RHS
Reproductive Health Science

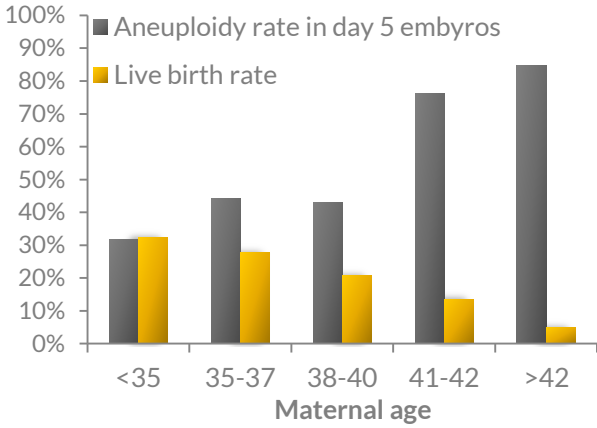
EmbryoCellect™

Pre-implantation Genetic Screening Kit

TECHNICAL INFORMATION

Aneuploidy

Whole chromosome aneuploidy has been shown to affect all chromosomes in IVF embryos. Aneuploidy is a significant cause of IVF failure, especially in women of advanced maternal age.



Harton et al, 2013 Fert Steril Dec 100 (6): 1695-703 & HFEA Fertility Treatment in 2012: trends and figures

Clinical studies show higher SET pregnancy rates with PGS

Scott et al. Fert Steril Sep 2014 102 (3): 660-661

Pre-implantation Genetic Screening (PGS)

Initial attempts to detect aneuploidy in IVF embryos used FISH screening for a limited subset of chromosomes (5-12 chromosomes only). Clinical data from these first attempts showed no benefit to IVF success rates.

This has changed dramatically since the introduction of advanced 24 chromosome pre-implantation genetic screening (PGS). PGS now assesses the loss or gain of any whole chromosomes.

PGS has been demonstrated to:

- reduce the time to pregnancy;
- reduce the incidence of miscarriage;
- achieve comparable single embryo transfer clinical pregnancy rates to unscreened multiple embryo transfer
- allow the selection of unaffected embryos for vitrification (freezing) avoiding the storage of aneuploid embryos; and
- overcome the maternal age impact on IVF success.

98 percent of aneuploid embryos fail to implant

Scott et al 2012 Fert Steril Apr 97 (4): 870-5

HOW EMBRYOCELLECT™ WORKS

EmbryoCellect™ has been designed to specifically screen for **whole chromosome aneuploidy**. It uses array Comparative Genomic Hybridisation (aCGH) to compare the number of chromosomes from a sample cell to a known reference sample. The samples are labelled and the relative fluorescence is measured for each chromosome by hybridisation to the EmbryoCellect™ microarray.

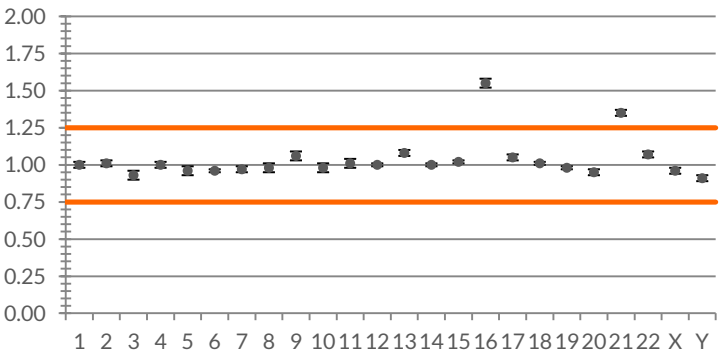
A sample is placed into a PCR tube and enzymatically lysed. The EmbryoCellect™ DOP-PCR then robustly amplifies the genome millions of times

The reference sample is labelled with red fluorescent dye

The test sample is labelled with green fluorescent dye

The test and reference are combined and they compete for binding positions on the EmbryoCellect™ microarray

After microarray scanning and rapid data analysis, the relative fluorescence signals of the test and reference are compared



Outliers indicating extra copies (trisomy) of chromosomes 16 and 21

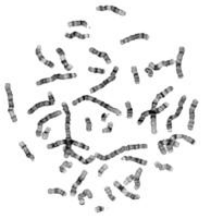
Equal number of chromosomes to the male reference

EmbryoCellect™ is for research use only and is not for use in diagnostic procedures.

This EmbryoCellect™ result was generated from a single fibroblast from a male cell line with trisomy for chromosomes 16 and 21 (48,XY,+16,+21).

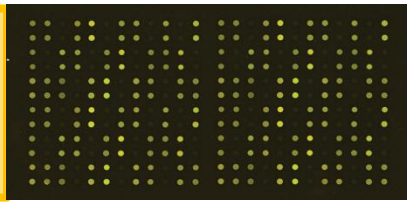
WHAT IS PRINTED ON THE EMBRYOCELLECT™ MICROARRAY?

Multiple copies of single human metaphase chromosomes are laser captured



The chromosomes are whole genome amplified using RHS DOP-PCR then repeat depleted

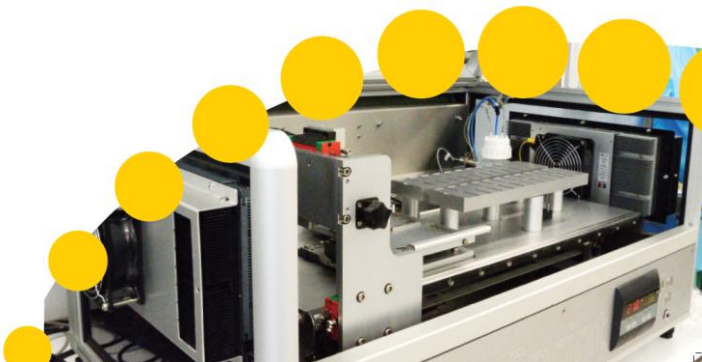
Whole chromosome paints containing on average 1.2million sequences per chromosome are printed on microarrays in replicates of 8 per microarray. There are 4 microarrays per slide.



The EmbryoCellect™ microarray product attributes

What is printed on the array:	Chromosome-specific PCR products from 120bp-4kb
How are the printed:	As a pooled library of sequences specific to each chromosome.
Number of array targets:	Over 35 million. On average 1.2m sequences per spot
Replicate targets on chip:	1 spot per chromosome. 8 replicates
Microarrays per slide:	4 allowing the testing of 4 samples at a time
Ease of analysis:	There is a single spot per chromosome
Software:	Excel Macro

EmbryoCellect™ has been specifically developed to screen for whole chromosome aneuploidy in single cells



Why EmbryoCellect™?

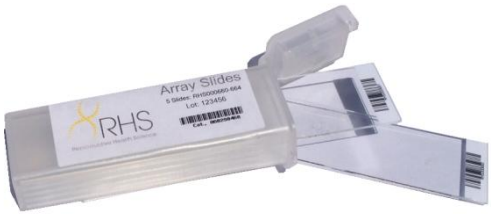
- The test is simple and robust;
- The results are easy to interpret;
- The raw scanner data is available; and
- The test has been validated for accuracy

Samples can be processed in batches as small as four

The EmbryoCellect™ kit contains enough reagents to test 20 samples

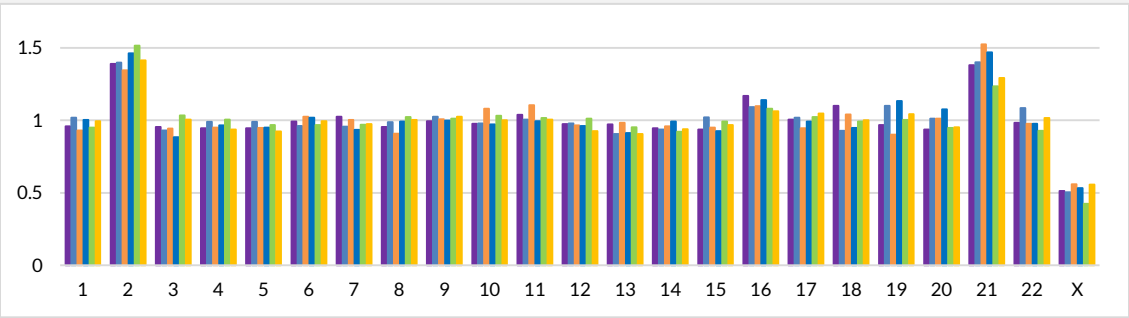
The EmbryoCellect™ kit contains:

- Cell lysis buffer and enzyme for test and reference
- Reference male gDNA
- Whole Genome Amplification (WGA) reagents
- Fluorescent labelling PCR reagents
- Five patented EmbryoCellect™ microarray slides with 4 microarrays per slide



EmbryoCellect™ has been validated using single cells from a range of euploid and aneuploid cell lines and tested on trophectoderm biopsies.

The microarray data has been further validated by Next Generation Sequencing on both the MiSeq and Ion Torrent sequencing platforms.



MiSeq reads for 48,XY,+2,+21 single cells normalized to 46,XX single cells

THE EMBRYOCELLECT™ WORKFLOW

Protocol Step	Explanation
Cell lysis	Following biopsy, a gentle but effective enzyme-based lysis procedure ensures robust cell lysis and a readily accessible DNA template for whole genome amplification. (15 mins)
Whole genome amplification	Whole genome amplification is performed using RHS's DOP-PCR, which has been optimised for the RHS microarray. DOP-PCR uses degenerate primers to initiate DNA amplification, binding across a broad range of different sequences scattered genome wide. (2.5 hrs)
Agarose gel assessment	Following amplification, the use of agarose gel electrophoresis is recommended to ensure that cell amplification has been successful. (30 mins)
Labelling PCR	Successfully amplified samples are fluorescently labelled by a second DOP-PCR. The test is labelled with a Cy3 equivalent dye and the reference with a Cy5 equivalent dye. (45 mins)
Clean-up and nanodrop Agarose gel assessment	Once purified, these labelled amplicons are again assessed using agarose gel electrophoresis and spectrophotometry to ensure adequate amplification and dye incorporation has occurred. (1 hour)
Hybridisation, Microarray washing, Microarray scanning and analysis	Samples are competitively hybridized to the RHS microarray. (3 hours to overnight). After incubation, the microarray is washed and scanned (1 hour). The ratio of test to reference dye intensity after normalization is determined using RHS proprietary software, providing the ploidy status of each chromosome in each sample.

EmbryoCellect™ Requirements

SPACE

A dedicated PCR-free laboratory equipped with laminar flow, dedicated pipettes and thermocycler or heating block (for cell lysis) is required for cell lysis, master mix set-up for whole genome amplification and labelling. It is imperative to maintain a clean, tidy work space limiting possible opportunities for DNA contamination. Agarose gel electrophoresis and handling of amplified DNA should be performed away from any master-mix set-up in a separate laboratory space.

STAFF TECHNICAL REQUIREMENTS

Experience in molecular biology and or PCR is desirable, however training a technician with experience in handling small starting materials is possible. The technician should be a diligent and tidy worker to avoid contamination issues. More experienced laboratory personnel are required to monitor and oversee general work flow, perform quality control checks and interpret the data, particularly assessing the quality and reliability of a result.

EQUIPMENT

The main equipment required when establishing a laboratory to use EmbryoCellect™ are:

- PCR machine;
- DNA purification system;
- Gel electrophoresis system
- Nanodrop Spectrophotometer;
- Centrifuge (4 -20°C);
- Hybridization Chamber;
- 37°C Incubation oven;
- Microarray high-speed centrifuge;
- Microarray slide scanner; and
- Other common laboratory equipment.

FOR ESTABLISHED aCGH LABORATORIES

If you already equipped to perform aCGH, EmbryoCellect™ does not require any different or additional equipment or human resources; only minimum training and adjustment to already established laboratory protocols.

About Reproductive Health Science Ltd

Reproductive Health Science Ltd (ASX:RHS) is a developer of advanced single cell genomic technologies with a focus on improving health and research outcomes.

Further background reading

Comparative genomic hybridization selection of blastocysts for repeated implantation failure treatment: a pilot study. Greco E, Bono S, Ruberti A, Lobascio AM, Greco P, Biricik A, Spizzichino L, Greco A, Tesarik J, Minasi MG, Fiorentino F. BioMed Research International 2014:457913

Diminished effect of maternal age on implantation after preimplantation genetic diagnosis with array comparative genomic hybridization. Harton GL, Munné S, Surrey M, Grifo J, Kaplan B, McCulloh DH, Griffin DK, Wells D; PGD Practitioners Group. Fertility and Sterility 2013 Dec;100(6):1695-703

Preimplantation genetic screening (PGS) with Comparative genomic hybridization (CGH) following day 3 single cell blastomere biopsy markedly improves IVF outcomes while lowering multiple pregnancies and miscarriages. Keltz MD, Vega M, Sirota I, Lederman M, Moshier EL, Gonzales E, Stein D. Journal of Assisted Reproduction and Genetics 2013 Oct;30(10):1339-9

Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. Scott RT Jr, Upham KM, Forman EJ, Zhao T, Treff NR. Fertility and Sterility 2013 Sep;100(3):624-30

Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. Scott RT Jr, Upham KM, Forman EJ, Hong KH, Scott KL, Taylor D, Tao X, Treff NR. Fertility and Sterility 2013 Sep;100(3):697-703

In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial. Forman EJ, Hong KH, Ferry KM, Tao X, Taylor D, Levy B, Treff NR, Scott RT Jr. Fertility and Sterility July 2013 100-7

Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study. Yang Z, Liu J, Collins GS, Salem SA, Liu X, Lyle SS, Peck AC, Sills ES, Salem RD. Molecular Cytogenetics 2012 May 2;5(1):24

Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study. Scott RT Jr, Ferry K, Su J, Tao X, Scott K, Treff NR. Fertility and Sterility 2012 Apr:97(4):870-5

RHS inventor publications

Gender determination and detection of aneuploidy in single cells using DNA array-based comparative genomic hybridization. Hu DG, Guan XY, Hussey N. Methods in Molecular Medicine 2007;132:135-51

Singleton births after routine preimplantation genetic diagnosis using exclusion testing (D4S43 and D4S126) for Huntington's disease. Jasper MJ, Hu DG, Liebelt J, Sherrin D, Watson R, Tremellen KP, Hussey ND. Fertility and Sterility 2006 Mar;85(3):597-602

Aneuploidy detection in single cells using DNA array-based comparative genomic hybridization. Hu DG, Webb G, Hussey N. Molecular Human Reproduction. 2004 Apr;10(4):283-6

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