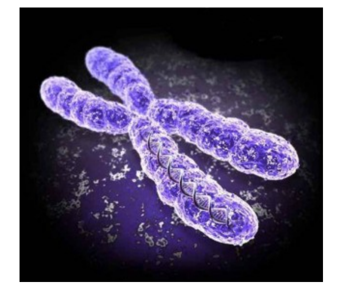
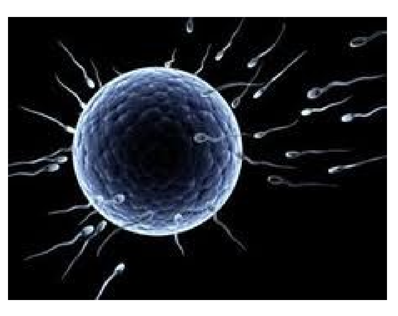




Utilization of genetic analyses in human assisted reproduction process

Mgr. Jan Smetana, Ph.D

Ústav Experimentální Biologie, PřF MU
Laboratoř molekulární cytogenetiky
OLG FN Brno

Assisted human reproduction (AHR)

- Assisted reproduction is the name given to medical procedures and methods in which germ cells or embryos are manipulated, including their storage, for the purpose of treating infertility in women or men
- The complex process is now mostly based on *in vitro* fertilization techniques
- In addition to couples with a diagnosis, couples with normal fertility also benefit, due to the risk of transmitting genetic defects or pathological markers
- Specialized centers - clinics, sanatoriums
- Goal - birth of healthy offspring = "infertility treatment"

Fertility

Fertility - definition

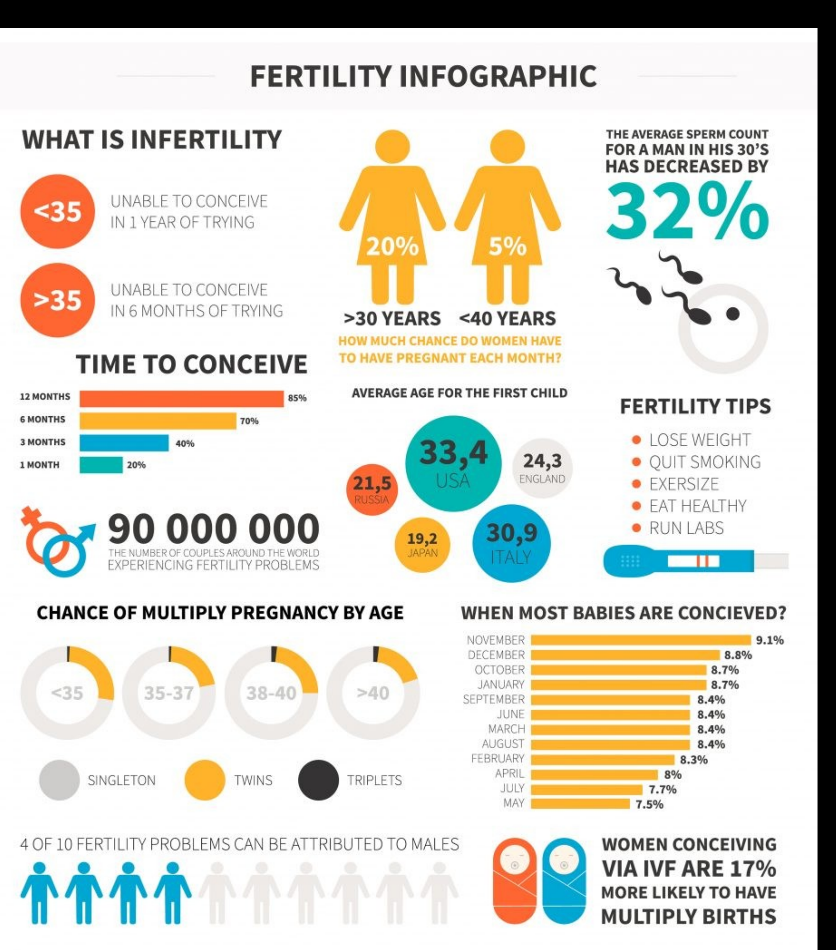
1) The ability of an individual to reproduce sexually

2) A complex trait that results from the ability of males and females to produce healthy offspring in optimal numbers over time

3) Demographic indicator expressing the average number of offspring per female

Infertility

Failure to achieve clinical pregnancy after 12 months or more of unprotected regular sexual intercourse (WHO)



INFERTILITY BY GENDER

Female 40-50% Male 30-40%

CAUSES

LIFESTYLE

- Increasing marital age
- Putting off having children because of career ambitions
- Rising alcohol and tobacco consumption
- Rising level of obesity

CLINICAL FACTORS

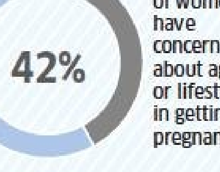
Increasing prevalence of medical conditions such as

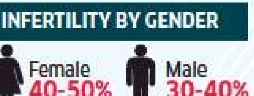
- PCOS
- Endometrial tuberculosis
- Sexually Transmitted Infections (STIs)

TREATMENT

- Lifestyle changes
- Usage of fertility monitors
- IVF

45% COUPLES WHO VISITED A DOCTOR WHEN TRYING TO CONCEIVE





42% of women have concerns about age or lifestyle in getting pregnant

45% Feel either anxiety or stress in trying to conceive

Men with spouse >30 have the highest pressure to have a baby

64% OF THE MEN FEEL PRESSURE TO HAVE A CHILD IF THEIR WIFE IS OVER 30

ISSUES IN TREATMENT

Lack of awareness about ovulation-tracking products like fertility monitors

55% 75%

1% Percentage of infertile couples who seek treatment

TEXT: SALIHA NASLINE

Causes of female infertility

Anovulation

Pelvic adhesions

Ovarian factor

Tubal factor

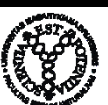
Uterine factor

Cervical factor

ovarian factor - the ovary does not form or does not release a quality viable egg

tubal factor - damage to the fallopian tubes, missing fallopian tubes, obstructed fallopian tubes

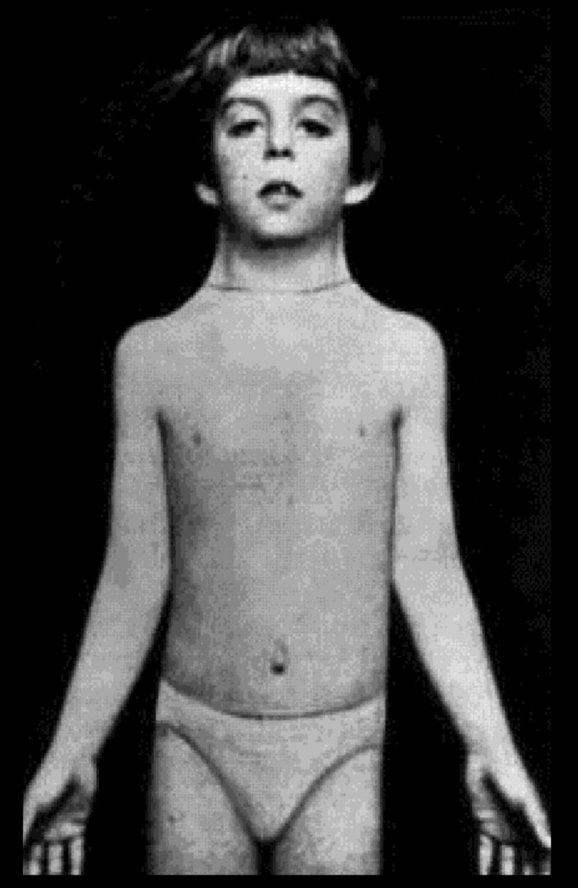
endometriosis - presence of uterine lining outside the uterine cavity



Genetic causes of female infertility

Chromosome aberrations – structural ornumerical

- Turner syndrome - 45, X
- "Superwomen" - 47, XXX
- Aneuploidy in gametes
- Robertsonian translocations, centromeric fusion acrocentric chromosomes (13-15, 21, 22)



Mutations - genes affecting blood clotting

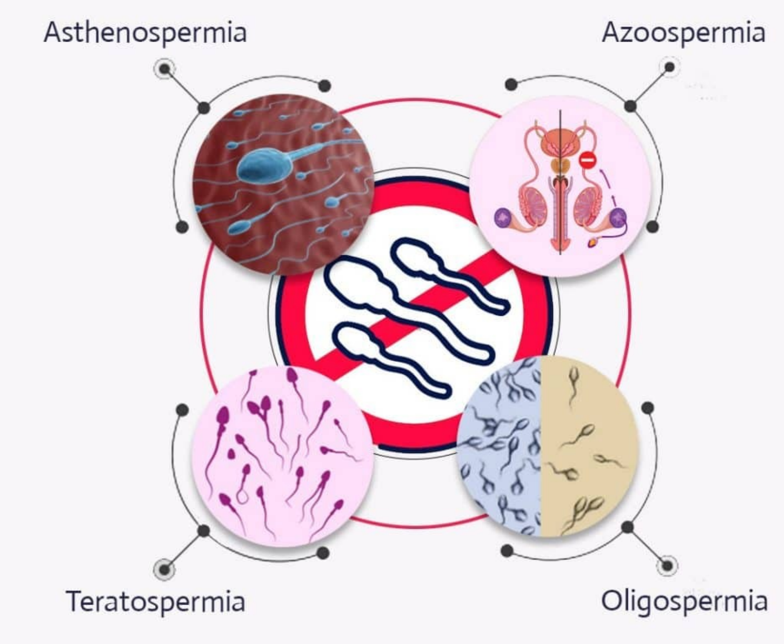
- *MTHFR* (1p36.3)
- Leiden mutation (F5 - 1q23),
- G20210A in the thrombin gene
- CFTR

Causes of male infertitlity

Male factors

poor sperm function – sperm incompetence to penetrate and fertilize a woman's egg

- **Oligozoospermia**
  - ($<15*10^6$ in ejaculate)
- **Asthenozoospermia**
  - lack of motility
- **Teratozoospermia**
  - abnormal morphology
- **Azoospermia**
  - absence of sperm in the ejaculate



Genetic causes of male infertility

Chromosomal aberrations

- Klinefelter syndrome - 47, XXY
- Males - 47, XYY
- Structural abnormalities chr. Y
  - Deletion in (Yp)(11.3) - SRY - disorder of genitourinary development
  - Deletion Yq11 - AZF - azoosperima factor = disorder of sperm development

- Autosomal translocations, Y/A, Robertsonian translocations - centromeric fusion of acrocentric chromosomes (13-15, 21, 22)
- Aneuploidy in gametes (X,Y, 21, 13,18)

Gene mutations

Cystic fibrosis - F508 mutation in CFTR1, 97% of men infertile

History of IVF

- **17th century-** van Graaf - Graaf follicles, van Leeuwenhoek - observation of mammalian sperm
- **19th century** - first scientific papers on in vitro fertilization in animalsSchenk (1878), W. Heape - birth of 6 rabbit pups after vitro fertilization (1890).
- **1944** - Rock, Menkin - in vitro fertilization of human oocyte
- **1951** - Austin, Chang - the fertilizing ability of sperm is essential for its previous residence in the female genital tract (sperm capacitation)
- **2nd half of the 20th century - Cambridge University - R.G. Edwards**
- Description of oocyte maturation and in vitro fertilization, possibility of
- embryo culture
- **1971 - Steptoe, Purdy:** Nature - possibility of *in vitro* culture of human embryos
- to the blastocyst stage
- **Late 70s** - improvements in culture media, laparoscopic techniques, Cryopreservation
- **1978 - Lancet** - clinical applications of in vitro fertilization
- L. Brown 2010 - R.G. Edwards - Nobel Prize

A. van Leeuwenhoek

R.G. Edwards

Evening News

Meet Louise, the world's first test-tube arrival

SUPERBABE

P.C. Steptoe

IVF centers in Brno

L'ART Gynecology, Laboratory Assisted...

UNICA Brno

Fakultní nemocnice Brno - Porodnice

IVF clinic ReproGenesis

Reprofit International Brno

REPROMEDA Biology Park

Brno

BRNO-KRÁLOVO POLE

BRNO-ŽABOVŘESKY

BRNO-SEVER

BRNO-JUNDROV

BRNO-LÍŠEŇ

BRNO-VINOHRADY

BRNO-NOVÝ LÍSKOVEC

BRNO-JIH

BRNO-SLATINA

BRNO-BOHUNICE

Hvězdárna a planetárium Brno

Galerie Vaňkovka

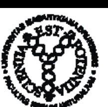
Ústřední hřbitov Brno

Černovická terasa

Hádecká planinka

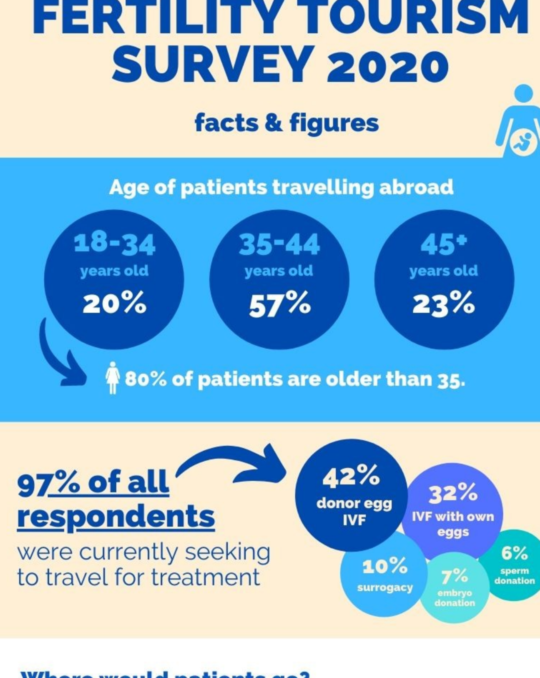
Obřany

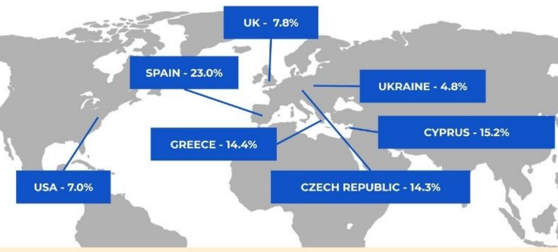
Proč tato reklama? Mapová data ©2017 Google Slovensko Smluvní podmínky Odeslat zpětnou



IVF centers in Czech rep.

- There are currently over 45 registered IVF centres in the Czech Republic (6x Brno, 8x Prague)
- Private IVF centers, (gynecology, obstetrics, reproductive medicine, genetics, biochemistry)
- Annually over 20,000 IVF cycles
- Over 50% covered by health insurance companies
- Specialization on foreign clientele - "reproductive tourism„





IVF process

In Vitro Fertilization

① egg production stimulated by hormone therapy

② eggs retrieved from ovary

③ sperm sample provided

④ eggs and sperm combined to allow fertilization

⑤ fertilized eggs introduced into uterus

Infographic: FDA/Renée Gordon

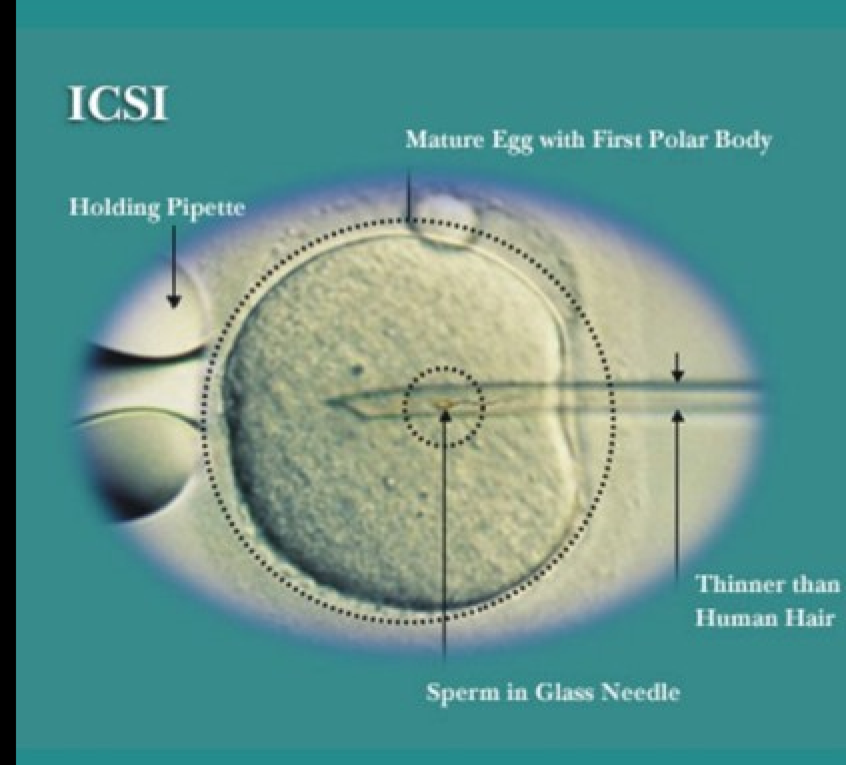
Methods of AHR

1. **Intrauterine insemination (IUI)**
= concentrated, purified sperm are introduced through a special catheter into the uterine cavity during ovulation

2. **In vitro fertilization (IVF)**
= classical method of ectopic fertilization, in which sperm are cultured with oocytes in vitro.

3. ICSI - intracytoplasmic sperm injection
through the zona pellucida into the egg

4. PICSI - enhanced ICSI
allows only the sperm to be selected and injected into the oocyte mature sperm through the attachment to the oocyte complex (hyaluronan)



Chirurgical sperm aspiration

Table 2 - Sperm retrieval techniques, acronyms and indications.

Technique	Acronym	Indications
Percutaneous epididymal sperm aspiration	PESA	Obstructive azoospermia
Microsurgical epididymal sperm aspiration	MESA	Obstructive azoospermia
Open epididymal fine-needle aspiration	ND	Obstructive azoospermia
Percutaneous testicular sperm aspiration; percutaneous testicular fine-needle aspiration	TESA; TEFNA	Obstructive azoospermia; Failed epididymal retrieval in OA cases; Epididymal agenesis in CAVD cases; Favorable testicular histopathology[1] in NOA cases; Previous successful TESA/TEFNA attempt in NOA cases
Testicular sperm extraction (single or multiple biopsies)	TESE	Obstructive azoospermia; Failed epididymal retrieval in OA cases; Failed TESA/TEFNA in OA cases; Non-obstructive azoospermia
Single seminiferous tubule biopsy	ND	Obstructive azoospermia; Failed epididymal retrieval in OA cases; Failed TESA/TEFNA in OA cases; Non-obstructive azoospermia
Microsurgical testicular sperm extraction	Micro-TESE	Non-obstructive azoospermia

OA: obstructive azoospermia; NOA: non-obstructive azoospermia. CAVD: congenital absence of the vas deferens. ND: not defined.
[1]Hypospermatogenesis

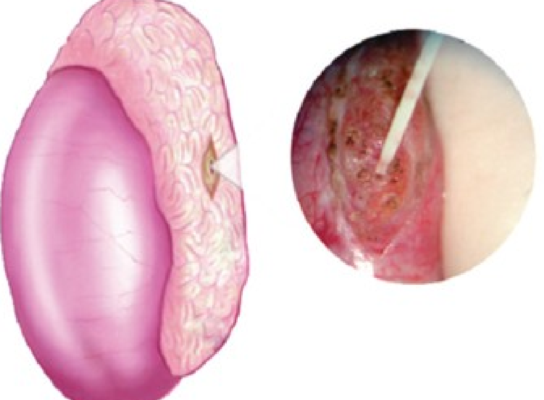
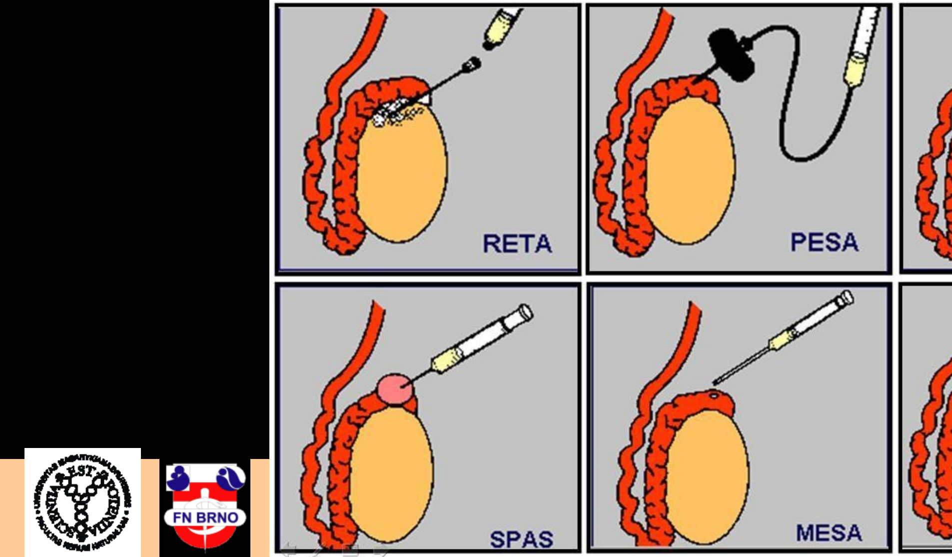


Figure 6 - Microsurgical epididymal sperm aspiration (MESA). After exposure of the testis and epididymis, a dilated epididymal tubule is dissected and opened. The fluid is aspirated, diluted with sperm medium and sent to the laboratory for examination.



Hormonal stimulation – harvesting of oocytes

Minimal stimulation

Gn[1]	Gn[1]+CC (100 mg)

Antagonist protocol

Gn[1]	Gn[1]+Cetorelix (0.25 mg)

Agonist long protocol

Triptorelin (0.1 mg)	Triptorelin (0.1 mg)+ Gonadotropin (Gn)[1]

Oocytes retrieval (post 36 hours)

HCG (post 14th day or when ≥2 follicles =18 mm)

21 30 1 2 6 15 16.5

[1]Gn dose varies among patients

GnRH - gonadorelin, gonadotropin-releasing hormone

CC - clomiphene citrate, synthetic estrogen, ovulation support

IVF cycle

- In the process of an **IVF cycle**, we usually obtain **several embryos**...
  - Ideally, a **single embryo** transfer is performed
- x **selection**...which is „the best“ - morphology, **genetics?**

Genetic aberrations and IVF

IVF News

Organon

IVF News, Volume 11, No 2, 2000. IVF News is a newsletter offered to you by N.V. Organon. For further information, please contact: N.V. Organon, PO Box 20, 5340 BH Oss, The Netherlands, www.fertility-net.com

INSIDE

Page 2
- *ESHRE's IVF monitoring program*
- *Cost-effectiveness of gonadotropins*

Page 3
- *New regimes in ovarian stimulation*
- *The potential for blastocyst transfer*

Page 4
- *Consumers take the stage at ESHRE: future directions described*

Around 70 percent of embryos in older patients are chromosomally abnormal

Pre-implantation genetic diagnosis for aneuploidy is 'living up to its promise'

Pre-implantation genetic diagnosis, technology pioneered more than a decade ago by Handyside and colleagues at the Hammersmith Hospital in London, UK, is finally living up to initial expectations, thereby bringing new hope to couples formerly at high risk of having a baby affected by a genetic abnormality.

Dr Santiago Munné of Saint Barnabas Medical Center in New Jersey, USA, said that application of the technique has already dramatically reduced the number of miscarriages in older mothers and of babies born with an inherited chromosomal condition. In reviewing progress so far for ESHRE, Dr Munné reported that pre-implantation genetic diagnosis (PGD) for aneuploidy (currently also known as pre-implantation genetic screening (PGS) in Europe) and for translocations is now available in most developed countries. He added, however, that large-scale PGD for these conditions is still confined to a total of only half a dozen centers in the Netherlands, the USA, Italy, Belgium and the UK.

Work conducted by Dr Munné has already shown that the risk of aneuploidy rises up to 70-fold as

Dr Luca Gianaroli: 'Pregnancy rates three times higher in PGD cycles'

Dr Santiago Munné: 'Risk of aneuploidy increases 70-fold as women age'

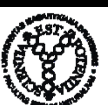
over 38 years as many as 70 percent of embryos are aneuploid and 50 percent are monosomies or trisomies.

a valuable tool to overcome the necessity of transferring more than two embryos in poor prognosis patients without negatively

User-friendliness steers developments at Organon

a **large proportion** of embryos, regardless of the age of the mother, are **aneuploid** (**54% under the age of 35, 82% aged 40 and over)**

Reason = **<u>disruption during meiosis</u>**



Chromosomal aberrations in embryos

MI MII

Hyperhaploid gametes (n = 24)

Hypohaploid gametes (n = 22)

MI non-disjunction

a

MI MII

Normal gametes (n = 23)

Hyperhaploid gamete (n = 24)

Hypohaploid gamete (n = 22)

MII non-disjunction

b

- **~ 90% of aneuploidies occur during meiosis I in women**

= gradual **degradation** of **cohesin** leads to violation of bivalent **integrity**

Aberration of segregation during Meiosis I

Preimplantation genetic analyses (PGA)

PGT-M: Preimplantation genetic testing of monogenic diseases

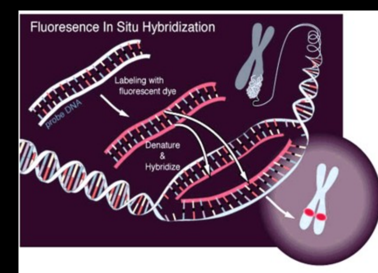
- Previously PGD = monogenic diseases
- Sex selection in X-linked diseases
- Congenital structural abnormalities (Robertsonian translocations, balanced translocations

PGT-A: Preimplantation genetic testing for aneuploidies

- Screening of the most common congenital chromosomal aneuploidies

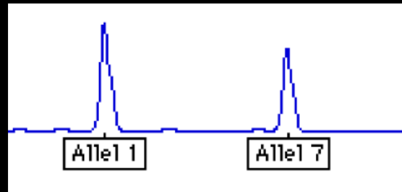
PGA methods

1. **Molecular cytogenetics (I-FISH, CGH)**
   - aneuploidy, translocations, microdeletion syndromes, etc.



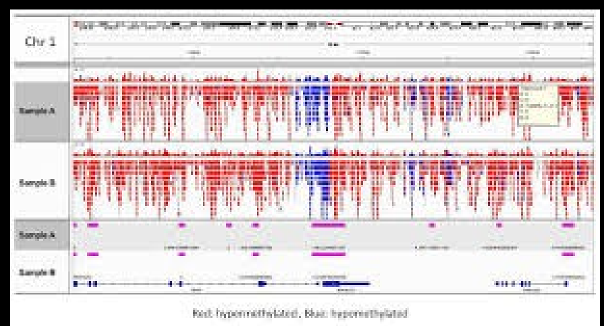
2. **PCR - monogenic diseases**

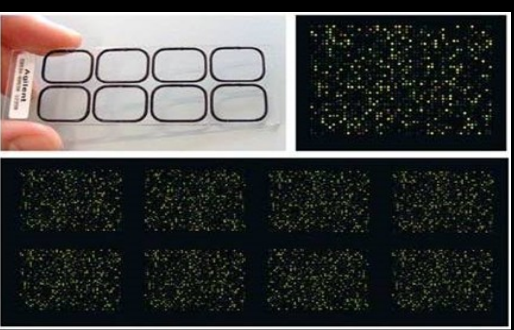
   - specific mutations - CF, thalassaemia, sickle cell anemia, hemophilia, DMD.....
   - QF PCR - +13,16,18,21, X,Y



3. **Screening techniques -** "PGD 2.0" - whole genome coverage

   - array-CGH (DNA chips) - numerical and structural CHA
   - SNP chips - KARYOMAPPING
   - NGS - comprehensive approach, PGD+PGS combination





PGA - biological material

X Y

XY XY 0 X X X XY Y XY

X

Y

Oplození *in vitro*

Biopsie embrya

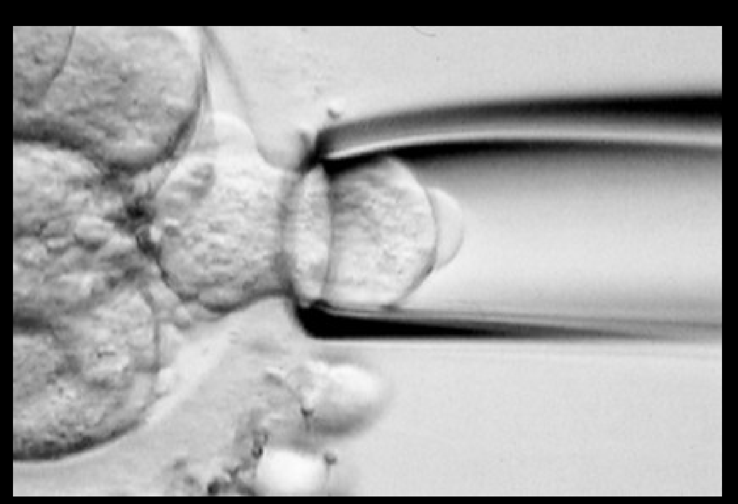
Genetický test

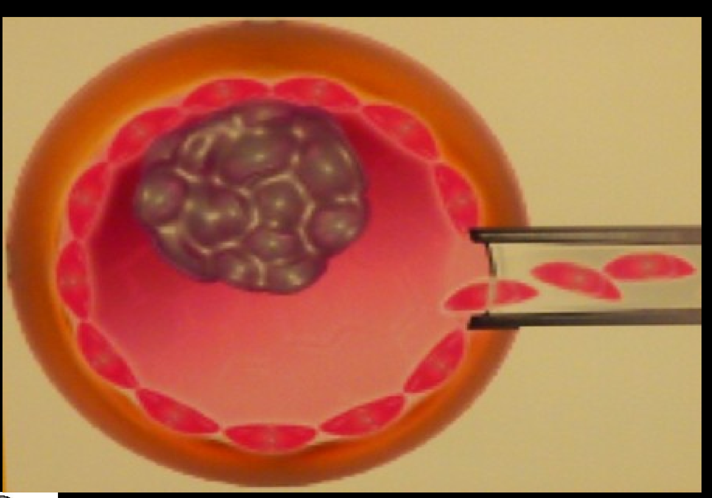
Polární tělísko

Blastomera (3. den)

Blastocysta (5-6. den)

Embryo biopsy difference between 3rd and 5th harvest day





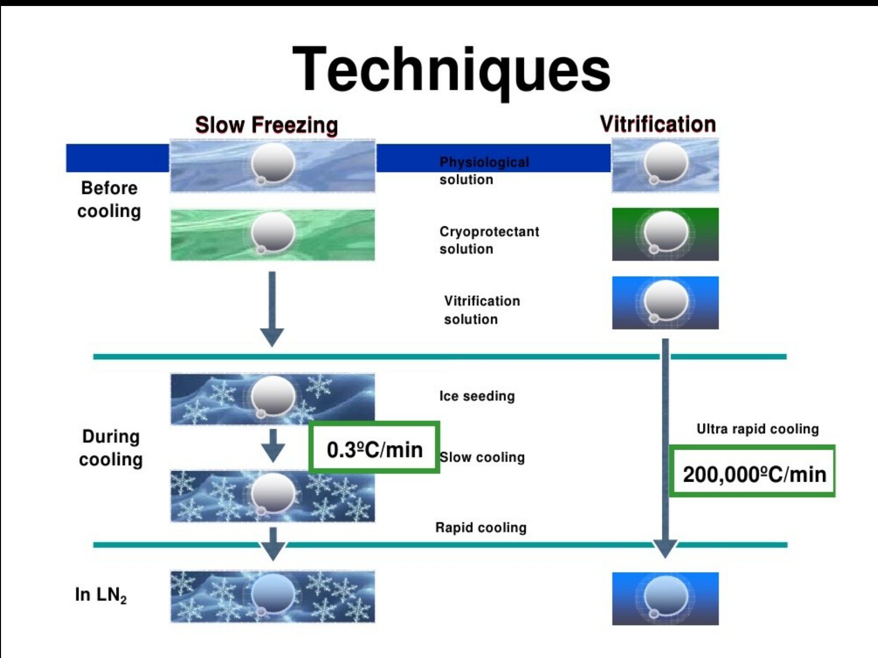
<u>**Day 3 embryo biopsy (blastomeres)**</u>
- analysis of 1 - 2 cells
- 30 - 60% loss of implantation potential
- higher risk of mosaicism
- time constraint (24 hours)

<u>**Embryo biopsy day 5-6 (blastocyst)**</u>
- analysis of **5 - 10 cells**
- less risk of mosaicism
- possibility of embryo **vitrification** = sufficient time for examination
- not all embryos reach the stage blastocysts = slection

Vitrification of embryos

Vitrification

- modern method of effective **cryopreservation of embryos**, oocytes and sperm
- **Superfast freezing** of biological **material** with a mixture of suitably selected cryoprotectants (sucrose, dimethylsulfoxide) **at -196C**
- **Viablity** after thawing approx. **98%**



Chromosomal aberrtaions in embryos

Multiple chromosome aberrations (aneuploidy)

- are the **most common** genetic alteration in human **embryos**
- **aneuploidies** often occur in **morphologically normal** developing embryos (A. Mertzanidou, 2013)
- **reduce** the success rate of **assisted reproductive** techniques

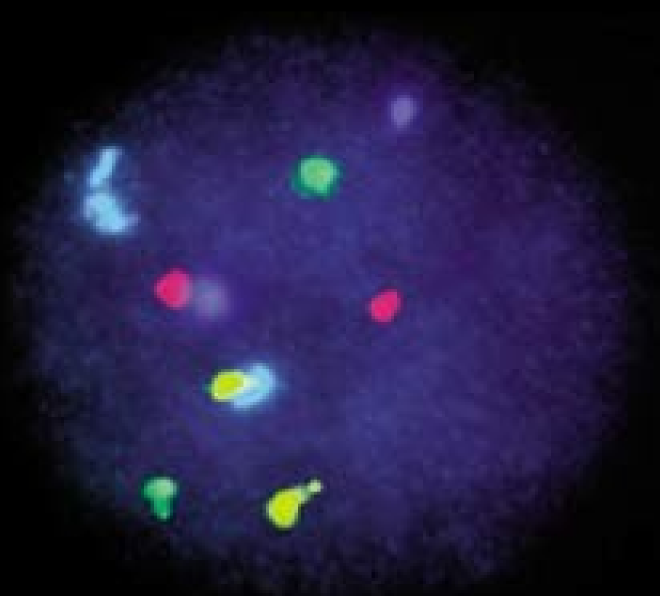
Structural chromosome aberrations

- **postzygotic mitotic** disorders are very **common** in embryos
- **Chromosomal instability** - duplication, amplification, deletion, UPD - has been demonstrated in **up to 70% of embryos** using SNP chips (Vanneste et al., 2009)

SCREEINING OF MERE ANEUPLOIDIES IN EARLY EMBRYOS IS NOT ENOUGH!

PGA with the use of I-FISH

Screening - AneuVysion Vysis MultiVysion Probe Panel (13,18,21,X,Y,16,22)



SpectrumGreen 21
SpectrumRed 13
SpectrumBlue X
SpectrumGold Y
SpectrumAqua 18

Multiple chromosomes on one cell - repeated FISH (FISH - evaluation, washout, new FISH - evaluation)

Preimplantation genetic screening: a systematic review and meta-analysis of RCTs

S. Mastenbroek*, **M. Twisk**, **F. van der Veen**, **and S. Repping**

Center for Reproductive Medicine, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands

*Correspondence address. Tel: +31-20-5663090; E-mail: s.mastenbroek@amc.uva.nl

Submitted on December 31, 2009; resubmitted on January 10, 2011; accepted on January 31, 2011

BACKGROUND: Preimplantation genetic screening (PGS) has increasingly been used in the past decade. Here we present a systematic review and meta-analysis of RCTs on the effect of PGS on the probability of live birth after IVF.

METHODS: PubMed and trial registers were searched for RCTs on PGS. Trials were assessed following predetermined quality criteria. The primary outcome was live birth rate per woman, secondary outcomes were ongoing pregnancy rate, miscarriage rate, multiple pregnancy rate and pregnancy outcome.

RESULTS: Nine RCTs comparing IVF with and without PGS were included in our meta-analysis. Fluorescence *in situ* hybridization was used in all trials and cleavage stage biopsy was used in all but one trial. PGS significantly lowered live birth rate after IVF for women of advanced maternal age (risk difference: −0.08; 95% confidence interval: −0.13 to −0.03). For a live birth rate of 26% after IVF without PGS, the rate would be between 13 and 23% using PGS. Trials where PGS was offered to women with a good prognosis and to women with repeated implantation failure suggested similar outcomes.

CONCLUSIONS: There is no evidence of a beneficial effect of PGS as currently applied on the live birth rate after IVF. On the contrary, for women of advanced maternal age PGS significantly lowers the live birth rate. Technical drawbacks and chromosomal mosaicism underlie this inefficacy of PGS. New approaches in the application of PGS should be evaluated carefully before their introduction into clinical practice.

PGA with I-FISH does not improve IVF success.....why?

Problems of PGA I

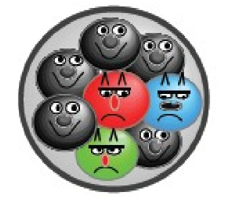
examination of single cell - possibility of **diagnostic error ?**

Problems of PGA II

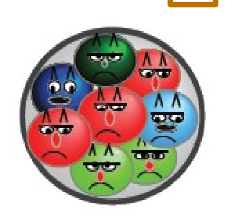
EMBRYA:

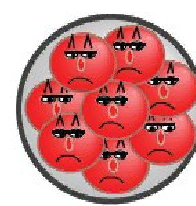
normal (all diploid cells)

One cell does not have to represent the whole embryo !!!



Mosaic (diploid + aneuploid cells)





abnormal (all cells abnormal)

chaotic (each cell contains different number of chromosomes)

Problems of PGA I – structural CHAs

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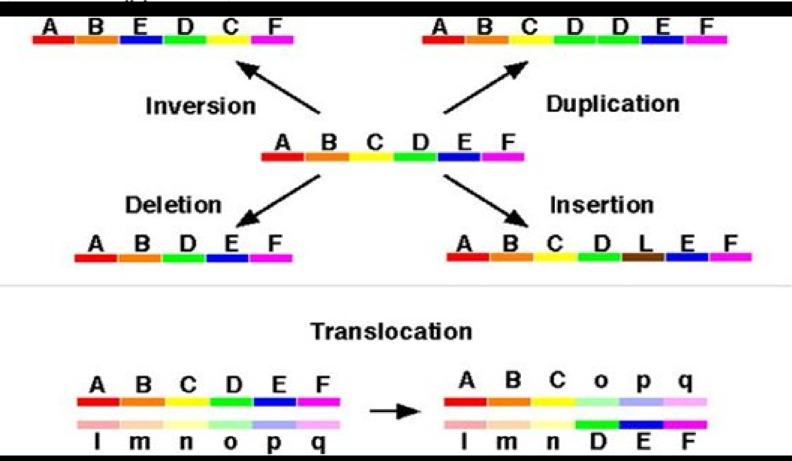
Journal home > Archive > Technical Report > Full Text

Technical Report

Nature Medicine **15**, 577 - 583 (2009)
Published online: 26 April 2009 | doi:10.1038/nm.1924

Chromosome instability is common in human cleavage-stage embryos

Evelyne Vanneste[1,2,9], Thierry Voet[1,9], Cédric Le Caignec[1,3,4], Michèle Ampe[5], Peter Konings[6], Cindy Melotte[1], Sophie Debrock[2], Mustapha Amyere[7], Miikka Vikkula[7], Frans Schuit[8], Jean-Pierre Fryns[1], Geert Verbeke[5], Thomas D'Hooghe[2], Yves Moreau[6] & Joris R Vermeesch[1]



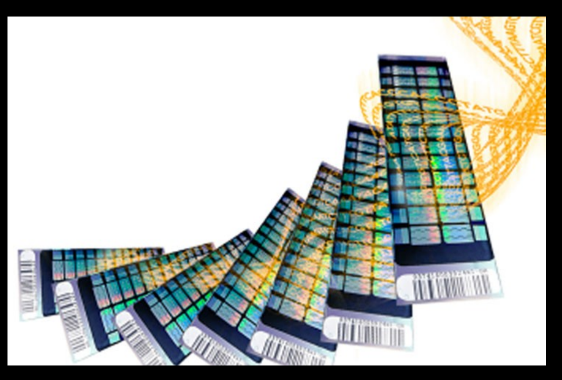
It's not enough to investigate aneuploidy!

Whole genome testing!

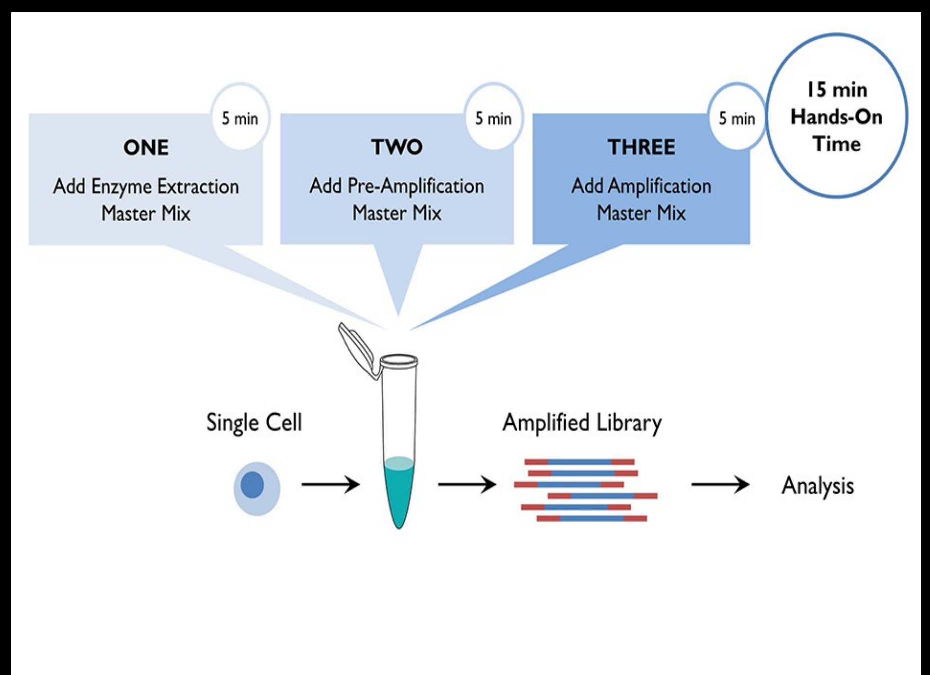
- **structural aberrations** (deletions, duplications, UPD etc...) also occur in embryos ...post-zygotic **mitotic disorders** are **more frequent** than meiotic ones...

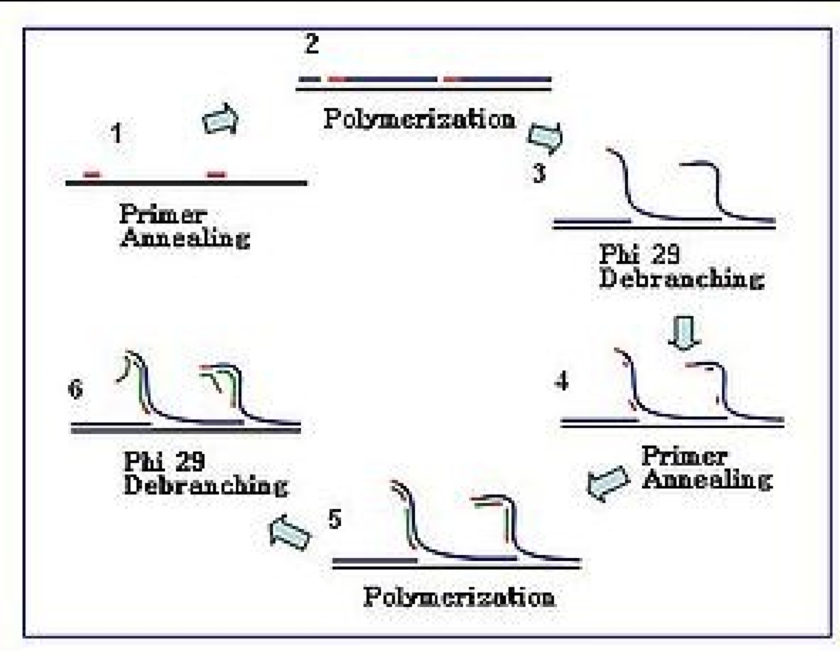
Use of whole genome screening techniques in PGA

- Isolation of one - several cells + **whole genome amplification**
-
- Use of array-CGH microarray techniques, SNP chips, NGS
- Possibility to examine the whole genome - necessary in a short time interval (24 h) X frozen embryos (vitrification)



DNA amplification - key step in complex PGA





Genomic methods – **hunderts ng of DNA** needed, = **10^6cells**

XX

trophoectoderm aspirate **20 cells = pg DNA**, DNA amplification required

Zheng et al. / J Zhejiang Univ-Sci B (Biomed & Biotechnol) 2011 12(1):1-11 1

Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
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www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Review:

Whole genome amplification in preimplantation genetic diagnosis*

Ying-ming ZHENG, Ning WANG, Lei LI, Fan JIN[†‡]

(*Department of Reproductive Endocrinology, Women's Hospital, School of Medicine, Zhejiang University, Hangzhou 310006, China*)

[†]E-mail: jinfan@zju.edu.cn

Received June 1, 2010; Revision accepted Sept. 29, 2010; Crosschecked Dec. 8, 2010

Human Reproduction, Vol.25, No.4 pp. 1066–1075, 2010
Advanced Access publication on January 24, 2010 doi:10.1093/humrep/dep452

human reproduction **ORIGINAL ARTICLE** ***Reproductive genetics***

Preclinical validation of a microarray method for full molecular karyotyping of blastomeres in a 24-h protocol

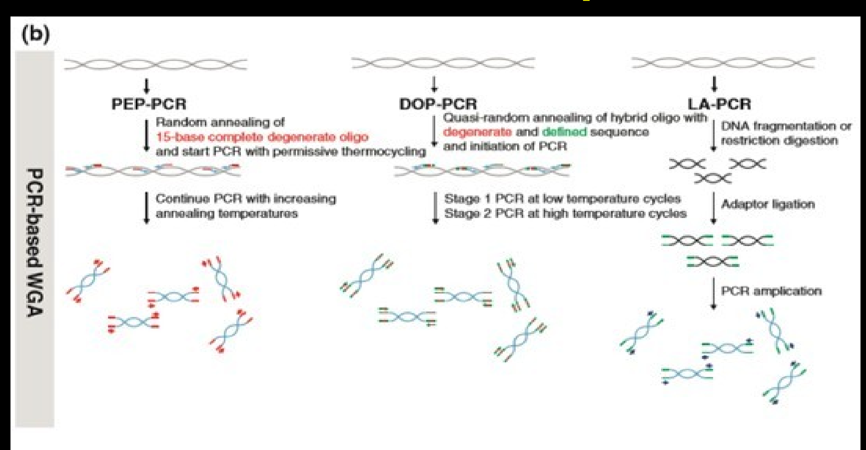
D.S. Johnson[1,8], G. Gemelos[1], J. Baner[1,2], A. Ryan[1], C. Cinnioglu[1], M. Banjevic[1], R. Ross[3], M. Alper[4], B. Barrett[4], J. Frederick[5], D. Potter[1,5], B. Behr[6], and M. Rabinowitz[1,7]

[1]Gene Security Network, Inc., 2686 Middlefield Road, Suite C, Redwood City, CA 94063, USA [2]Genome Technology Center, Stanford University, 318 Campus Drive, Stanford, CA 94305, USA [3]La Jolla IVF, 9850 Genesee Avenue No. 610, La Jolla, CA 92037, USA [4]Boston IVF, 130 Second Avenue, Waltham, MA 02451, USA [5]Huntington Reproductive Center, 23961 Calle de la Magdalena, Suite 503, Laguna Hills, CA 92653, USA [6]Obstetrics and Gynecology, Stanford University Medical Center, 900 Welch Road, Palo Alto, CA 94304, USA [7]School of Engineering, Aeronautics and Astronautics, Stanford University, Stanford, CA 94305, USA

[8]Correspondence address. E-mail: djohnson@genesecurity.net

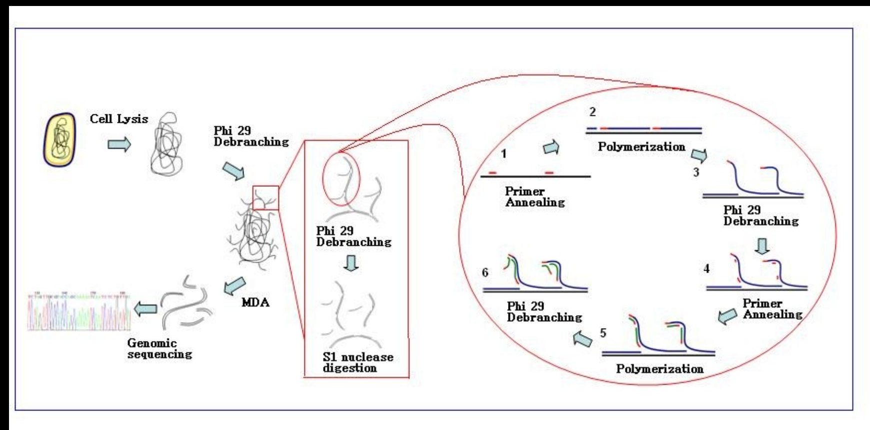
Single cell WGA principy – PCR

PCR based WGA amplification



Advantages:
higher yield, simple protocol, less time consuming
XXX
creates artifacts, ADO
Applications: array-CGH, QF - PCR

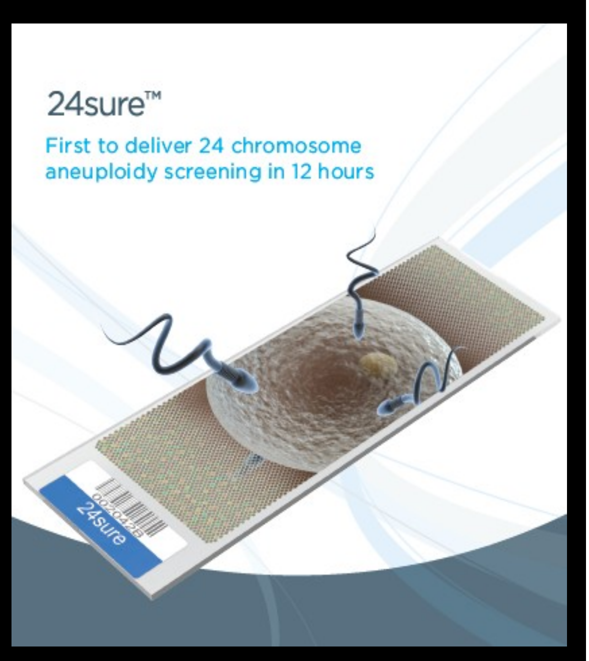
Multiple displacement amplification

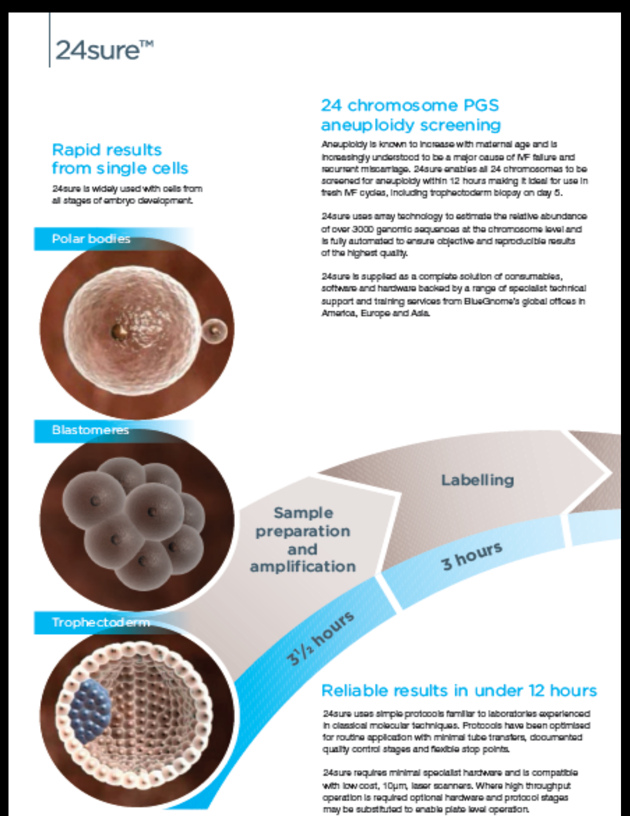


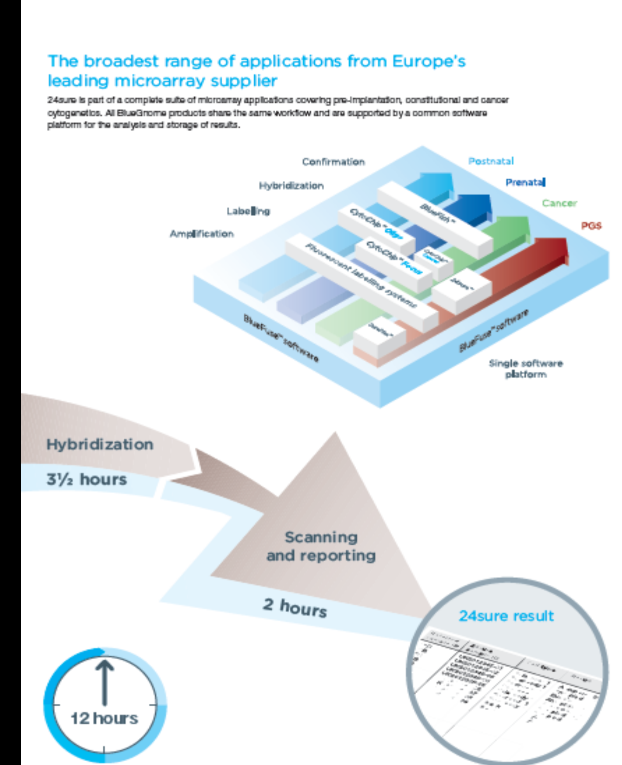
Circular amplification using the thermostable phage mutant Phí29
Advantages - lower ADO rate, no amplification products x more challenging, lower yield
Applications: NGS, methylation analysis (PWS/AS)

BAC array CGH – PGS in 12 hours

Aneuploidy and structural changes (deletions, duplications) in the whole genome ; Resolution ~ 5 Mbp







Metodika screeningových technik u PGT

Array-cgh workflow

Sample

Fluorescent Labels

Apply to Microarray

Scan

Fluorescent Signal

Cy5
Cy3

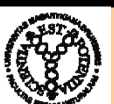
Red
Green

Amp
Del

Test
Ref

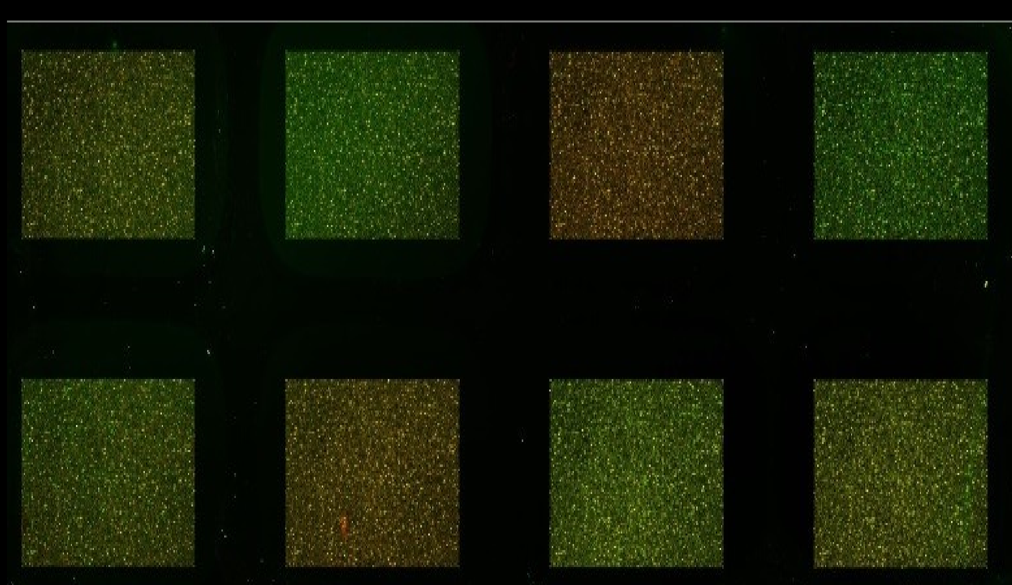
-16X -4X -2X 1 +2X +4X +16X

Interpret Data



PGA using high-resolution array-CGH

Material:	cells from trophoectoderm of 5-day-old embryos
Amplification protocol:	PicoPLEX WGA Kit (Rubicon Genomics, USA)
Microarrays:	8x15K - CytoSure™Single Cell Aneuploidy Array, OGT UK
	8x60K - Agilent SurePrint G3 Oligo CGH Microarray
Software:	CytoSure Interpret Software, Genomic Workbench



Comparison of chromosome 19 profile on Agilent and OGT platform

Agilent 8x60k | **OGT 8x15**

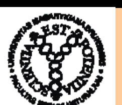
Higher false positivity of the 15k platform, most commonly chr. 11, 16 and 19

Porovnání profilu chromozomu 22 na platformě Agilent a OGT

Agilent 8x60k

OGT 8x15

Higher density of 60k microarrays gives more robust results compared to 15k



Thanks to WGA… Story of 400 Embryos

Preimplantation genetic analysis

Oligonucleotide DNA microarrays platforms:

CytoSure Single Cell Aneuploidy Array	8x15K (OGT) (Resolution: 250kb)	n=222
SurePrint G3 Human CGH Microarray Kit	8x60K (Agilent) (Resolution: 41kb)	n=178
		n=400

Analysis success: 90% (400/442)

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%

34% — ABNORMAL n=138

66% — „NORMAL" n=262

STRUCTURAL CNVs: 17%, 3%, ANEUPLOIDIES: 80%

Pregnancy Rate (PR):	56%
Clinical Pregnancy Rate (cPR)	46%

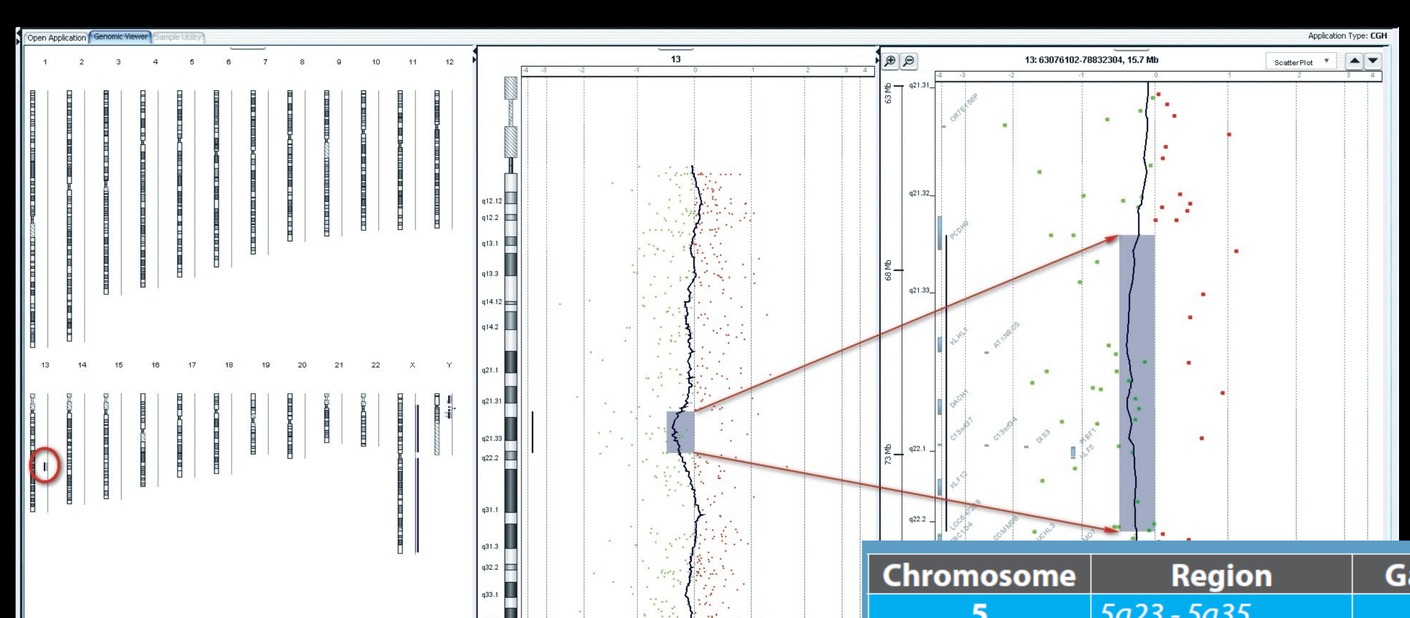
Mikulášová A. et al, SLG konference 2014, Praha

Results of PGS array-CGH screening

- **most common monosomy**: chromosome 22 (7.7%; 5/65), 7, 8 and 18 (6.1% each; 4/65)
- **most common trisomy**: chromosome 15, 21 and 22 (4.6% each; 3/65)

INCIDENCE
0,12
0.1
0.08
0.06
0.04
0.02
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X
CHROMOSOME
MONOSOMY
TRISOMY

Results of PGS array-CGH screening



Visualization of 8.4 Mb segmental deletion in chromosome 13q21.32 - q22.2 affecting loci of *CDH9, KLHL1, ATXN8OS, DACH1, C13orf37, C13orf34, DIS3, PIBF1, KLF5, KLF1 gene.*

Chromosome	Region	Gain	Loss	Size (Mbp)
5	*5q23 - 5q35*		+	56,9
5	*5q21.1 - 5q35*	+		79,5
8	*8q24.21 - 8q24.3*	+		23,5
8	*8p23.2 - 8p11.22*	+		43,3
8	*8q24.22 - q24.3*		+	130,5
9	*9p23-p21.3*		+	15,1
9	*9q21.1 - 9q34.3*		+	69,8
13	*13q21.32 - q22.2*		+	8,4
13	*13q21.33-13q34*	+		43,6
13	*13q12.11 - 13q33.1*	+		102,5
14	*14q21-14q32*	+		57,2
16	*16p13.3- 16p11.1*		+	34,8
17	*17p13.3 - 17p11.2*	+		24,4

Biol Reprod. 2012 Dec 27;87(6):148. doi: 10.1095/biolreprod.112.103192. Print 2012 Jun.

DNA microarray reveals that high proportions of human blastocysts from women of advanced maternal age are aneuploid and mosaic.

Liu J, Wang W, Sun X, Liu L, Jin H, Li M, Witz C, Williams D, Griffith J, Skorupski J, Haddad G, Gill J.

Key Laboratory of Major Obstetrics Diseases of Guangdong Province, Guangzhou Medical College, Guangdong, China.

Hum Reprod. 2013 Jan;28(1):256-64. doi: 10.1093/humrep/des362. Epub 2012 Oct 9.

Microarray analysis reveals abnormal chromosomal complements in over 70% of 14 normally developing human embryos.

Mertzanidou A, Wilton L, Cheng J, Spits C, Vanneste E, Moreau Y, Vermeesch JR, Sermon K.

Faculty of Medicine and Pharmacy, Research Group Reproduction & Genetics, Vrije Universiteit Brussel, 1090 Brussels, Belgium.

Human Reproduction, Vol.26, No.4 pp. 941–949, 2011
Advanced Access publication on February 2, 2011 doi:10.1093/humrep/der004

human reproduction

CASE REPORT *Reproductive genetics*

PGD for a complex chromosomal rearrangement by array comparative genomic hybridization

E. Vanneste[1,2], C. Melotte[1], T. Voet[1], C. Robberecht[1], S. Debrock[2], A. Pexsters[3], C. Staessen[4], C. Tomassetti[2], E. Legius[1], T. D'Hooghe[2], and J.R. Vermeesch[1,*]

Karyomapping – PGA-M

Karyomapping: comprehensive linkage-based PGD
(harnessing the power of ~280,000 genome-wide SNPs

Father (Carrier)
Mother (Carrier)

Child
Affected

Embryo 1
Unaffected

Embryo 2
Carrier

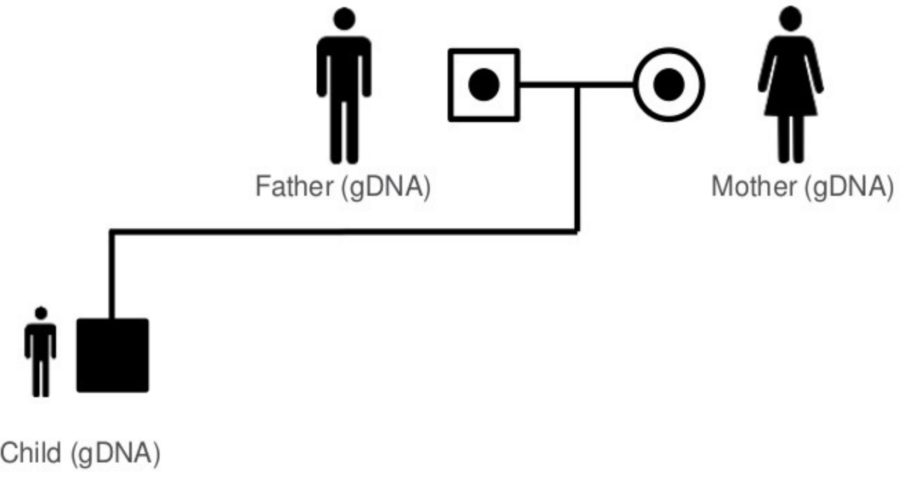
Embryo 3
Carrier

Embryo 4
Affected

illumina

Karyomapping – PGA-M

Karyomapping – Patient assessment and testing

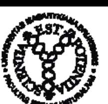


- Obtain genomic DNA from **parents**
- Obtain genomic DNA from **reference** (known genotype status e.g. affected child, siblings or other relatives)

illumina

Karyomapping – PGA-M

PGD condition	Mode of inheritance	Gene/Locus	Phenotype MIM number	Chr	Region	SNP coverage		
						5'	Gene/ Locus	3'
Crigler Najar Syndrome	AR	UGTIA1	218800	2	234,668,918-234,681,944	261	2	372
Bardet Biedl Syndrome 3	AR	ARL6	209900	3	97,483,594-97,517,372	280	6	212
Huntington Disease	AD	HTT	143100	4	3,076,407-3,245,686	315	14	256
Facioscapulohumeral muscular dystrophy	AD	FSHD	158900		183,200,000-191,154,276	291	853	0
Spinal Muscular Atrophy	AR	SMN1	253300	5	70,220,767-70,248,838	29	7	98
Osteopetrosis-infantile Malignant	AR	OSTM1	259720	6	108,362,612-108,395,940	221	7	113
Polycystic Kidney Disease	AR	PKHD1	263200	6	51,480,144-51,952,422	274	78	342
Cystic Fibrosis	AR	CFTR	219700	7	117,120,016-117,308,718	93	34	55
Congenetial Lipodystrophy type 1	AR	AGPAT2	608594	9	139,567,594-139,581,910	304	2	211
Beta-thalassemia	AR	HBB	613985	11	5,246,695-5,248,300	201	14	305
Sickle cell Anemia	AR	HBB	603903	11	5,246,695-5,248,300	201	14	305
Smith Lemli Optiz	AR	DHCR7	270400	11	71,145,456-71,159,476	213	4	277
Breast cancer predisposition (BRCA2)	AD	BRCA2	612555	13	32,889,616-32,973,808	207	7	151
Retinoblastoma	AD	RB1	180200	13	48,877,883-49,056,026	294	14	195
Propinic Acedimuia	AR	Alpha PCCA	606054	13	100,741,268-101,182,690	299	46	258
Li-Fraumeni syndrome	AD	TP53	151623	17	7,571,719-7,590,867	250	2	283
Breast Cancer 1	AD	BRCA1	604370	17	41,196,311-41,277,499	156	25	340
Peutz-Jeghers syndrome	AD	STK11 (LKB1)	175200	19	1,205,797-1,228,433	137	4	307
Familial hypercholesterolemia	AD	LDLR	143890	19	11,200,037-11,244,505	281	12	281
Myotonic dystrophy type 1	AD	DMPK	160900	19	46,272,974-46,285,814	136	0	108
Bardet Biedel Syndrome	AR	MKKS / BBS6	209900	20	10,385,427-10,414,886	324	3	274
Duchene Muscular Dystrophy	XR	DMD	310200	X	31,137,344-33,357,725	226	320	66
Xq deletion				X	131,336,145-132,612,743	156	38	152
Fragile-X Syndrome	XD	FMR1	300624	X	146,993,468-147,032,646	279	8	259
X-linked myotubular myopathy	XR	MTM1	310400	X	149,737,046-149,841,615	255	17	356
Incontinentia pigmenti	XD	IKBKG	308300	X	153,770,458 -153,793,260	340	4	246
					Range	29-340	0-853	0-372



Karyomapping – PGA-M

Karyomapping - Diagnostic Laboratory Process

Whole Genome Amplification of samples using SureMDA (2.5 hrs)

Kit = 96 reactions

↓

Process DNAs - Infinium HumanKaryomap-12 DNA analysis kit (20 hrs)

Kit = 24 samples (12 per run)

↓

Scan using iScan (0.5 hr)

↓

Import scan data in to BlueFuse multi v4.0 (karyomapping module), Analyse results, Report (~1 hr)

illumina

Karyomapping - PGA-M

https://els-jbs-prod-cdn.jbs.elsevierhealth.com/cms/attachment/87c0990f-7465-402a-a562-20be1ed5580d/rbmo1376-fig-0001.jpg

B

karyomapping Log R ratio

Chromosomal Position

C

Karyomapping B-Allele frequency chart

Chromosomal Position

D

Chromosome 11

E

Chromosome 22

F

Translocation	Chromosome	Breakpoint detected (N)	Breakpoint position (±SD)
46,XX,t(9;12)(q21.34;q21.32)	9	7	chr9:98,732,527 (±60,810)
	12	7	chr12:88,604,484 (±1,061,262)
46,XX,t(11;22)(q23.3q11.2)	11	1	chr11:11,6628,381
	22	1	chr22:20168614

Kubeciek et al, Incidence and origin of meiotic whole and segmental chromosomal aneuploidies detected by karyomapping. 2018. https://doi.org/10.1016/j.rbmo.2018.11.023

Karyomapping - PGA-M

Format: Abstract ▾ Send to ▾

Reprod Biomed Online. 2017 Sep;35(3):264-271. doi: 10.1016/j.rbmo.2017.06.004. Epub 2017 Jun 15.

Karyomapping: a single centre's experience from application of methodology to ongoing pregnancy and live-birth rates.

Ben-Nagi J[1], Wells D[2], Doye K[3], Loutradi K[3], Exeter H[3], Drew E[3], Alfarawati S[2], Naja R[2], Serhal P[3].

⊕ Author information

Abstract

This study aimed to determine whether karyomapping can be applied to couples requiring preimplantation genetic diagnosis (PGD) for single gene disorder (SGD) and/or chromosomal rearrangement. 75/82 (91.5%) and 6/82 (7.3%) couples were referred for autosomal SGD and X-linked disease, respectively. One couple (1.2%) was referred for SGD and chromosomal rearrangement. Of 608 embryos, 146 (24%, 95% CI 21-28) day-3 and 462 (76%, 95% CI 72-79) blastocyst biopsies were performed. A total of 81 embryo transfers were performed; 16/81 (20%) were following day-3 embryo biopsy, 65/81 (80%) were following blastocyst biopsy and cryopreserved embryo transfer. Of 81 embryo transfers with known pregnancy outcome, 51 (63%, 95% CI 52-73) were on-going pregnancies, 6/81 (7%, 95% CI 3-15) resulted in first trimester miscarriages and 24/81 (30%, 95% CI 21-40) were failed implantations. Of the 51 on-going pregnancies, 15 (29%, 95% CI 19-43) couples had a singleton live birth at the time of write up. There have been no reports of abnormal prenatal, genetic testing or diagnosis of phenotype at birth. Karyomapping is reliable, efficient and accurate for couples requiring PGD for SGD and/or chromosomal rearrangement. Additionally, it provides aneuploidy screening, minimising risks of miscarriage and implantation failure.

Copyright © 2017 Reproductive Healthcare Ltd. Published by Elsevier Ltd. All rights reserved.

KEYWORDS: Embryo biopsy; Karyomapping; Preimplantation genetic diagnosis

PMID: 28648921 DOI: 10.1016/j.rbmo.2017.06.004

Format: Abstract ▾ Send to ▾

Genet Med. 2014 Nov;16(11):838-45. doi: 10.1038/gim.2014.45. Epub 2014 May 8.

Genome-wide karyomapping accurately identifies the inheritance of single-gene defects in human preimplantation embryos in vitro.

Natesan SA[1], Bladon AJ[1], Coskun S[2], Qubbaj W[2], Prates R[3], Munne S[3], Coonen E[4], Dreesen JC[5], Stevens SJ[5], Paulussen AD[5], Stock-Myer SE[6], Wilton LJ[6], Jaroudi S[7], Wells D[7], Brown AP[1], Handyside AH[8].

⊕ Author information

Abstract

PURPOSE: Our aim was to compare the accuracy of family- or disease-specific targeted haplotyping and direct mutation-detection strategies with the accuracy of genome-wide mapping of the parental origin of each chromosome, or karyomapping, by single-nucleotide polymorphism genotyping of the parents, a close relative of known disease status, and the embryo cell(s) used for preimplantation genetic diagnosis of single-gene defects in a single cell or small numbers of cells biopsied from human embryos following in vitro fertilization.

METHODS: Genomic DNA and whole-genome amplification products from embryo samples, which were previously diagnosed by targeted haplotyping, were genotyped for single-nucleotide polymorphisms genome-wide detection and retrospectively analyzed blind by karyomapping.

RESULTS: Single-nucleotide polymorphism genotyping and karyomapping were successful in 213/218 (97.7%) samples from 44 preimplantation genetic diagnosis cycles for 25 single-gene defects with various modes of inheritance distributed widely across the genome. Karyomapping was concordant with targeted haplotyping in 208 (97.7%) samples, and the five nonconcordant samples were all in consanguineous regions with limited or inconsistent haplotyping results.

CONCLUSION: Genome-wide karyomapping is highly accurate and facilitates analysis of the inheritance of almost any single-gene defect, or any combination of loci, at the single-cell level, greatly expanding the range of conditions for which preimplantation genetic diagnosis can be offered clinically without the need for customized test development.

PMID: 24810687 PMCID: PMC4225458 DOI: 10.1038/gim.2014.45

[Indexed for MEDLINE] Free PMC Article

Format: Abstract ▾ Send to ▾

Klin Onkol. 2016;29 Suppl 1:S93-9.

[Assisted Reproduction and Preimplantation Genetic Diagnosis in Patients Susceptible to Breast Cancer].

[Article in Czech]

Veselá K, Kocur T, Horák J, Horňák M, Oráčová E, Hromadová L, Veselý J, Trávník P.

Abstract

BACKGROUND: Assisted reproduction, as well as pregnancy itself, in patients with breast cancer or other hereditary type of cancer, is a widely discussed topic. In the past, patients treated for breast cancer were rarely involved in the discussion about reproductive possibilities or infertility treatment. However, current knowledge suggests, that breast cancer is neither a contraindication to pregnancy, nor to assisted reproduction techniques. On the contrary, assisted reproduction and preimplantation genetic diagnosis methods might prevent the transmission of genetic risks to the fetus.

AIM: In this review we summarize data concerning pregnancy risks in patients with increased risk of breast cancer. In addition, we introduce current possibilities and approaches to fertility preservation prior to assisted reproduction treatment as well as novel methods improving the safety of fertility treatment. In the second part of this review, we focus on karyomapping--an advanced molecular genetic tool for elimination of germinal mutations in patients with predisposition to cancer. Moreover, the rapid development of preimplantation genetic diagnosis methods contributes to detection of both chromosomal aneuploidy and causal mutations in a relatively short time-span.

PMID: 26691949

[Indexed for MEDLINE]

Karyomapping – PGA-M

Benefits

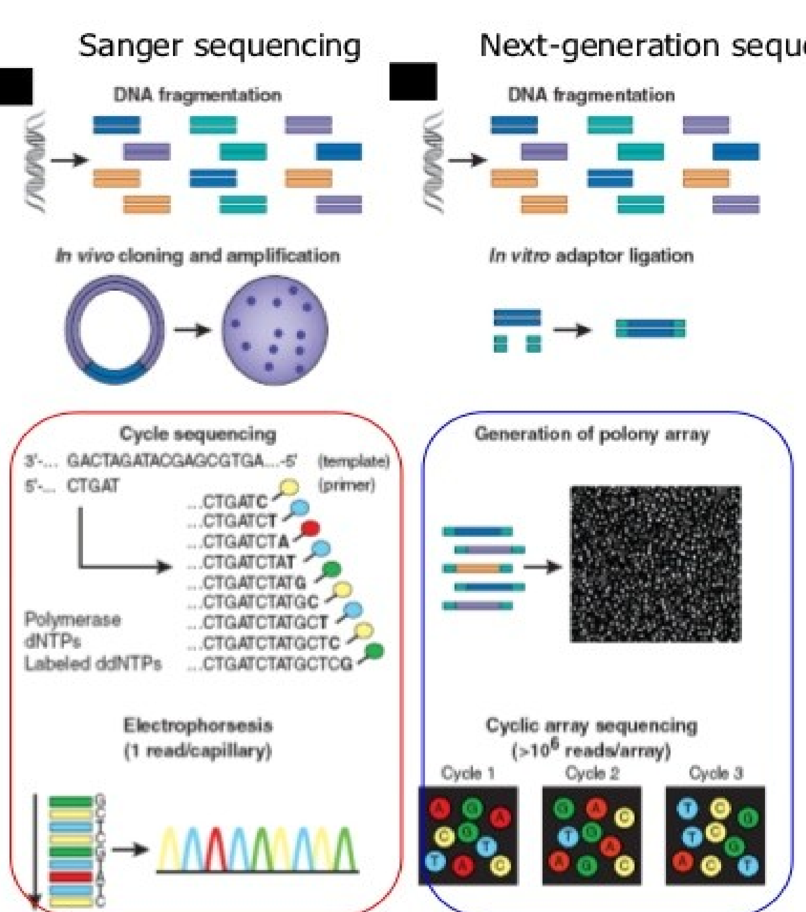
- **Fast and efficient** method for complex PGA-M if we have a suitable reference
- Detection of **structural deletions, aneuploidies** and **monogenic diseases**

Disadvantages

- Patented technology (Illumina), no competition for chemistry, costly, closed system
- Potential for problems in the absence of reference DNA

Next generation (massive parallel) sequnincg in PGS

Next-generation DNA sequencing



Advantages:

- Construction of a sequencing library → clonal amplification to generate sequencing features

  ✓ No in vivo cloning, transformation, colony picking...

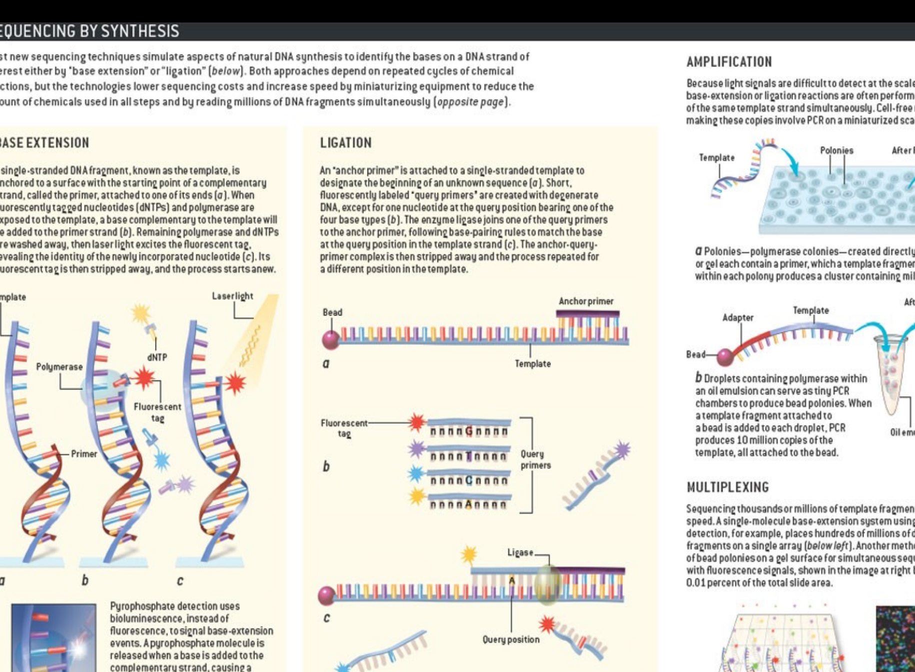
- Array-based sequencing

  ✓ Higher degree of parallelism than capillary-based sequencing

Massive parallel sequencing technology (MPS) in IVF

- **NGS technologies** are starting to make their **way into PGS**
- Processing of a **larger number** of samples in **one** experiment compared to microchip techniques
- Currently used in large IVF clinics mainly for **screening** of **aneuploidies** x possibility of a comprehensive view (**ploidy**, **structural changes, mutations**)
- Most often a form of **closed systems** - Illumina, Ion Torrent, or a form of library preparation (e.g. Agilent, Roche, etc.)

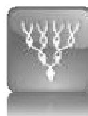
VeriSeq PGS (Illumina)



- Sequenicng by synthesis
- Anueploididy detection in 12 hours
- Up to 24 samples, resolution 16 Mbp

VeriSeq PGS (Illumina)

VeriSeq PGS

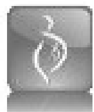


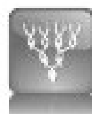
- **Massively parallel sequencing approach – 25 million reads per MiSeq run**
- **Multiplex up to 24 samples per run by using indexing**
- **800K to 1M reads per sample**
- 36nt read length
- Reads are mapped and grouped into bins (median size 1 Mbp)
- Count number of reads per bin
- Algorithms to correct for technical and GC biases
- Normalisation within sample, assuming median bin count across all autosomes corresponds to copy number 2
- Number of fragments from each bin is proportional to its copy number
  - **A trisomy chromosome will have 1.5x more counts than a disomy chromosome**

illumina

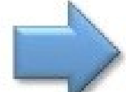
VeriSeq PGS (Illumina)

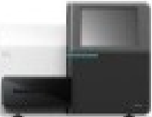
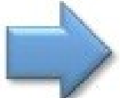
VeriSeq PGS Workflow





Sample Preparation	Library Preparation	MiSeq Instrument	BlueFuse Analysis
• Total 2.5 hours • Hands-on 45 mins	• Total 3.5 hours • Hands-on 1.5 hours	• Total 5.5 hours • Hands-on 10 mins	• Total 10 mins • Hands-on <1 min



- 2 hours 30 min hands-on time
- Total protocol of approximately 12 hours for 12 to 24 samples

illumina

VeriSeq PGS (Illumina)

BlueFuse analytický SW

BlueFuse software provides a complete solution for analyzing, storing, and reporting VeriSeq results. A. Sample database shows experimental information. B. Profiles for the sample (top) and DecisionTrack information (bottom). C. Karyotype chart for whole-genome view (top) and region view with the opportunity to annotate (bottom). D. Reports per embryo or per cycle (embryo report shown).

VeriSeq PGS (Illumina)

Hum Reprod. 2014 Dec;29(12):2802-13. doi: 10.1093/humrep/deu277. Epub 2014 Oct 21.

Application of next-generation sequencing technology for comprehensive aneuploidy screening of blastocysts in clinical preimplantation genetic screening cycles.

Fiorentino F[1], Bono S[2], Biricik A[2], Nuccitelli A[2], Cotroneo E[2], Cottone G[2], Kokocinski F[3], Michel CE[3], Minasi MG[4], Greco E[4].

Author information

Abstract

STUDY QUESTION: Can next-generation sequencing (NGS) techniques be used reliably for comprehensive aneuploidy screening of human embryos from patients undergoing IVF treatments, with the purpose of identifying and selecting chromosomally normal embryos for transfer?

SUMMARY ANSWER: Extensive application of NGS in clinical preimplantation genetic screening (PGS) cycles demonstrates that this methodology is reliable, allowing identification and transfer of euploid embryos resulting in ongoing pregnancies.

WHAT IS KNOWN ALREADY: The effectiveness of PGS is dependent upon the biology of the early embryo and the limitations of the

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Mol Cytogenet. 2015 Jun 16;8:38. doi: 10.1186/s13039-015-0143-6. eCollection 2015.

Application of next-generation sequencing for 24-chromosome aneuploidy screening of human preimplantation embryos.

Zheng H[1], Jin H[2], Liu L[2], Liu J[1], Wang WH[3].

Author information

Abstract

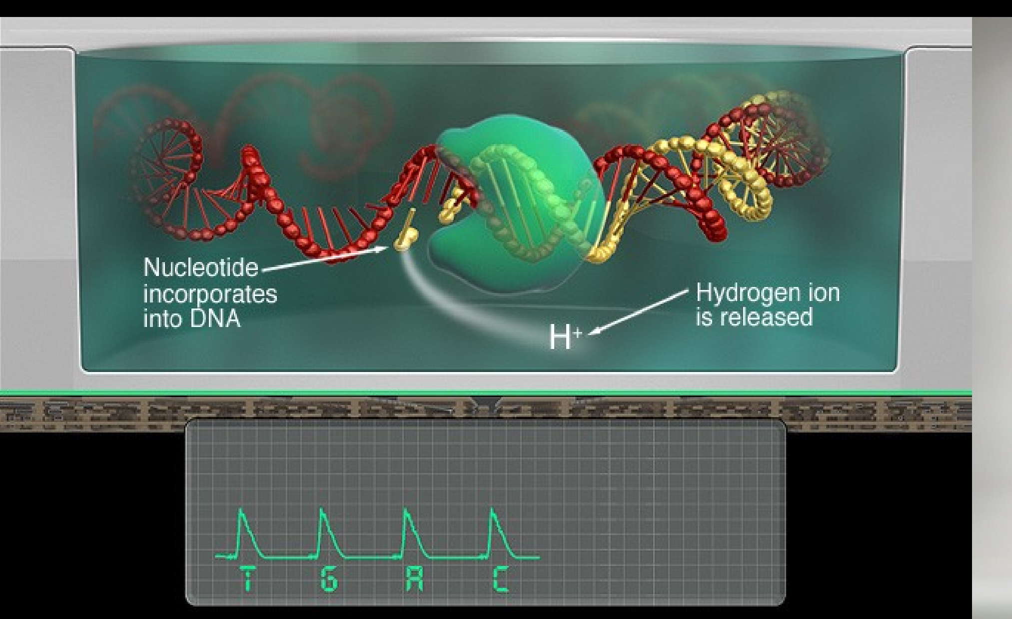
BACKGROUND: Aneuploidy is a leading cause of repeat implantation failure and recurrent miscarriages. Preimplantation genetic screening (PGS) enables the assessment of the numeral and structural chromosomal errors of embryos before transfer in patients undergoing in vitro fertilization. Array comparative genomic hybridization (aCGH) has been demonstrated to be an accurate PGS method and in present thought to be the gold standard, but new technologies, such as next-generation sequencing (NGS), continue to emerge. Validation of the new comprehensive NGS-based 24-chromosome aneuploidy screening technology is still needed to determine the preclinical accuracy before it might be considered as an alternative method for human PGS.

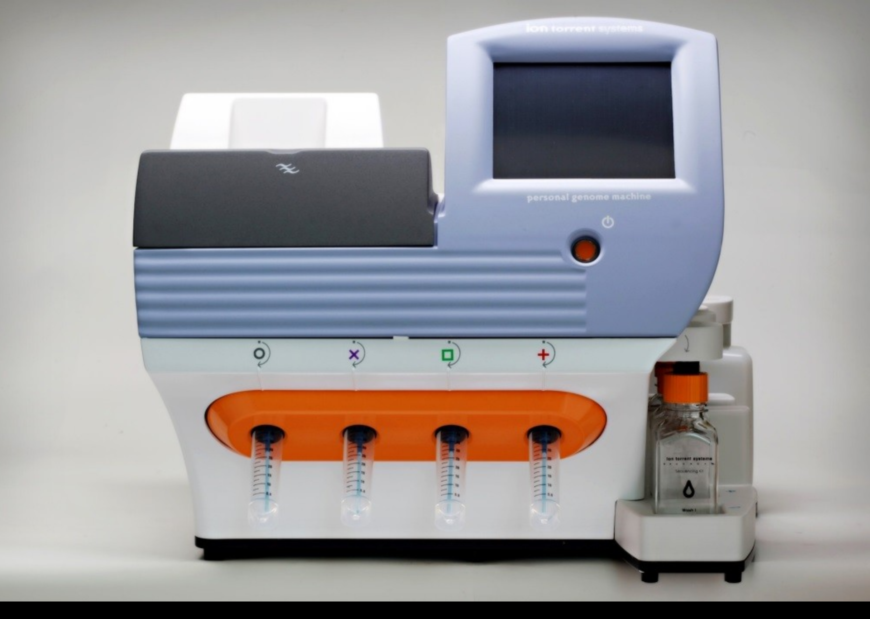
Ion Torrent Semiconuctor Sequencing

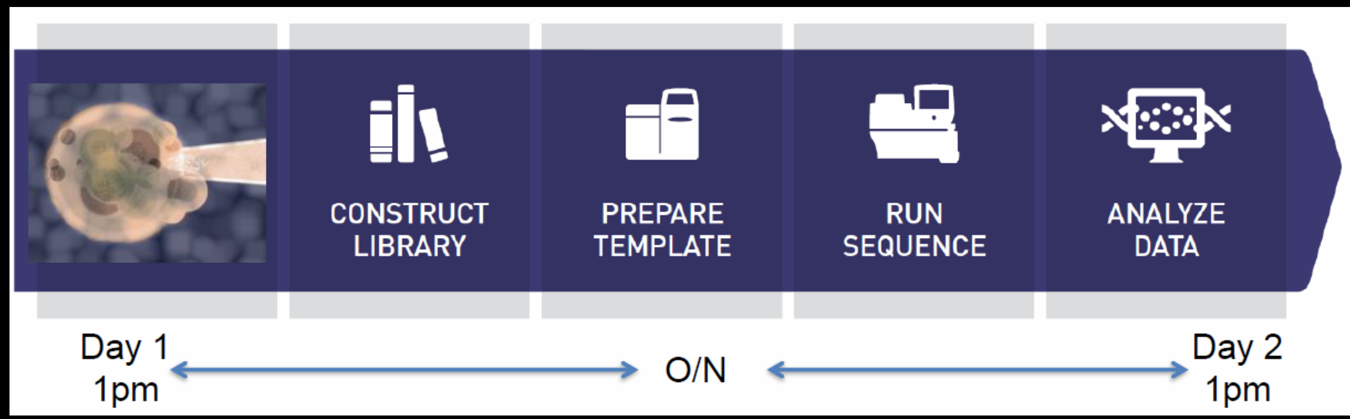
Principle and Elements of Semiconductor Sequencing

Simple Natural Chemistry of Sequencing-by-Synthesis with H^+ release detection

Ion Torrent Aneuploidy Analysis
(Life Tech Inc.)



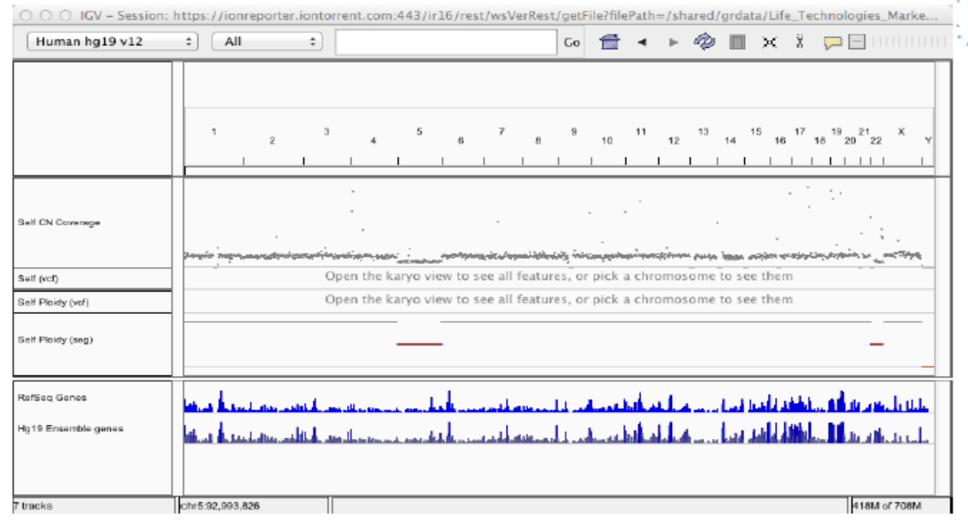




Ion Torrent Aneuploidy Analysis

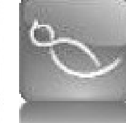
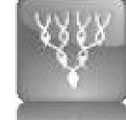
- "Semiconductor" sequencing
- Based on the detection of the pH change that occurs when H+ is released during base binding to deoxyribose

- Protocol within 24 hours
- Resolution ~10 Mbp
- Cost $70/embryo for 32 embryos analyzed together

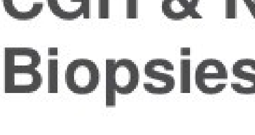


NGS in IVF

- Routine use is still hampered by cost and algorithm in laboratories (3 vs. 5 day embryos, vitrification technology, etc.)
- Advantages - more robust compared to array-CGH, higher capacity,
- Higher "dynamic interval" - detection of mosaicism
- Development - exome level detection - "all in" = CHA, mutations for monogenic diseases

Performance Comparison between array CGH & NGS on Day 5 Trophectoderm Biopsies



aCGH

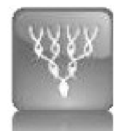
Log2 Ratio Ch1/Ch2

2.00
1.60
1.20
0.80
0.40
-0.00
-0.40
-0.80
-1.20
-1.60

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y

NGS

Improved dynamic range – Fiorentino (2014) ESHRE S07



Improved dynamic range of NGS enables enhanced detection and quantification of chromosomal mosaicism

aCGH

Log2 Ratio Ch1/Ch2

↑

Chromosomal Position

NGS

Copy Number

↑

Chromosomal Position

illumina

I

aCGH

NGS

2.19 Mb segmental duplication of 6p

II

2.53 Mb segmental deletion of 5q

III

1.81 Mb segmental deletion of 9p

Copy number

Chro... ...e position

Results of partial aneusomy detection

NGS in IVF

Display Settings: Abstract — Send to:

Fertil Steril. 2013 Apr;99(5):1377-1384.e6. doi: 10.1016/j.fertnstert.2012.12.018. Epub 2013 Jan 9.

Evaluation of targeted next-generation sequencing-based preimplantation genetic diagnosis of monogenic disease.

Treff NR[1], Fedick A, Tao X, Devkota B, Taylor D, Scott RT Jr.

Author information

Abstract

OBJECTIVE: To investigate the applicability of next-generation sequencing (NGS) to preimplantation genetic diagnosis (PGD); to evaluate semiconductor-based NGS for genetic analysis of human embryos.

DESIGN: Blinded.

SETTING: Academic center for reproductive medicine.

PATIENT(S): Six couples at risk of transmitting single-gene disorders to their offspring.

INTERVENTION(S): None.

MAIN OUTCOME MEASURE(S): Embryonic genotype consistency of NGS with two independent conventional methods of PGD.

RESULT(S): NGS provided 100% equivalent PGD diagnoses of compound point mutations and small deletions and insertions compared with both reference laboratory- and internally developed quantitative polymerase chain reaction (qPCR)-based analyses. Furthermore, NGS single-gene disorder screening could be performed in parallel with qPCR-based comprehensive chromosome screening.

CONCLUSION(S): NGS can provide blastocyst PGD results with a high level of consistency with established methodologies. This study and its design could serve as a model for further development of this important and emerging technology.

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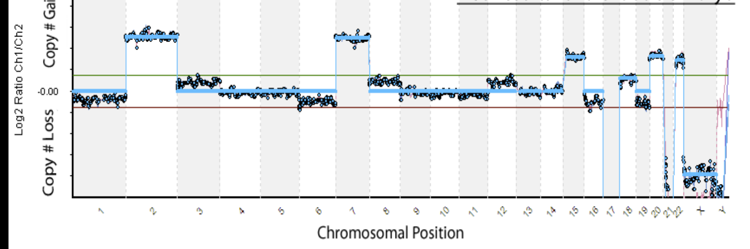

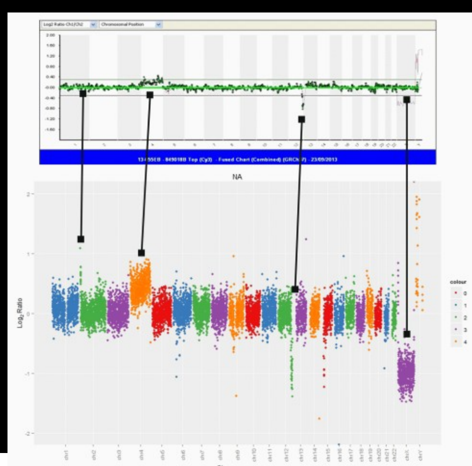

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Fertil Steril. 2014 May;101(5):1375-82. doi: 10.1016/j.fertnstert.2014.01.051. Epub 2014 Mar 6.

Development and validation of a next-generation sequencing-based protocol for 24-chromosome aneuploidy screening of embryos.

Fiorentino F[1], Biricik A[2], Bono S[2], Spizzichino L[2], Cotroneo E[2], Cottone G[2], Kokocinski F[3], Michel CE[3].

Author information

Abstract

OBJECTIVE: To validate a next-generation sequencing (NGS)-based method for 24-chromosome aneuploidy screening and to investigate its applicability to preimplantation genetic screening (PGS).

DESIGN: Retrospective blinded study.

SETTING: Reference laboratory.

PATIENT(S): Karyotypically defined chromosomally abnormal single cells and whole-genome amplification (WGA) products, previously analyzed by array comparative genomic hybridization (array-CGH), selected from 68 clinical PGS cycles with embryos biopsied at cleavage stage.

INTERVENTION(S): None.

MAIN OUTCOME MEASURE(S): Consistency of NGS-based diagnosis of aneuploidy compared with either conventional karyotyping of single cells or array-CGH diagnoses of single blastomeres.

RESULT(S): Eighteen single cells and 190 WGA products from single blastomeres, were blindly evaluated with the NGS-based protocol. In total, 4,992 chromosomes were assessed, 402 of which carried a copy number imbalance. NGS specificity for aneuploidy call (consistency of chromosome copy number assignment) was 99.98% (95% confidence interval [CI] 99.88%-100%) with a sensitivity of 100% (95% CI 99.08%-100%). NGS specificity for aneuploid embryo call (24-chromosome diagnosis consistency) was 100% (95% CI 94.59%-100%) with a sensitivity of 100% (95% CI 97.39%-100%).

CONCLUSION(S): This is the first study reporting extensive preclinical validation and accuracy assessment of NGS-based comprehensive aneuploidy screening on single cells. Given the high level of consistency with an established methodology, such as array-CGH, NGS has demonstrated a robust high-throughput methodology ready for clinical application in reproductive medicine, with potential advantages of reduced costs and enhanced precision.

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NGS in IVF - problems?

1) **With more robust screening methods, the volume of data is increasing - interpretation?**
2) **Detection of mosaicism in embryos - transfer yes or no?**
3) **PGD 2.0 - does it really improve IVF outcomes?**

REVIEW Open Access

CrossMark

Is the hypothesis of preimplantation genetic screening (PGS) still supportable? A review

Norbert Gleicher[1,2,3,4*] and Raoul Orvieto[5]

Abstract

The hypothesis of preimplantation genetic diagnosis (PGS) was first proposed 20 years ago, suggesting that elimination of aneuploid embryos prior to transfer will improve implantation rates of remaining embryos during in vitro fertilization (IVF), increase pregnancy and live birth rates and reduce miscarriages. The aforementioned improved outcome was based on 5 essential assumptions: (i) Most IVF cycles fail because of aneuploid embryos. (ii) Their elimination prior to embryo transfer will improve IVF outcomes. (iii) A single trophectoderm biopsy (TEB) at blastocyst stage is representative of the whole TE. (iv) TE ploidy reliably represents the inner cell mass (ICM). (v) Ploidy does not change (i.e., self-correct) downstream from blastocyst stage. We aim to offer a review of the aforementioned assumptions and challenge the general hypothesis of PGS. We reviewed 455 publications, which as of January 20, 2017 were listed in PubMed under the search phrase < preimplantation genetic screening (PGS) for aneuploidy>. The literature review was performed by both authors who agreed on the final 55 references. Various reports over the last 18 months have raised significant questions not only about the basic clinical utility of PGS but the biological underpinnings of the hypothesis, the technical ability of a single trophectoderm (TE) biopsy to accurately assess an embryo's ploidy, and suggested that PGS actually negatively affects IVF outcomes while not affecting miscarriage rates. Moreover, due to high rates of false positive diagnoses as a consequence of high mosaicism rates in TE, PGS leads to the discarding of large numbers of normal embryos with potential for normal euploid pregnancies if transferred rather than disposed of. We found all 5 basic assumptions underlying the hypothesis of PGS to be unsupported: (i) The association of embryo aneuploidy with IVF failure has to be reevaluated in view how much more common TE mosaicism is than has until recently been appreciated. (ii) Reliable elimination of presumed aneuploid embryos prior to embryo transfer appears unrealistic. (iii) Mathematical models demonstrate that a single TEB cannot provide reliable information about the whole TE. (iv) TE does not reliably reflect the ICM. (v) Embryos, likely, still have strong innate ability to self-correct downstream from blastocyst stage, with ICM doing so better than TE. The hypothesis of PGS, therefore, no longer appears supportable. With all 5 basic assumptions underlying the hypothesis of PGS demonstrated to have been mistaken, the hypothesis of PGS, itself, appears to be discredited. Clinical use of PGS for the purpose of IVF outcome improvements should,

Preimplantation genetic screening 2.0: the theory

Joep Geraedts[1,*] and Karen Sermon[2]

[1]GROW School for Oncology and Developmental Biology, Maastricht University, Maastricht, The Netherlands [2]Research Group Reproduc and Genetics, Vrije Universiteit Brussel, Laarbeeklaan 101, Brussels 1090, Belgium

*Correspondence address. E-mail: joep.geraedts@mumc.nl

Submitted on February 26, 2016; resubmitted on April 25, 2016; accepted on May 16, 2016

ABSTRACT: During the last few years a new generation of preimplantation genetic screening (PGS) has been introduced. In this paper, an over view of the different aspects of this so-called PGS 2.0 with respect to the why (what are the indications), the when (which developmental stage, i.e which material should be studied) and the how (which molecular technique should be used) is given. With respect to the aims it is clear that PGS 2.0 can be used for a variety of indications. However, the beneficial effect of PGS 2.0 has not been proved yet in RCTs. It is clear that cleavage stage is not the optimal stage for biopsy. Almost all advocates of PGS 2.0 prefer trophectoderm biopsy. There are many new methods that allow the study of complete aneuploidy with respect to one or more of the 24 chromosomes. Because of the improved vitrification methods, selection of fresh embryos for transfer is more and more often replaced by frozen embryo transfer. The main goal of PGS has always been the improvement of IVF success. However, success is defined by different authors in many different ways. This makes it very difficult to compare the outcomes of differen studies. In conclusion, the introduction of PGS 2.0 will depend on the success of the new biopsy strategies in combination with the analysis of all 24 chromosomes. It remains to be seen which approach will be the most successful and for which specific groups of patients.

After PGA…

After PGD, the results should be consulted with a clinical geneticist

+

Follow-up with prenatal genetic diagnosis should be performed

Thank you for attention