EMBRYOTEST PLUS™

Result Report for Preimplantation Genetic Testing (PGT-A).

The following report lists the results of the patient's embryo biopsies in **General Data** hereunder. The herein does not represent any diagnosis or suggestion. It only informs the findings retrieved by processing using Next Generation Sequencing (NGS) of each sample.

Patient Name:		**Date:**	
Medical Center:		**Batch ID:**	
Doctor:		**Sample No.:**	
Email:		**Phone(s):**	

GENERAL DATA

REQUESTED STUDY

There are normally 23 pairs of chromosomes in each cell of our body. An aneuploidy means a change in the number of chromosomes. **EmbryoTest Plus™ (ETP)** analyzes the 46 chromosomes of human embryos to detect possible gains or losses of genetic material (aneuploidies) during their cellular division. The Next Generation Sequencing technique is used to screen the chromosomes. It represents a very useful test as it supports Assisted Reproductive Technology (ART) and reproductive medicine.

RATIONALE

Since alterations in the set of chromosomes can cause failed implantations in ART cycles, spontaneous abortion, and chromosomal abnormalities in newborn babies, **EmbryoTest Plus™** allows the selection of those chromosomally normal embryos from all evolutionary embryos of a patient. This increases their reproductive possibilities and the chances of evolving correctly leading to a healthy child.

EmbryoTest Plus™ increases the chances of transferring embryos free of chromosomal abnormalities to the mother. Aneuploidies can cause spontaneous abortion leading to congenital defects and intellectual disability in newborn babies or they are not compatible with life. However, within the viable chromosomal aneuploidies that can be detected, there are the most common syndromes, non-sexual, such as Down Syndrome, Edwards Syndrome, and Patau Syndrome.

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METHODOLOGY

EmbryoTest Plus™ consists of a series of steps with quality controls in each one of them.

1. Embryo Biopsy: It consists of extracting some embryonic stem cells for genetic study. They are evaluated according to internationally established criteria to determine embryo viability.
2. Whole Genome Amplification (WGA): It consists of making genetic material, or genome, copies of the embryonic stem cell to have a greater quantity of work material for the test.
3. WGA Quality Control: It is made through the electrophoresis technique, which allows the visualization of amplified genetic material quality in the previous step. Likewise, a fluorometric method is used to quantify the amplified genome to continue the process.
4. Library Generation: DNA fragment synthesis compatible with the flow cell using WGA.
5. Cluster Generation: It is a "bridge amplification" process that generates copies of each one of the fragments attached to the flow cell, leading to clusters.
6. Next-Generation Sequencing: It is a sequencing process by synthesis.
7. File Generation: It is a process in which the sequencing generates database files (FASTQ) and genome assembly (BAM).
8. Result Analysis: FASTQ and BAM are analyzed through mathematical algorithms to generate graphs that allow the semiquantitative and qualitative analysis of aneuploidies.
9. These kinds of tests pose a risk of false positive/negative results that occur by sample contamination, codification problems, rare genetics that interferes with the analysis, technical issues, and human error. Among all these factors, the chance of a false result that is not associated with mosaicism is about 2%.
10. On the other hand, errors in result interpretation commonly occur by biological reasons called "mosaicisms", present in approximately 5% of embryo biopsies.
11. Embryonic mosaicism means that the embryo has different cell lines. These may or may not have the same ploidy (chromosome number) All the above is summarized in the below results table.

RESULTS TABLE

No.	ID Doctor	Cycle (Cycle ID)	PIN	Embryo	Quality Control	Sex	Result
1	BV	12345	YG	1	Yes	NA	CA
2	BV	12345	YG	4	Yes	Feminine (XX)	Abnormal: seg(chr4)3.7x, (chr9)3.0x
3	BV	12345	YG	5	Yes	Feminine (XX)	Abnormal: Low-level mosaic (chr7)1.65x

PIN: Personal Identification Number, CA = Complex Abnormal = When there are more than 5 aneuploidies present in embryonic stem cells.
chr = chromosome, x = copy number, seg = segmentation.

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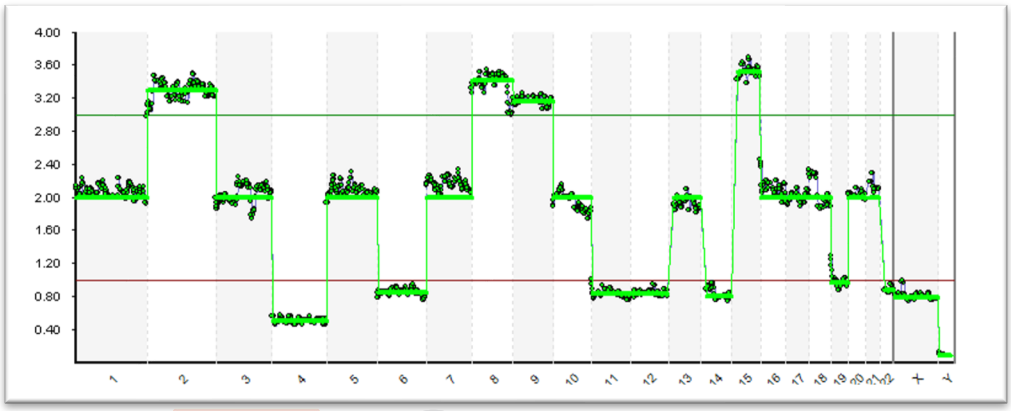
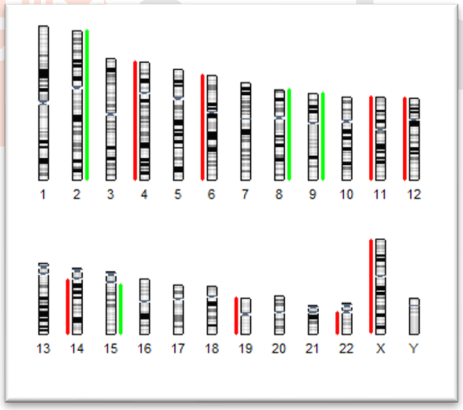
GRAPHS

No.	ID Doctor	Cycle (Cycle ID)	PIN	Embryo	Quality Control	Sex	Result
1	BV	12345	YG	1	Yes	NA	CA





Observations: It is a complex abnormal sample.

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Patient: YG
Doctor: BV
Clinic: Semper Genomics
Date: 03-23-2023

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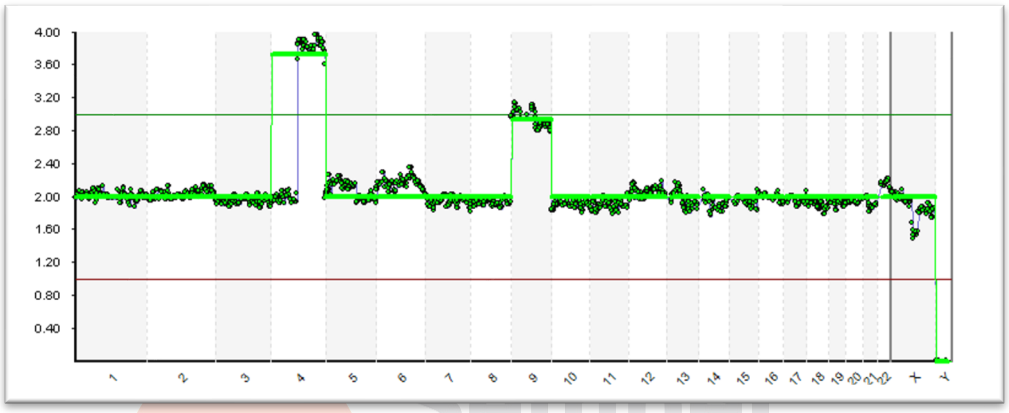
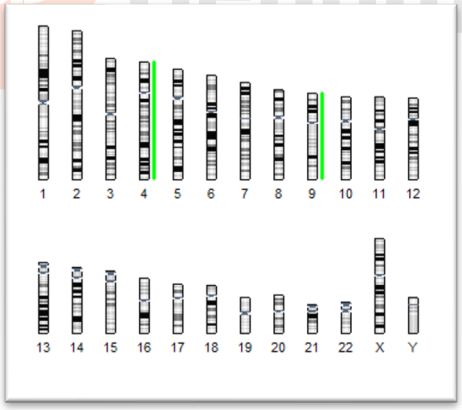
No.	ID Doctor	Cycle (Cycle ID)	PIN	Embryo	Quality Control	Sex	Result
2	BV	12345	YG	4	Yes	Feminine (XX)	Abnormal: seg(chr4)3.7x, (chr9)3.0x





Observations: Segmental chromosomal gain (3.7x) and gain in chromosome 9 (3.7x).

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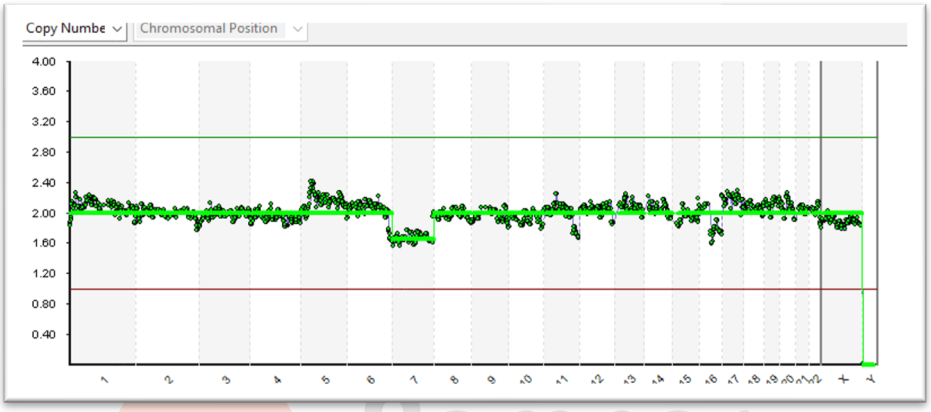
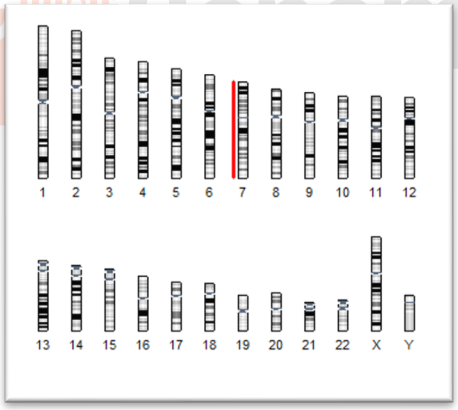
No.	ID Doctor	Cycle (Cycle ID)	PIN	Embryo	Quality Control	Sex	Result
1	BV	12345	YG	5	Yes	Feminine (XX)	Anormal: low-level mosaic (chr7)1.65x





Observations: The sample has a low-level mosaic in chromosome 7 (1.65x)

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RESULT INTERPRETATION

Analysis software generates a graph that represents the number of copies of each analyzed embryo chromosome, and it compares it with a reference normal human genome. An embryo is considered normal when any chromosome has differences from the reference genome. An embryo is considered abnormal when there is the presence of one aneuploidy in any of the chromosomes, either a loss (-) or a gain (+) in the graph. The presence of more than five (5) aneuploidies is considered a complex aneuploidy. An embryo that was not detected does not provide information.

If the loss or gain of genetic material does not encompass the whole chromosome, this is recorded as partial or segmental loss or gain. Mosaicism is the presence of two or more independent cell lines in the same sample. In the report, it is interpreted as *normal* if the copy number result of autosomal chromosomes is 1.8-2.2x, low-level mosaic if the result is in a range of 2.2-2.5x (gain) or 1.5-1.8x (loss), high-level mosaic if the result is in a range of 2.5-2.8x (gain) or 1.2-1.5x (loss), aneuploidy if 2.8x or more (gain), 1.2x or less (loss).

TECHNIQUE LIMITATIONS

EmbryoTest Plus™ is not a test for the detection of monogenetic or specific mutations. It is neither possible the detection of specific structural chromosomal rearrangements.

DATA PRIVACY

The test results will be protected under strict privacy, and they can be provided by email only to the assigned people herein. The data and records privacy will be 90 calendar days from the sending of results to the client.

TERMINATION OF SERVICE

Semper Genomics will terminate the service in accordance with the results 72 hours after the file sending if no notification is received from the client.

REFERENCES

Fiorentino F, Bono S, Biricik A, et al. Application of next-generation sequencing technology for comprehensive aneuploidy screening of blastocysts in clinical preimplantation genetic screening cycles. Hum Reprod. 2014;29(12):2802–2813.

Yang Z, Liu J, Collins GS, Salem SA, et al. Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis patients: results from a randomized pilot study. Mol Cytogenetic. 2012;5: 24.

Nidhee M Sachdev, Susan M Maxwell, Andria GBesser, James A Grifo, Diagnosis and clinical management of embryonic mosaicism, Fertil Steril, 2017 Jan;107(1):6-11. PMID: 27842993

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Performed by	Reviewed by	Approved by
Signature	Signature	Signature
Bárbara Verduzco Garza Technical Manager	QFB Fabiola Morales Mandujano Health Manager	Saúl Karr de León Quality Manager

*This document DOES NOT represent a diagnosis. For decision-making based on your medical history, you must reach out to your doctor.

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