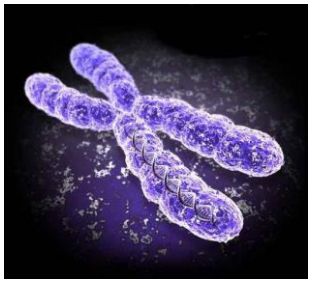
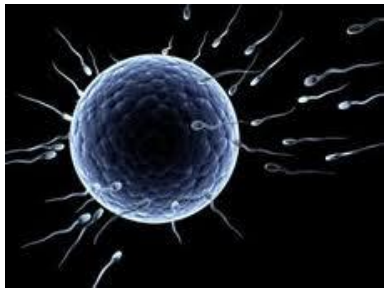




# Možnosti preimplantačních genetických analýz na prahu nového milénia


***Mgr. Jan Smetana, Ph.D***

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

# Asistovaná reprodukce (ART)

- Asistovaná reprodukce je označení pro lékařské postupy a metody, při kterých dochází k manipulaci se zárodečnými buňkami nebo s embryi, včetně jejich uchovávání, a to za účelem léčby neplodnosti ženy nebo muže
- Komplexní proces je dnes většinou založen na **technikách *in vitro* fertilizace**
- Kromě párů s diagnózou využívají i páry s normální fertilitou, ale riziko přenosu genetické vady nebo patologických markerů
- Specializovaná centra - kliniky, sanatoria
- Cíl – narození **zdravého potomka = „léčba neplodnosti“**

# Plodnost (Fertilita)

**Plodnost (fertilita)**

= schopnost jedince se pohlavně množit

= komplexní vlastnost, která je výsledkem schopností samců a samic poskytovat zdravé potomstvo v optimálním počtu za určitý čas

= demografický ukazatel vyjadřující průměrný počet potomků na jednu ženu

# Plodnost (Fertilita) v ČR

- Pravděpodobnost otěhotnění při nechráněném styku u zdravého páru na jeden menstruační cyklus je cca **25%**
- Průměrný věk prvorodiček v ČR **26,3** roku
- Průměrná zdravá žena do 35 let s pravidelným nechráněným pohl. stykem má šanci otěhotnět cca **16%,** tj. průměrně **otěhotnění lze dosáhnout 1/6 ovulačních cyklů**
- **50%** žen (20-34 let) otěhotní do **dvou měsíců, 80% do půl roku**

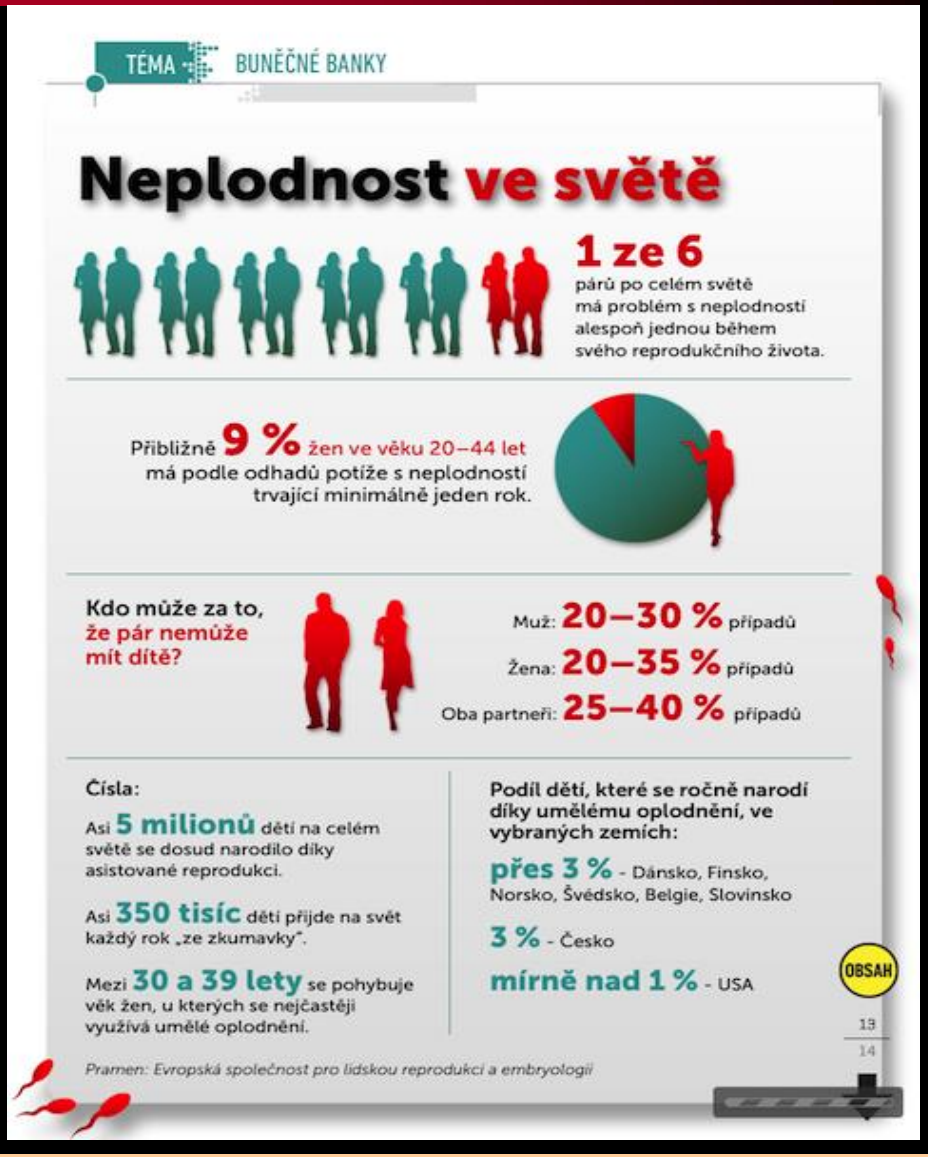
*(Zdravotnická statistika MZCR, www.uzis.cz)*

# Neplodnost (Infertilita)

**Neplodnost (Infertilita)**

= neschopnost dosáhnout klinického těhotenství **po 12 a více měsících** nechráněného pravidelného pohlavního styku (WHO)

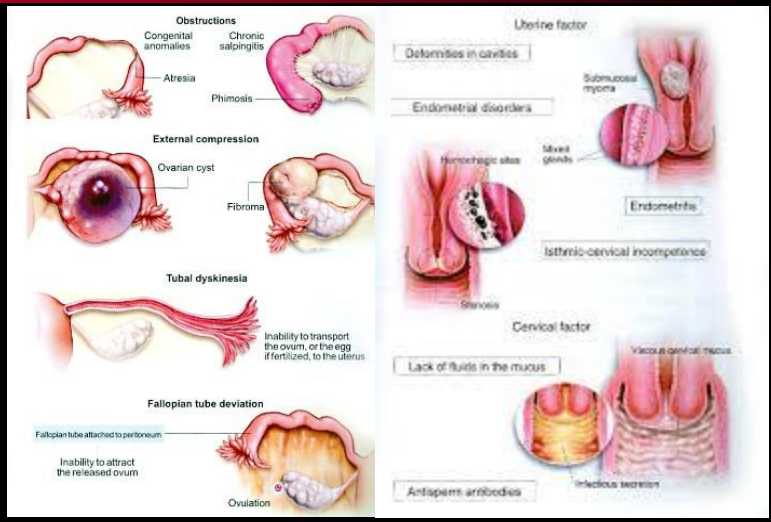
- V ČR ~ 20-25 % párů



# Příčiny neplodnosti

## Ženské faktory

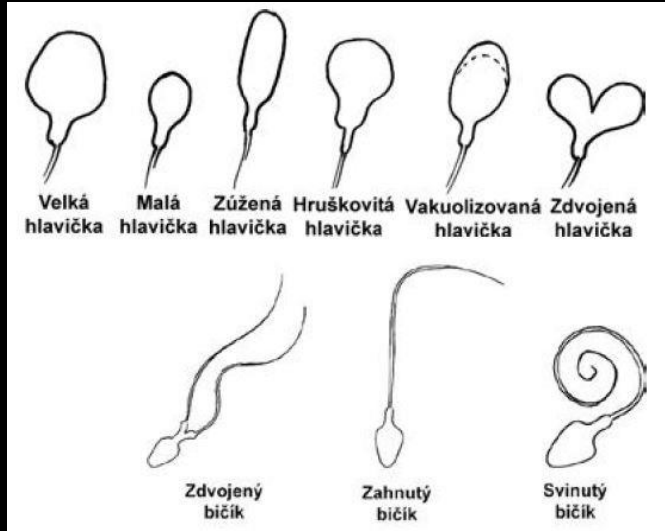
- *ovariální faktor* - vaječník nevytvoří nebo neuvolní kvalitní životaschopné vajíčko
- *tubární faktor* - poškození vejcovodů, chybění vejcovodů, neprůchodné vejcovody
- *endometrioza* - přítomnost děložní sliznice mimo dutinu děložní




## Mužské faktory

- *špatná funkce spermie* - neschopnost spermie proniknout a oplodnit vajíčko ženy

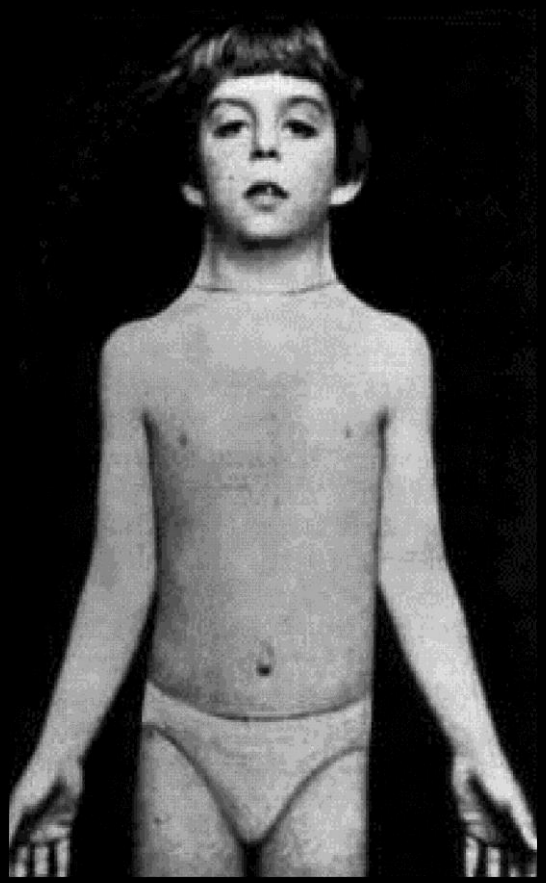
- Oligozoospermie (<15*10$^6$ v ejakuklátu)
- Astenozoospermie (nedostatečná pohyblivost)
- Teratozoospermie (špatná morfologie)
- Azoospermie (nepřítomnost spermií v ejakulátu)
- **„grand sperm zero“ - cca 2060**




*Obr. Příklady patologických morfologických nálezů (zdroj: ZDRAVÁ SPERMIE – prevence neplodnosti)*

# Genetické příčiny neplodnosti u žen

1) **Chromozomové aberace** - strukturní i početní změny
   Turnerův syndrom – 45, X
   „Superženy“ – 47, XXX
   Aneuploidie v gametách
- Robertsonské translokace, centromerická fúze akrocentrických chromozomů (13-15, 21, 22)

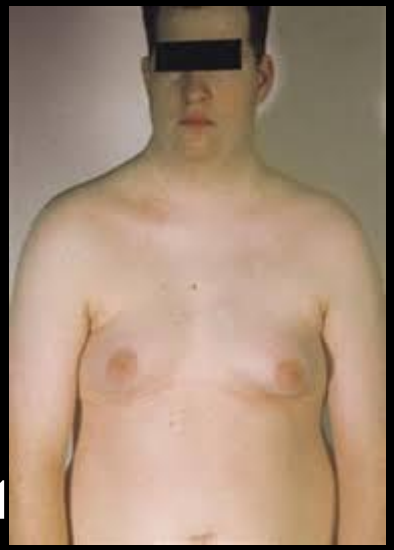


2) **Mutace** - geny ovlivňující srážení krve
   *MTHFR* (1p36.3)
   Leidenská mutace (*F5* – 1q23),
   G20210A v genu pro trombin
   CFTR

# Genetické příčiny neplodnosti u můžů

## Chromozomové aberace

- Klinefelterův syndrom - **47, XXY**
- Muži - 47, XYY
- Strukturní abnormality chr. Y
  delece v (Yp)(11.3) – *SRY* – porucha vývoje pohl. ústrojí
  delece Yq11 – *AZF* – azoosperima factor
  = porucha vývoje spermií
- Translokace autozomů, Y/A, Robertsonské translokace
  – centromerická fúze akrocentrických chromozomů (13-1
- Aneuploidie v gametách (X,Y, 21, 13,18)



## Genové mutace

Cystická fibróza – mutace F508 v *CFTR1*, 97% mužů neplodných

•

# Historie IVF

- 17st- **van Graaf -** Graafovy folikuly**, van Leeuwenhoek -** pozorování savčích spermií
- 19st - první veděcké práce o oplození ***in vitro na zvířatech***
  - Schenk (1878), W. Heape – porod 6 králičích mláďat po vitro oplození (1890).
- 1944 – ***Rock, Menkin*** – oplození lidského oocytu *in vitro*
- *1951* – ***Austin, Chang*** - pro oplozovací schopnost spermie je nezbytný její předchozí pobyt v genitálním traktu samice (kapacitace spermií)

A. van Leeuwenhoek

- **<u>2. pol 20st – Cambridge University - R.G. Edwards</u>**
  - popis zrání oocytů a oplození *in vitro*, možnost kultivace embryí
- 1971 – Steptoe, Purdy: Nature – možnost *in vitro* kultivace lidských embryí do stádia blasystocysty
- 70. léta – vylepšení kultivačních médií, laparoskopických technik, kryokonzervace
- 1978 – Lancet – klinické aplikace oplození *in vitro* - L. Brownová
- 2010 - **R.G. Edwards - Nobelova cena za embryologii**

**R.G. Edwards**



**P.C. Steptoe**

# Historie IVF Č(SS)R

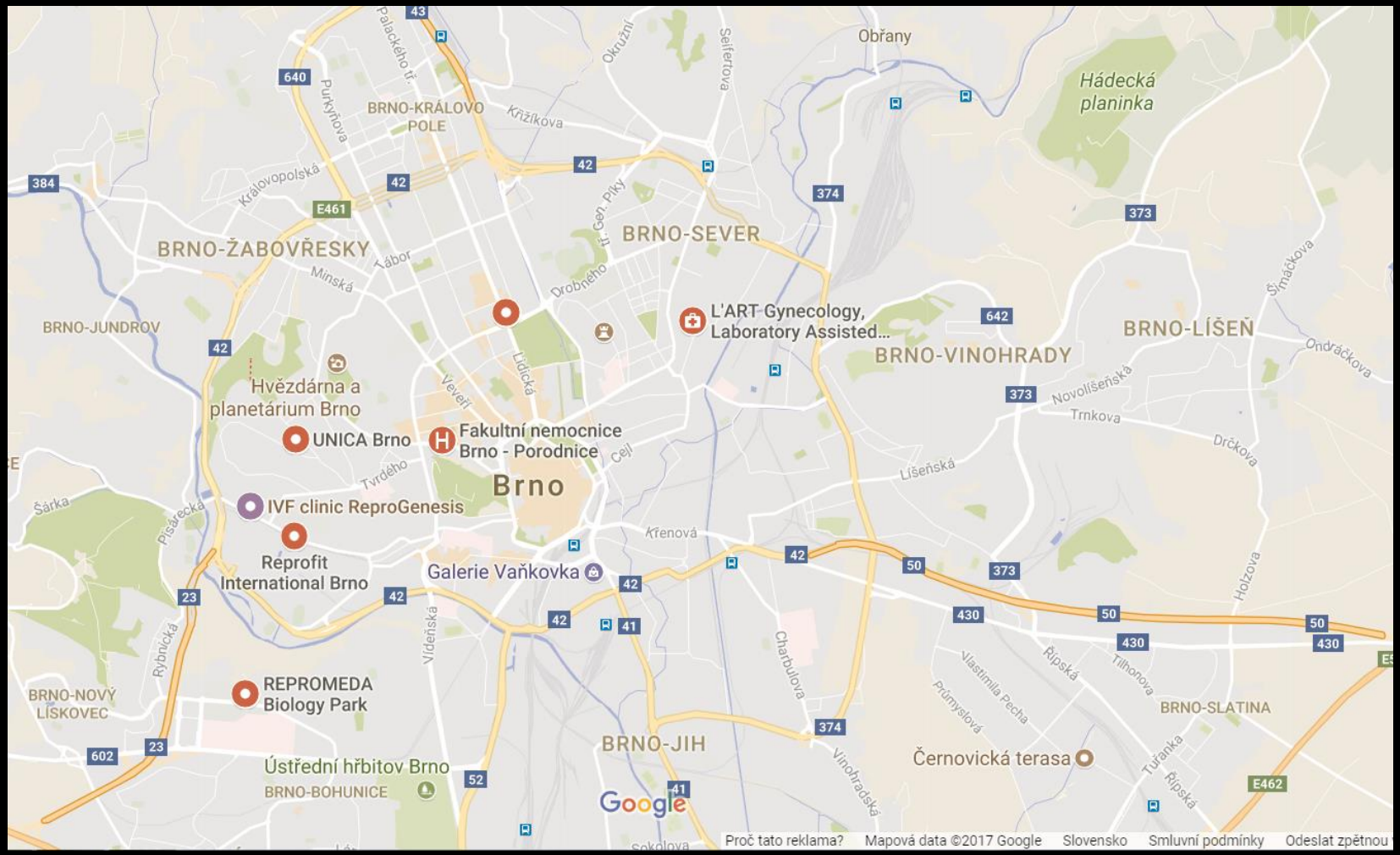
| 25. 7. 1978 | 29. 10. 1984 | 4. 1. 1988 | 2001 | V současné době |
|---|---|---|---|---|
| První dítě na světě počaté metodou in vitro se narodilo třicetileté Angličance Lesley Brownové. Šlo o holčičku jménem Louisa. | První dítě v České republice narozené po početí in vitro. Stalo se tak na gynekologicko-porodnické klinice FN v Brně, která je dodnes jedním z nejvýznamnějších center asistované reprodukce ve střední Evropě. | Porod prvních dvojčat ze zkumavky v ČR | První těhotenství po použití PGD v ČR | se rodí v ČR „ze zkumavky“, tedy po metodách asistované reprodukce (AR), kolem 2 % dětí ze všech, což je téměř 2000 ročně. Ve světě počet takto narozených dětí již překročil milión. |

Prof. L. Pilka

MUDr. J. Tesarik

Prof. P. Trávník

# IVF kliniky v Brně

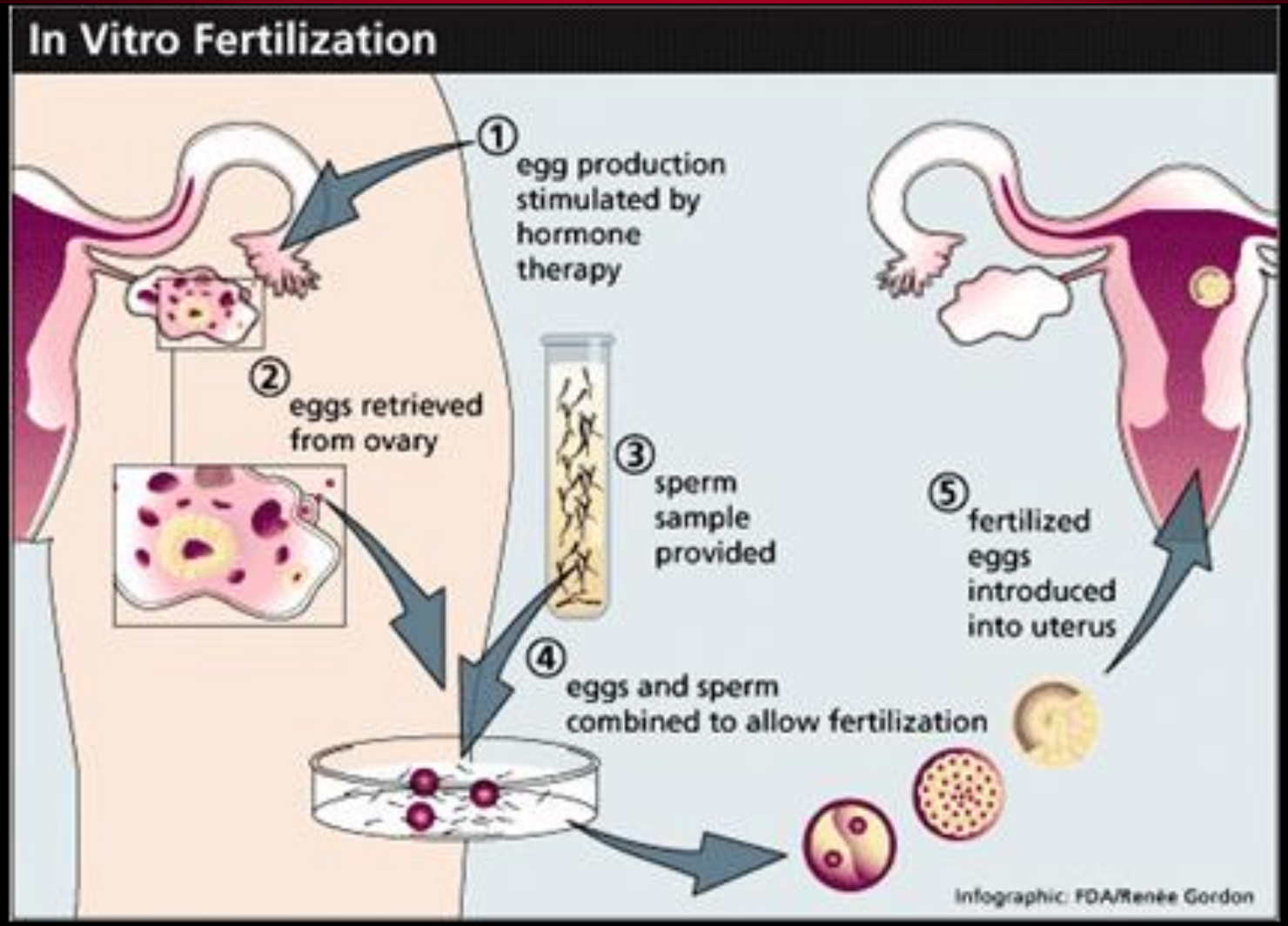


# IVF kliniky

- V ČR v současné době **41** registrovaných IVF center (6x Brno, 8x Praha)
- Specializovaná centra asistované reprodukce (gynekologie, porodnictví, reprodukční medicína, genetika, biochemie)
- Většinou soukromá centra x spolupráce s akademickým a zdravotnickým sektorem
- 2016 – **17998** IVF cyklů, **52**% hrazeno ZP
- Specializace na zahraniční klientela - **„reprodukční turistika“**

  *„CT2 – dovolená ve dvou – dítě v ceně“*

# Co obnáší IVF



# Metody asistované reprodukce

## Intrauterinní inseminace (IUI)

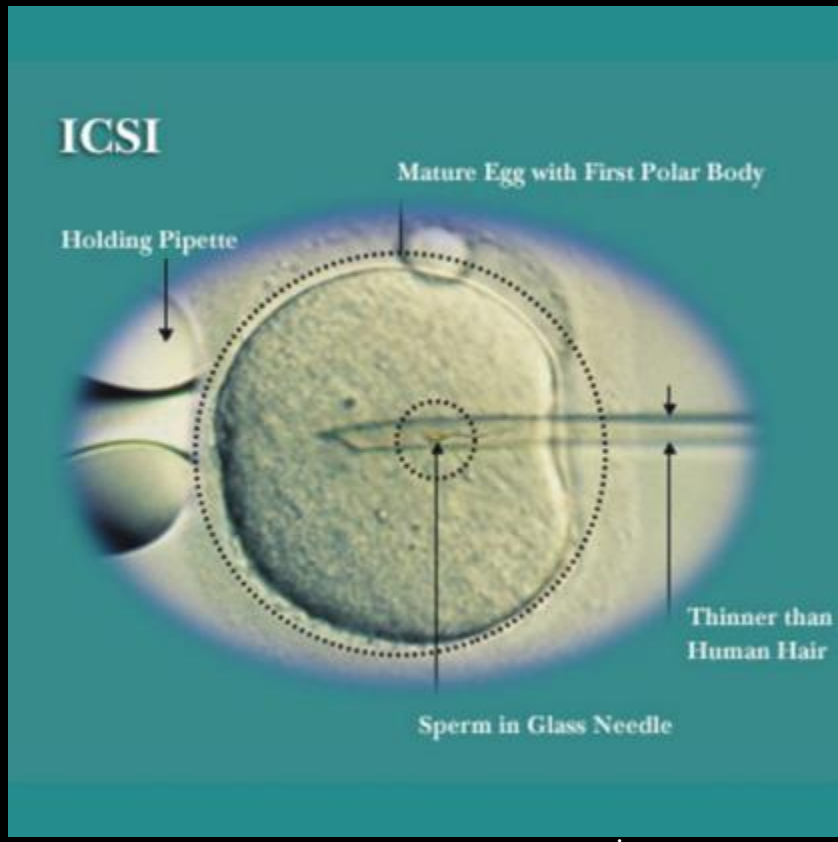
= zkoncentrované pročištěné spermie se zavádějí speciálním katetrem do dutiny děložní v období ovulace

## In vitro fertilizace (IVF)

= klasická metoda mimotělního oplodnění, při které jsou spermie kultivovány s oocyty in vitro.

***ICSI*** - intracytoplasmatická injekce spermií skrz obal (zona pellucida) do vajíčka

***PICSI*** - zdokonalená ICSI
umožňuje vybrat a vpravit do oocytu pouze zralou spermii přes vazbu na oocytární komplex (hyaluronan)




www.gipom.com

# Chirurgická aspirace spermií

**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;<br>Failed epididymal retrieval in OA cases;<br>Epididymal agenesis in CAVD cases;<br>Favorable testicular histopathology[1] in NOA cases;<br>Previous successful TESA/TEFNA attempt in NOA cases |
| Testicular sperm extraction (single or multiple biopsies) | TESE | Obstructive azoospermia;<br>Failed epididymal retrieval in OA cases;<br>Failed TESA/TEFNA in OA cases;<br>Non-obstructive azoospermia |
| Single seminiferous tubule biopsy | ND | Obstructive azoospermia;<br>Failed epididymal retrieval in OA cases;<br>Failed TESA/TEFNA in OA cases;<br>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

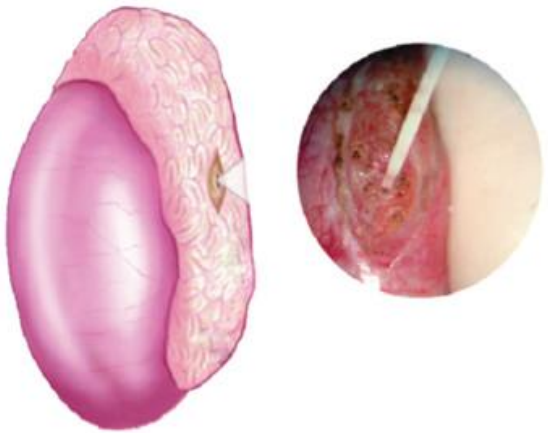
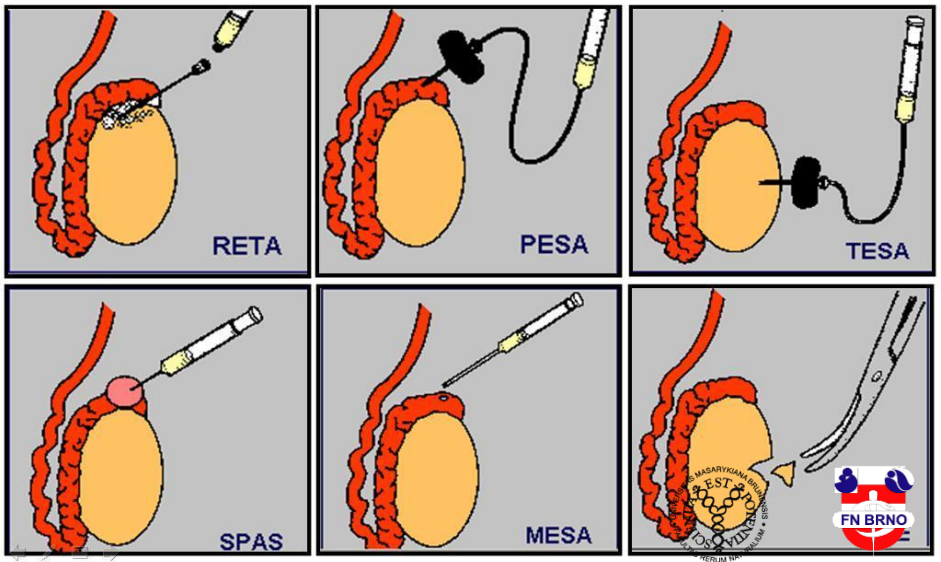
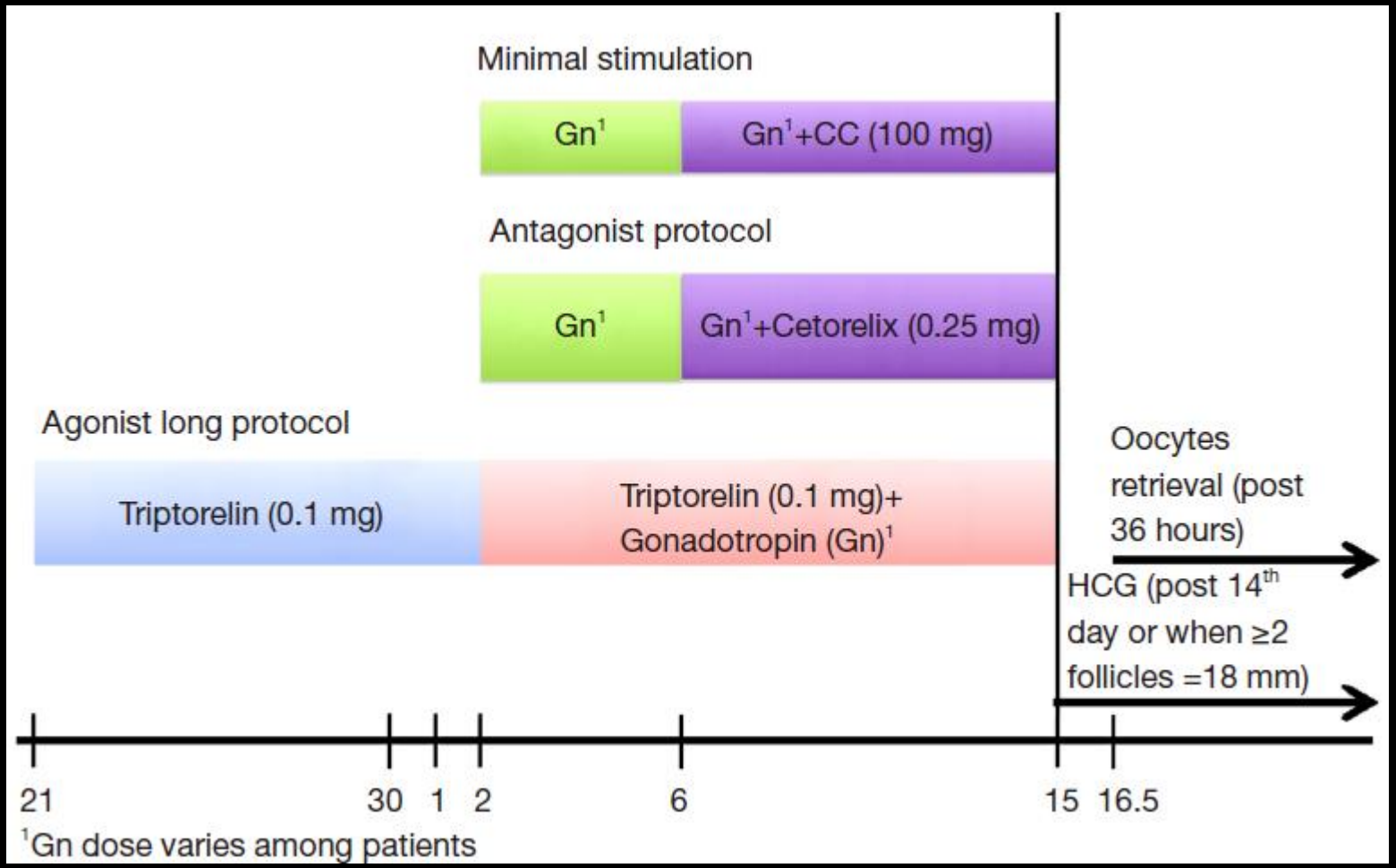


**Figure 6** - Microsurgical epididymal sperm aspiration (MESA). After exposure of the testis and epididymis, a dilated epididymal tubule is dissected and opened. The fluid is aspirated, diluted with sperm medium and sent to the laboratory for examination.

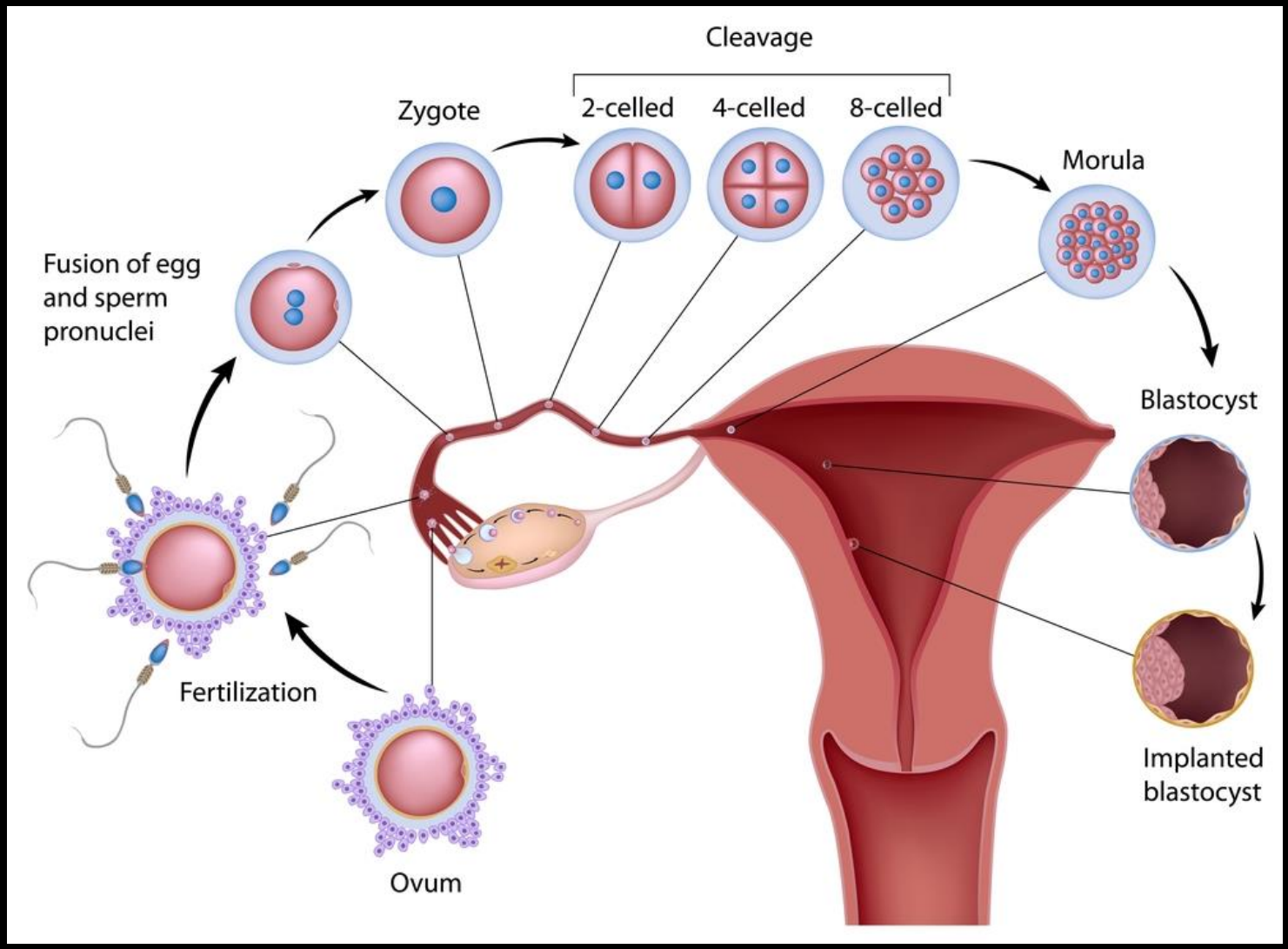


# Hormonálni stimulace – zisk oocytů

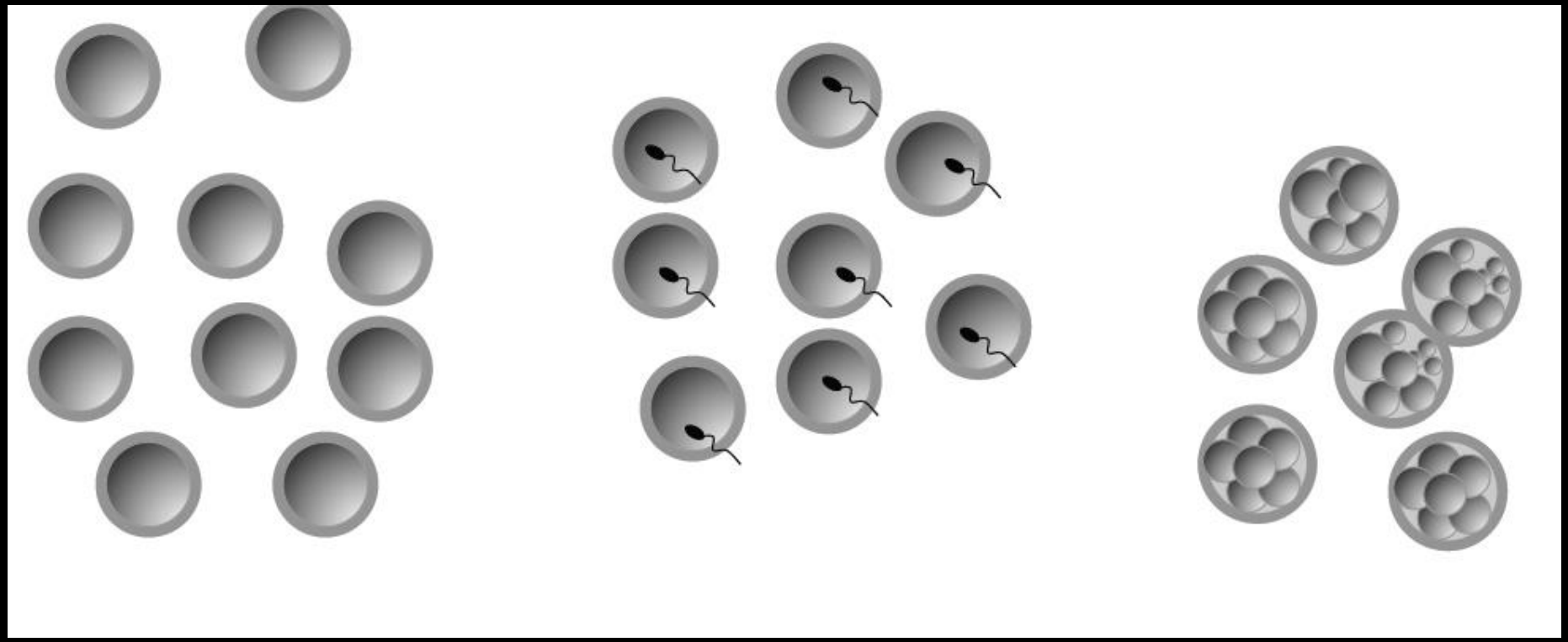



**GnRH** - gonadorelin, **hormon** uvolňující gonadotropin
**CC** - clomiphene citrate, syntetický estrogen, podpora ovulace

# IVF cyklus


Cleavage
Zygote
2-celled
4-celled
8-celled
Morula
Blastocyst
Fusion of egg and sperm pronuclei
Fertilization
Ovum
Implanted blastocyst

# IVF cyklus



- při IVF cyklu získáme obvykle několik embryí…
  - ideální je provést transfer **jednoho** embrya

x výběr … **morfologie, genetika ?**

# Genetické abnormality

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



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

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

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

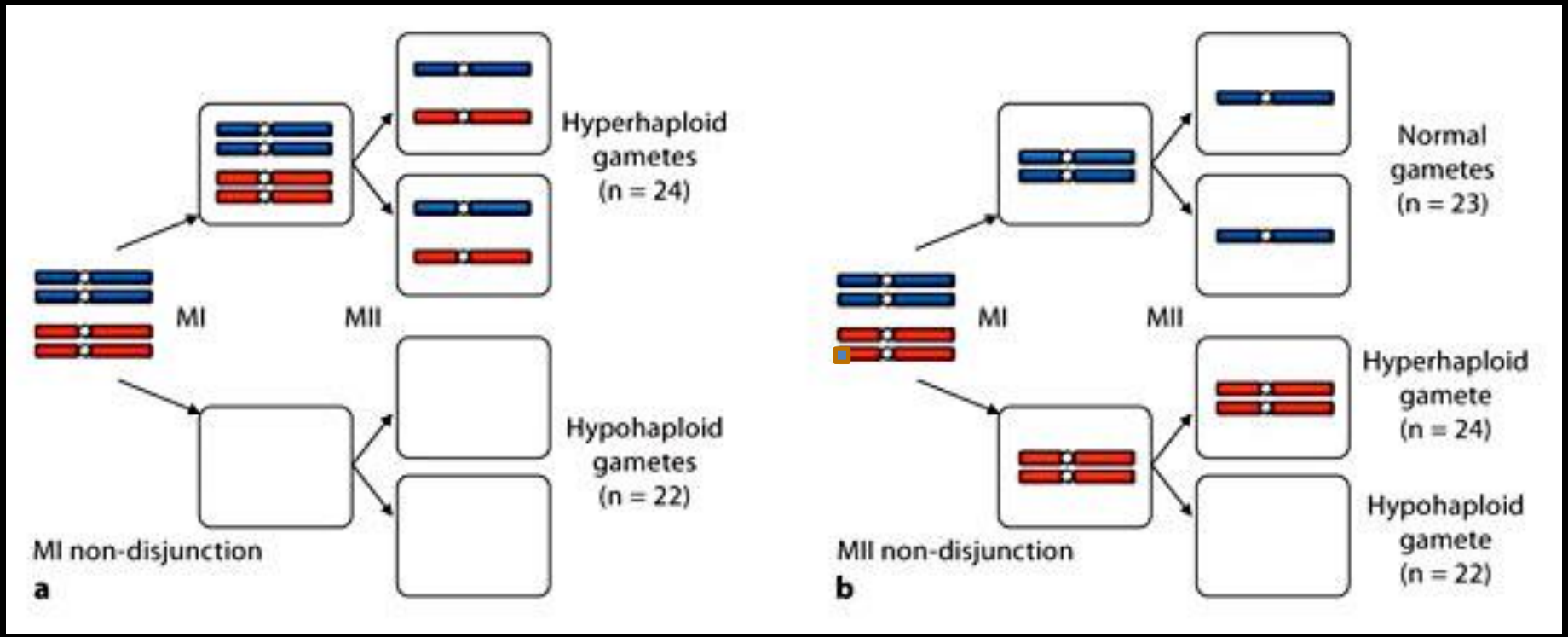
a valuable tool to overcome the necessity of transferring more than two embryos in poor prognosis patients, without negatively

User-friendliness steers developments at Organon

- velká část embryí bez ohledu na věk matky je aneuploidní **(54 % ve věku pod 35 let, 82 % ve věku 40 let a více)**

**Důvod = poruchy meiózy**

# Chromozomové aberace u embryí




- **~ 90 % aneuploidií vzniká v průběhu meiózy I u žen**

= postupná degradace kohezinu (porušení integrity bivalnetů

= absence distálních crossing – porušení segreagce během MI

J Med Genet. 2011 Jul;48(7):433-7. doi: 10.1136/jmg.2010.088070. Epub 2011 May 26.

**Array comparative genomic hybridisation on first polar bodies suggests that non-disjunction is not the predominant mechanism leading to aneuploidy in humans.**

Gabriel AS, Thornhill AR, Ottolini CS, Gordon A, Brown AP, Taylor J, Bennett K, Handyside A, Griffin DK.

School of Biosciences, University of Kent, Canterbury CT2 7NJ, UK.

# Předčasné rozdělení sesterských chromatid v MI je více než desetkrát častější příčinou vzniku aneuploidie, než klasická nondisjunkce !

Oocyte at Anaphase I following non-disjunction (smaller chromosome)

Oocyte at Anaphase I following precocious separation of sister chromatid (smaller chromosome)

Gabriel A S et al. J Med Genet 2011;48:433-437

# Genetické analýzy používané u IVF

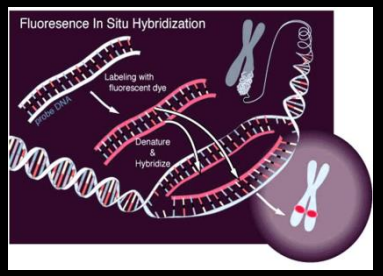
1. **Preimplantační genetické testování monogenních onemocnění - PGT-M** (PGD)
   - Dříve PGD = monogenní choroby
   - volba pohlaví u X-vázaných chorob
   - vrozené strukturní abnormality (Robertsonské translokace, balancované translokace)

2. **Preimplantační genetické testování aneuploidií PGT-A (PGS)**
   - detekce nejčastějších vrozených početních chromozomových aberací - aneuploidií

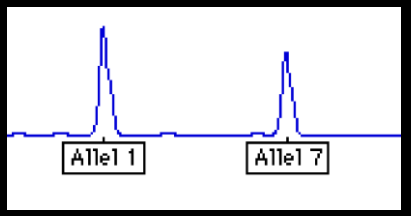
## PREIMPLANTAČNÍ GENETICKÉ TESTOVÁNÍ (PGT)

# PGA – diagnostické metody

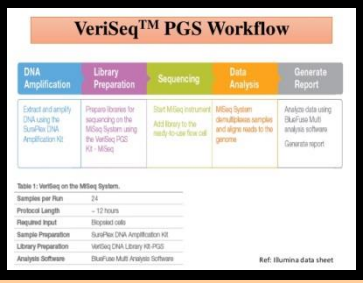
a) **Molekulární cytogenetika (I-FISH, CGH)**
- aneuploidie, translokace, mikrodeleční syndromy aj

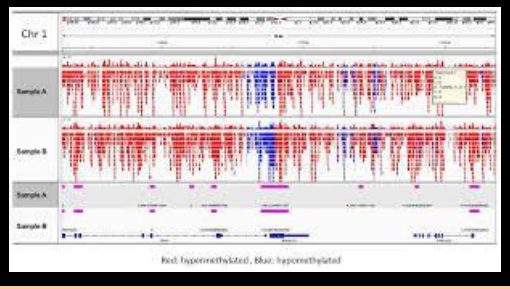


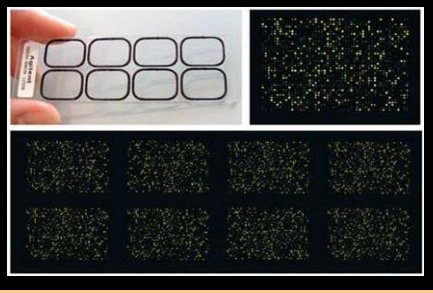

b) **PCR – monogenní choroby**
- specifické mutace - CF, thalasemie, srpkovitá anémie, hemofilie, DMD…..
- QF PCR - +13,16,18,21, X,Y



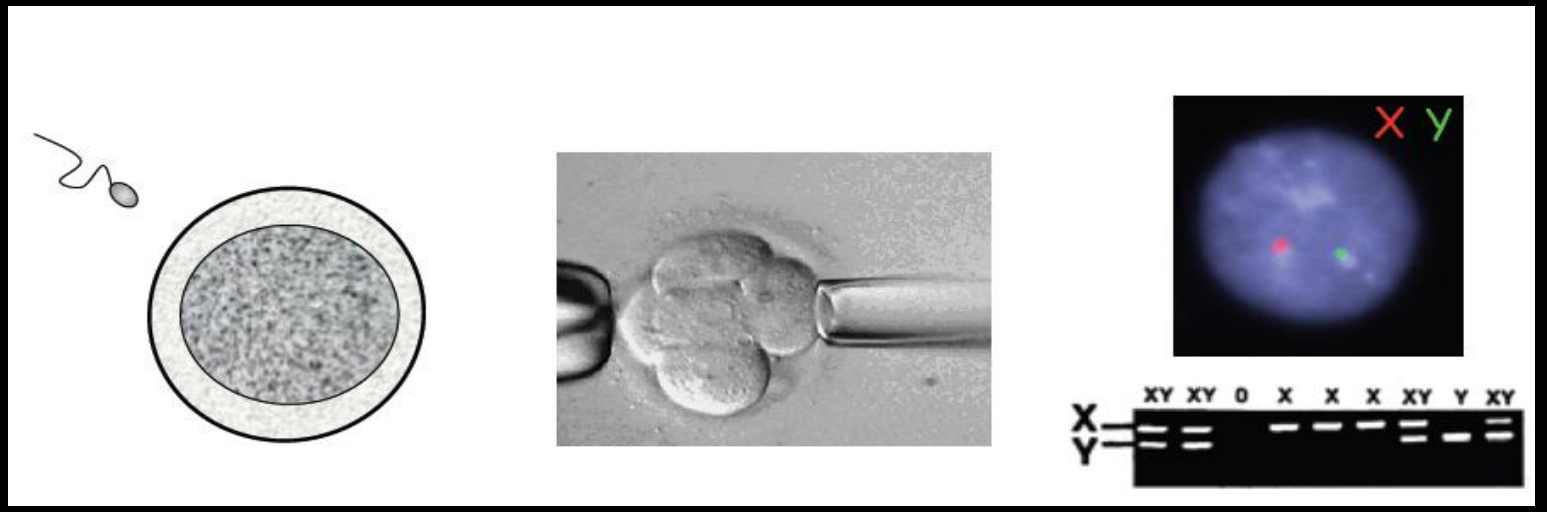

c) **Screeningové techniky** – „PGD 2.0“ - celogenomové pokrytí
- array-CGH (DNA čipy) - početní i strukturní CHA
- SNP čipy – KARYOMAPPING
- NGS - komplexní přístup, spojení PGD+PGS



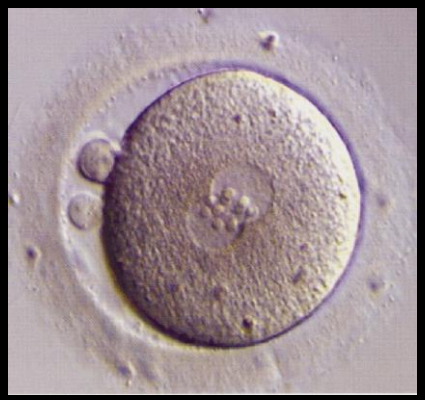



# PGA – vstupní biologický materiál




Oplození *in vitro* | Biopsie embrya | Genetický test

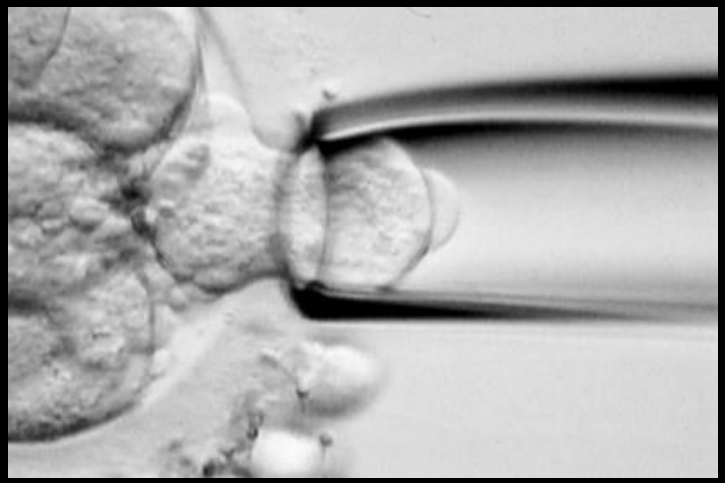


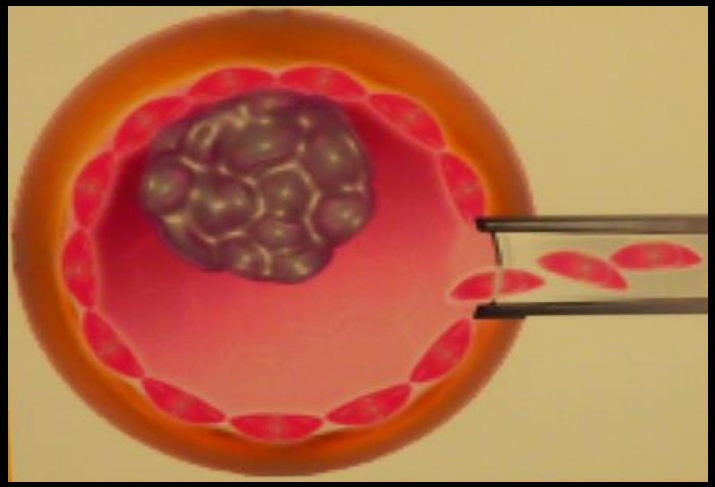
Polární tělísko

Blastomera (3. den)

Blastocysta (5-6. den)

# Biopsie embryí - rozdíly mezi 3. a 5. denním odběrem





**Biopsie embrya 3. den (blastomery)**

- analýza 1 – 2 buněk
- **30 - 60 % ztráta implantačního potenciálu**
- vyšší riziko mozaicismu
- časová tíseň (24 hod)

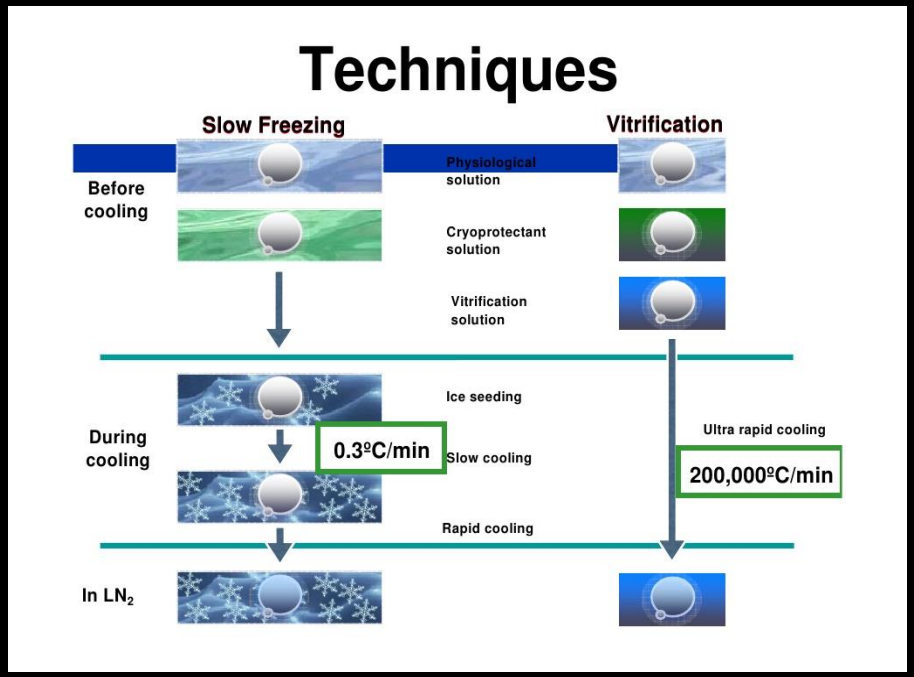
**Biopsie embrya 5.- 6. den (blastocysta)**

- analýza 5 – 10 buněk
- menší riziko mozaicismu
- možnost **vitrifikace embryí** po odběru – dostatek času pro vyšetření
- ne všechna embrya dosáhnou stádia blastocysty

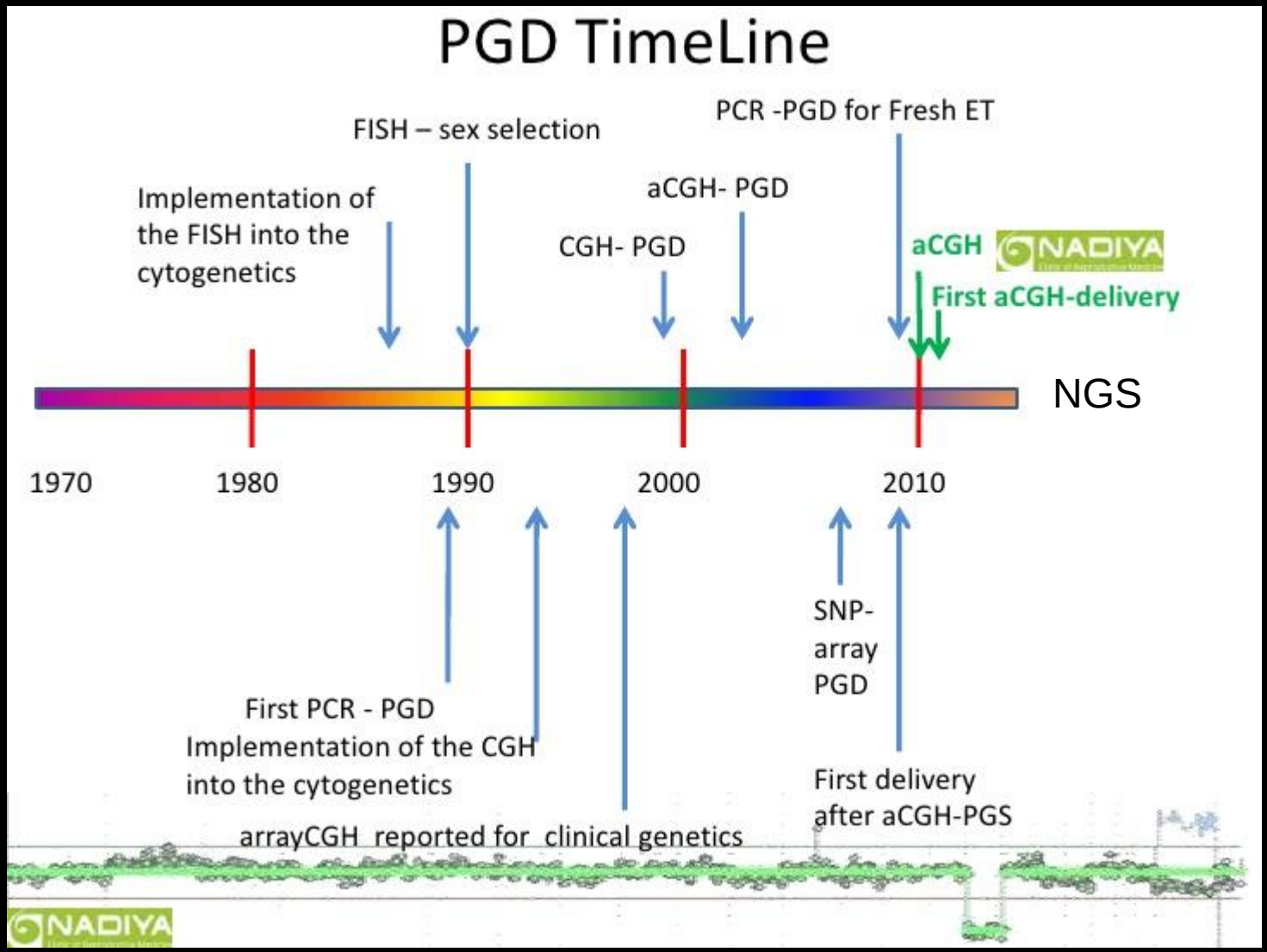
# Kryokonzervace a vitrifikace embryí

## Vitrifikace

- moderní metoda efektivní kryokonzervace embryí, oocytů i spermií
- Superrychlé zamražení biol. materiálu se směsí vhodně zvolených kryoprotektiv(sacharózy, dimethysulfoxidu) **na -196C**
- Viablita po rozmažení cca 98%




# Preimplantační genetický screening/diagnostika



# Chromozomové abnormality u embryí

## *Početní chromozomové aberace (aneuploidie)*

- jsou nejčastější genetickou změnou u lidských embryí
- aneuploidie se často vyskytují i u morfologicky normálně se vyvíjejících embryí *(A. Mertzanidou, 2013)*
- snižují úspěšnost metod asistované reprodukce

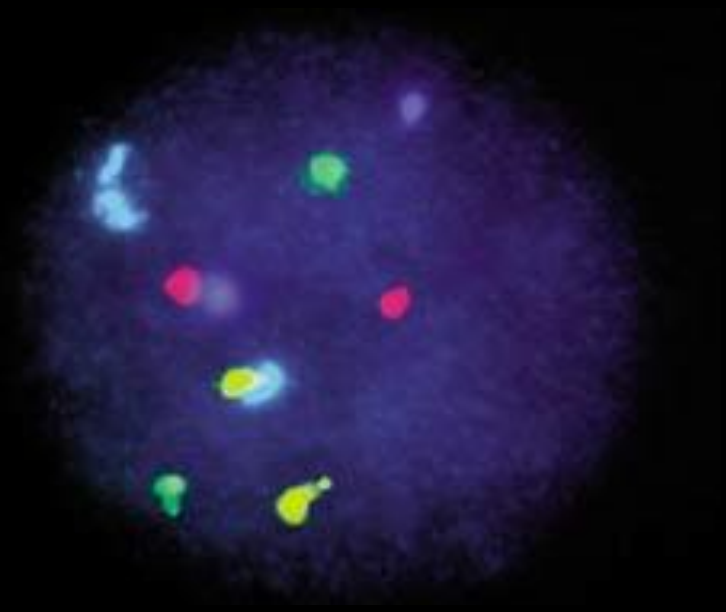
## *Strukturní chromozomové aberace*

- postzygotické mitotické poruchy jsou u embryí velmi časté
- **až u 70 % embryí** byla pomocí SNP čipů prokázána chromozomová nestabilita – duplikace, amplifikace, delece, UPD *(Vanneste et al., 2009)*

**SCREEINING POUHÝCH ANEUPLOIDIÍ U RANÝCH EMBRYÍ NESTAČÍ!**

# Preimplantační genetický screening/diagnostika pomocí techniky 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

Více chromozomů na jedné buňce – opakovaná FISH (FISH – zhodnocení, odmytí, nová FISH – zhodnocení)

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

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*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.

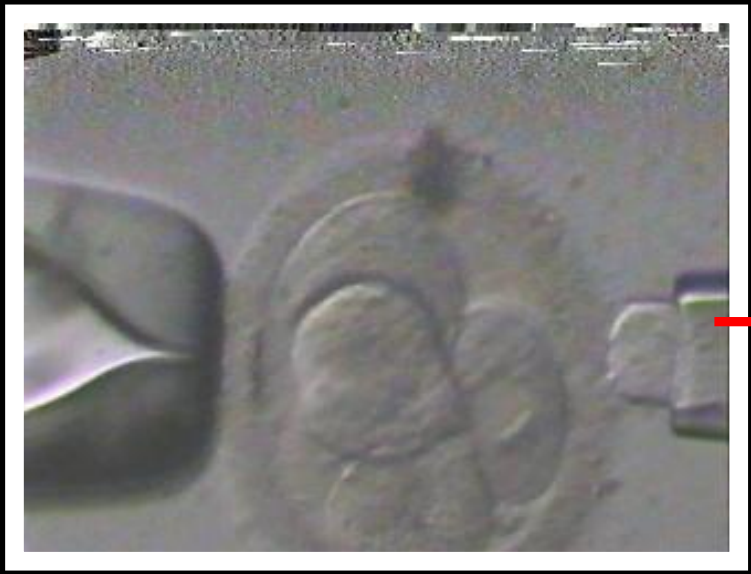
**RESULTS:** Nine RCTs comparing IVF with and without PGS were included in our meta-analysis. Fluorescence *in situ* hybridization was used in all trials and cleavage stage biopsy was used in all but one trial. PGS significantly lowered live birth rate after IVF for women of advanced maternal age (risk 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.

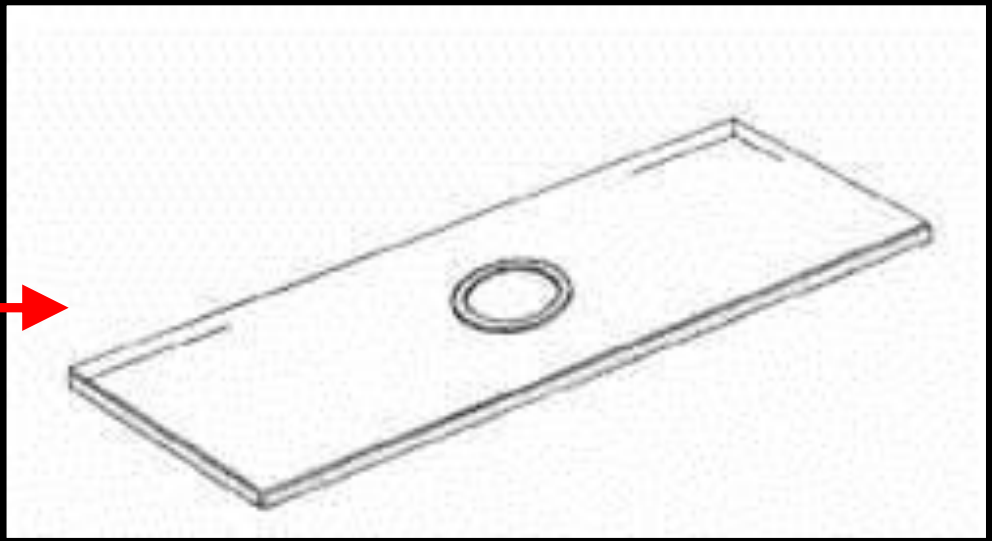
**CONCLUSIONS:** There is no evidence of a beneficial effect of PGS as currently applied on the live birth rate after IVF. On the contrary, for women of advanced maternal age PGS significantly lowers the live birth rate. Technical drawbacks and chromosomal mosaicism underlie this inefficacy of PGS. New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

**PGS pomocí I-FISH nezlepšuje úspěšnost IVF…..proč?**

# Problémy PGA I

- vyšetření 1 buňky - možnost diagnostického omylu ?





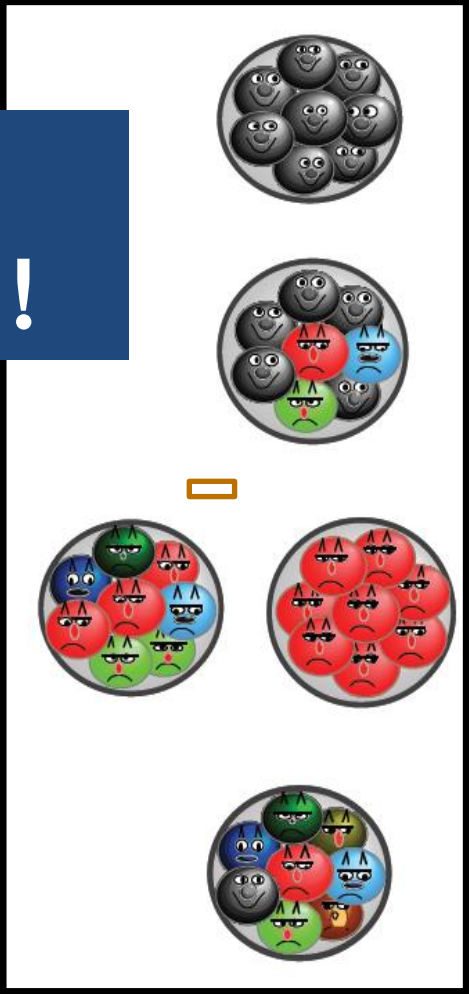
# Problémy PGA II

## EMBRYA:

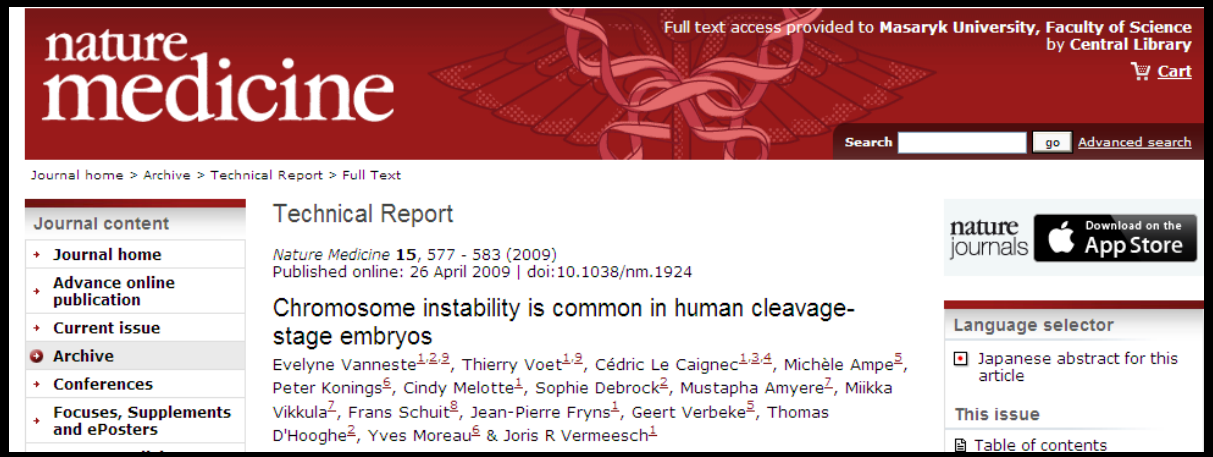
- **normální** (všechny buňky diploidní)

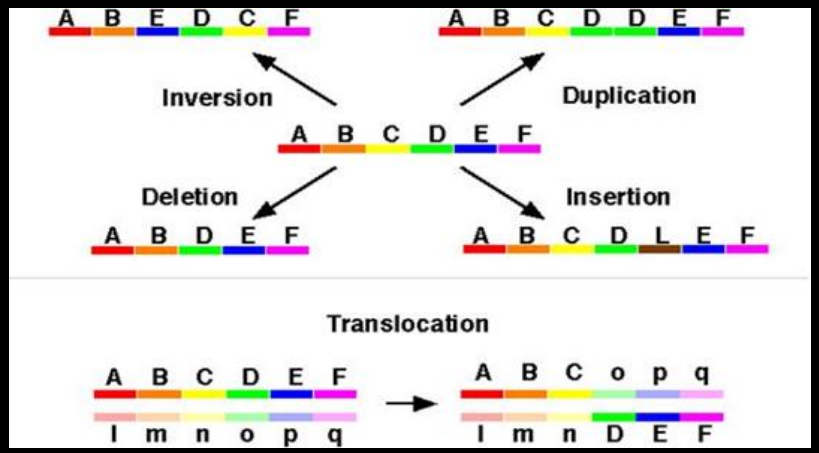
**Jedna buňka nemusí reprezentovat celé embryo !!!**

- **mozaika** (diploidní + aneuploidní buňky)
- **abnormální** (všechny buňky abnormální)
- **chaotické** (každá buňka obsahuje jiný počet chromozomů)



# Problémy PGA III – strukturní CHA



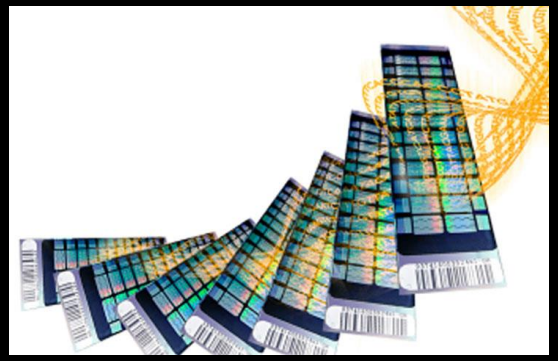




**Nestačí vyšetřit aneuploidie ! Celogenomové vyšetření !**

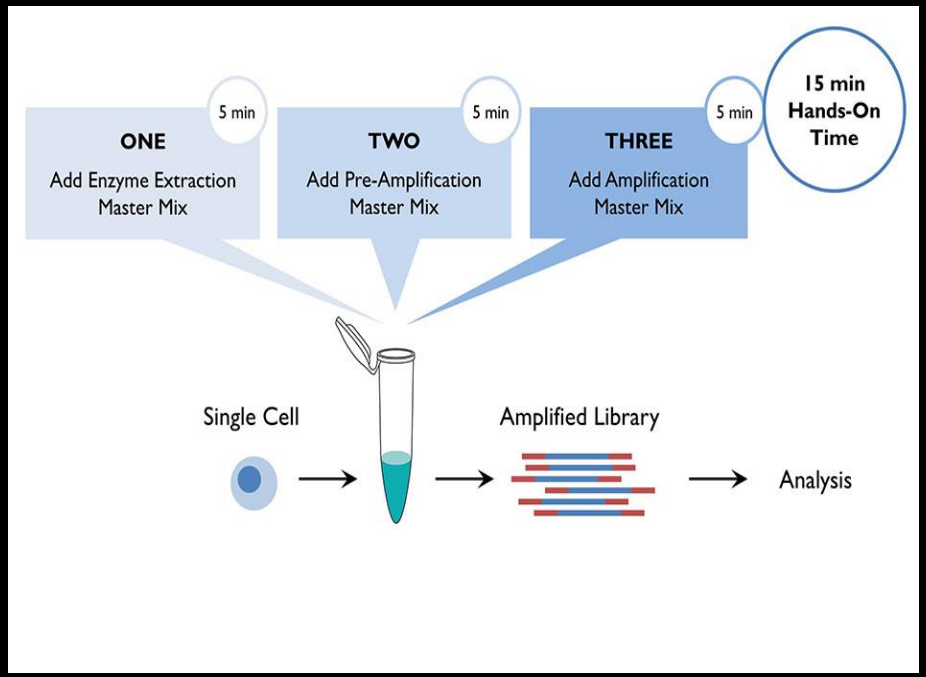
- u embryí se vyskytují též **strukturní aberace** (delece, duplikace, UPD atd…) …postygotické mitotické poruchy mitózy jsou **četnější** než meiotické…

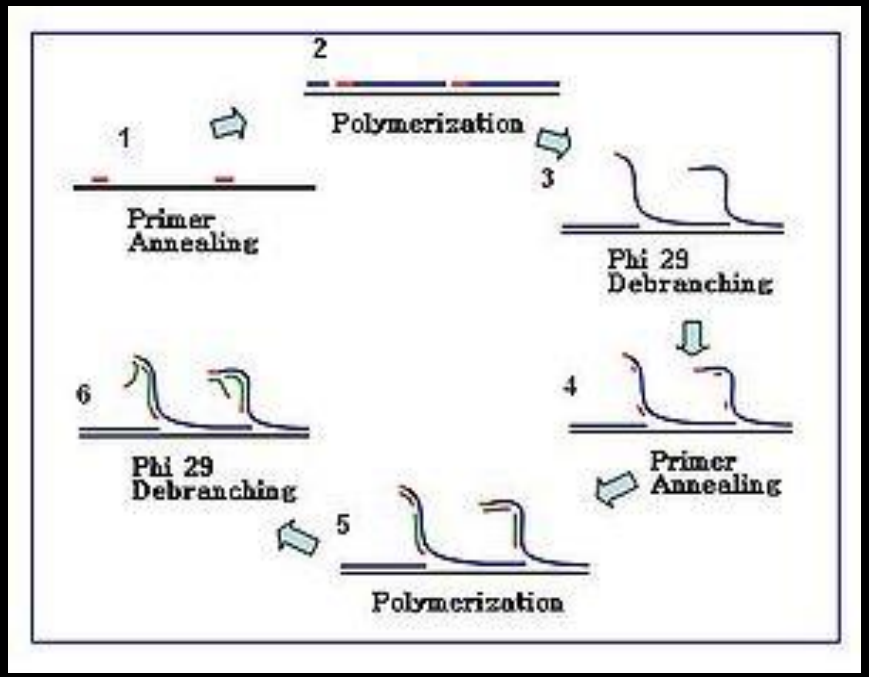
# Využití celogenomových screeningových technik v PGA

- Izolace 1 – několika buněk + celogenomová amplifikace
- Využití mikročipových technik array-CGH, SNP čipy, NGS
- Možnost vyšetřit celý genom – nutno v krátkém časovém intervalu (24 h) X zamražená embrya (vitrifikace)

# Amplifikace DNA - klíčový krok PGA




Genomové metody – potřeba ng DNA, = $10^6$buněk
XX aspirát trofoektodermu 20 buněk = pg DNA, nutnost amplifikace DNA

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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†‡

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**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*

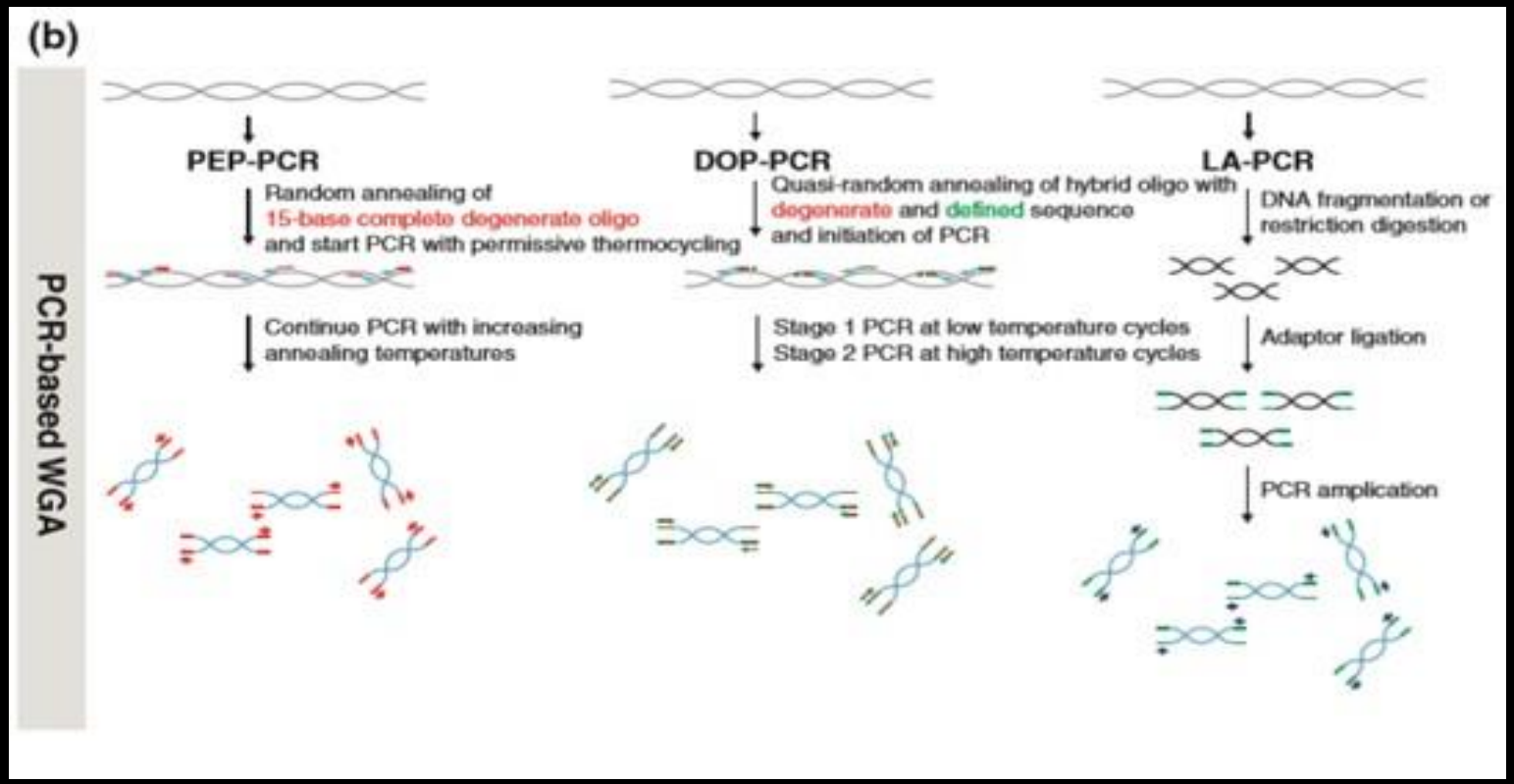
# Preclinical validation of a microarray method for full molecular karyotyping of blastomeres in a 24-h protocol

**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

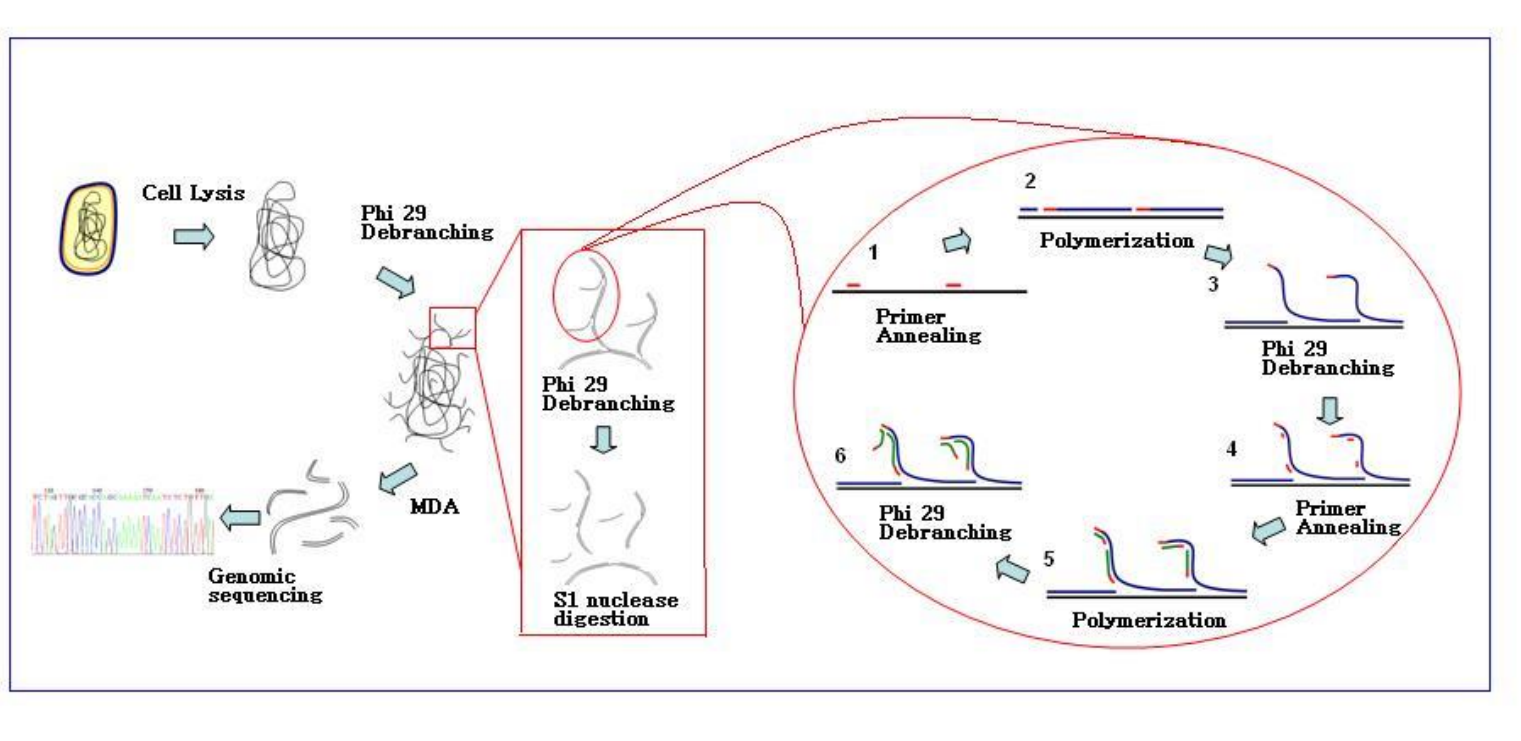
[8]Correspondence address. E-mail: djohnson@genesecurity.net

# Single cell WGA principy – PCR



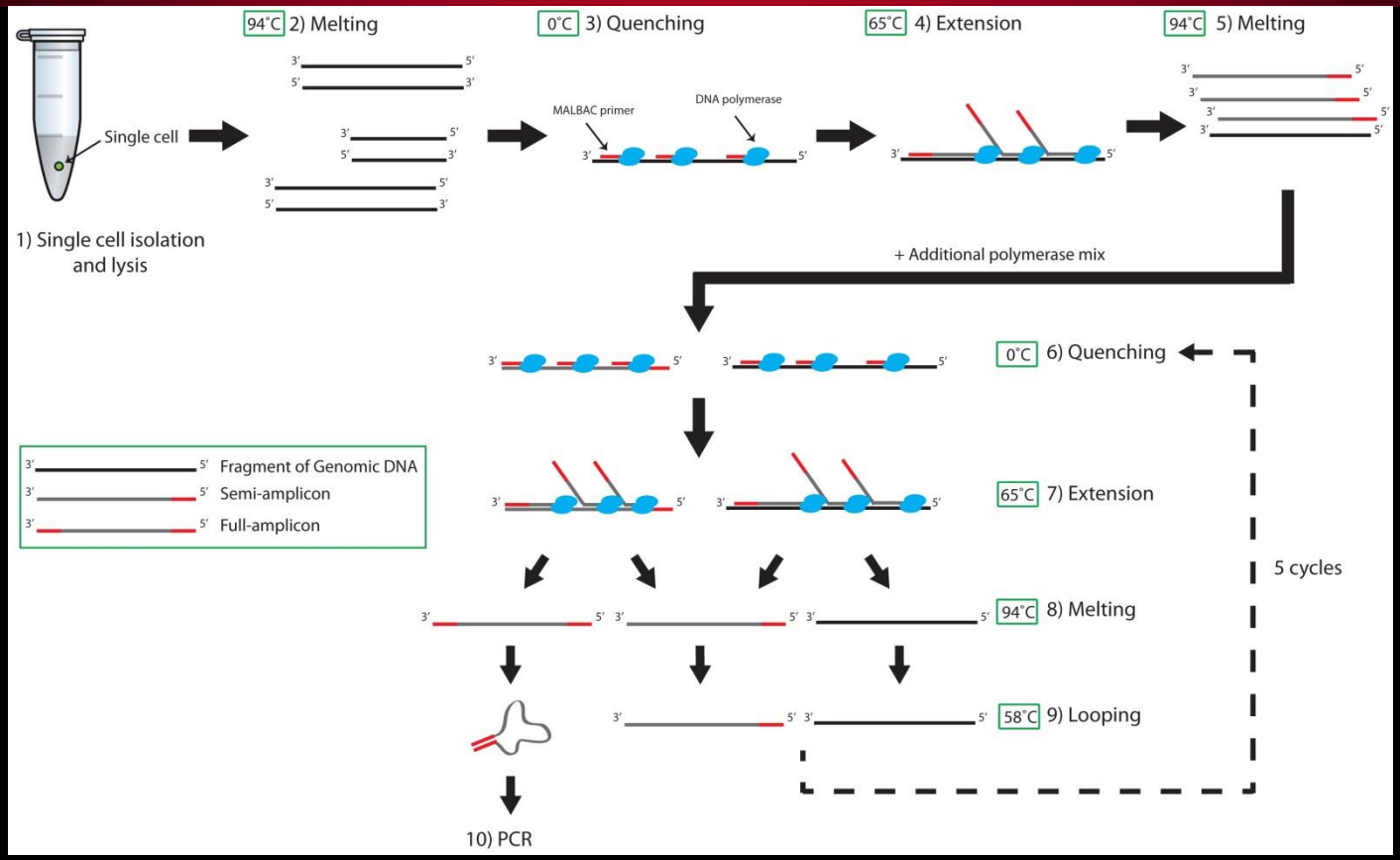

Výhody: vyšší výtěžnost, jednoduchý protokol, časově méně náročné
X tvoří artefakty, ADO
Aplikace: array-CGH, QF - PCR

# Single cell WGA principy - MDA




Amplifikace „kruhem“ pomocí termostabilního mutanta fága Phí29
Výhody – menší míra ADO, nejsou produkty amplifikace x náročnější, menší výtěžek
Aplikace: NGS, metylační analýzy (PWS/AS)

# Single cell WGA principy - MALBAC

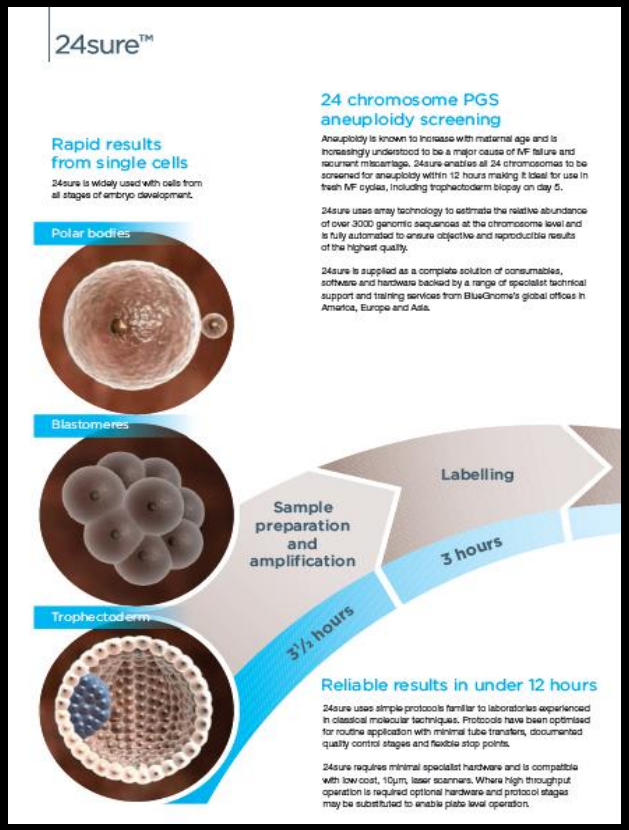


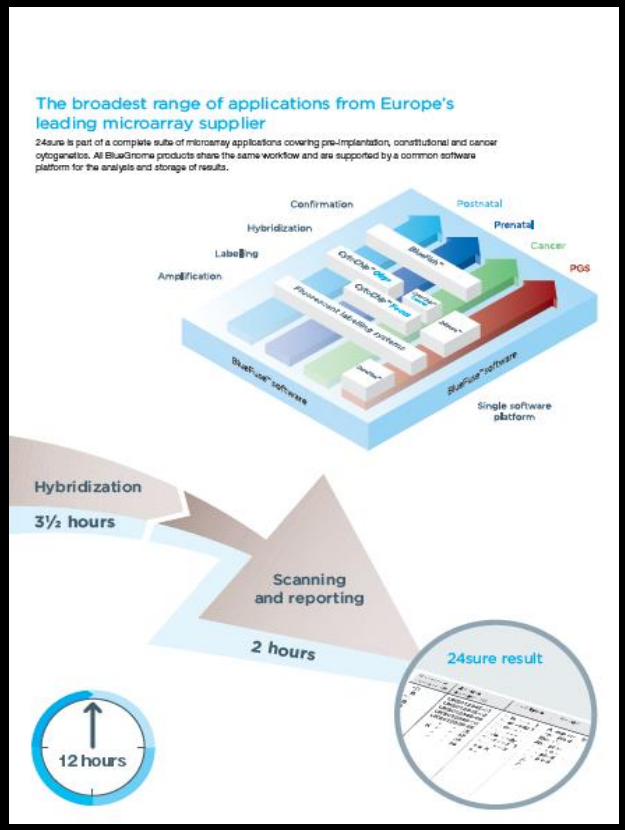

MALBAC - Multiple Annealing Looped Base Amplification Cycles
Kombinace PCR a MDA, dnes standard pro DNA i RNA sekvenování

https://youtu.be/CaFq9cnfTZI

# BAC array CGH-za 12 hodin

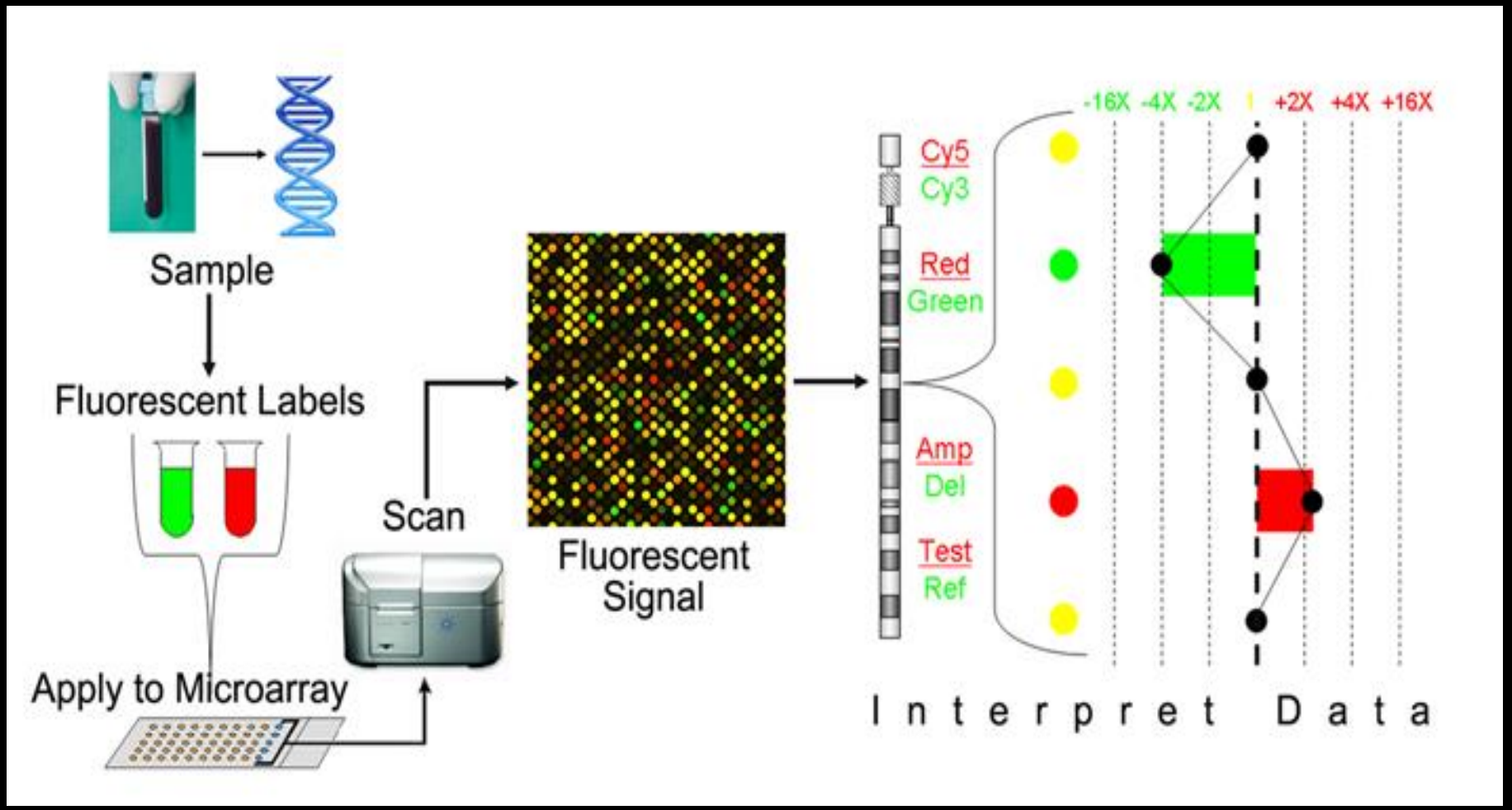
**Aneuploidie i strukturní změny (delece, duplikace) v celém genomu**
**Rozlišení ~ 5 Mbp**

# Metodika screeningových technik u PGD

## Array-cgh workflow



# Oligo array-CGH pro PGD

## Agilent Technologies

**1. Agilent SurePrint Mikročipy**

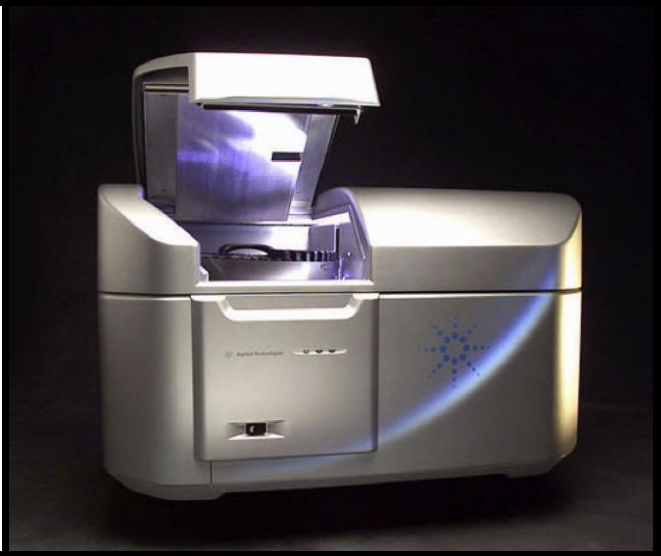
- 8x60k mikročipy
- Rozlišení až 1 Mbp,. 2 - 5 Mbp standard

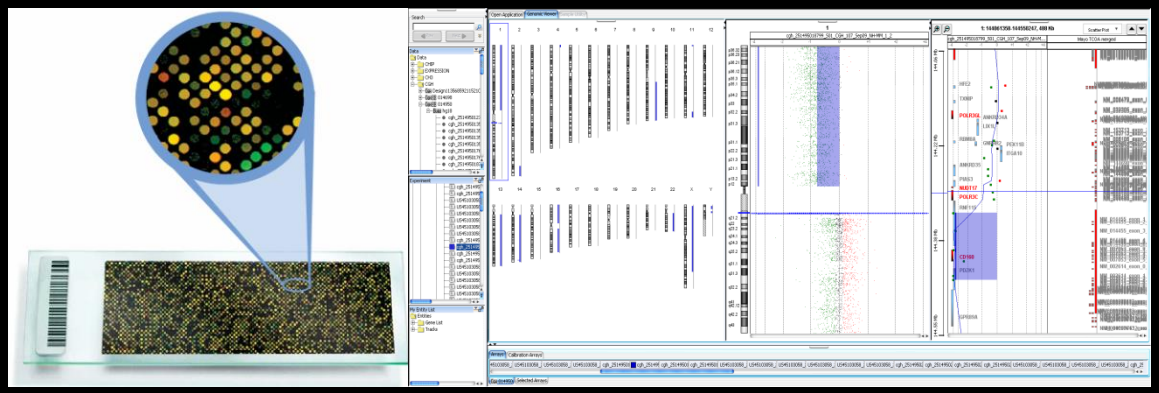
**2. High Resolution Scanner**

- Rozlišení 10- 2 mm
- Až 48 microarrays / cyklus
- 15 min / 1 array (60k array)

**3. Agilent Genomic Workbench**

- Kritéria pro analýzu
- Vizualizace dat
- Tvorba protokolů, správa dat



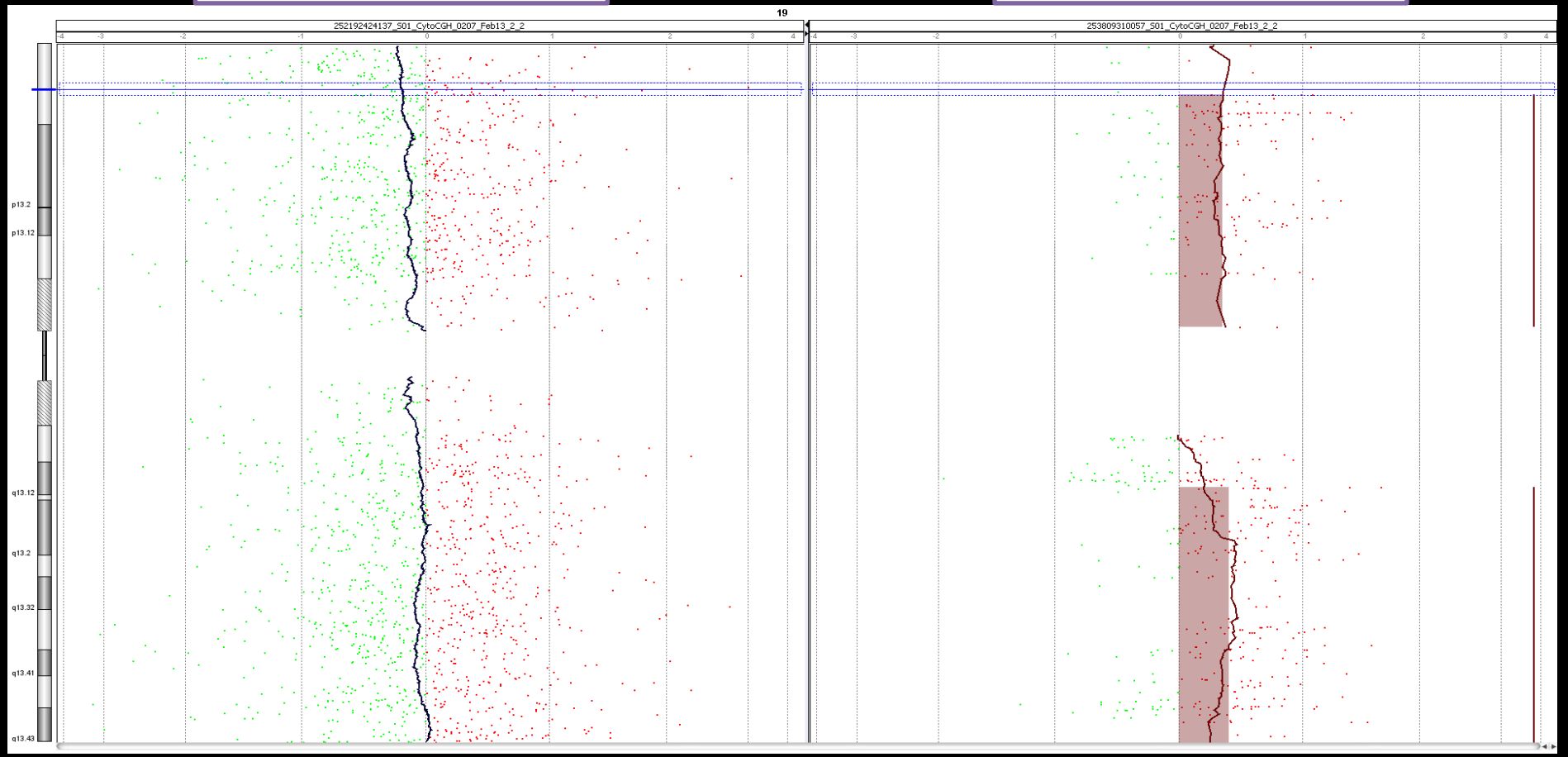
# Preimplantační genetická analýza pomocí high-resolution array-CGH

- **Materiál:** buňky z **trofoektodermu** 5-denních embryí
- **Amplifikační protokol:** 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
- **Kontrola:** hodnocení 4 zaškolení pracovníci

# Porovnání profilu chromozomu 19 u na platformě Agilent a OGT

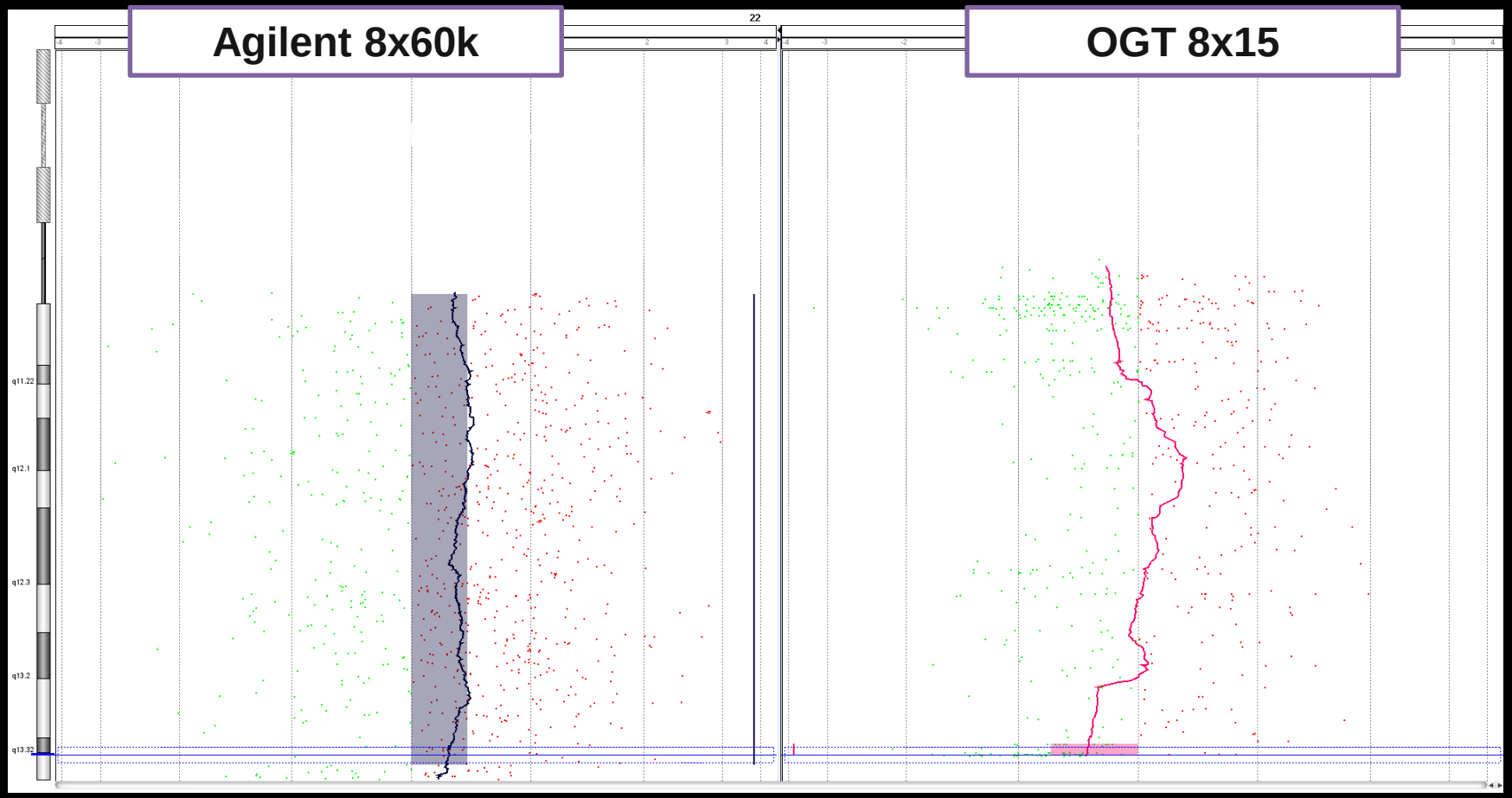
**Agilent 8x60k**

**OGT 8x15**



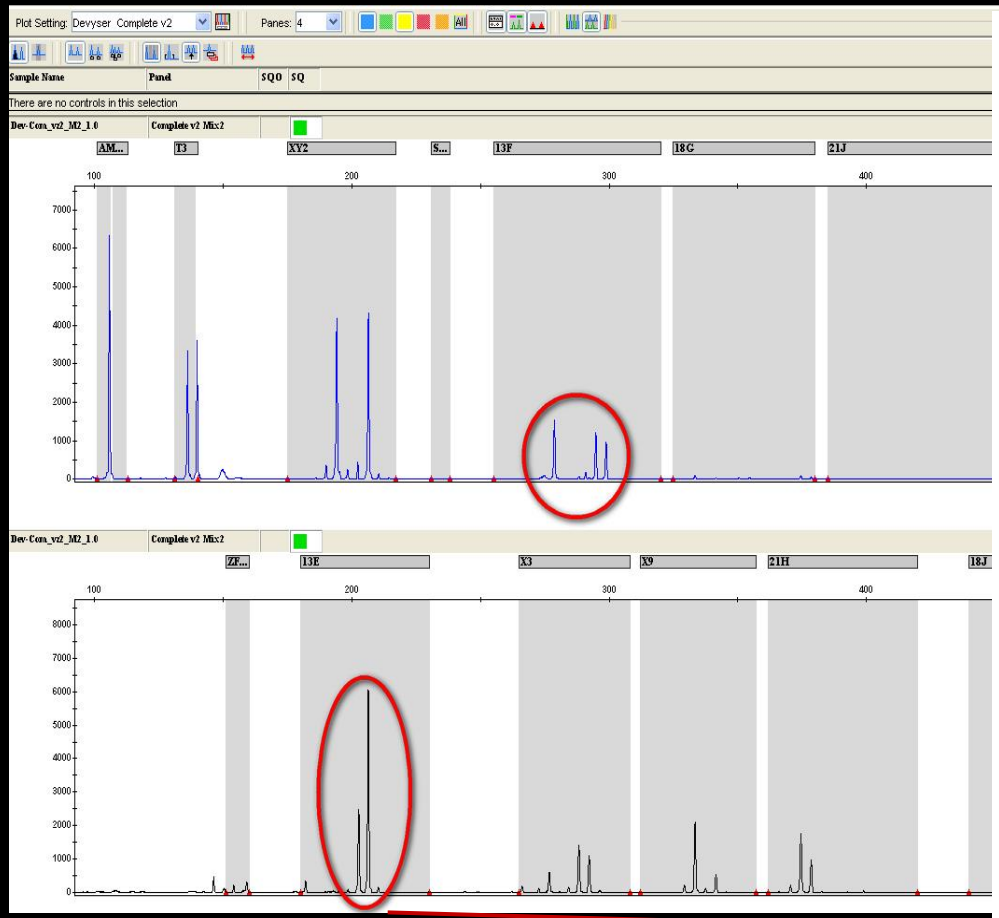

Vyšší **falešná pozitivita** 15k platformy, nejčastěji chr. 11, 16 a 19

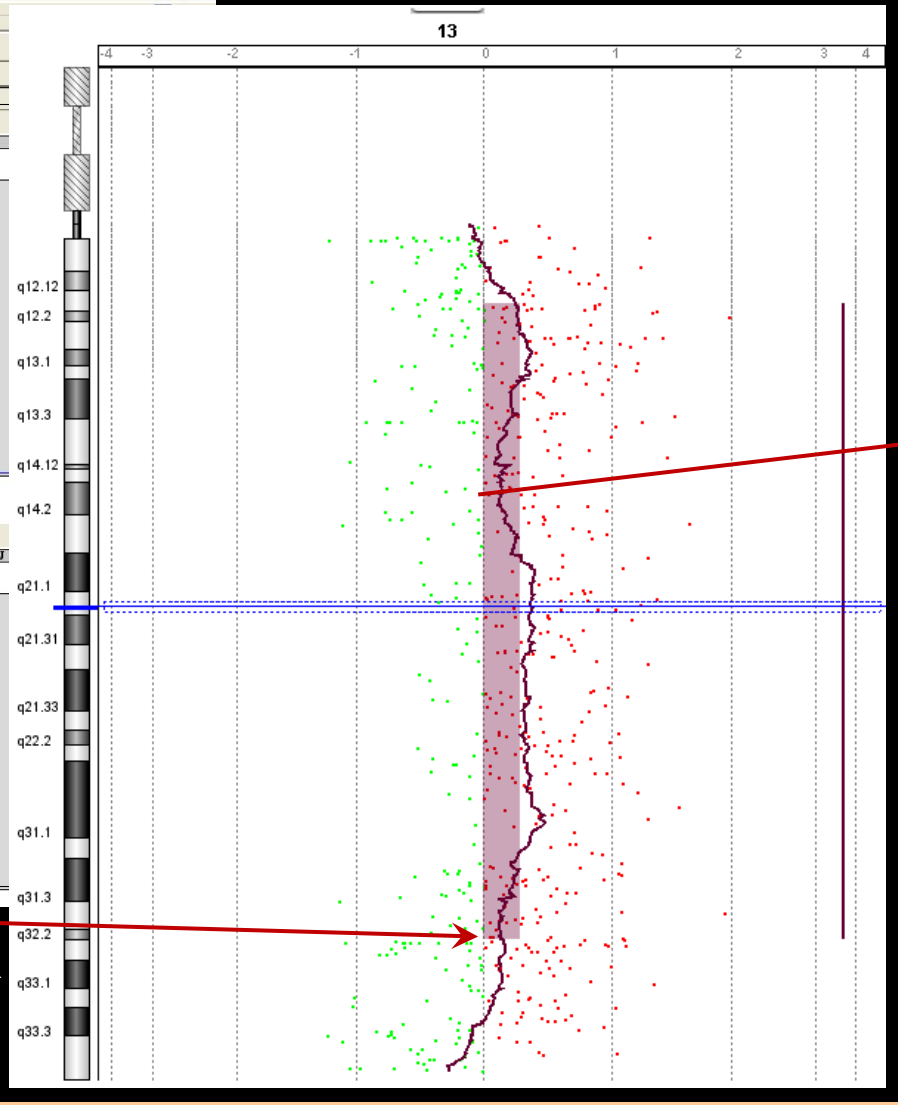
# Porovnání profilu chromozomu 22 na platformě Agilent a OGT




Vyšší hustota 60k microarrays dává **robustnější výsledky** v porovnání s 15k

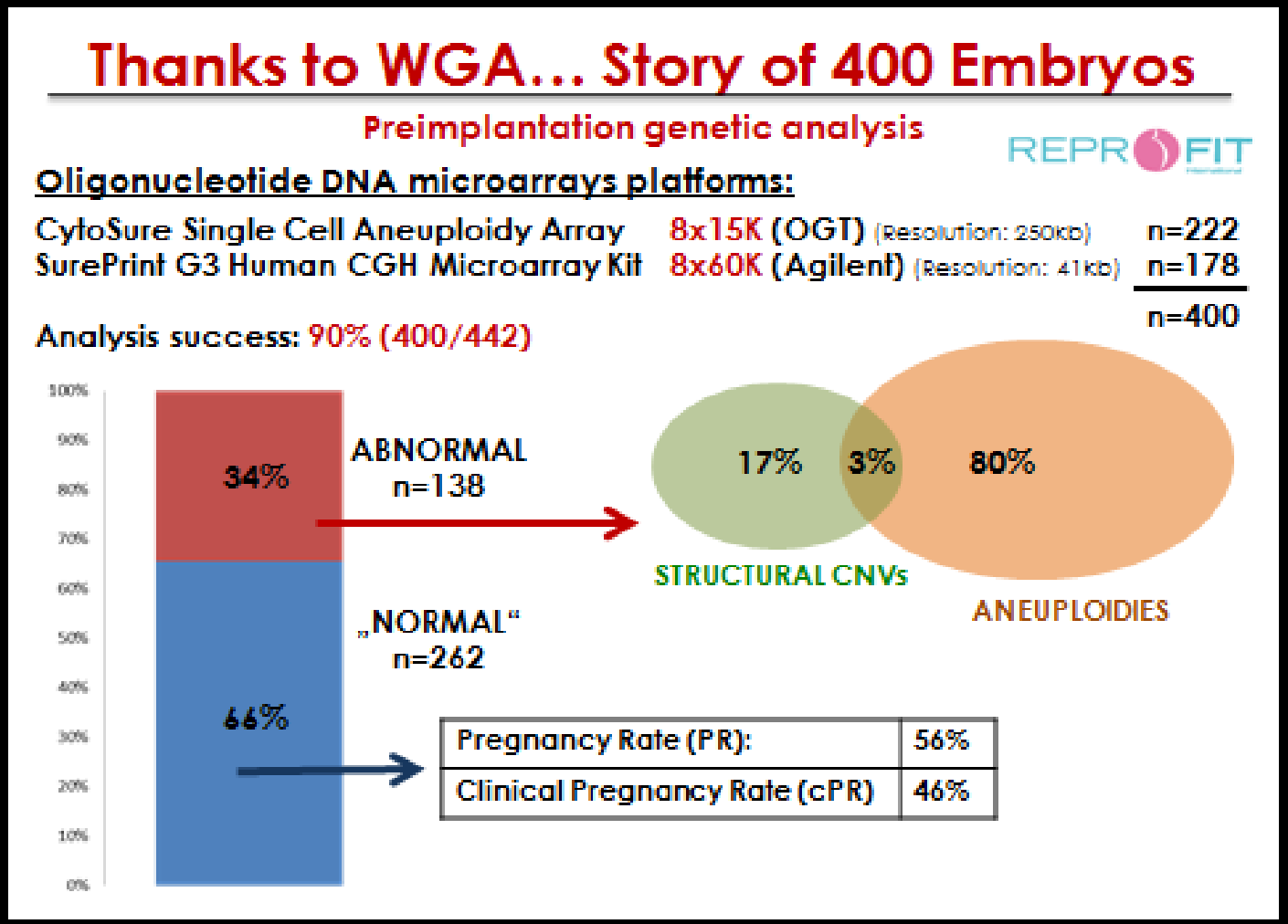
# Mozaicismus a verifikace výsledků u PGA




Embryo s mozaikou +13, potvrzeno QF PCR

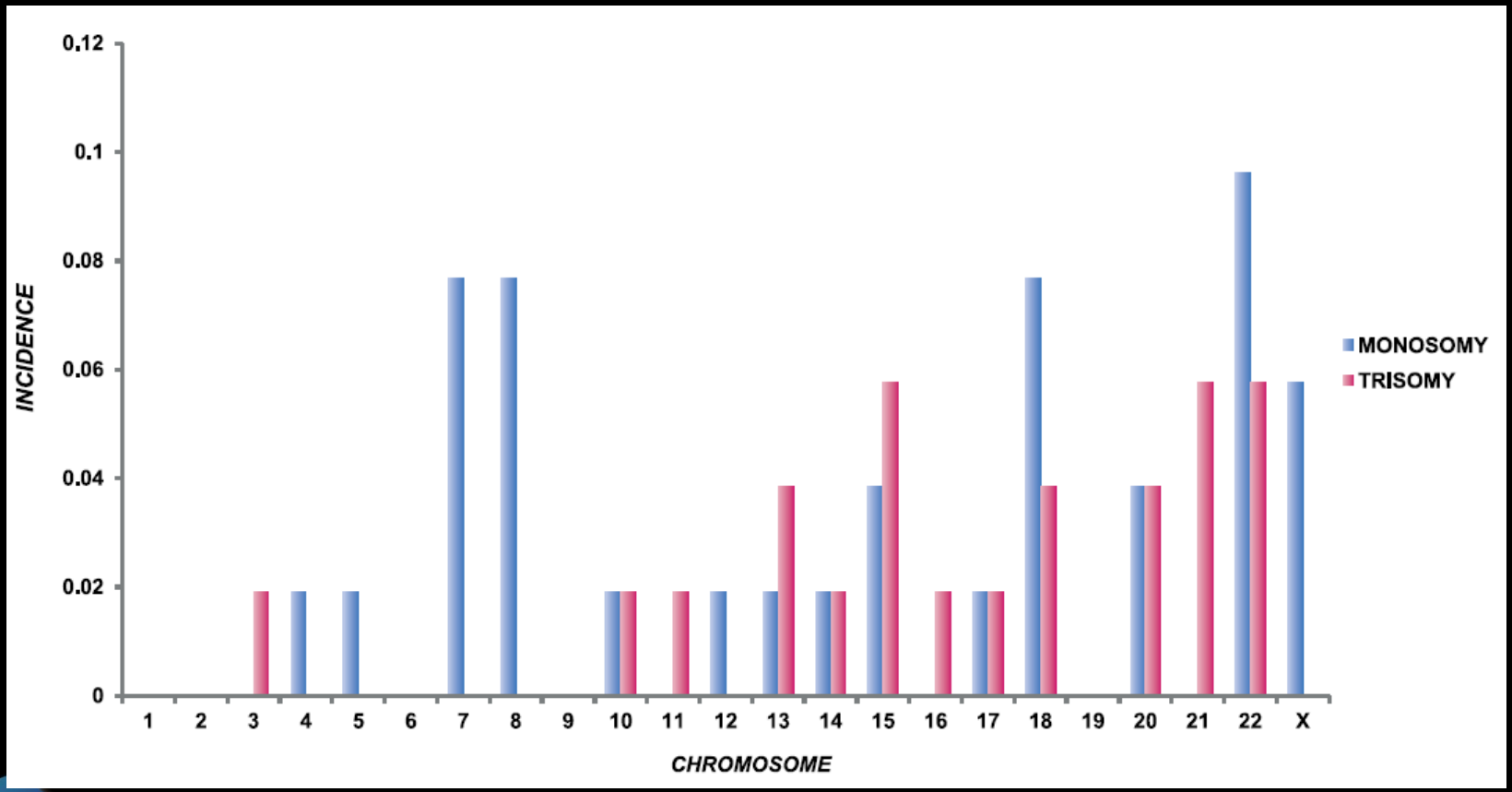
# Preimplantační genetická analýza pomocí high-resolution array-CGH



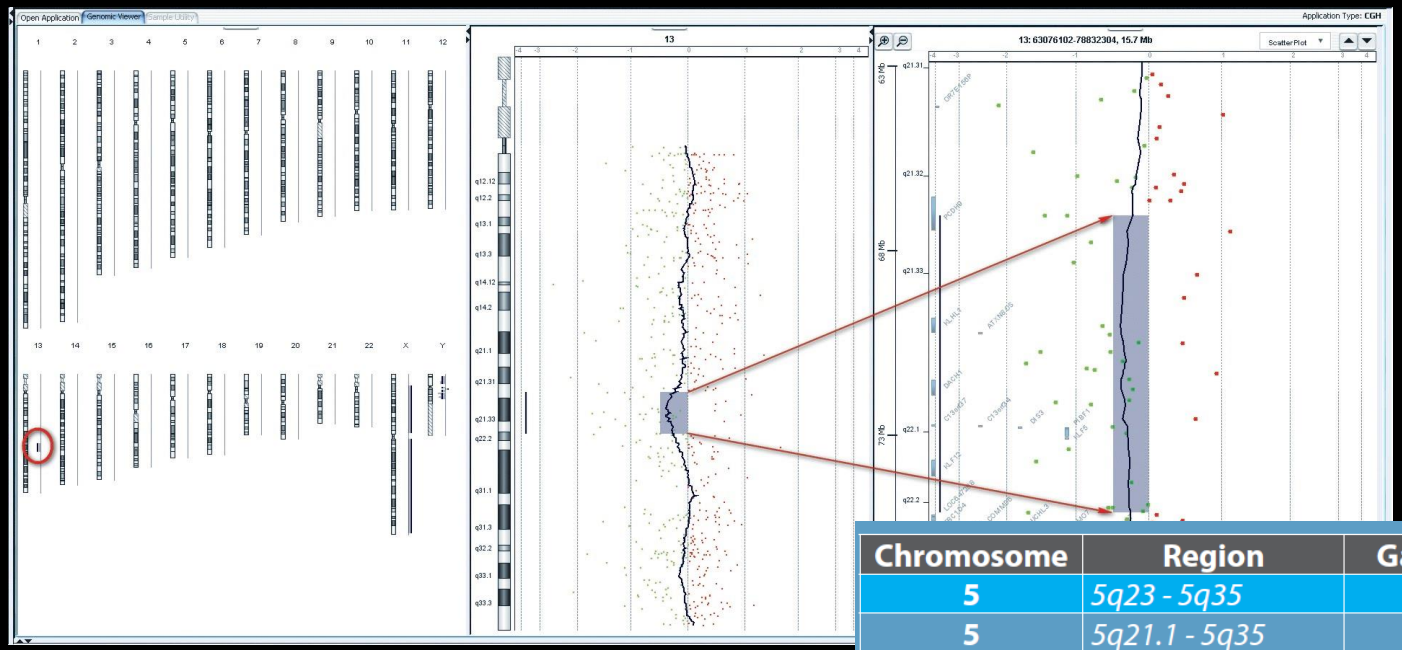

*Mikulášová A. et al, SLG konference 2014, Praha*

# Výsledky PGS I.

- nejčastější **monozomie:** chromozom **22** (7.7 %; 5/65), **7, 8 a 18** (po 6.1%; 4/65)
- nejčastější **trizomie:** chromozom **15, 21 a 22** (po 4,6 %; 3/65)



# Výsledky PGS II.



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

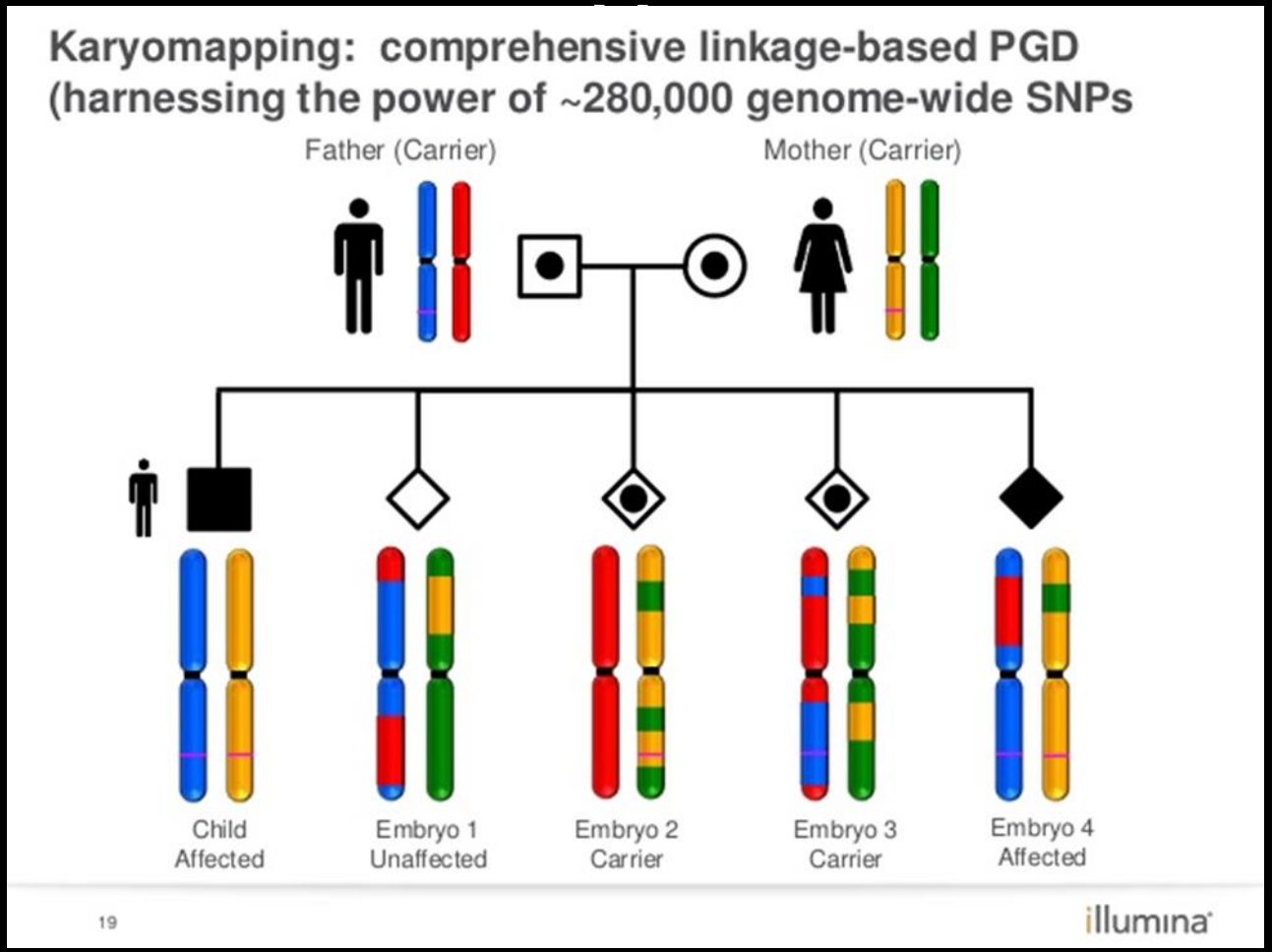
CASE REPORT *Reproductive genetics*

## 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,*]**

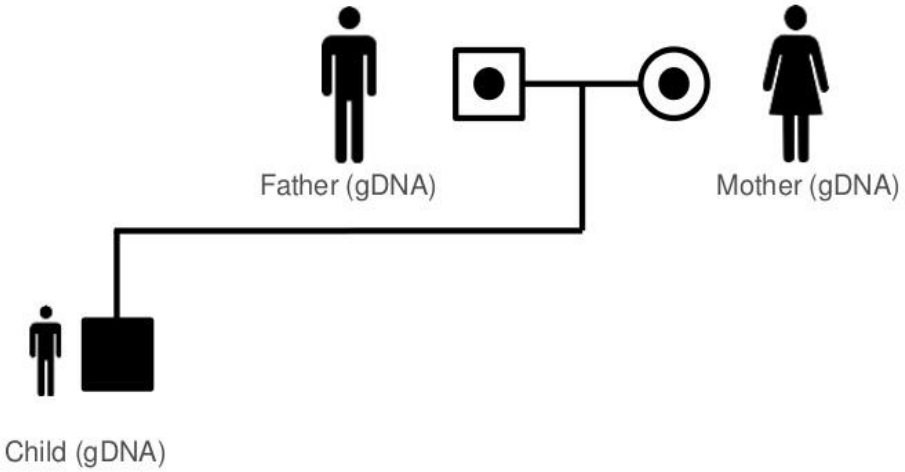
# Karyomapping – SNP profilování

SNP profilování rodičů + embryí = komplexní pohled, monogenní choroby + detekce aneuploií



# Karyomapping – SNP profilování

## 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)



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# Karyomapping – SNP profilování

| 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 – SNP profilování

## 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)



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| Informativnost | Genotyp | | |
|---|---|---|---|
| | Otec | Matka | Embryo |
| Paternální informativní SNP | AB | AA | AB = "key"<br>BB = "key"*<br>AA = "non–key" |
| | AB | BB | AB = "key"<br>AA = "key"*<br>BB = "non–key" |
| Maternální informativní SNP | AA | AB | AB = "key"<br>BB = "key"*<br>AA = "non–key" |
| | BB | AB | AB = "key"<br>AA = "key"*<br>BB "non–key" |

## Tabulka 1:

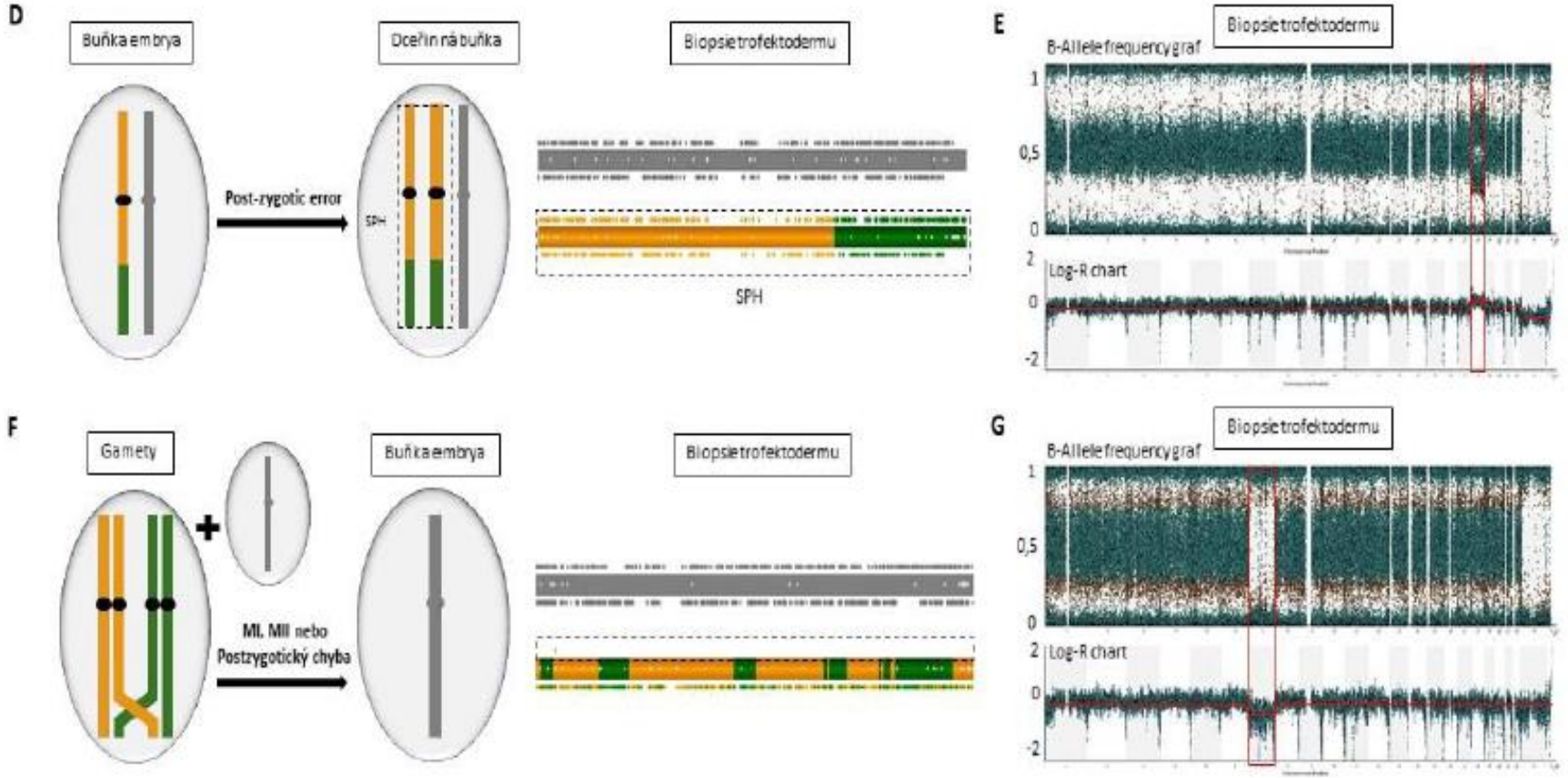
### Infromativní SNP

*Paternální informativní SNP jsou znázorněny modře (key SNP) a červeně (non–key SNP), zatímco maternální zeleně (key SNP) a žlutě (Non-key SNP). Barvy informativních SNP byly vybrány, aby korespondovaly s výstupem ze software BlueFuse Multi.*

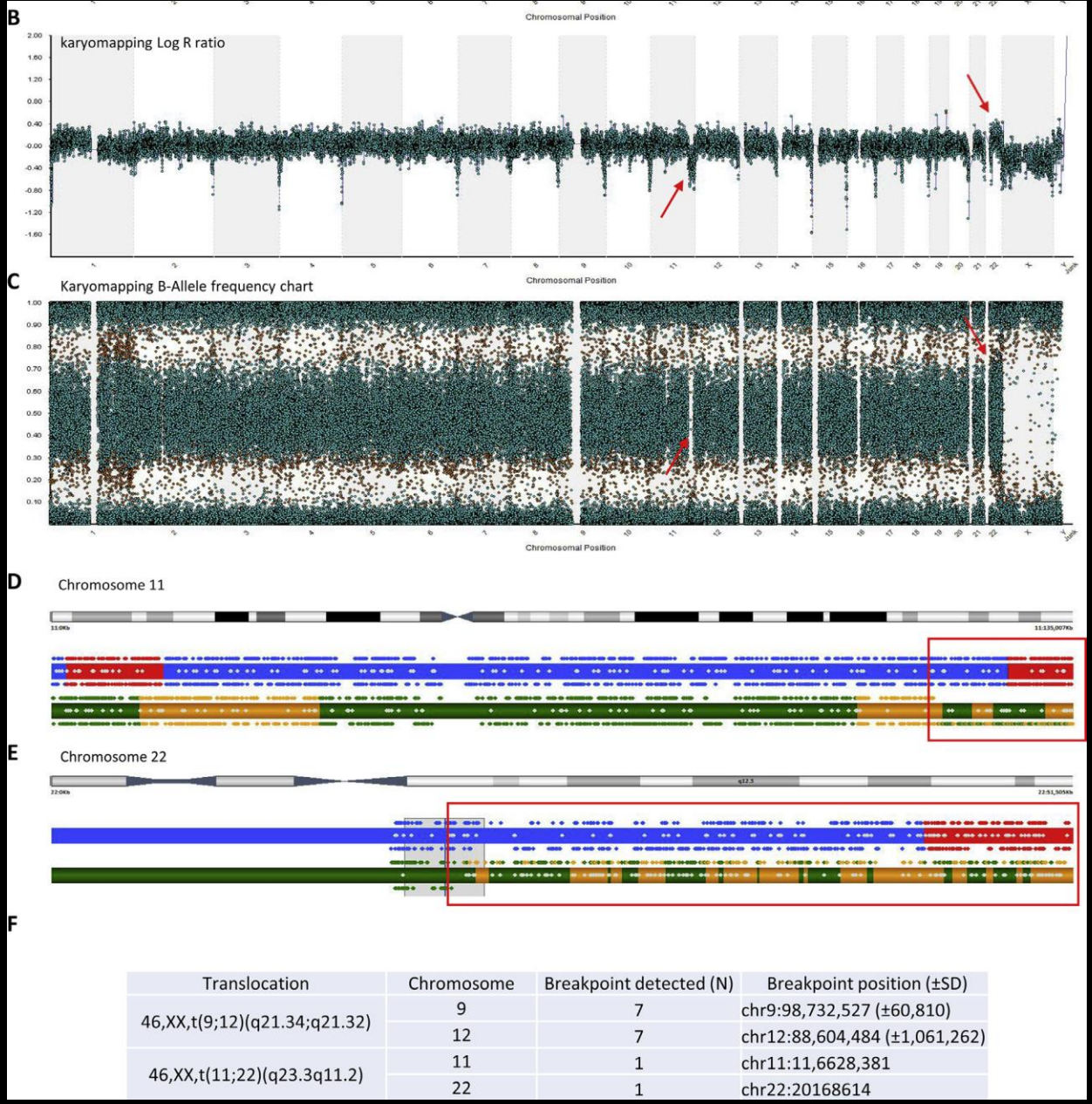
**Genotypy, které se u embryí mohou vyskytnout pouze v případě ztráty alely (ADO) neinformativního rodiče.*

*Zdroj: vlastní*

KUBÍČEK, David. Využití metod preimplantační genetické diagnostiky v reprodukční medicíně [online]. Brno, 2019 [cit. 2019-04-23]. Dostupné z: <https://is.muni.cz/th/qlmgj/>




*mitotické trizomie (na obrázku) a MII chyby, u které nedošlo k rekombinaci, budou u vzorky přítomny dva identické chromozomy (vzor SPH po celém chromozomu, přerušovaný box). **(E)** Všechny typy trizomií jsou také potvrzeny zvýšenou intenzitou Log R ratio a charakteristickým rozdvojením AB heterozygotních SNPs do dvou subpopulací genotypů ABB, respektive AAB (červený box). **(F)** V případě monozomií není možné rozlišit, zda vznikly meioticky nebo až mitotickou ztrátou chromozomu. Ztráta chromozomu je vyjádřena ztrátou informativních SNP (přerušovaný box). **(G)** Ztráta heterozygotnosti je zobrazena i ztrátou heterozygotních SNPs na B- allele frequency chartu (BAF) a sníženou intenzitou na log R chartu (červený*




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 – SNP profilování

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], Dove 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.



**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 – SNP profilování

**Výhody**

- Rychlá a efektivní metoda pro komplexní PGD pokud máme vhodnou referenci
- Detekce strukturních delecí a aneuploidií dvou meiotických chromozomů

**Nevýhody**

- Patentováno, není konkurence pro chemii, finančně náročné, uzavřený systém
- Možnost problémů při absenci referenční DNA

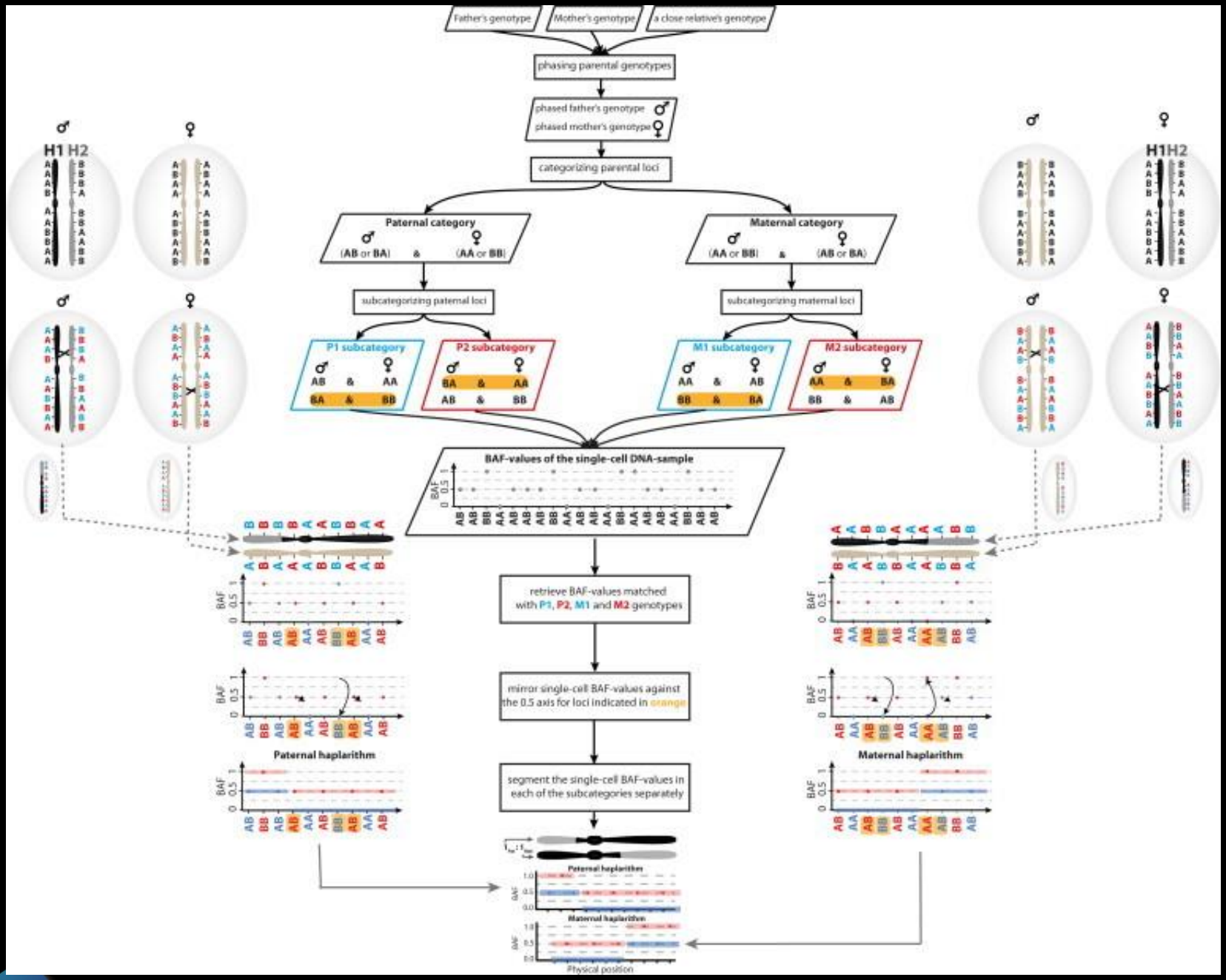
# Haplarithmisis

- 2016 – Vermeesch et al. – Nová koncepce analýzy halptypů pro potřeby PGD
- Haplarithmisis – z řeckého „počítání haplotypů“

- Princip – celogenomová analýza haplotypů + detekce nebalancovaných změn v rámci jedné buňky

- Analýza genotypu rodičů + blízký příbuzný

- Bioinformační algoritmus **siCHILD** (single-cell haplotyping and imputation of linked disease variants)
  = identifikace recesivních alel přenašečů, strukturních změn i aneuploidií
  = lze diagnostikovat meiotické aberace od mitotických


Father's genotype
Mother's genotype
a close relative's genotype
phasing parental genotypes
phased father's genotype
phased mother's genotype
categorizing parental loci
Paternal category
(AB or BA) & (AA or BB)
Maternal category
(AA or BB) & (AB or BA)
subcategorizing paternal loci
subcategorizing maternal loci
P1 subcategory
P2 subcategory
M1 subcategory
M2 subcategory
BAF-values of the single-cell DNA-sample
retrieve BAF-values matched with P1, P2, M1 and M2 genotypes
mirror single-cell BAF-values against the 0.5 axis for loci indicated in orange
segment the single-cell BAF-values in each of the subcategories separately
Paternal haplarithm
Maternal haplarithm
BAF
Physical position

# Haplarithmisis

Format: Abstract Send to

Hum Reprod. 2017 Mar 1;32(3):687-697. doi: 10.1093/humrep/dex011.

## Principles guiding embryo selection following genome-wide haplotyping of preimplantation embryos.

Dimitriadou E[1], Melotte C[1], Debrock S[2], Esteki MZ[1], Dierickx K[3], Voet T[1,4], Devriendt K[1], de Ravel T[1], Legius E[1], Peeraer K[2], Meuleman C[2], Vermeesch JR[1].

Author information


**Abstract**

**STUDY QUESTION:** How to select and prioritize embryos during PGD following genome-wide haplotyping?

**SUMMARY ANSWER:** In addition to genetic disease-specific information, the embryo selected for transfer is based on ranking criteria including the existence of mitotic and/or meiotic aneuploidies, but not carriership of mutations causing recessive disorders.


Format: Abstract Send to

Hum Reprod. 2017 Sep 23:1-10. doi: 10.1093/humrep/dex286. [Epub ahead of print]

## Genome stability of bovine in vivo-conceived cleavage-stage embryos is higher compared to invitro-produced embryos.

Tšuiko O[1,2,3,4], Catteeuw M[5], Zamani Esteki M[6], Destouni A[1], Bogado Pascottini O[5], Besenfelder U[7], Havlicek V[7], Smits K[5], Kurg A[4], Salumets A[2,3,8,9], D'Hooghe T[10], Voet T[6,11], Van Soom A[5], Robert Vermeesch J[1].

Author information


**Abstract**

**STUDY QUESTION:** Is the rate and nature of chromosome instability (CIN) similar between bovine in vivo-derived and in vitro-cultured cleavage-stage embryos?

**SUMMARY ANSWER:** There is a major difference regarding chromosome stability of in vivo-derived and in vitro-cultured embryos, as CIN is significantly lower in in vivo-derived cleavage-stage embryos compared to in vitro-cultured embryos.


Format: Abstract Send to

Genome Res. 2016 May;26(5):567-78. doi: 10.1101/gr.200527.115. Epub 2016 Apr 12.

## Zygotes segregate entire parental genomes in distinct blastomere lineages causing cleavage-stage chimerism and mixoploidy.

Destouni A[1], Zamani Esteki M[2], Catteeuw M[3], Tšuiko O[4], Dimitriadou E[1], Smits K[3], Kurg A[5], Salumets A[6], Van Soom A[3], Voet T[7], Vermeesch JR[1].

Author information


**Abstract**

Dramatic genome dynamics, such as chromosome instability, contribute to the remarkable genomic heterogeneity among the blastomeres comprising a single embryo during human preimplantation development. This heterogeneity, when compatible with life, manifests as constitutional mosaicism, chimerism, and mixoploidy in live-born individuals. Chimerism and mixoploidy are defined by the presence of cell lineages with different parental genomes or different ploidy states in a single individual, respectively. Our knowledge of their mechanistic origin results from indirect observations, often when the cell lineages have been subject to rigorous selective pressure during development. Here, we applied haplarithmisis to infer the haplotypes and the copy number of parental genomes in 116 single blastomeres comprising entire preimplantation bovine embryos (n = 23) following in vitro fertilization. We not only demonstrate that chromosome instability is conserved between bovine and human cleavage embryos, but we also discovered that zygotes can spontaneously segregate entire parental genomes into different cell lineages during the first post-zygotic cleavage division. Parental genome segregation was not exclusively triggered by abnormal fertilizations leading to triploid zygotes, but also normally fertilized zygotes can spontaneously segregate entire parental genomes into different cell lineages during cleavage of the zygote. We coin the term "heterogoneic division" to indicate the events leading to noncanonical zygotic cytokinesis, segregating the parental genomes into distinct cell lineages. Persistence of those cell lines during development is a likely cause of chimerism and mixoploidy in mammals.





PMID: 27197242 PMCID: PMC4864459 DOI: 10.1101/gr.200527.115

# Haplarithmisis

**Výhody**

- Komplexní metoda kombinující výhody SNP profilování, vazbovou analýzu a aCGH
- „Otevřený“ systém – lze použít data jak z SNP mikročipů Illumina a Affimetrix, tak i NGS

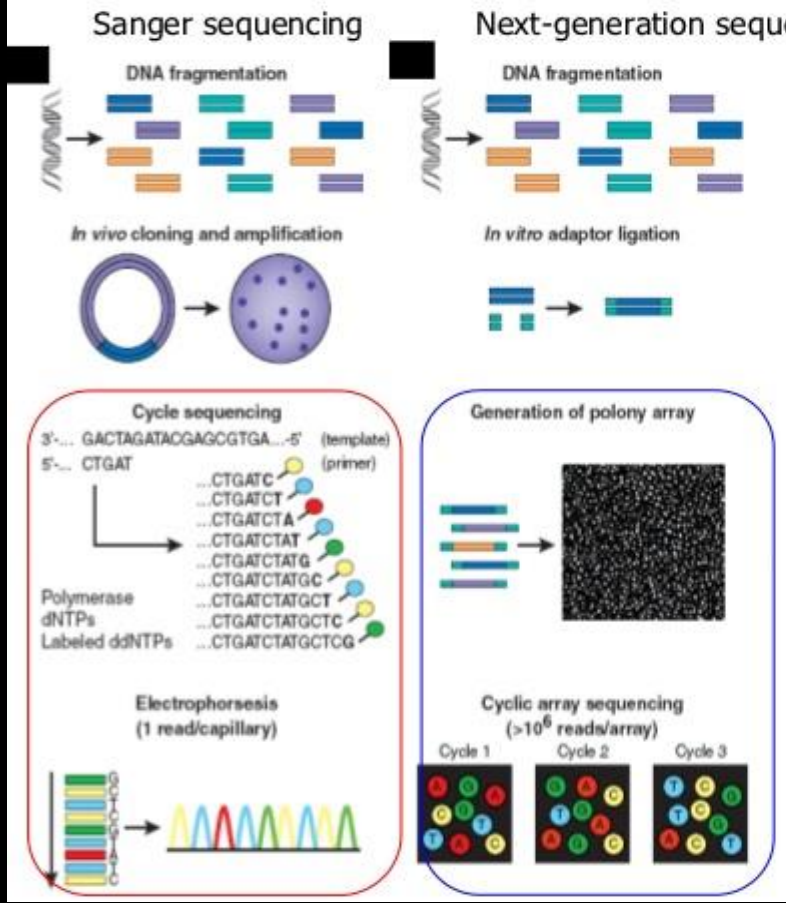
**Nevýhody**

- Experimentálně a laboratorně velmi náročné
- Potřeba solného bioinformatického zázemí
- V současné době doména University Hospitals Leuven (prof Veermeesh

Případné zjednodušení algoritmu má velký potenciál i pro menší laboratoře a IVF centra

# Technologie masivního paralelního sekvenování

## Next-generation DNA sequencing




### Advantages:

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

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

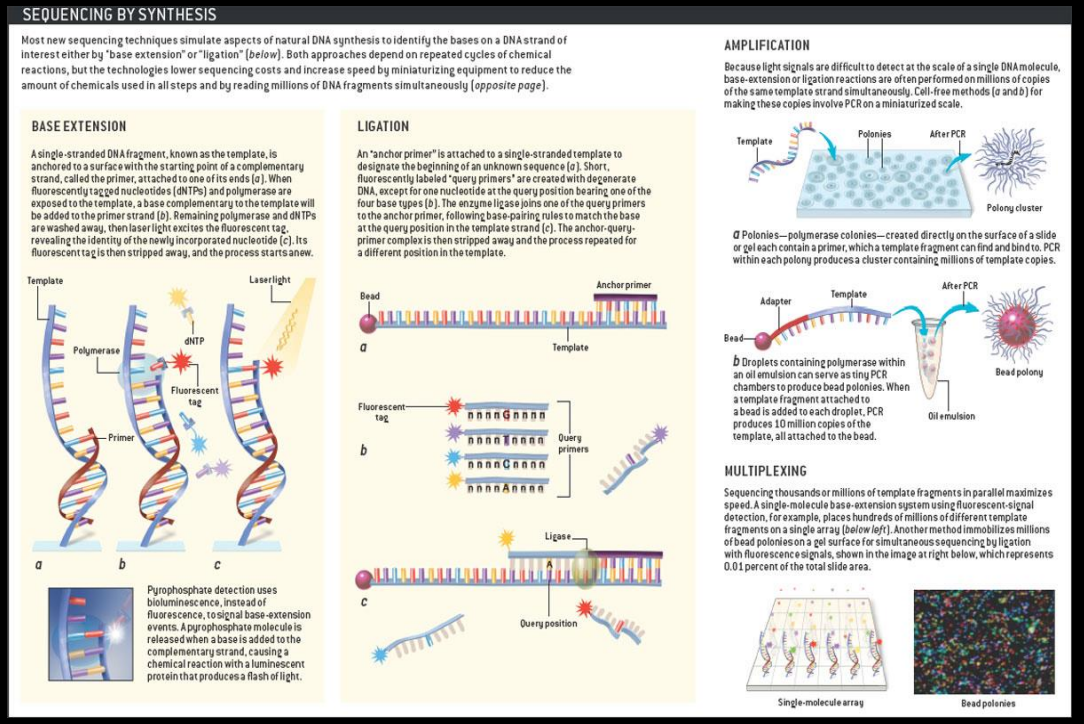
- Array-based sequencing

  ✓ Higher degree of parallelism than capillary-based sequencing

# Technologie masivního paralelního sekvenování (NGS) v IVF

- NGS technologie se pomalu začínají prosazovat i v rámci PGS
- Zpracování většího množství vzorků v jednom experimentu v porovnávání s mikročip. technikami
- V současné době používány na velkých IVF klinikách hlavně pro screening aneuploidií x možnosti komplexního pohledu (ploidie, strukturní změny, mutace)
- Nejčastěji forma uzavřených systémů – Illumina, Ion Torrent, nebo forma přípravy knihoven (např Agilent, Roche apod)

# VeriSeq PGS (Illumina)




- Sekvenace syntézou
- Detekce aneuploidií za 12 hod.
- Až 24 vzorků, rozlišení 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**



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# VeriSeq PGS (Illumina)

## VeriSeq PGS Workflow

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

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



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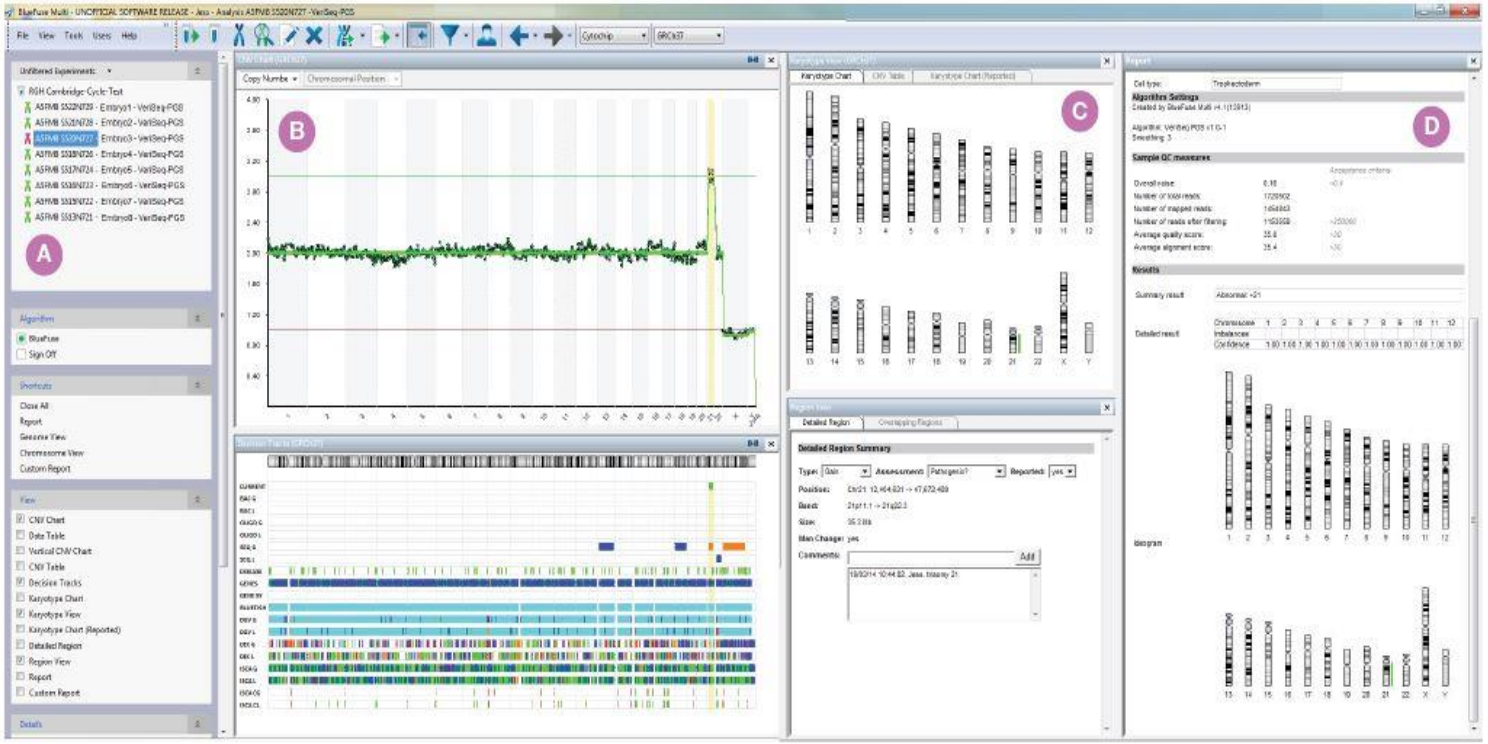
# VeriSeq PGS (Illumina)

## 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

Format: Abstract Send to

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.

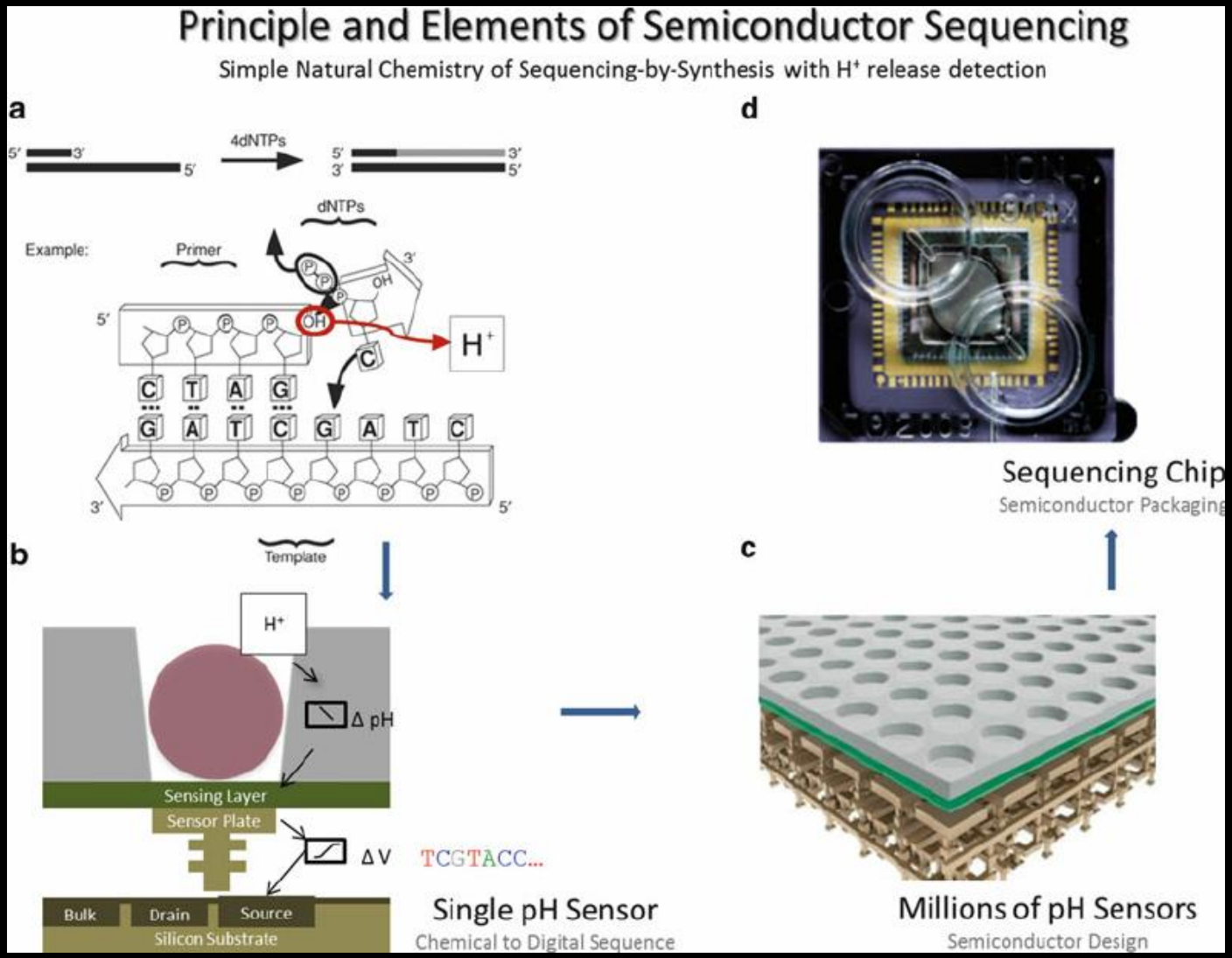
Zheng H[1], Jin H[2], Liu L[2], Liu J[1], Wang WH[3].

Author information

**Abstract**

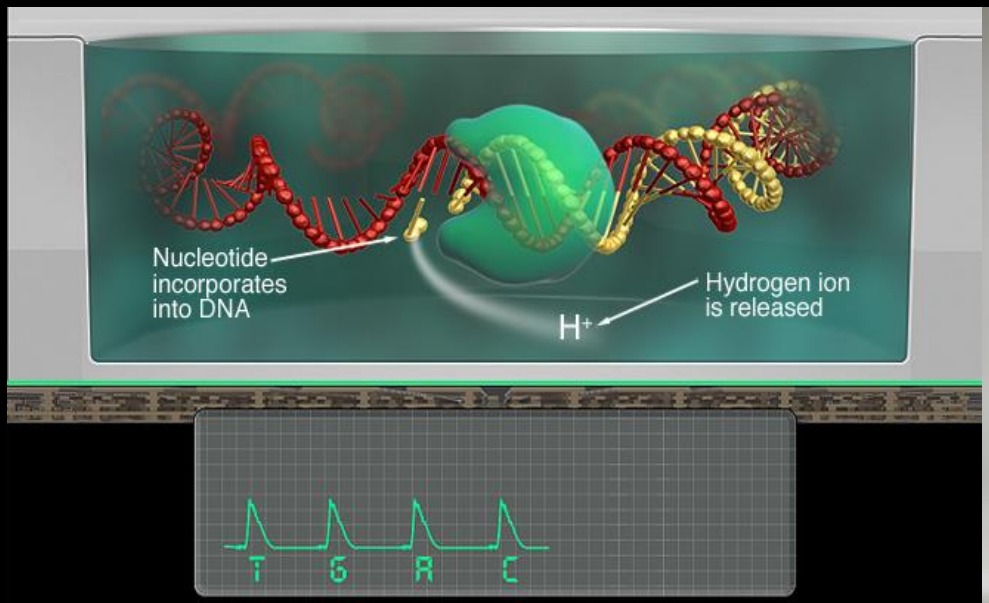
**BACKGROUND:** Aneuploidy is a leading cause of repeat implantation failure and recurrent miscarriages. Preimplantation genetic screening (PGS) enables the assessment of the numeral and structural chromosomal errors of embryos before transfer in patients undergoing in vitro fertilization. Array comparative 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.

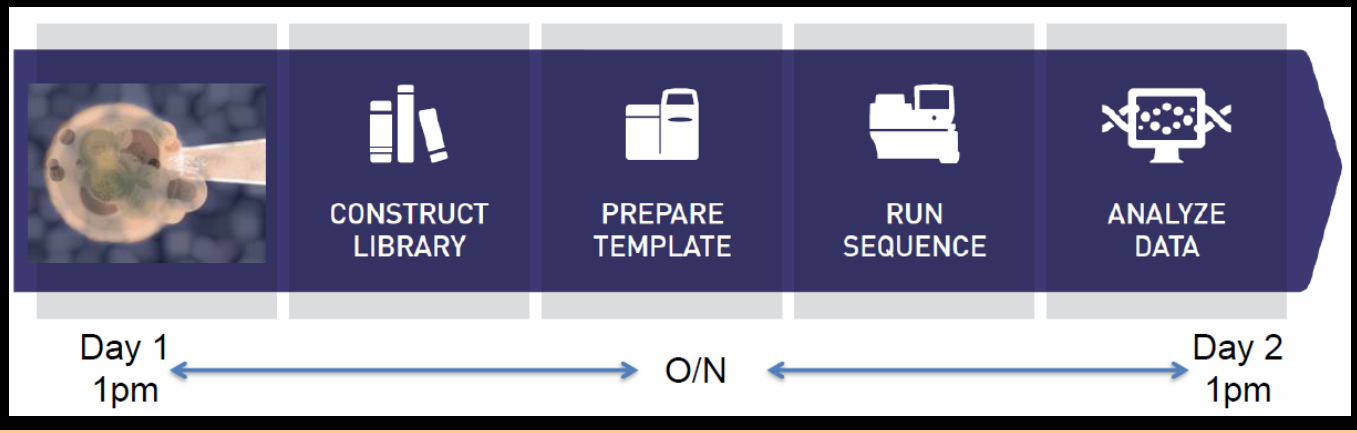
# Ion Torrent Semiconuctor Sequencing



# Ion Torrent Aneuploidy Analysis

## (Life Tech Inc.)

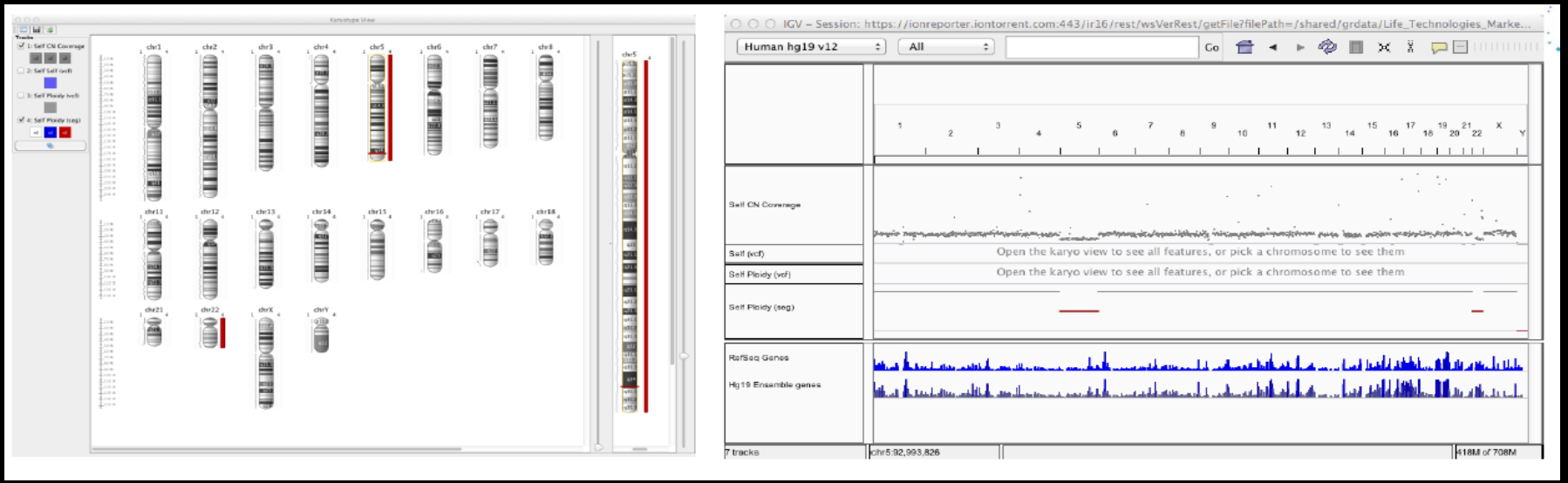


# Ion Torrent Aneuploidy Analysis

- „Polovodičové“ sekvenování
- Založeno na detekci změny pH, která nastává při uvolnění H během navázání báze na deoxyribózu

- Protokol do 24 hodin
- Rozlišení ~ 10 Mbp
- Cena 70$ / embryo při 32 společné analýze 32 embryí



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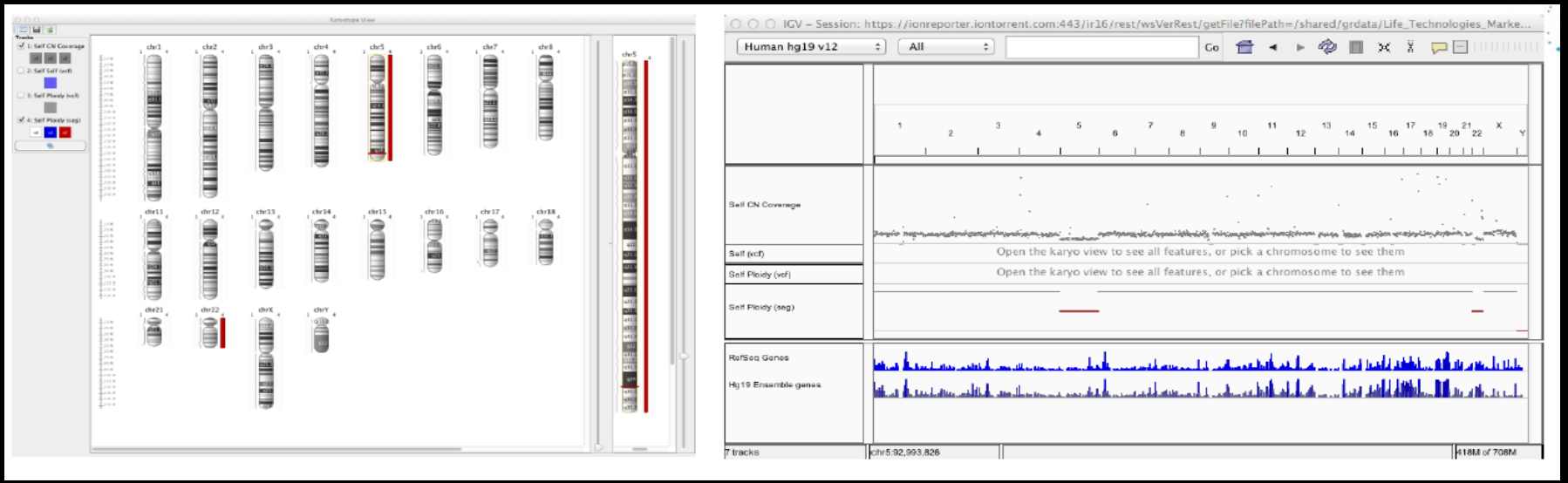
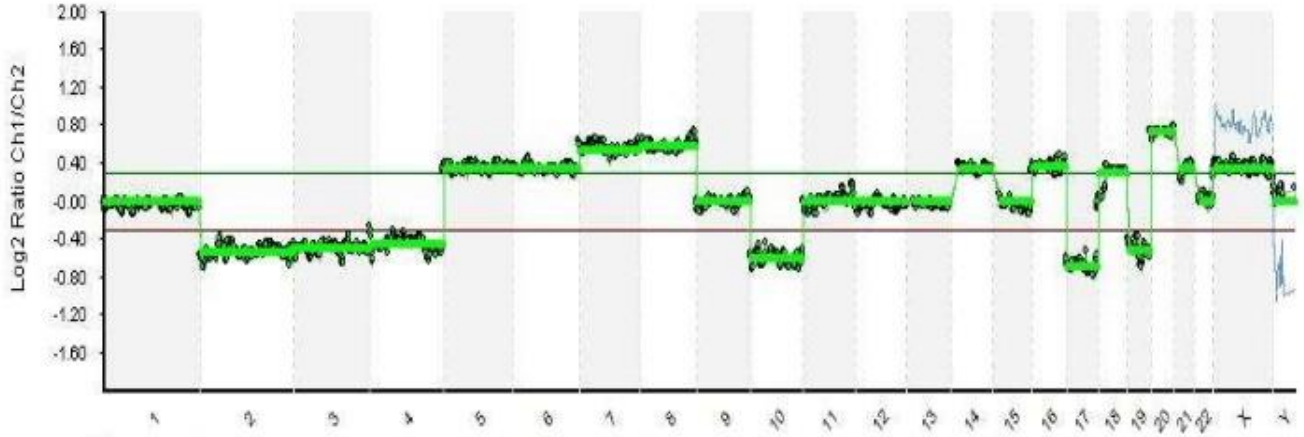


# NGS u PGD

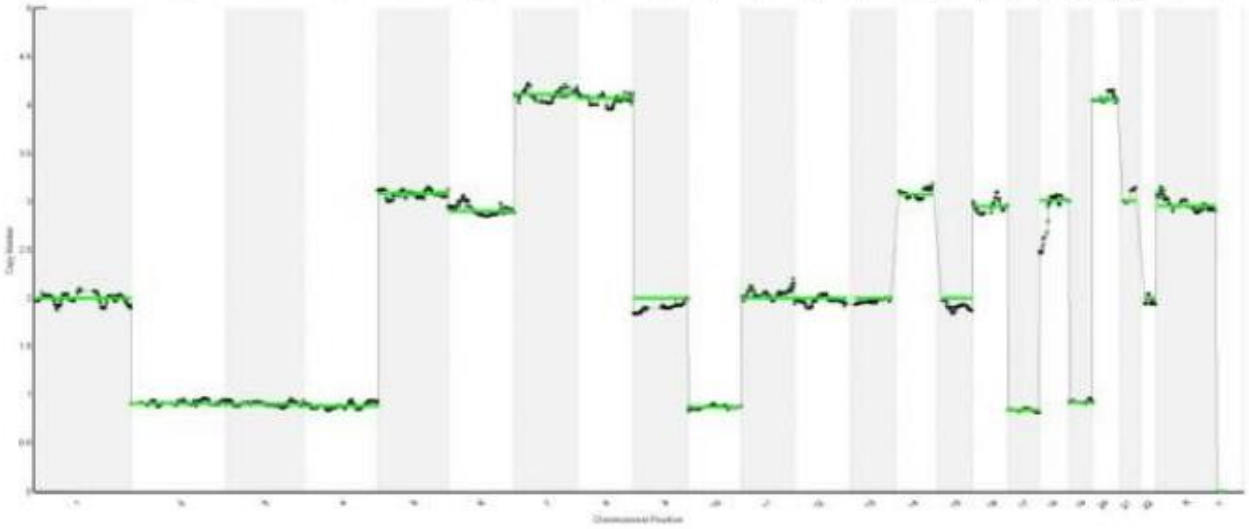
- Technologie budoucnosti
- Rutinnímu využití zatím brání cenové náklady a algoritmus v laboratořích (3 vs. 5 denní embrya, technologie vitrifikace apod.)
- Výhody - robustnější v porovnání s array-CGH, větší kapacita,
- vyšší „dynamický interval“ - detekce mozaicismu
- Vývoj – detekce na exomové úrovni – „all in“
  = CHA, mutace pro monogenní choroby

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

aCGH



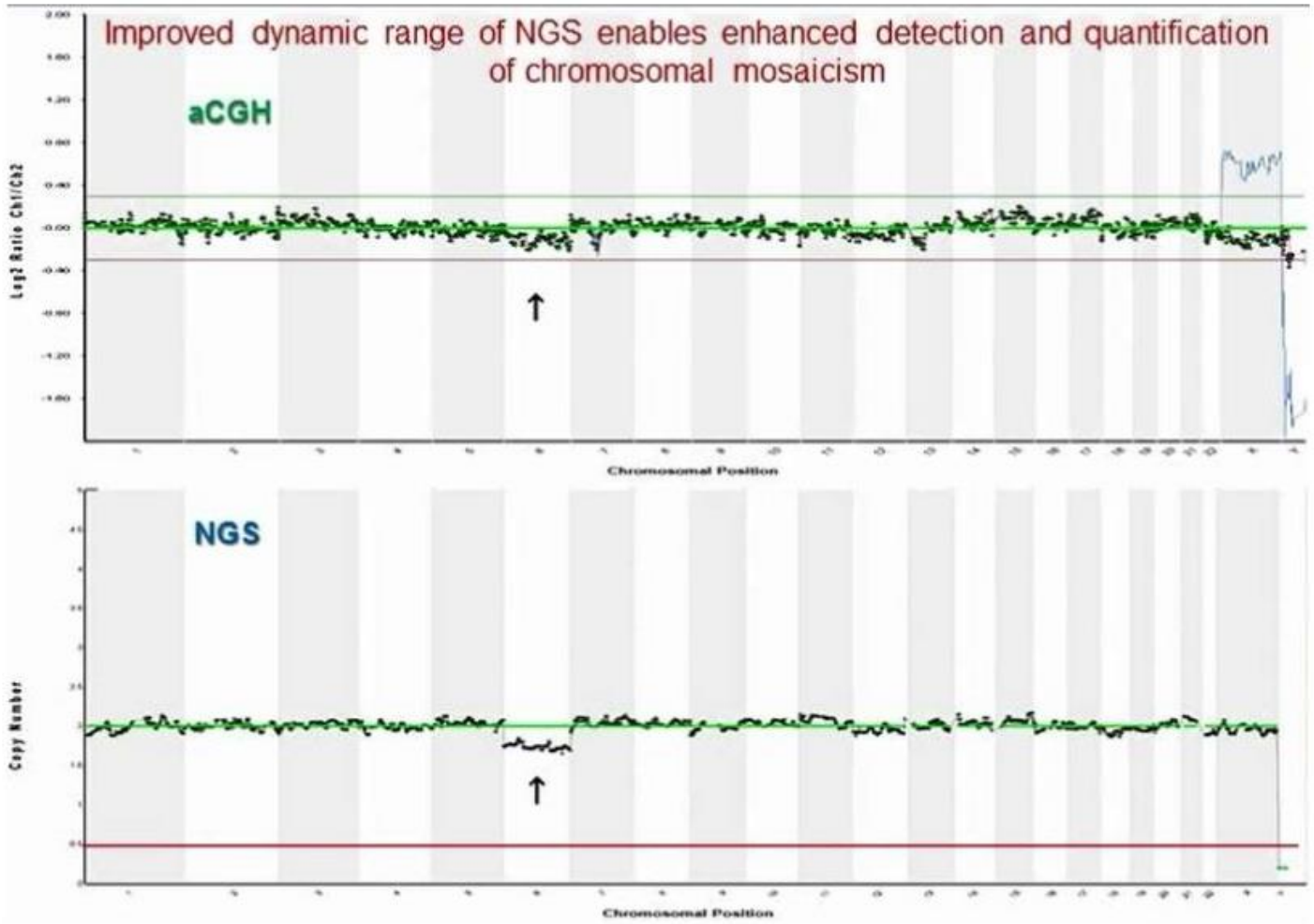

NGS





illumina

# Improved dynamic range – Fiorentino (2014) ESHRE S07



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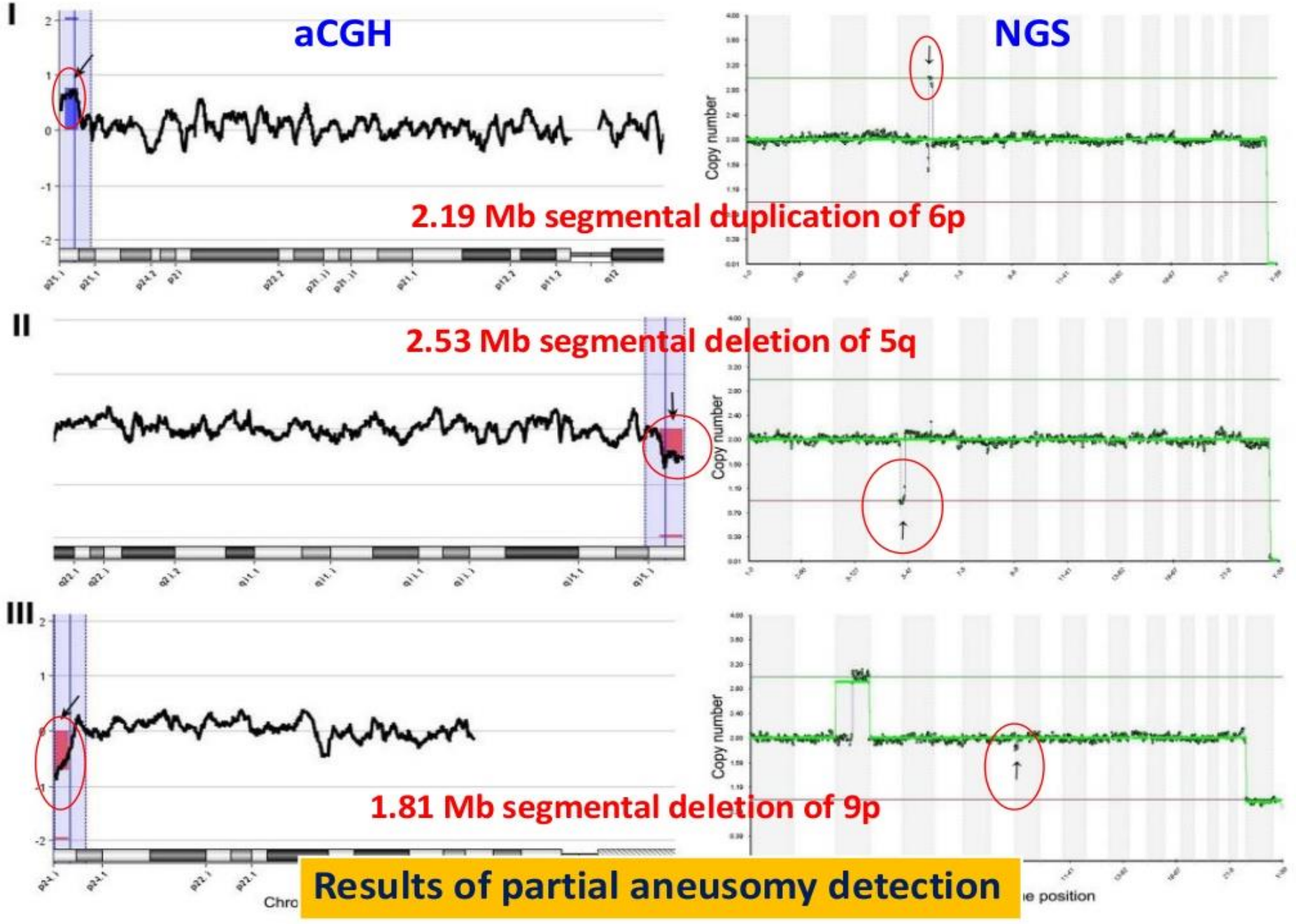



**Results of partial aneusomy detection**

# NGS u PGD

## Agilent OneSeq Target Enrichment

Detekce CNAs, LOH a mutací v rámci jednoho experimentu

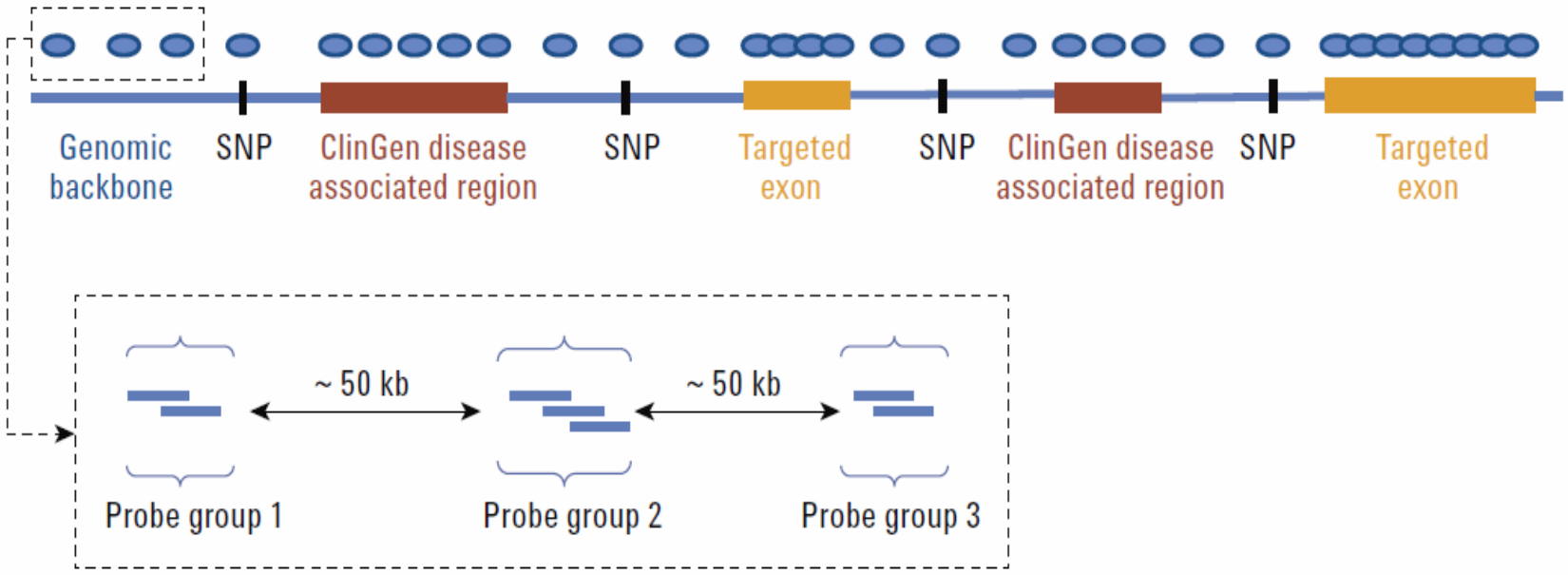



**Figure 1.** Bait design schema used for OneSeq target enrichment.

# NGS u PGD

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.

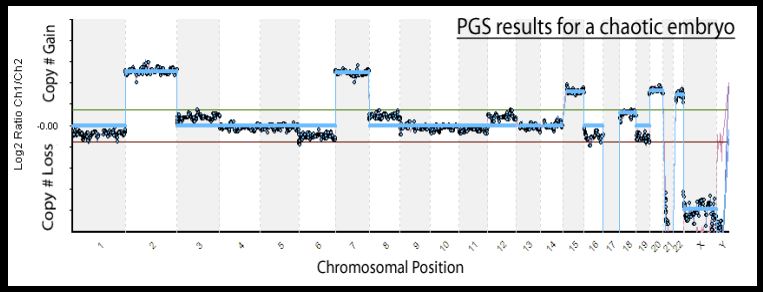
**INTERVENTION(S):** None.

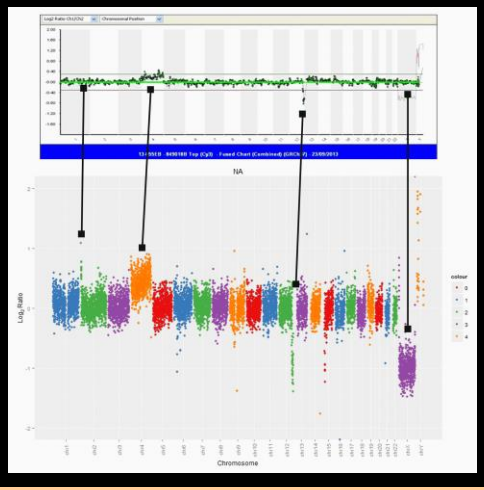
**MAIN OUTCOME MEASURE(S):** Embryonic genotype consistency of NGS with two independent conventional methods of PGD.

**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.






Display Settings: Abstract Send to:

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%).

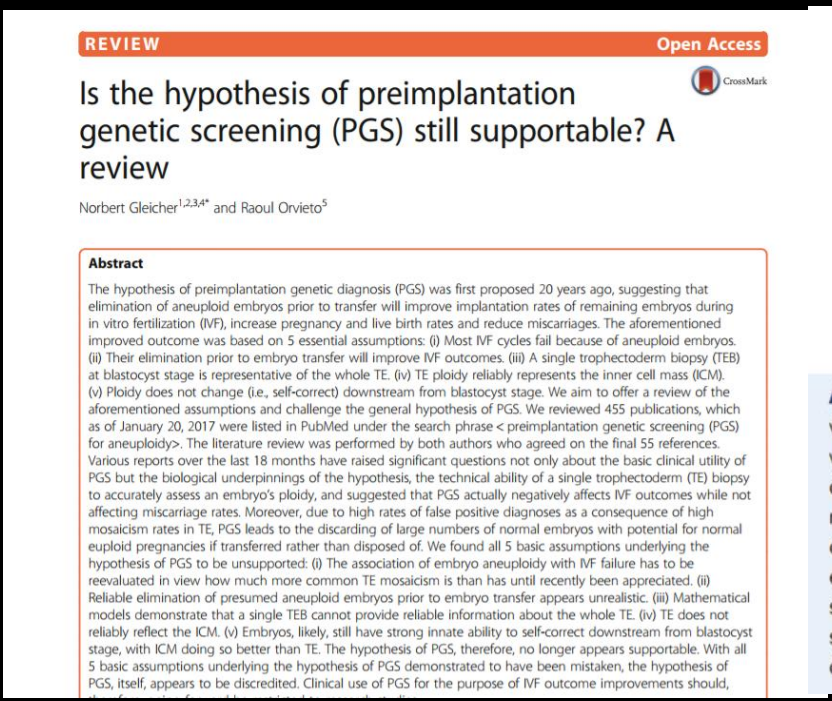
**CONCLUSION(S):** This is the first study reporting extensive preclinical validation and accuracy assessment of NGS-based comprehensive aneuploidy screening on single cells. Given the high level of consistency with an established methodology, such as array-CGH, NGS has demonstrated a robust high-throughput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

# NGS u PGD - problémy?

1) S robustnějšími metodami screeningu narůstá objem dat – interpretace?

2) Detekce mozaicismu u embryí – transfer ano či ne?

3) PGD 2.0 – zlepšuje skutečně IVF výsledky?



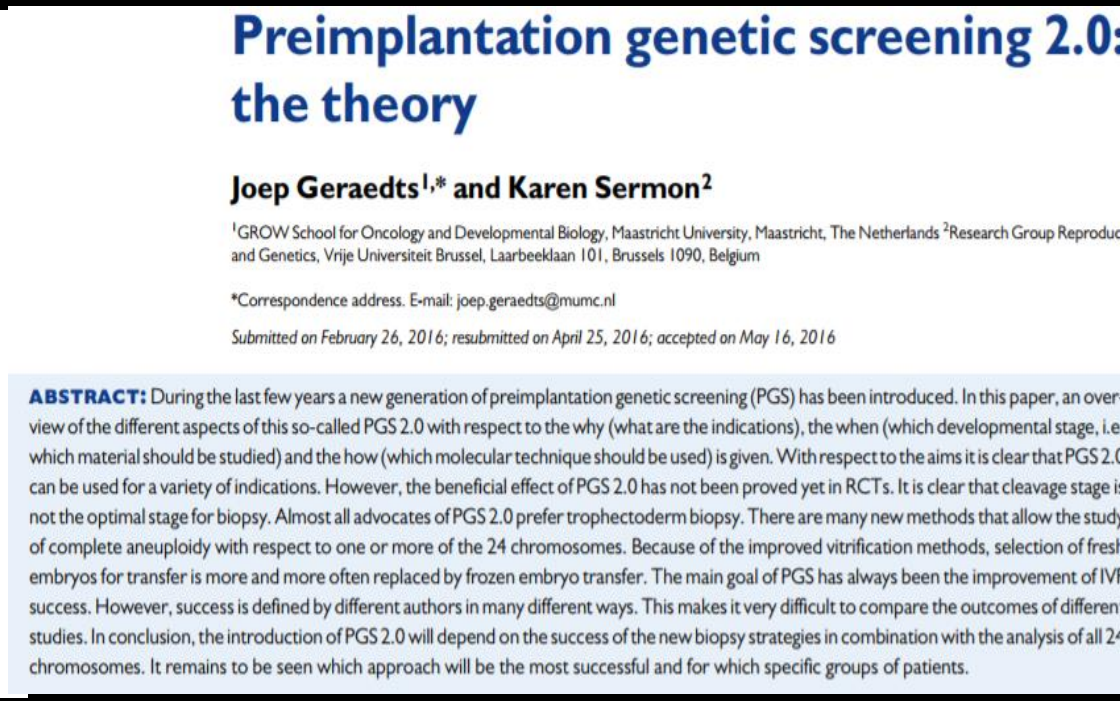
REVIEW Open Access

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]**

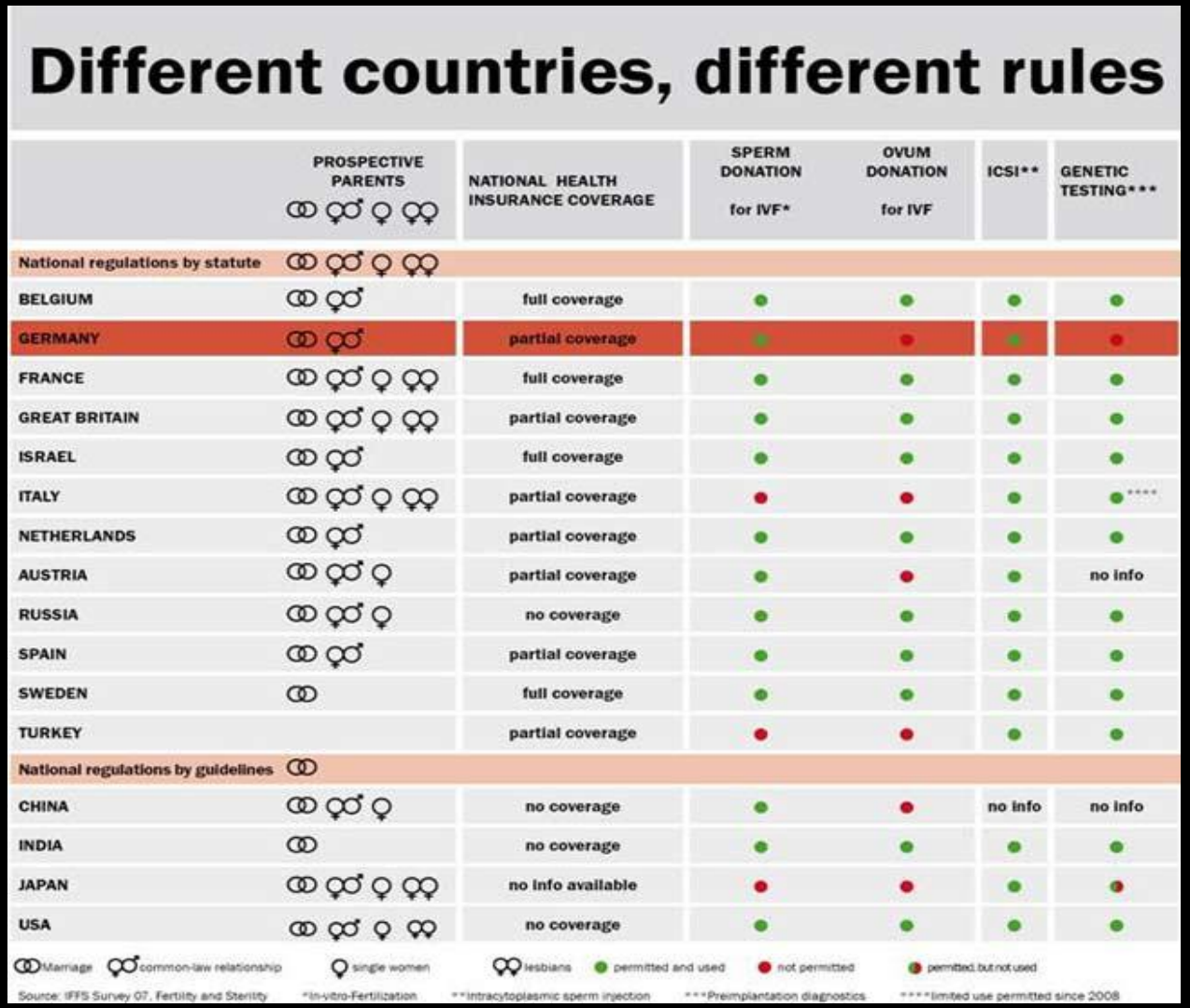
[1]GROW School for Oncology and Developmental Biology, Maastricht University, Maastricht, The Netherlands [2]Research Group Reproduc and Genetics, Vrije Universiteit Brussel, Laarbeeklaan 101, Brussels 1090, Belgium

*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.

# Legislativa a IVF



## Different countries, different rules

| | PROSPECTIVE PARENTS | NATIONAL HEALTH INSURANCE COVERAGE | SPERM DONATION for IVF* | OVUM DONATION for IVF | ICSI** | GENETIC TESTING*** |
|---|---|---|---|---|---|---|
| National regulations by statute | | | | | | |
| BELGIUM | | full coverage | ● | ● | ● | ● |
| GERMANY | | partial coverage | ● | ● | ● | ● |
| FRANCE | | full coverage | ● | ● | ● | ● |
| GREAT BRITAIN | | partial coverage | ● | ● | ● | ● |
| ISRAEL | | full coverage | ● | ● | ● | ● |
| ITALY | | partial coverage | ● | ● | ● | ●**** |
| NETHERLANDS | | partial coverage | ● | ● | ● | ● |
| AUSTRIA | | partial coverage | ● | ● | ● | no info |
| RUSSIA | | no coverage | ● | ● | ● | ● |
| SPAIN | | partial coverage | ● | ● | ● | ● |
| SWEDEN | | full coverage | ● | ● | ● | ● |
| TURKEY | | partial coverage | ● | ● | ● | ● |
| National regulations by guidelines | | | | | | |
| CHINA | | no coverage | ● | ● | no info | no info |
| INDIA | | no coverage | ● | ● | ● | ● |
| JAPAN | | no info available | ● | ● | ● | ● |
| USA | | no coverage | ● | ● | ● | ● |

Marriage · common-law relationship · single women · lesbians · ● permitted and used · ● not permitted · ● permitted, but not used

Source: IFFS Survey 07, Fertility and Sterility · *In-vitro-Fertilization · **Intracytoplasmic sperm injection · ***Preimplantation diagnostics · ****limited use permitted since 2008

- http://www.pharmaceutical-int.com/article/co2-incubator-for-in-vitro-fertilisation.html

# Etické aspekty

- Pokrok technologií asistované reprodukce a prenatální a preimplantační diagnostiky vyžaduje **úpravu etických a právních norem**, které by bránily jejich **zneužití** a umožnili naopak jejich využití v prevenci
- Zabránění neodůvodněným genetickým manipulacím….volba pohlaví ?

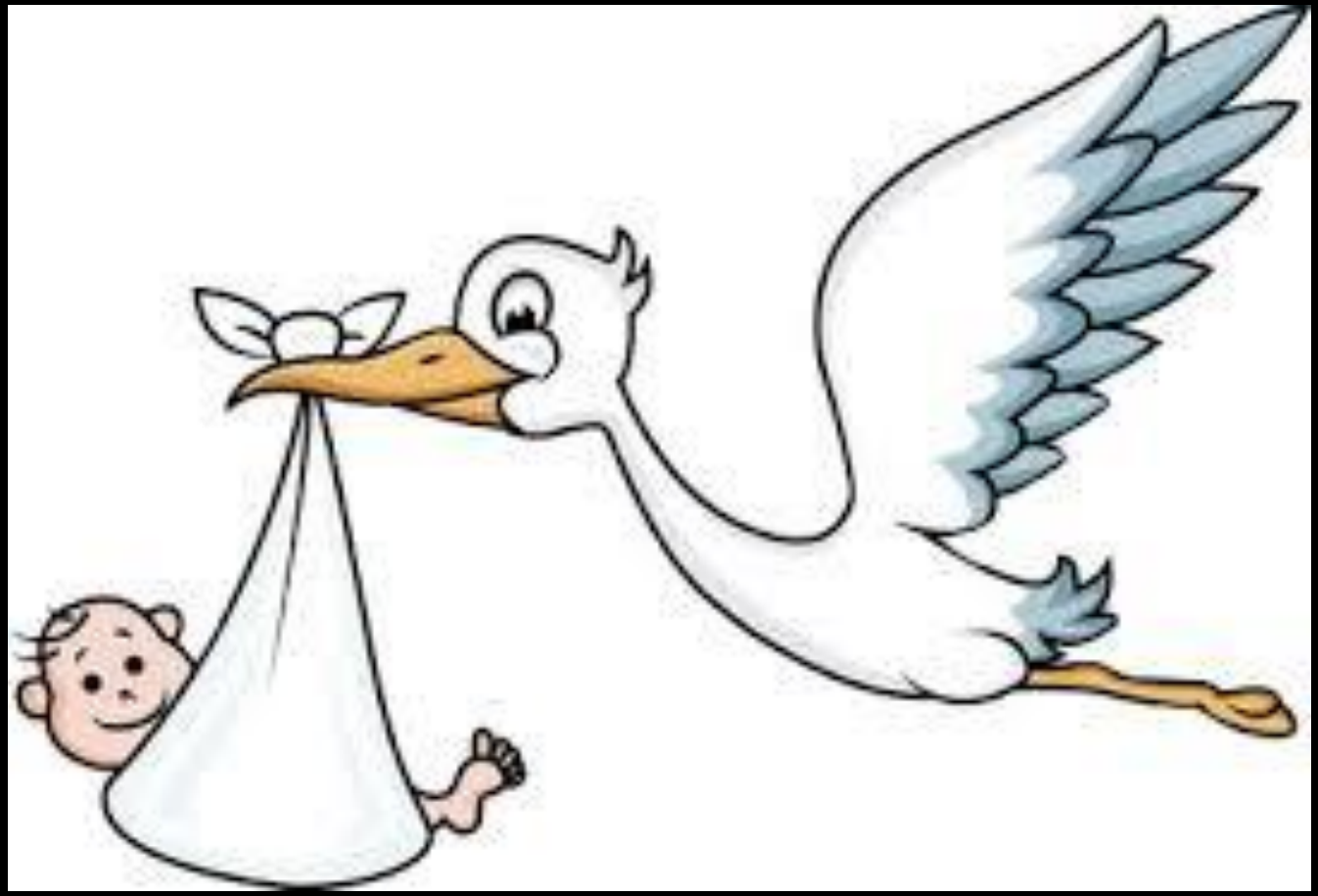
# Po PGD…

Po provedení PGD by měly být výsledky konzultovány s klinickým genetikem

+

Měla by být provedena kontrola pomocí prenatální genetické diagnostiky

# Rozhodnutí vždy přísluší rodině.



# Děkuji za pozornost