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FINAL RESULTS OF A MULTICENTER STUDY COMPARING CELL-FREE DNA AND TROPHECTODERM BIOPSIES IN 2539 HUMAN BLASTOCYSTS.

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Title:

FINAL RESULTS OF A MULTICENTER STUDY COMPARING CELL-FREE DNA AND TROPHECTODERM BIOPSIES IN 2539 HUMAN BLASTOCYSTS.

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Preferred Presentation Type:

Oral or Poster

Study Type:

Prospective Observational/ Cohort

Category - Subcategory(ies):

Genetics: PGT

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ACCME Disclosure

Nothing to disclose. No off-label or otherwise non-approved product use.

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Abstract Text:

OBJECTIVE: This study aimed to evaluate the intrinsic and extrinsic factors that can have an impact in the concordance rate, when testing for chromosomal abnormalities in cell-free DNA (cfDNA) and trophoctoderm (TE) biopsies obtained from the same blastocysts.

MATERIALS AND METHODS: We carried out a prospective study to investigate the concordance of cfDNA present in spent blastocyst medium with the corresponding TE biopsy in 10 IVF clinics. A total of 2539 day-6/7 human blastocysts from 716 patients underwent media collection and TE biopsy from April 2018 to December 2022. Embryos were cultured in routine conditions up to day 4, when embryos were washed, transferred to a new 10µl medium droplet, and cultured for at least a further 48 hours. Then, culture media were collected and frozen at -20°C. Assisted hatching, TE biopsy and vitrification were performed after media collection. All samples were analyzed by next generation sequencing (NGS) using the Ion ReproSeq PGS Kit (ThermoFisher Scientific) and the Ion Chef plus the Ion S5 XL Sequencer. R (version 4.2.1) was used for the statistical analysis. A multivariate logistic regression analysis was performed to identify the variables affecting the concordance rate, their adjusted odds ratio (aOR) and confidence interval (CI).

RESULTS: From the 2539 embryos analyzed, we obtained a result in 2208 cfDNA-TE pairs. In 1726 of them, both cfDNA/TE were euploid or aneuploid, corresponding to a ploidy concordance rate of 78.2%. It was not statistically different between the 10 participating centres (73.8-83.1%, p=0.12). The multivariate analysis showed that only the number of NGS reads in the media was significantly related to the concordance rate. See table below:

| Variable | aOR | 95% CI | p-value |
|-----------------------------------|------|-------------|---------|
| Female age | 1.03 | 0.98 - 1.08 | 0.27 |
| Body mass index | 1.00 | 0.97 - 1.04 | 0.85 |
| No. previous implantation failure | 1.02 | 0.90 - 1.18 | 0.73 |
| No. previous miscarriages | 1.10 | 0.93 - 1.31 | 0.29 |
| No. previous live birth | 1.08 | 0.88 - 1.34 | 0.49 |

| | | | |
|---|------|-------------|------|
| Oocyte origin (own/donated) | 0.73 | 0.19 - 2.27 | 0.61 |
| No. MII oocytes | 0.96 | 0.91 - 1.02 | 0.19 |
| Type of fertilization (ICSI/IVF) | - | - | 0.53 |
| No. 2PN | 1.04 | 0.97 - 1.12 | 0.26 |
| Culture conditions (media and incubator used) | - | - | 0.67 |
| No. blastocysts analyzed | 1.05 | 0.99 - 1.11 | 0.14 |
| Embryo quality | 0.79 | 0.52 - 1.19 | 0.26 |
| Expansion degree | - | - | 0.68 |
| Day of media collection | 1.33 | 0.34 - 5.34 | 0.68 |
| No. NGS reads | 1.19 | 1.01 - 1.40 | 0.04 |
| Media result (euploid/aneuploid) | 1.09 | 0.79 - 1.49 | 0.61 |

CONCLUSIONS: Only the number of NGS reads in the media was significantly related to the concordance rate, showing that sample quality (cfDNA concentration in the culture media) deeply impacts in the results.

IMPACT STATEMENT: Embryo cfDNA analysis shows very robust results independently of the patient infertility background, stimulation response, culture conditions and blastocyst quality. Therefore, it can be widely applied as a non-invasive approach.

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Biographical Sketch Dr. Denny Sakkas received his undergraduate training at the University of Melbourne, Australia and received his Doctorate of Philosophy at Monash University, Melbourne, Australia. He serves as Deputy Editor of Human Reproduction. He is currently Chief Scientific Officer at Boston IVF and Associate Professor at the Department of Obstetrics, Gynecology and Reproductive Sciences at the Yale University School of Medicine.

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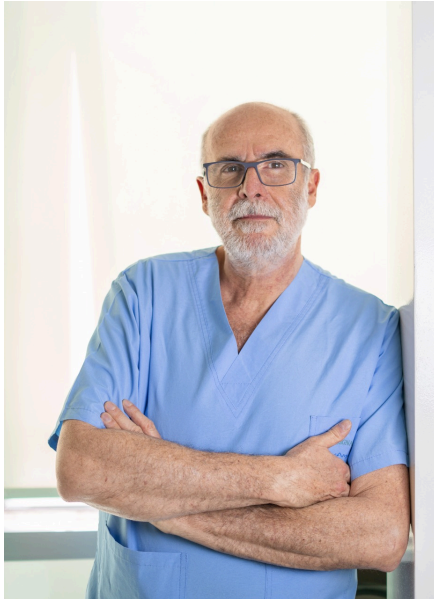
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Yes

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| Igenomix R&D | Full-Time Company Employee Relationship Began - Tuesday, February 24, 2015 Relationship Ended - | Self | |

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Biographical Sketch Carlos Simón is Professor of Ob/Gyn at the University of Valencia, Spain; Senior Lecturer PT, BIDMC Harvard University, Boston, MA, USA, and Adjunct Clinical Professor at Baylor College of Medicine, USA. His main clinical and scientific interest is in the understanding of the human embryonic implantation process, a critical process to the survival of the species, considering as key elements the embryo, the maternal endometrium, and the cross-communication between them. In human endometrial research, his group identified the transcriptomic signature of human endometrial receptivity using microarray technology (PMID: 20619403) and its confirmation by single-cell RNA seq (PMID: 32929266). Clinical translation of this work resulted in the creation of the endometrial receptivity analysis (ERA) for the diagnosis of the personalized window of implantation in infertile patients. His team provided evidence of a decidualization defect in the endometrium of women with severe preeclampsia, a pathology detectable at the time of delivery and persisting for years (PMC: 28923940), further discovering the footprint encoding this defect (PMC: 8553341). Also, they demonstrated that the human uterine cavity is not sterile, by identifying the existence of the endometrial microbiome (PMID: 27717732) and its functional implications in pregnancy outcome in infertile patients (PMID: 34980280). Further, they investigated the existence and the functional proof of concept of human endometrial stem cells. Today, these findings are being translated to the first advanced cellular therapy of Asherman Syndrome ((PMID: 27005892)(EudraCT Number: 2016-003975- 23)). For the human embryo, his created a prediction model for aneuploidy in early embryo development revealed by single-cell analysis (PMID: 26151134), deciphered the clinical impact of embryo mosaicism (PMID: 34798051), and discovered the origin, and composition of human embryo-cell free DNA (PMID: 29471395) and its clinical translation (PMID: 32470458)). His team derived, characterized, and registered 10 human embryonic stem cell lines in the Spanish National Stem Cell Bank. (PMID: 20018958). His pioneering work in this field made possible the creation of the Valencia Node of the Spanish Stem Cell Bank in 2004. Finally, the cross-communication between maternal endometrium and the embryo (PMID: 29390102) has been addressed by discovering that maternal microRNAs

(miRNAs) that might act as transcriptomic modifier of the pre-implantation embryo (PMID: 26395145). His commitment to excellence in research is demonstrated by the publication of 534 papers (Pubmed) in peer-reviewed journals with an accumulated impact factor of 3,814.94. His papers have received a total of 47,701 citations. His Google Scholar is 125. He is editor of 21 books in English, Spanish, and Portuguese, and supervisor of 38 PhD Thesis. His work has been awarded by several scientific societies including SGI/SRI (see SRI related CV) and institutions including the Rey Jaime I Medical Research Award 2011, the ASRM Distinguished Research Award 2016, and the Lilly Foundation Biomedical Research Award 2021.

Within the past 2 years, have you or your spouse/partner had any potential COI?

No

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Biographical Sketch Trained in Science and Embryology at the University of Valencia, Spain, Dr Carmen Rubio specialized in cytogenetic studies in human reproduction, partly at the University of Barcelona. She completed her PhD in the field of Reproductive Genetics and post-doctoral research included research in male and female meiosis at the laboratory of Drs. Patricia Hunt and Terry Hassold (Washington State University, USA). She has published more than 100 papers in the main peer-reviewed specialist journals in the field, books chapters as well as numerous lectures at conferences worldwide. She is appointed as lecturer in post-graduate courses in Reproductive Genetics, supervising PhD students, and she is an active member of ESHRE, ASRM and board member of the PGDIS society. Currently she is the head of the Research & Development department at Igenomix (Vitrolife group). Her main current research interests after 30 years working in this field, are non-invasive approaches of genetic testing, being particularly active in the study of chromosomal abnormalities in the cell-free DNA released into the culture medium as a non-invasive approach for the assessment of embryo viability.

Within the past 2 years, have you or your spouse/partner had any potential COI?

Yes

| Organization Name | Relationship Type | Who has this Relationship? | |
|----------------------------|--|----------------------------|--|
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