (Illumina)	NGS (Illumina)
Li et al. (2018)	40	97.5 (39/40)	Ploidy: 76.3 (29/38)[c] Full: 47.4 (18/38) Partial: 28.9 (11/38)	13.2 (5/38)	10.5 (4/38)	Ploidy: 78.9 (30/38)[c] Full: 55.3 (21/38) Partial: 23.7 (9/38)	15.8 (6/38)	5.3 (2/38)	25 (25)	Small breech in the zona pellucida	D3–D5	MALBAC (Yikon)	NGS (Illumina)
Jiao et al. (2019)	62	98.4 (61/62)	Ploidy: 98.3 (58/59)[c] Full: 88.1 (52/59) Partial: 10.2 (6/59)	–	1.7 (1/59)	Ploidy: 96.7 (59/61) Full: 88.5 (54/61) Partial: 8.2 (5/61)	3.3 (2/61)	–	12 (10)	AH, vitrification D5/6; artificial shrinkage	14 h culture after warming	MALBAC (Yikon)	NGS (Illumina)
Zhang et al. (2019b)[d]	32	87.5 (28/32)	–	–	–	Ploidy: 70.4 (19/27)[c] Full: 66.7 (18/27) Partial: 3.7 (1/27)	14.8 (4/27)	7.4 (2/27)	Approximately 20–30 (approximately 20–30)	Vitrification D2/3; artificial shrinkage	D4–D5 D5–D6	MALBAC (Yikon)	NGS (Illumina)
Kuznyetsov et al. (2020)	102	88.2 (90/102)	Ploidy: 88.9 (80/90) Full: 78.9 (71/90) Partial: 10.0 (9/90)	3.3 (3/90)	7.8 (7/90)	–	–	–	25 (5)	AH on D4; artificial shrinkage	D4–D6	SurePlex (Illumina)	NGS (Illumina)
Chen et al. (2020)	26	100 (26/26)	Ploidy: 100 (26/26)[e] Full: 76.9 (20/26) Partial: 23.1 (6/26)	–	–	Ploidy: 100 (26/26)[f] Full:80.8 (21/26) Partial: 19.2 (5/26)	–	–	15 (10)	TE biopsy D5/6, vitrification; artificial shrinkage	15 h culture after warming	MALBAC (Yikon)	NGS (Illumina)
Li et al. (2021)[g]	41	95.1 (39/41)	Ploidy: 76.3 (29/38)[c,e] Full: 68.4 (26/38) Partial: 7.9 (3/38)	7.9 (3/38)	15.8 (6/38)	Ploidy: 87.2 (34/39) Full: 84.6 (33/39) Partial: 2.6 (1/39)	10.3 (4/39)	2.6 (1/39)	15 (10)	TE biopsy D5/6, vitrification; laser collapse	14–18 h culture after warming	MALBAC (Yikon)	NGS (Illumina)
Sialakouma et al. (2021)	40	100 (40/40)	Ploidy: 81.8 (27/33)[h] Full: 63.6 (21/33) Partial: 18.2 (6/33)	3.0 (1/33)	15.2 (5/33)	Ploidy (all full): 100 (4/4)[i]	–	–	10 (–)	Artificial shrinkage	D3–D5/6	SurePlex (Illumina)	NGS (Thermo Fisher)

REVIEW

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BIOGRAPHY

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TE Biopsy/Embryo versus Spent Blastocyst Media+Blastocoel Fluid Aspiration:

- Varied amplification rates ranging from 87.5% to 100%.
- Varied concordance rates ranging from 76.3% to 100%.
- Varied drop volume ranging from 10ul to 25ul.
- Varied time in culture including D3-D5, D4-D5, D5-D6, D4-D6 etc..
- The number of samples analyzed in all cases was low and the results were heterogeneous.
- This approach of including blastocoel fluid aspiration did not provide an advantage to the analysis of the spent media alone.

Reasons for Disparate Results?

Experimental variables:

- Culture media volume: 10-20ul
- Timing of media drop collections: Day 5/6/7 of blastocyst development
  (D5 = higher maternal DNA contamination)
- Interval of culture time: 24-48 hours
- Method for media drop collection (DNA contamination)
- Blastocyst developmental stage and quality relative to DNA yield/degradation
- PGT lab testing platforms including; DNA amplification, NGS, diagnostic algorithms etc..

More clinical studies are needed to evaluate, optimize and standardize these variables!

Journal Pre-proof

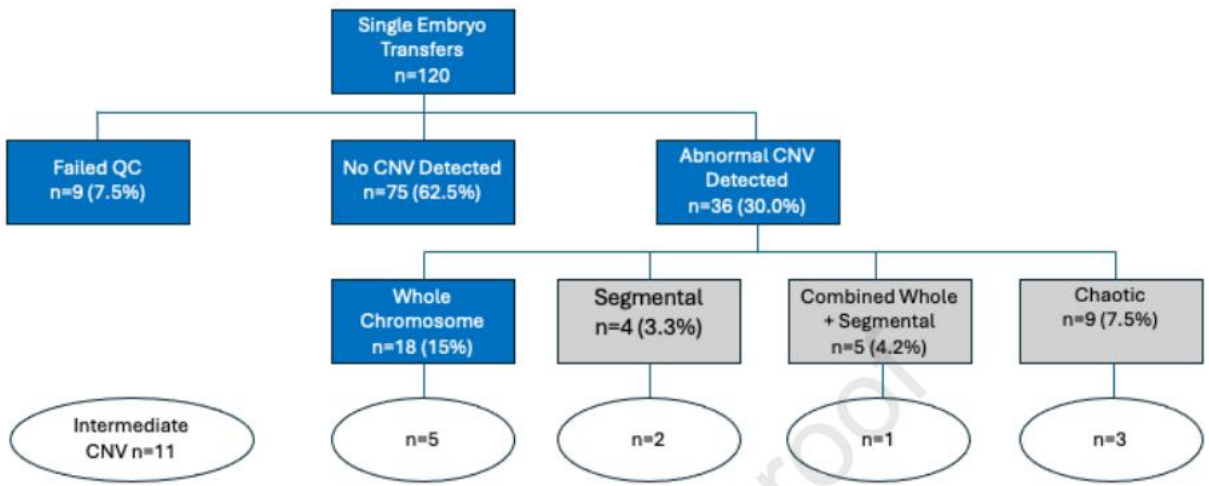
A pilot study to investigate the clinically predictive values of copy number variations detected by next generation sequencing of cell free DNA in spent culture media.

Gary Nakhuda, MD, Sally Rodriguez, ScM, CGC, Sophia Tormasi, BSc, TS, Catherine Welch, MBA, TS

Table 1: Patient & cycle characteristics.

	Median	**IQR**
Age	32	30-34
Partner age	34	32 - 37.8
Oocytes retrieved	16	11- 23
M2 oocytes	13	8 -18
2PN	10	7-14
Blastocysts vitrified	6	3 - 9

Figure 1: NGS interpretations for 120 single embryo transfers



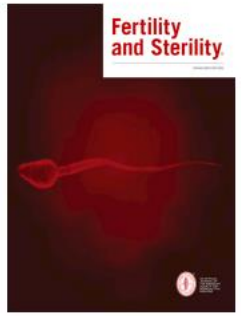


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Table 2: Clinical outcomes stratified by NGS interpretation.

NGS Interpretation (n=120)	**Implantation %(n)**	**Clinical pregnancy %(n)**	**Sustained implantation, NPV %(n)**	**Total SAB %(n)**
Failed QC (9)	66.7 (6)	66.7 (6)	66.7 (6)	0
No CNV (75)	78.6 (59)	64 (48)	57.3 (43)	27.1 (16)
Abnormal CNV(36)	63.9 (23)	44.4 (16)	41.2 (15)	34.7 (8)
Abnormal CNV Stratified				
Whole chromosome (18)	50 (9)	11.1 (2)	5.6 (1)	88.9 (8)
Segmental (4)	100 (4)	100 (4)	100 (4)	0
Combined (5)	80 (4)	80 (4)	80 (4)	0
Chaotic (9)	66.7 (6)	66.7 (6)	66.7 (6)	0

Table 3: Prognostic accuracy of subcategories of CNV abnormalities stratified by NGS interpretation.

NGS Interpretation	**PPV % [95% CI]**	**Specificity % [95% CI]**	**Sensitivity % [95% CI]**	**Relative Risk [95% CI]***
Any abnormal CNV (36)	58.3 [42.2-73.3]	74.4 [61.6-83.6]	39.6 [27.6-53.1]	1.37 [0.93-2.0] p=0.122
Abnormal CNV Stratified				
Whole chromosome (18)	94.4 [76.8-99.4]	97.7 [88.2-99.6]	34.7 [22.9-48.6]	2.21 [1.66-2.94] p<0.001
Segmental (4)	0 (0) [0-44.5]	91.5 [80.1-96.6]	0 [0-10.7]	0.26 [0.02-3.57] p=0.312
Combined (5)	20 (1) [2.3-62.9]	91.5 [80-96.6]	3 [0.54-15.3]	0.47 [0.08-2.76] p=0.319
Chaotic (9)	33.3 (3) [10.4-65.2]	87.8 [75.8-94.3]	8.6 [2.9-22.4]	0.78 [0.3-2.04] p=0.592

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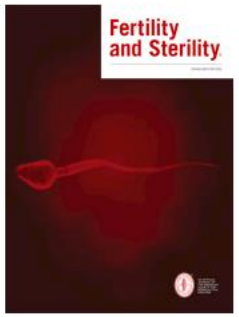

Summary: The results of this prognostic accuracy study of niPGT-A revealed a high PPV when aneuploidy of whole chromosomes were detected, suggesting that SCM may provide a reliable source for aneuploidy screening.

Although niPGT-A poses no direct harm to the embryo, valid concerns regarding unintended disposal of viable embryos due to unreliable or poorly predictive results are still relevant. This concern is underscored by the data which demonstrates that CNV other than whole chromosome abnormalities were not associated with significant PPV.

Optimizations of technical aspects of niPGT-A, including sample collection, pre-analytical processing, DNA amplification and sequencing, bioinformatics, and careful interpretations of CNV profiles are necessary before clinical utilization.

Ultimately, randomized controlled trials are required to not only validate the predictive value of niPGT-A for embryonic reproductive potential, but also to demonstrate that utilization will actually improve outcomes.

Non-Invasive PGT-A: Future Directions

- NIPGT is an exciting area of research and approach to aneuploidy screening
- Ongoing research and larger sample size studies are essential to answer the question: Is the media drop PGT-A result concordant with the embryo's chromosome constitution?
- Other important questions remaining:
  - What is the source of the cell-free DNA?
  - Is there preferential apoptosis of aneuploid cells that could lead to discordance and/or mosaicism?
  - Impact and challenge of external DNA contamination?
  - If standard embryology practices are required to be altered for NIPGT success will that burden IVF lab practices, impact the embryo and thus remove any cost benefit?

Thank you!

Questions?