Analysis of spent culture medium

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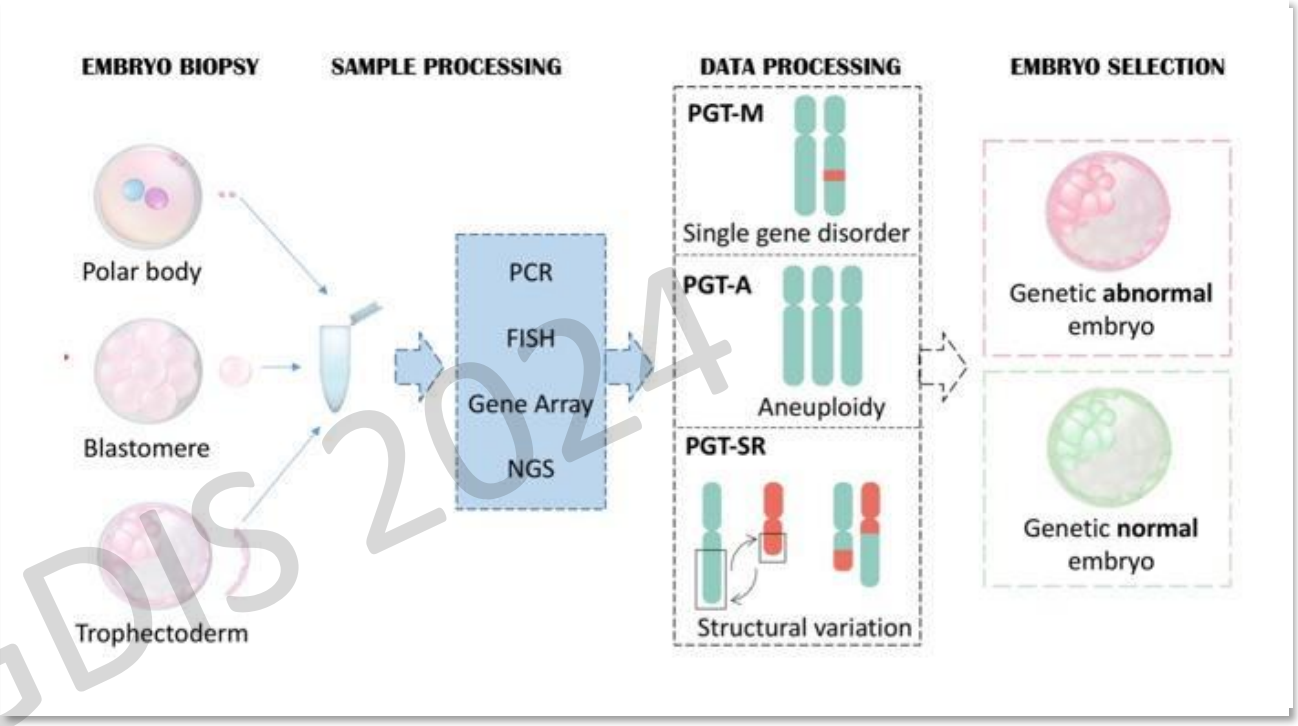
Outline

- Why analysis spent culture medium
- Part I: Non-invasive PGT-A (niPGT-A)
- Part II: Metabolic profiling

Biopsy hurts the embryo



What are the down sides of PGT?

- Increases costs IVF
- Need elective cryopreserving embryos, delay ET until results back
- Invasive procedure
  - Embryo biopsy may affect embryo development
  - Lead to potential loss of embryo
  - Long –term effect of embryo biopsy not defined at this time
  - Reported to have 5% relative reduction in live birth rate due to damage and also false positive results from inherent technical errors

Hypotheses to analysis spent culture medium

- Embryos release DNA, RNA, and protein molecules into its surroundings (handling and/or culture media)
- The **identities** and **quantities** of these molecules reflect the **health** and **birth potential** of the embryo

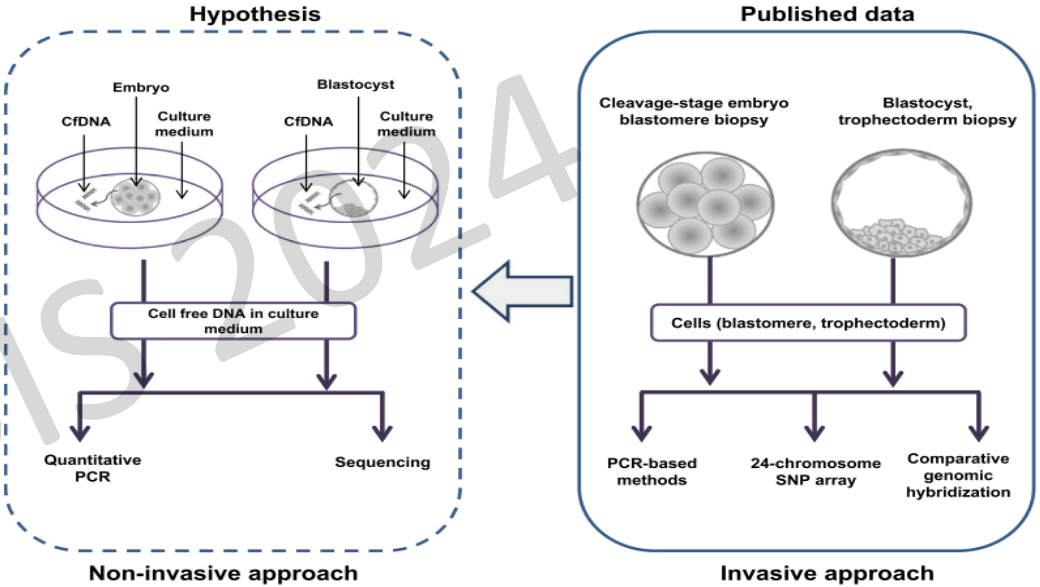


Figure from the proof-of-concept paper: Assou S, *et al. Med Hypotheses* 2014

Human embryo reveals nuclear DNA

Please cite this article in press as: Domingo-Muelas et al., Human embryo live imaging reveals nuclear DNA shedding during blastocyst expansion and biopsy, Cell (2023), https://doi.org/10.1016/j.cell.2023.06.003

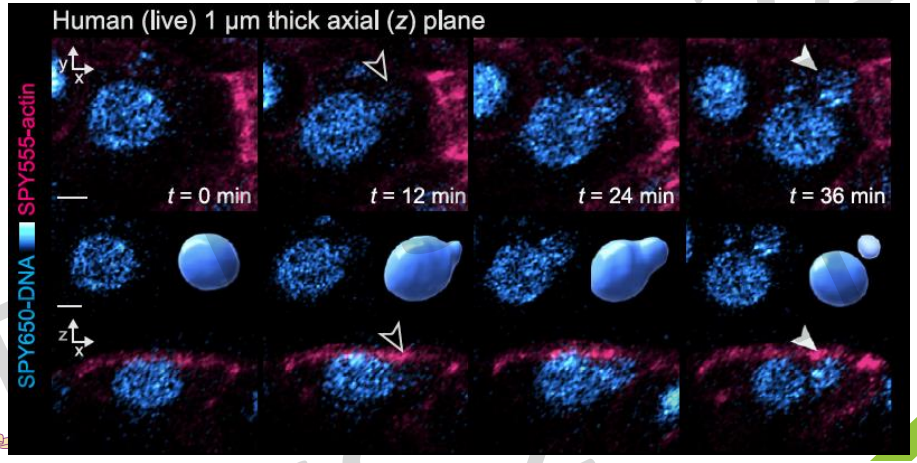
Cell

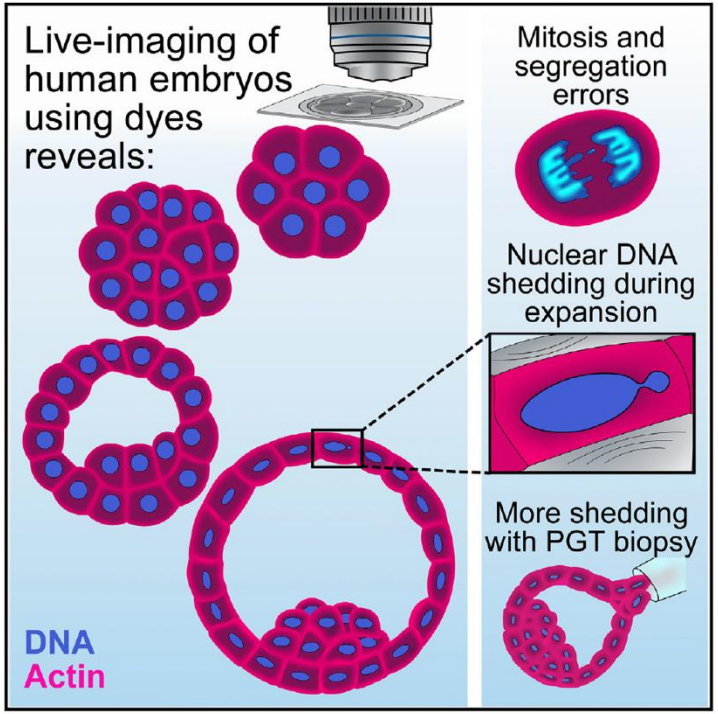
CellPress

Article

Human embryo live imaging reveals nuclear DNA shedding during blastocyst expansion and biopsy

Ana Domingo-Muelas,[1,2,10] Robin M. Skory,[1,3,10] Adam A. Moverley,[1,4] Goli Ardestani,[5] Oz Pomp,[1] Carmen Rubio,[6] Piotr Tetlak,[1] Blake Hernandez,[1] Eric A. Rhon-Calderon,[1] Luis Navarro-Sánchez,[6] Carmen M. García-Pascual,[6] Stephanie Bissiere,[1] Marisa S. Bartolomei,[1] Denny Sakkas,[5,*] Carlos Simón,[2,7,8,9,*] and Nicolas Plachta[1,11,*]





Analysis spent culture medium

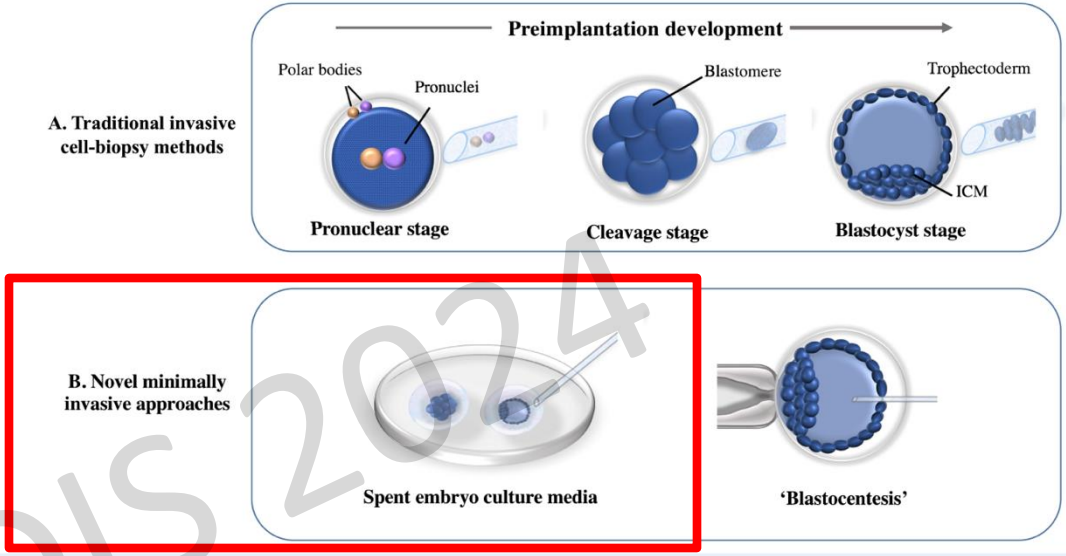


Figure 1 Methods of obtaining embryonic DNA for preimplantation genetic testing. (**A**) Traditional invasive methods comprise biopsy of both polar bodies, blastomere(s) from cleavage stage embryos, or trophectoderm cells from blastocyst stage embryos. (**B**) Novel minimally invasive approaches of obtaining embryonic genetic material for analysis include analysis of DNA in spent embryo culture media and the blastocoel fluid of blastocyst embryos.

Leaver M and Wells D. Hum Reprod Update. 2020

Part I: Non-invasive PGT-A (niPGT-A)

PNAS

Noninvasive chromosome screening of human embryos by genome sequencing of embryo culture medium for in vitro fertilization

Juanjuan Xu[a,1], Rui Fang[b,1], Li Chen[a,1], Daozhen Chen[b], Jian-Ping Xiao[b], Weimin Yang[b], Honghua Wang[b], Xiaoqing Song[b], Ting Ma[c], Shiping Bo[c], Chong Shi[c], Jun Ren[c], Lei Huang[d,e,f,g], Li-Yi Cai[b,2], Bing Yao[a,2], X. Sunney Xie[d,g,h,2], and Sijia Lu[c,2]

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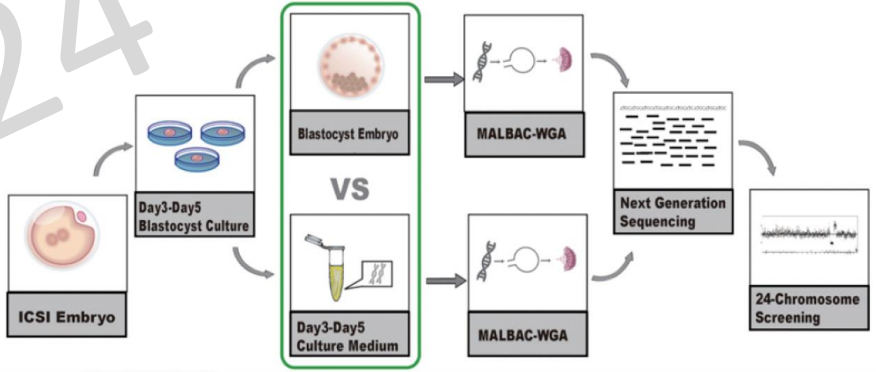
	Number	Ratio(%)
Sensitivity	15/17	88.2%
Specificity	21/25	84.0%
PPV	15/19	78.9%
NPV	21/23	91.3%

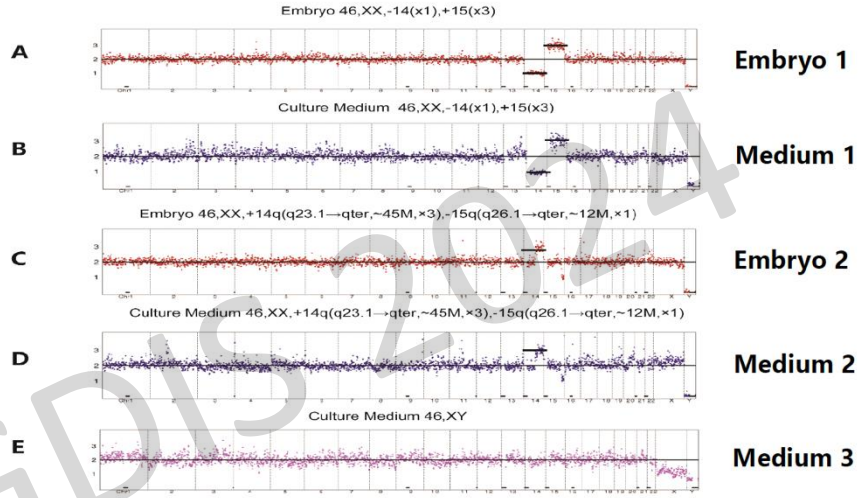
PPV: pos. predictive value
NPV: neg. predictive value



Screenshot

First clinical case

IVF Cycles	Wife	Husband	No. of Blastocyst Embryos	No. of Transferrable Embryos	Clinical Outcome
3	Premature ovarian failure	t(14;15)(q22;q24)	3	1	Healthy boy was born at 38^{+4} wk



Xu J., et. al., PNAS, 2016 Oct 18;113(42):11907-1191.

Non-invasive PGT-A (niPGT-A)

Spent Embryo culture medium (SCM)

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

Journal of Assisted Reproduction and Genetics
https://doi.org/10.1007/s10815-019-01517-7

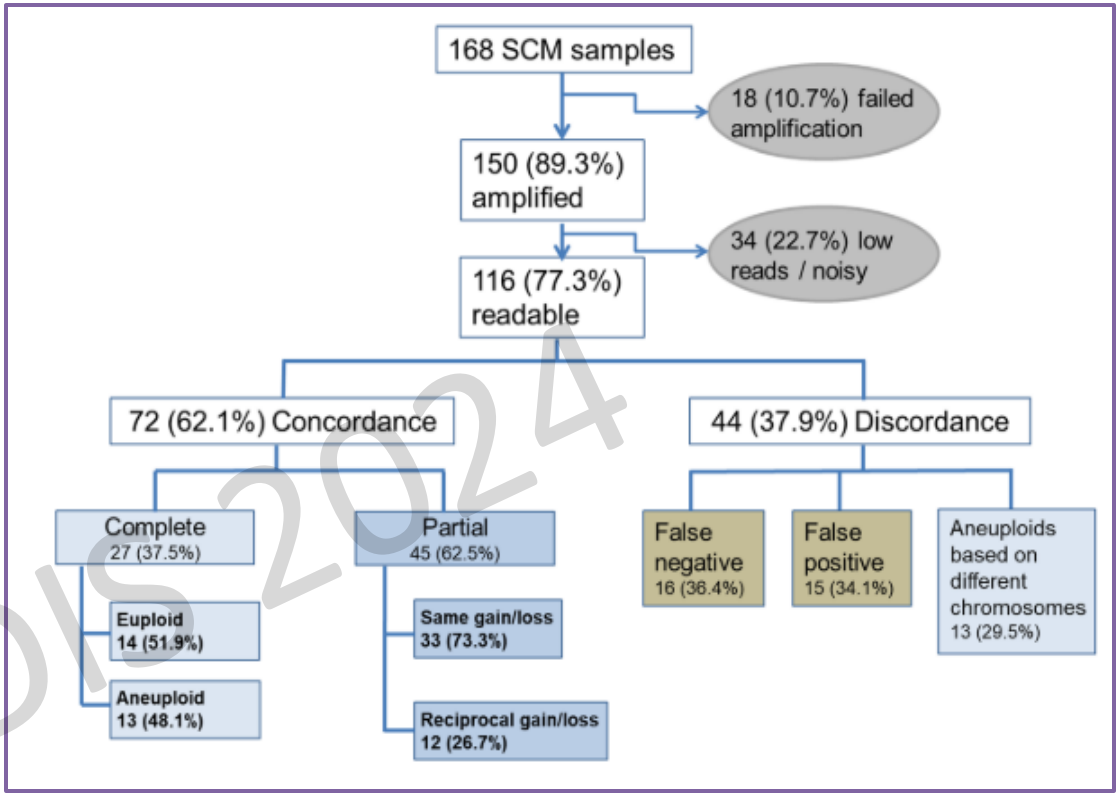
ASSISTED REPRODUCTION TECHNOLOGIES

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]

Received: 10 February 2019 / Accepted: 30 June 2019

PGT-A vs niPGT-A concordance rate



PGT-A concordance 62% between SCM and trophectoderm (TE)

Overview of current niPGT-A application

Table IV niPGT-A analysis of SCM.

Study	Sample details		Analysis				Concordance (%)		
	Media analyzed	Sample number	Amplification method	Amplification rate (%)	Cytogenetic method	Sequencing platform	Overall ploidy (aneuploid or euploid)	Full karyotype	Per single chromosome
Spent media only									
Shamonki et al. (2016)	D3-5/6	57	Repli-G (Qiagen)	97	aCGH (Agilent Technologies)	Not applicable	–	–	–
Xu et al. (2016)	D3-5	42	MALBAC (Yikon Genomics)	100	NGS (NEB Ultra DNA Kit, NEB)	HiSeq 2500 (Illumina)	*Normal (euploid) vs'abnormal' (inc. mosaicism and segmentals):* WE: 86	–	–
Liu et al. (2017)	D1-5	88	MALBAC (Yikon Genomics)	91	NGS (Library prep method not specified)	HiSeq 2500 (Illumina)	*Normal (euploid) vs'abnormal' (inc. mosaicism and segmentals):* TE: 84	*Including >40% mosaicism and large copy number variation:* TE: 65. Cells of arrested/ degenerated embryos (which didn't reach blastocyst stage): 44	–
Feichtinger et al. (2017)	D1-5	22	SurePlex (Illumina)	82	aCGH (24Sure, Illumina)	Not applicable	*Euploid vs aneuploid:* PB: 72	–	PB: 49% of single chromosomal aneuploidies concordant with SCM
Ho et al. (2018)	D1-3 D1-5	41	PicoPLEX (Rubicon Genomics), using 20 cycles instead of 14	D1-3 = 39 D1-5 = 80	NGS	Ion S5 Sequencer (Life Technologies)	*Euploid vs aneuploid:* D1-3 SCM vs WE: 56 D1-5 SCM vs WE: 46	–	–
Vera-Rodriguez et al. (2018)	D3-5	56	SurePlex (Illumina), then a second round using Ion Reproseq (TherrmoFisher)	91	NGS (Ion Reproseq, ThermoFisher)	Ion PGM instrument (ThermoFisher)	*Both aneuploid:* TE: 30	*Including segmental aneuploidies and mosaicism:* TE: 16	*Whole chromosome aneuploidies only:* TE: 5
Fang et al. (2019)	D3-5/6	170	MALBAC (Yikon Genomics)	97	NGS (NEB Ultra DNA Kit, NEB)	HiSeq 2500 (Illumina)	—	—	–
Huang et al. (2019)	D5-6 and D6/7	52	NICSwift—modified MALBAC (Yikon Genomics)	92	NGS (NEBNext Ultra II DNA kit, NEB)	HiSeq 2500 (Illumina)	*Euploid vs aneuploid:* WE: 94	*Including segmental aneuploidies and mosaicism:* WE: 83	–
Yeung et al. (2019)	D3-5/6	168	SurePlex (Illumina)	89	NGS (VeriSeq, Illumina)	MiSeq (Illumina)	*Euploid vs aneuploid:* TE: 73	Autosomal chromosomes: TE: 62 Sex chromosomes: TE: 82	-
Rubio et al. (2019)	D4-5/6/7	115	Modified version of IonReproseq (ThermoFisher)	95	NGS (Ion Reproseq, ThermoFisher)	Ion S5TM XL system (ThermoFisher)	*Euploid vs aneuploid:* D4-5 SCM vs TE = 63 D5-6/7 SCM vs TE = 84 Overall vs TE = 79	*Including segmental aneuploidies:* D4-5 SCM vs TE = 41 D5-6/7 SCM vs TE = 72 Overall vs TE = 64	–

- Currently by niPGT-A, not all groups can achieve 100% amplification rate.
- The highest sensitivity is 88.6% and 87.5% by Kuznyetsov, V. *et al*.
- An estimated >20% to 80% of instances involve contamination with maternal genetic material

Table from Leaver M, Wells D. *Hum Reprod Update*, 2019;1–27.

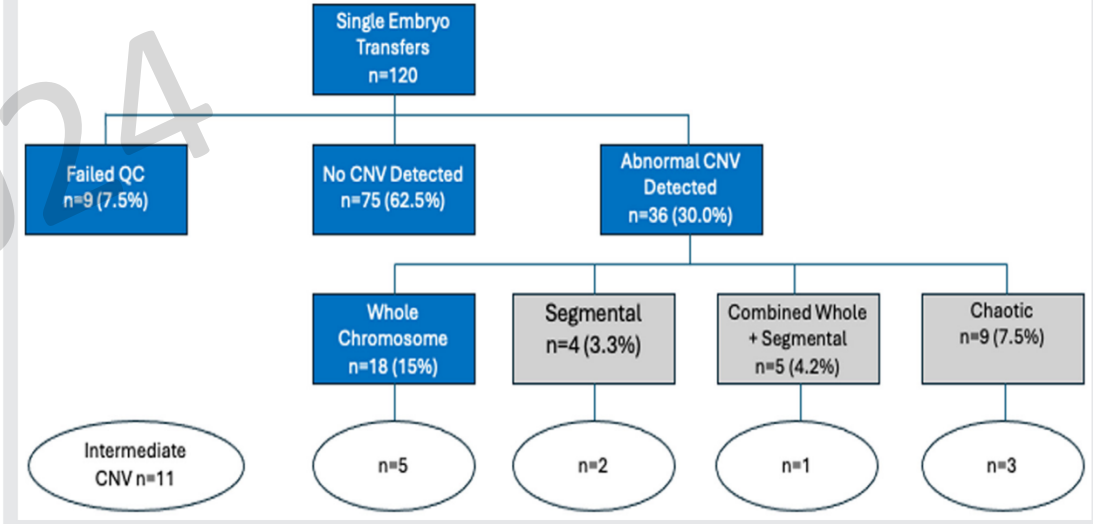
ASSISTED REPRODUCTION

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

Gary Nakhuda, M.D.,[a] Sally Rodriguez, Sc.M., C.G.C.,[b] Sophia Tormasi, B.Sc. and Catherine Welch, M.B.A., T.S.[b]

-woman of age 35 years or younger at the time of oocyte retrieval

-NOT all CNV abnormalities apparent on NGS profiles are equally predictive of nonviability.
-Sex chromosome: consistent results in 74.1% (n = 40/54).



NGS interpretations for 120 single embryo transfers. CNV, copy number variation; NGS, next-generation sequencing; QC, quality control.

Nakhuda. Study of predictive values of niPGT-A. Fertil Steril 2024.

Noninvasive preimplantation genetic testing for aneuploidy in spent culture medium as a substitute for trophectoderm biopsy

Carmen Rubio, Ph.D.,[a,b] Catherine Racowsky, Ph.D.,[c] David H. Barad, M.D., M.S.,[d,e] Richard T. Scott Jr., M.D., H.C.L.D.,[f,g] and Carlos Simon, M.D., Ph.D.[h,i]

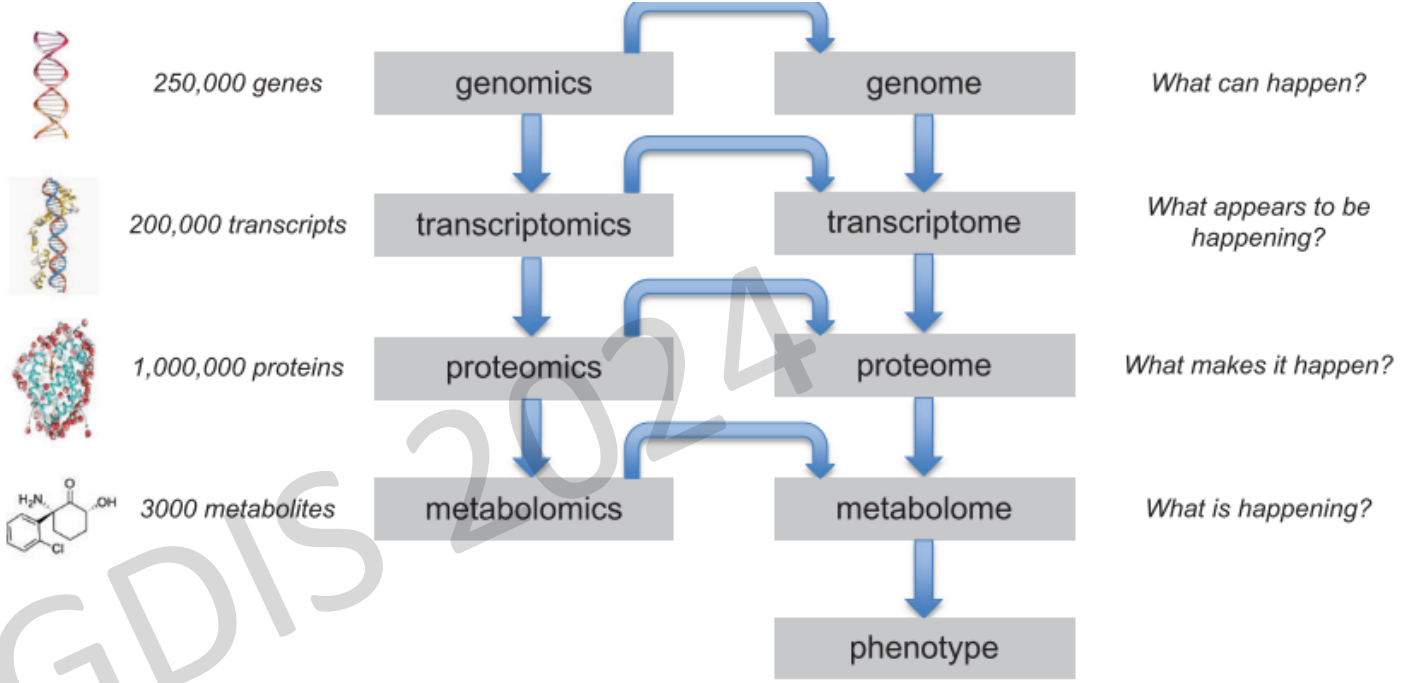
Pros and cons:

1. How reliable is embryo cfDNA versus teDNA as a representative of embryo chromosomal constitution?

2. Is embryo cfDNA secretion related to embryo chromosomal self-correction and/or apoptosis?

3. What are the reasons that the noninvasive model will prevail or not over the invasive model?
4. niPGT-A still an expensive approach

The Multi-omics approach?



Application to predict Repeated Implantation Failure (RIF)

compared between embryos from RIF patients (n=35) and oocyte donors as controls (n=15)

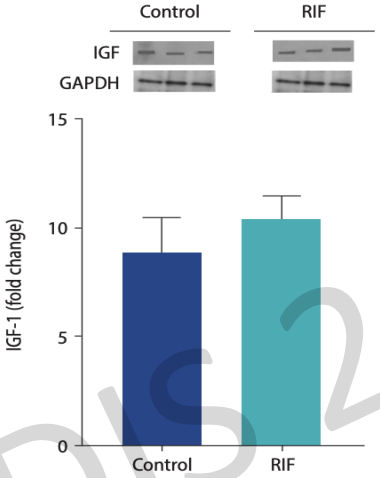


Figure 1. The insulin-like growth factor 1 (IGF-1) protein expression in culture medium from the repeated implantation failure (RIF) and control groups. Values are presented as mean±standard deviation of at least three replicates. GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

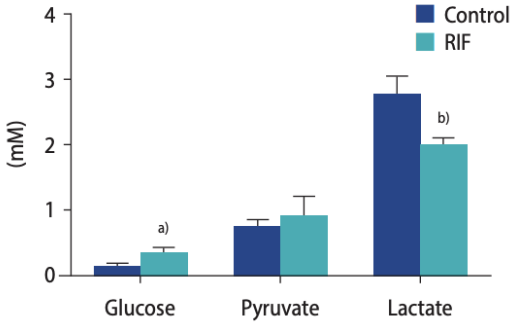
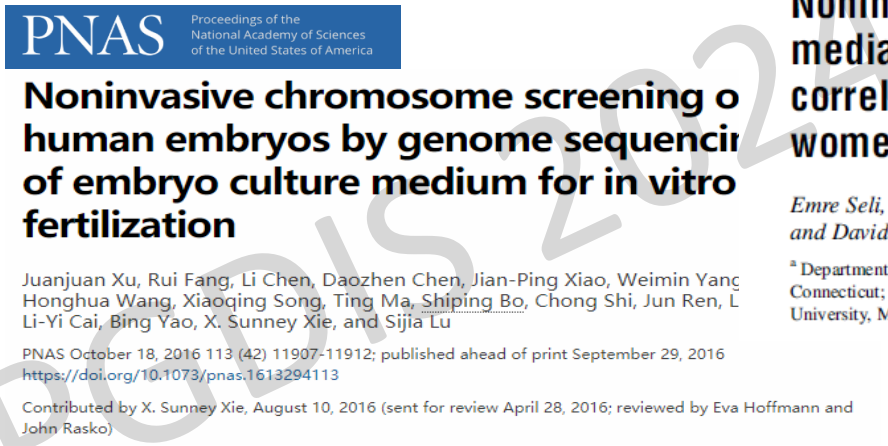


Figure 2. Glucose, pyruvate, and lactate concentrations in the repeated implantation failure (RIF) and control groups. Values are presented as mean±standard deviation of three replicates. [a]$p<0.05$; [b]$p<0.01$.

Metabolic profiling-(niPGT?)

Hypothesis

- Embryo scecrets DNA、RNA and protein into the spend culture medium
- The identities and quantities of these molecules reflect the **health** and **birth potential** of the embryo



PNAS Proceedings of the National Academy of Sciences of the United States of America

Noninvasive chromosome screening o human embryos by genome sequencir of embryo culture medium for in vitro fertilization

Juanjuan Xu, Rui Fang, Li Chen, Daozhen Chen, Jian-Ping Xiao, Weimin Yang Honghua Wang, Xiaoqing Song, Ting Ma, Shiping Bo, Chong Shi, Jun Ren, L Li-Yi Cai, Bing Yao, X. Sunney Xie, and Sijia Lu

PNAS October 18, 2016 113 (42) 11907-11912; published ahead of print September 29, 2016 https://doi.org/10.1073/pnas.1613294113

Contributed by X. Sunney Xie, August 10, 2016 (sent for review April 28, 2016; reviewed by Eva Hoffmann and John Rasko)

Noninvasive metabolomic profiling of embryo culture media using Raman and near-infrared spectroscopy correlates with reproductive potential of embryos in women undergoing in vitro fertilization

Emre Seli, M.D.,[a] Denny Sakkas, Ph.D.,[a] Richard Scott, M.D.,[b] Shing C. Kwok,[c] Scott M. Rosendahl,[c] and David H. Burns, Ph.D.[c]

[a] Department of Obstetrics, Gynecology, and Reproductive Sciences, Yale University School of Medicine, New Haven, Connecticut; [b] Reproductive Medicine Associates, Morristown, New Jersey; and [c] Department of Chemistry, McGill University, Montreal, Quebec, Canada

Seli et al., 2007 Fertil Steril. 88(5):1350-1357.
Xu et al., 2016 Proc Natl Acad Sci. 113(42):11907-11912.

Previous Embryonic Metabolomic Profiling

A list of the 92 metabolites found in the spent media of day 3 human embryos.

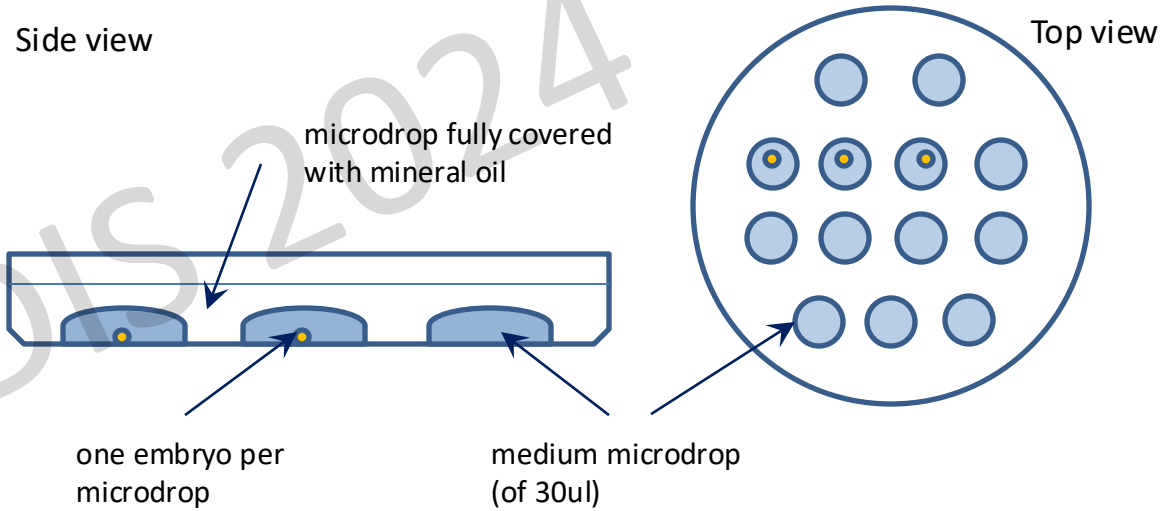
Super pathway	Metabolite	Fold change T vs. N	*P* value
Peptide	Glycylglutamine	1.01	.2668
Carbohydrate	Sucrose	1.67	.6042
	Glucose	1.31	.5945
	Pyruvate	0.96	.9970
	Lactate	1.01	.3787
Energy	Citrate	0.76	.5763
Lipid	Linolenate [alpha or gamma; (18:3n3 or 6)]	0.95	.7597
	Caproate (6:0)	1.14	.0382
	Heptanoate (7:0)	0.99	.8784
	Caprylate (8:0)	1.02	.1597
	Pelargonate (9:0)	0.98	.8312
	Caprate (10:0)	0.95	.8317
	Laurate (12:0)	1.04	.4924
	Myristate (14:0)	0.92	.0672
	Myristoleate (14:1n5)	1.05	.8060
	Oleate (18:1n9)	0.93	.1038
	Linoleate (18:2n6)	0.92	.0609
	Dihomo-linoleate (20:2n6)	0.93	.1673
	Arachidonate (20:4n6)	0.93	.2915
	Choline	1.01	.6761
	Glycerophosphorylcholine	1.01	.5283
	Dehydroisoandrosterone sulfate (DHEAS)	1.02	.7595
	Epiandrosterone sulfate	1.00	.5858
	Androsterone sulfate	1.06	.0229
	4-androsten-3beta, 17beta-diol disulfate 1	1.01	.4111
	4-androsten-3beta, 17beta-diol disulfate 2	1.05	.2181
	Pregnen-diol disulfate	1.01	.8393
	Pregn steroid monosulfate	0.97	.2769

Metabolomic profiling by liquid chromatography mass spectroscopy of 15 SCMs from Trisomy 21 Day 3 embryos and 15 controls.

Sánchez-Ribas I, *et al*. Fertil Steril 2012

Methods & Materials

- SCM sample collection
  - Single embryo culture
  - Sequential (G-2) or One-step (G-TL) medium
  - Collect the culture medium and store at -20 °C

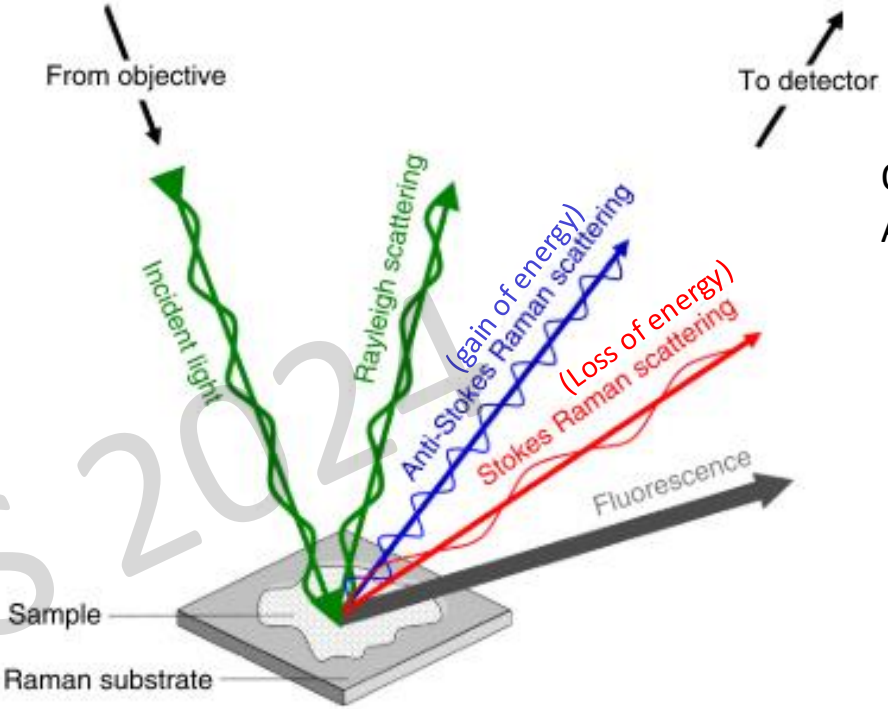


Hypothesis

- Non-invasive Preimplantation Genetic Testing
  - Spent Culture Med

To assess the Aneuploidies or implantation potential of an embryo by Metabolomic Profiling by Raman spectroscopy?

What is Raman spectroscopy?

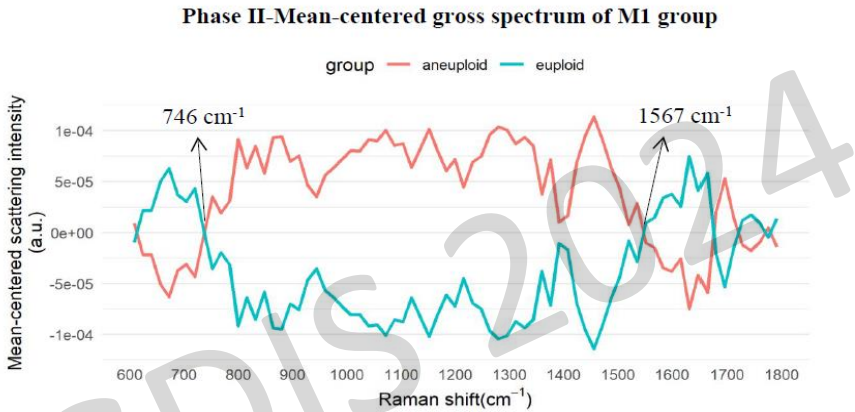


Obtain instant result in
A few seconds

Butler HJ, et al. Nat Protoc. 2016

Objectives

Is Raman spectroscopy combining machine learning applicable for different types of culture media as a first-tier non-invasive screening test for aneuploidies?



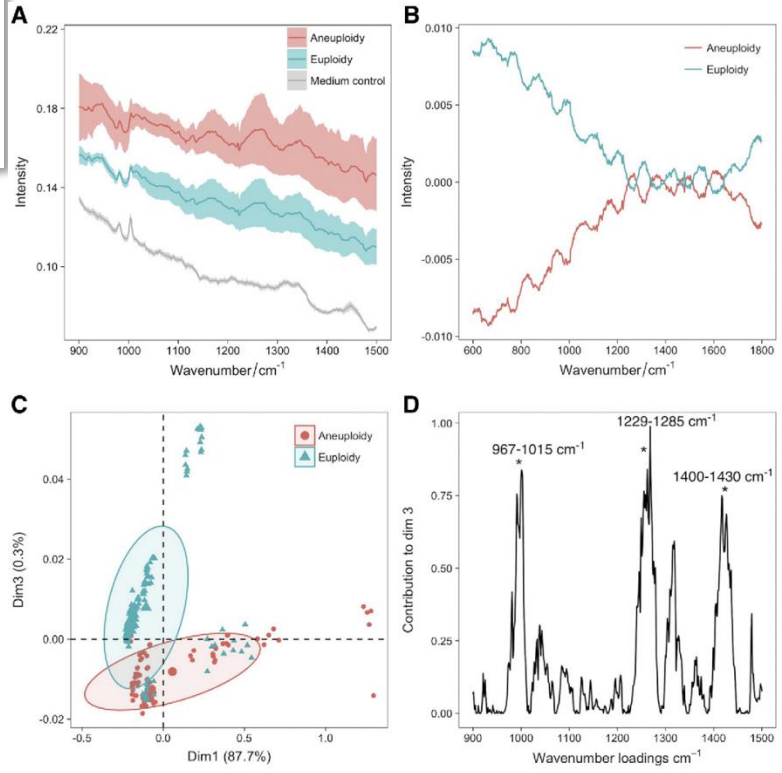
Differential scattering intensity across Raman spectrum, classified by PGT-A result on trophectoderm biopsies

Raman 200 machine

Raman profiling of embryo culture medium to identify aneuploid and euploid embryos

Unique embryonic metabolomic profiling

Mean-centered Raman spectra demonstrated differential intensities of SCMs from euploid and aneuploid among Day 5 embryos



Liang B, *et al.* Fertil Steril ,2019

Message from this paper:

- Proof-of-concept of Raman combing machine learning for ploidy prediction
- One type of medium
- Validation size need to be expanded
- Reproducibility

Confusion matrix and performance evaluation of kNN, RF, XGB, and stacking classification models for an independent testing set of 222 Raman spectra.

		Confusion matrix		Performance evaluation			
Model	**Actual class**	**Predicted euploidy**	**Predicted aneuploidy**	**Precision**	**Sensitivity**	**F1 score**	**Accuracy**
kNN	Euploidy	117	8	96.7%	93.6%	0.951	94.6%
	Aneuploidy	4	93	92.1%	95.9%	0.939	
RF	Euploidy	72	53	78.2%	57.6%	0.664	67.1%
	Aneuploidy	20	77	59.2%	79.4%	0.678	
XGB	Euploidy	116	9	92.8%	92.8%	0.928	91.9%
	Aneuploidy	9	88	90.7%	90.7%	0.907	
Stacking	Euploidy	121	4	96.0%	96.8%	0.964	95.9%
	Aneuploidy	5	92	95.8%	94.9%	0.953	

Note: Models were trained from a training set of 885 Raman spectra. kNN = k-nearest neighbors; RF = random forests; XGB = extreme gradient boosting. Stacking analysis is based on a first layer of kNN, RF, and XGB and a second layer of XGB.

Liang B, *et al.* Fertil Steril ,2019

Metabolic profiling predicts the blastocyst development potential

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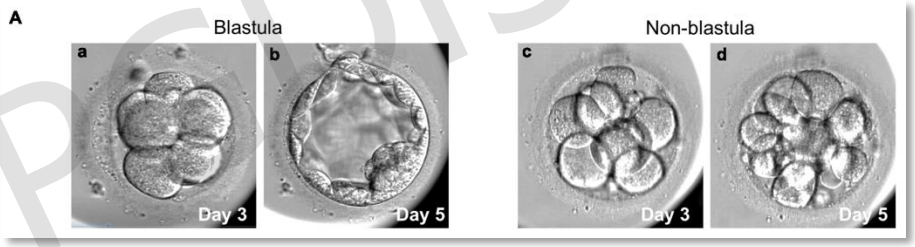
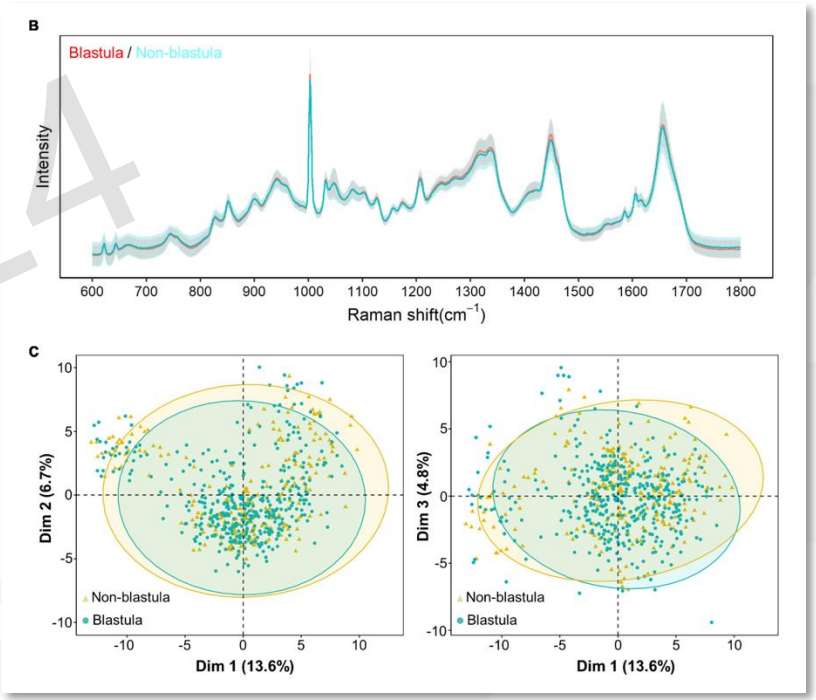
frontiers in Physiology

BRIEF RESEARCH REPORT
published: 19 November 2021
doi: 10.3389/fphys.2021.777259

Non-invasive Metabolomic Profiling of Embryo Culture Medium Using Raman Spectroscopy With Deep Learning Model Predicts the Blastocyst Development Potential of Embryos

prediction sensitivity 73.35%

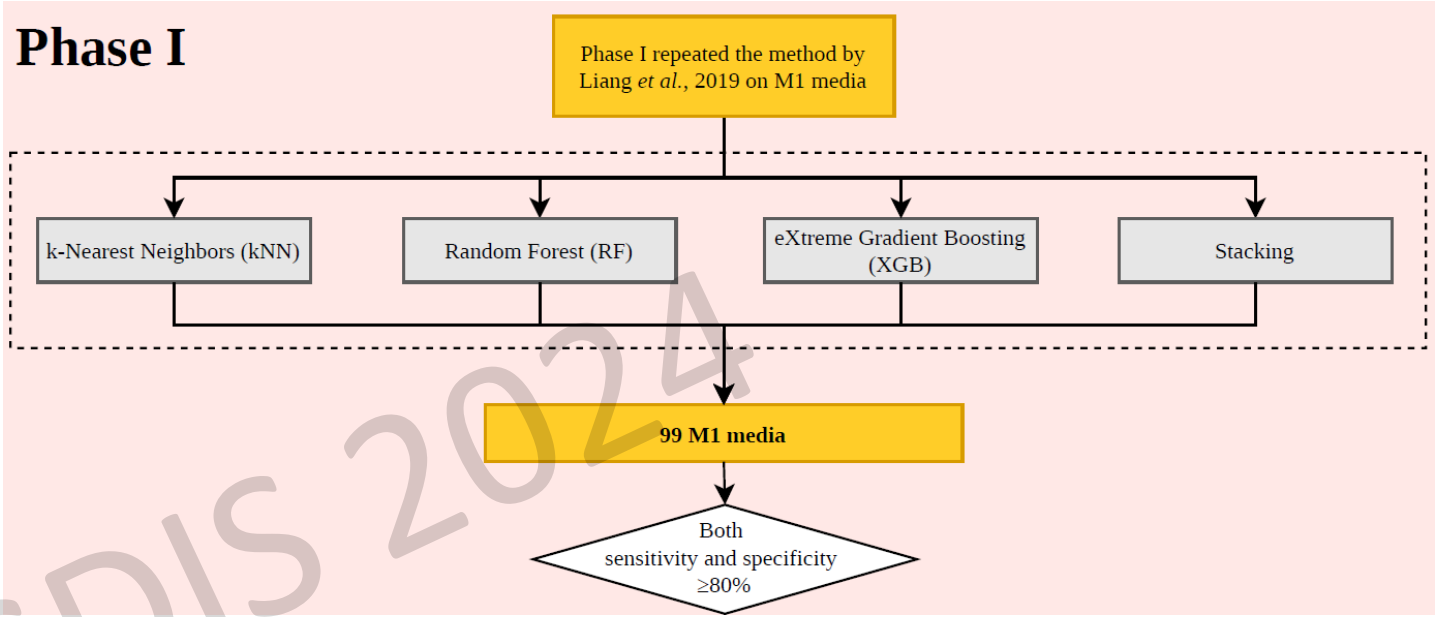




让更多家庭生育健康的孩子

Bo Liang and Liang Hu et al. Frontiers in Physiology . 2021

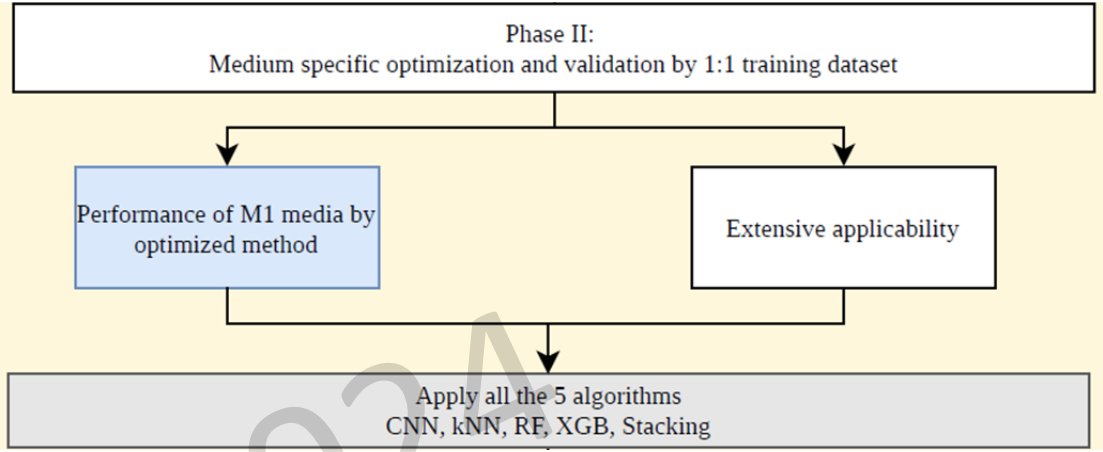
Reproducibility study



Phase I completely repeat the method by Liang *et al.* to test the reproducibility.

Liang B, *et al.* Fertil Steril ,2019

Aim: Optimization and Validation Study



Optimization by:

1. Testing wet lab improved quality control standard
2. Algorithms optimized by re-seeking hyperparameters
3. Euploid & aneuploid for training re-shuffled and retrained
4. Added Convolutional Neural Networks (CNN)

Phase II – Optimization and Validation

Re-trained & tested M1 media

Phase II – M1 Training (30 euploid vs. 30 aneuploid)					
	CNN	kNN	RF	XGB	Stacking
Sensitivity	86.7% (26/30)	93.3% (28/30)	76.7% (23/30)	86.7% (26/30)	93.3% (28/30)
Specificity	83.3% (25/30) ✓	76.7% (23/30)	76.7% (23/30)	100.0% (30/30) ✓	83.3% (25/30) ✓

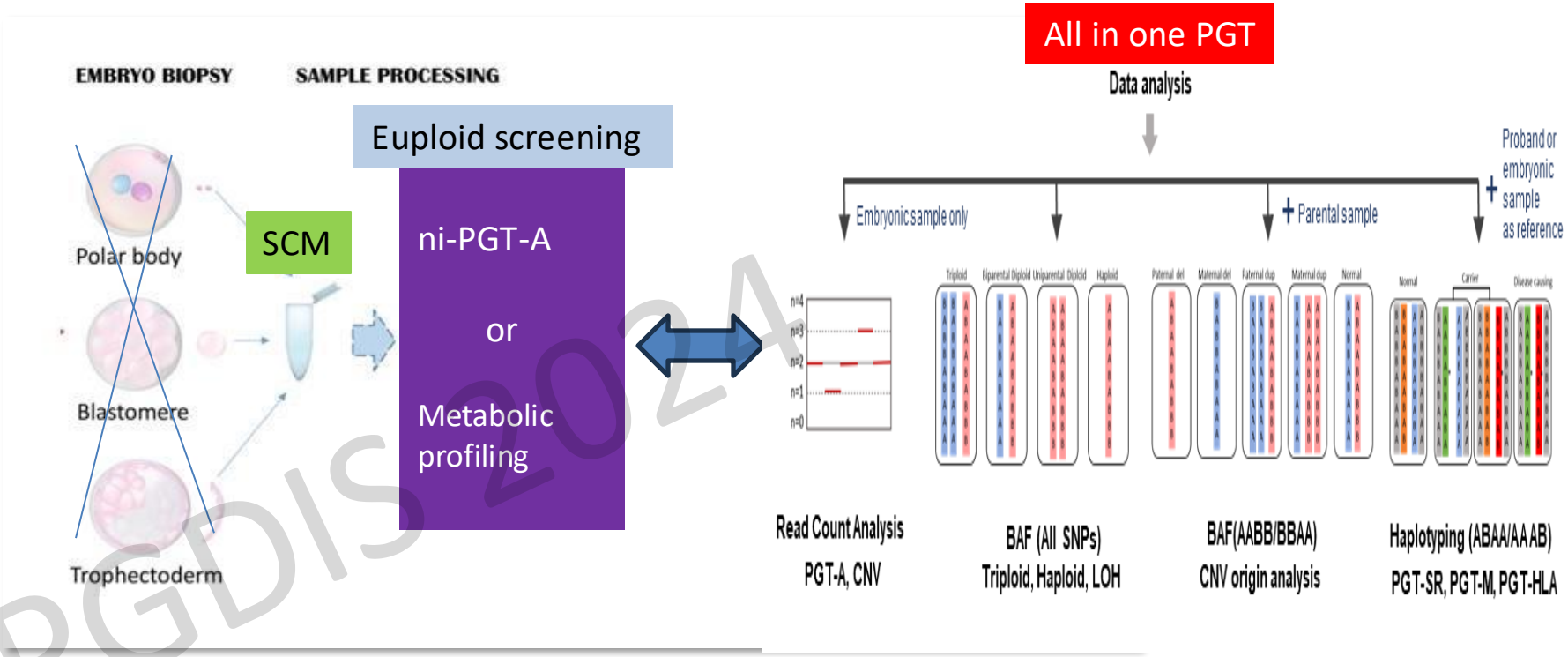
Stacking out	Phase II – M1 Testing (43 euploid vs. 56 aneuploid)				
Sensitivity	75.0% (42/56)	73.2% (41/56)	69.6% (39/56)	76.8% (43/56)	80.4% (45/56) ✓
Specificity	83.7% (36/43)	83.7% (36/43)	81.4% (35/43)	100.0% (43/43)	81.4% (35/43)

Table 3. Performance of Raman spectroscopy pregnancy outcome.

	Euploid transfers by TE biopsy (N=102)	Euploid transfers by Raman* (hypothetical, N=97)		
	n (%)	n (%)	Sensitivity	Specificity
Implantation failure	32 (31.4%)	30 (30.9%)	93.8%	95.7%
Biochemical pregnancy	8 (11.4%)	8 (11.9%)	72.7%	100%
Miscarriage (per clinical pregnancy)	12 (19.4%)	11 (18.6%)	91.7%	96.0%
Ongoing pregnancy/Live birth	50 (49.0%)	48 (49.5%)	96.0%	100%

*: Stacking algorithm was adopted here considering the best performance shown in our current study.

New algorithm for PGT-A



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Thank You!!

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THANK YOU

PGT and BEYOND...