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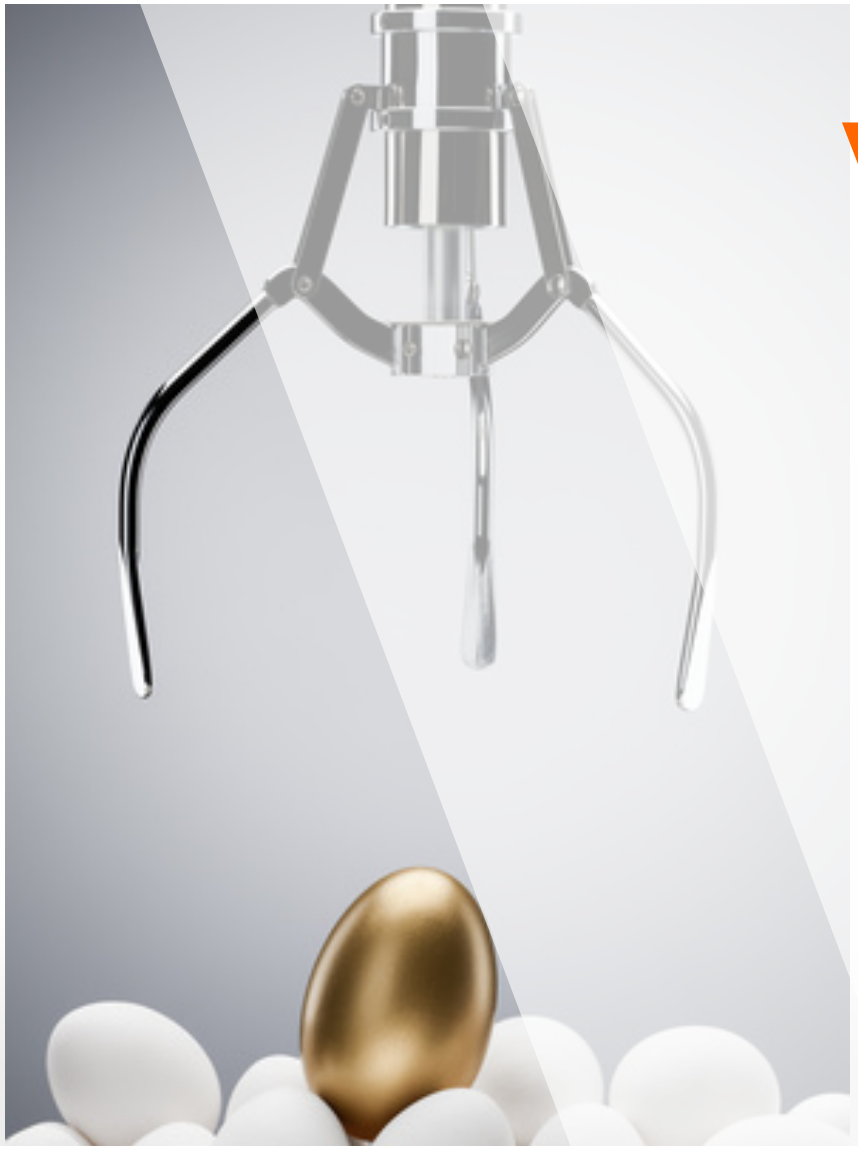
PGS 2.0

Willem Verpoest, Brussels PGD



TERMINOLOGIE

PGD preimplantation genetic diagnosis (PGT –M and SC)

1. autosomal dominant/recessive and X-linked diseases – monogenic (PGT-M)
2. chromosomal translocations (Robertsonian/reciprocal) (PGT-SC)

Methods for the identification of healthy v. unhealthy embryos in a fertile population (65%; Verpoest *et al.*, 2009)

PGS preimplantation genetic screening (PGT-A)

Methods for the identification of euploid embryos in an infertile population (Fragouli *et al.*, 2008)

OVERZICHT

1. nieuwe diagnostische technieken: een hele wereld opent zich
2. toepassing op alle niveau's: een nieuw paradigma in reproductieve en prenatale geneeskunde
3. huidige toepassingen
    1. Mendeliomen en exoom sequencing
    2. preimplantatie genetische diagnose
    3. preimplantatie genetische screening

OVERZICHT

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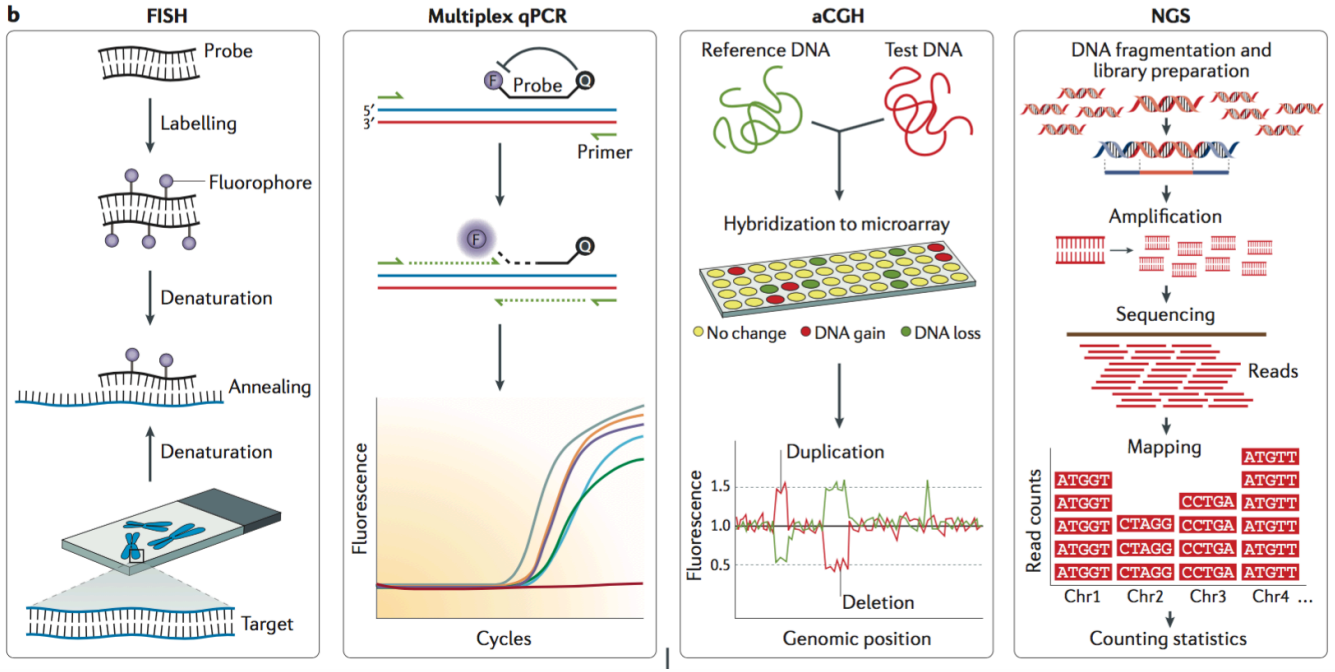
1. NIEUWE TECHNIEKEN

NIEUWE TECHNIEKEN



Voet, Vermeesch, Devriendt, Nat Genet 2016

NIEUWE TECHNIEKEN

single cell screening

- single cell array CGH
  - aCGH; only CNV
  - SNP arrays; CNV and genotyping
- single cell haplotyping
  - haploid genotyping
  - two genome wide haplotyping techniques
    1. karyomapping
    2. single cell haplotyping and imputation of linked disease variants (siCHILD)
- single cell sequencing
  - low depth sequencing

Table 2 | **Pre-implantation genetic diagnosis and screening tests**

	Microsequencing	QF-PCR	FISH	qPCR	aCGH	Genome-wide haplotyping	Low-coverage sequencing
Genetic lesion							
Monogenic disorders	+	+	–	–	–	+	–
Combination of monogenic and chromosomal disorders	–	–	–	–	–	+	–
Whole-chromosome aneuploidy	–	+/–*	+/–‡	+	+	+	+
Balanced chromosomal rearrangements	–	–	–	–	–	+	–
Unbalanced translocations	–	+/–*	+	+/–	+/–§	+	+
Complex rearrangements	–	–	+/–‡	±	+/–§	+	+/–§
Submicroscopic deletions	–	+/–*	+	–	–	+	–
Submicroscopic duplications	–	+/–*	–	–	–	+	–
Uniparental disomy	–	+/–*	–	–	–	+	–
Mechanistic origin of trisomies (mitotic vs meiotic)	–	–	–	–	–	+	–
Familially inherited	+	+	+	+	+	+	+
De novo mutations	+	–	+	+	+	–	+
Methodology							
WGA required	–	–	–	–	+	+	+

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NIEUWE TECHNIEKEN

Method	Duration of test	Complexity	Equipment cost	Reagent cost	Resolution	Pros and Cons
CGH	12–72 h	Medium	Medium	Low	Low	Low cost Skilled Labor intensive
Array CGH	12–24 h	Medium	Medium	Medium	Medium	Robust Scalable
Digital PCR	8 h	Medium	Medium	Low	Low	Low cost Scalable Rapid Polar body analysis only
Real-time quantitative PCR	4 h	Medium	Medium	Low	Low	Low cost Not scalable without additional equipment Multiple cell samples only
SNP microarray	16–72 h	High	High	Medium	High	Genome-wide analysis Quantitative and marker analysis Parental origin
Next-generation sequencing	15 h	High	High	Medium	Low	Scalable with multiplexing

Handyside FS 2013

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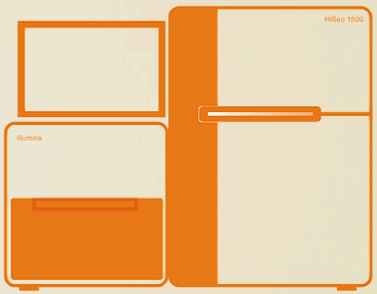
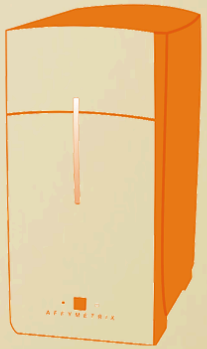
Genomics Solutions



MASSIVE PARALLEL SEQUENCING



ARRAY

VUB publicatie genomineerd als NAR 2016 Breakthrough Article

VUB publicatie genomineerd als NAR 2016 Breakthrough Article
VUB Today
M. Bonduelle & S. Van Dooren & D. Daneels & A. Gazzo

◄ 1/2 ►

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2. TOEPASSING OP ALLE NIVEAU'S: EEN NIEUW PARADIGMA IN REPRODUCTIEVE EN PRENATALE GENEESKUNDE

THE POWER OF PREDICTIVE CARE

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NIEUWE TECHNIEKEN

NIEUWE TECHNIEKEN

NIEUWE TECHNIEKEN

- 1/280 geboortes hebben een genetische aandoening
- 80% zonder een familiale voorgeschiedenis
-
- 2017 ACOG guidelines: carrier screening on 22 genes
- Deense spermabanken: 42 ziekten

author	# genes	% individuals	% couples
Plantinga et al 2016	50		0.69%
Haque et al	110		0.64%
Abuli et al 2016	368		3.03%
Cooper Genomics	314	45%	3.5%
iGenomix	600		5%

the 23andMe blog

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December 5, 2013

23andMe Provides An Update Regarding FDA's Review

Published by AnneW under Health and Traits, news

By Anne Wojcicki

After discussion with officials from the Food and Drug Administration today, 23andMe will comply with the FDA's directive and stop offering new consumers access to health-related genetic tests while the company moves forward with the agency's regulatory review processes.

23andMe has been giving consumers access to health information for six years and is committed to finding the right regulatory path for our customers. I am highly disappointed that we have reached this point and will work hard to make sure consumers have direct access to health information in the near future. Our goal is to work cooperatively with the FDA to provide that opportunity.

We also want to make clear that we stand behind the data we have generated for customers. Our lab partner adheres to strict quality standards that are part of the Clinical Laboratory Improvement Amendments of 1988 — known as CLIA. These are the same standards used in the majority of other health and disease-related tests. We decided several years ago to comply with CLIA guidelines to be consistent with other types of laboratory testing and to assure customers about the quality of data.

the 23andMe blog

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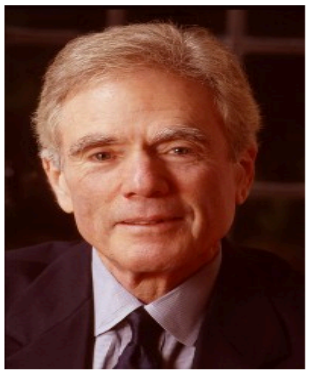
November 9, 2011

The Power of Predictive Care

Published by ScottH under Health and Traits

You are unique, but sometimes when you see your doctor you don't feel you're being treated that way.

The promise of personalized medicine is that what makes you, *you*, can also be used to tailor the health care plan that is most effective for *you*.

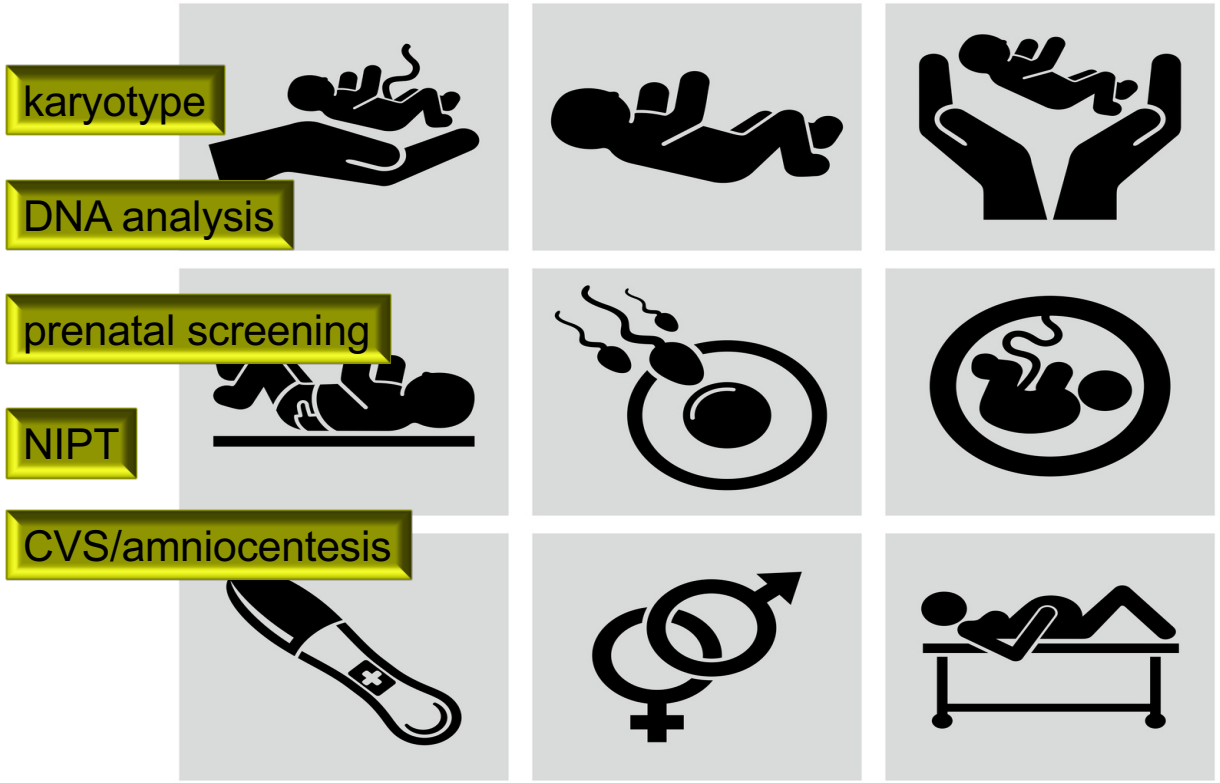


This is already happening, in part, because of the work of people like Dr. Ralph Snyderman, chancellor emeritus at Duke University and James B. Duke Professor of Medicine. Snyderman will be speaking early next year at the Personalized Medicine World Conference 2012 about bringing this approach into the clinic.

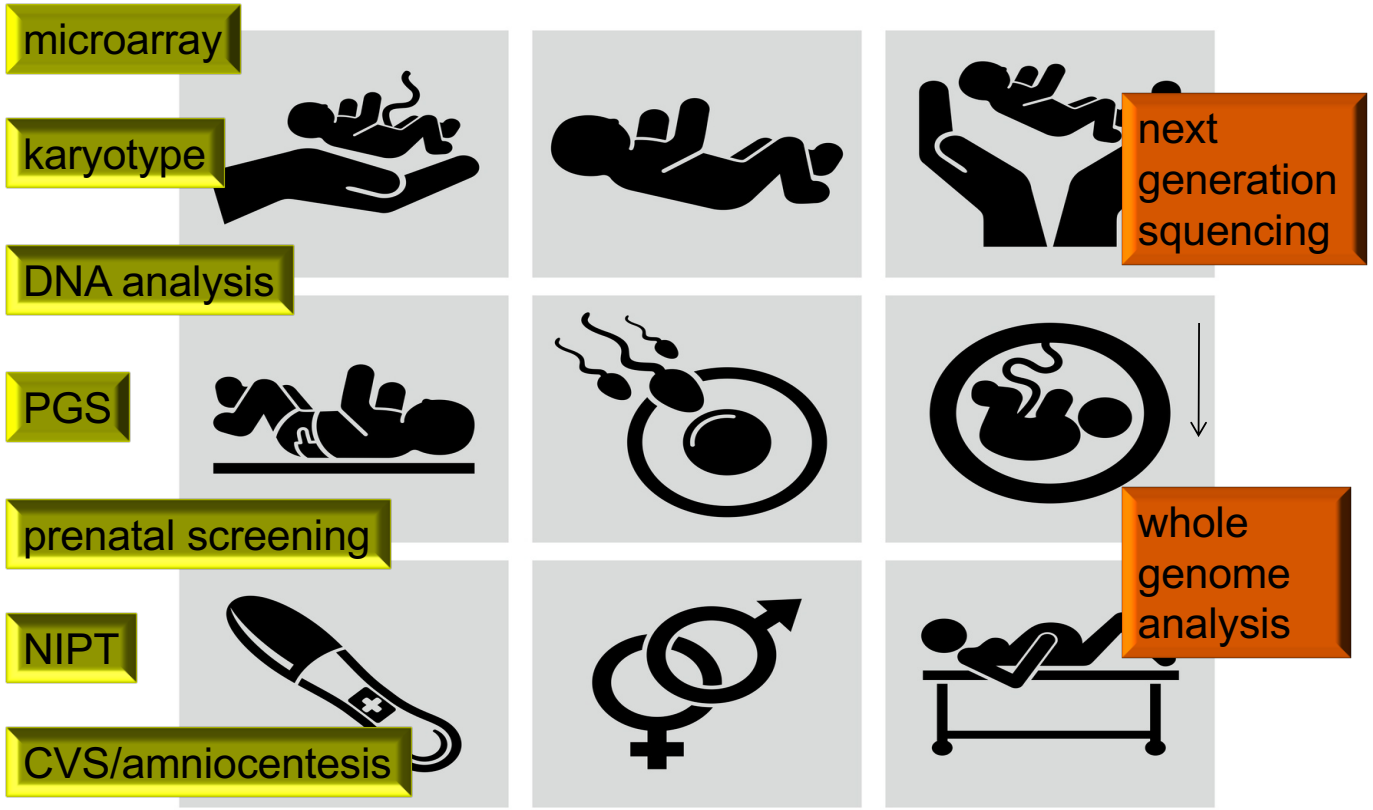
"As far as I'm concerned, the patient must be at the center of effective personalized medicine and prospective care," he said.

The former Dean of Duke's medical school and Chancellor for Health Affairs, Snyderman started talking about personalized and "prospective" health care more than a decade ago. At the time, he predicted that medicine would move away from traditional reactive methods for treating disease. Instead doctors would start using personalized data and health care planning to provide a more preventive, targeted, and individualized approach to health care.

van ouders tot kinderen



van ouders tot kinderen



3. PGS 2.0

RATIONALE VOOR PGS

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RATIONALE VOOR PGS

- meer aneuploidy in humane embryos (7-10% aneuploid)
- lage implantatie
- VEEL MISKRAMEN!! (70% aneuploid)

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8 November 2010 Last updated at 00:01 GMT

Share

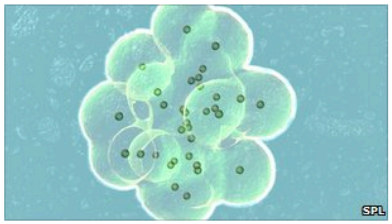
New test to dramatically increase chance of IVF success

By Pamela Rutherford
Reporter, BBC News

A new screening technique to test embryos could dramatically increase the chances of having a baby from IVF.

The test allows for any chromosomal abnormalities, the biggest cause of early pregnancy loss, to be picked up in embryos before they are reimplanted.

The UK-based researchers expect the technique to double or triple current IVF success rates.



The new technique allows the viability of embroys to be tested without damaging them

Trials of the technique are being lead by fertility specialists at CARE Fertility in Manchester.

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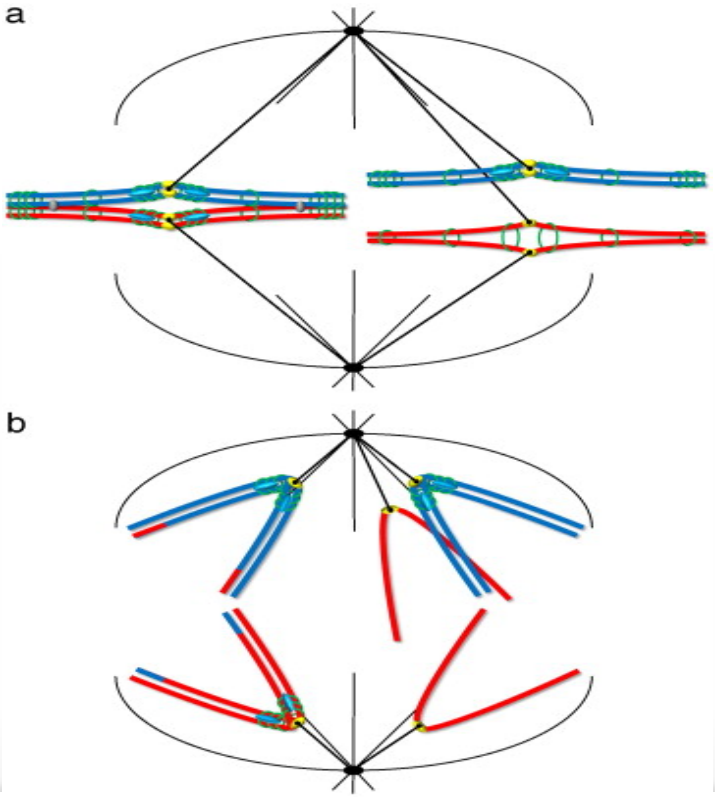
RATIONALE VOOR PGS

aneuploidie in oocyten

- etiologie
  1. non-disjunctie in meiosis I or meiosis II
  2. premature predivisie van zuster chromatiden in meiosis I

Gabriel *et al.*, 2011;
Handyside 2013;



TIMING VAN PGS

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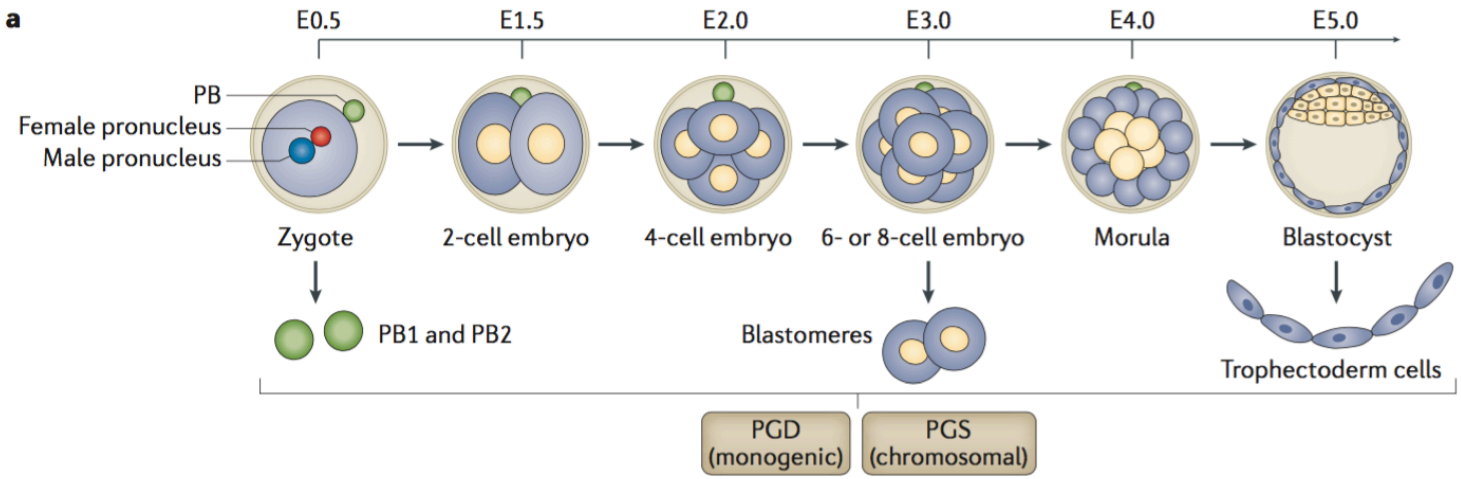
TIMING VAN PGS



Voet, Vermeesch, Devriendt, Nat Genet 2016

BEWIJS VOOR PGS

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polar body PGS

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Results of ESTEEM

an RCT to test preimplantation genetic testing for aneuploidy

Karen Sermon
ESTEEM Coordinator

SCIENCE MOVING PEOPLE MOVING SCIENCE

Conclusion

- ESTEEM is the largest intention-to-treat RCT to date on PGS
- The most important clinical implication is the lack of benefit of PB aCGH in women of AMA regarding increasing live birth delivery rate
- However, PB aCGH may avoid unnecessary embryo transfers and decrease miscarriage rates
- More multiple pregnancies were observed in the control group due to double embryo transfer policy

eshre
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BEWIJS VOOR PGS

cleavage stage biopsy/ aCGH

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CLEAVAGE STAGE BIOPSY

70% of embryos aneuploid

- Vanneste *et al.*, 2010; Mertzanidou *et al.*, 2013

claims that higher number of probes used, or target-specific probes will reduce false-positive rates

- Rubio *et al.*, 2013; Mir *et al.*, 2013

RUBIO, 2017

ORIGINAL ARTICLE: ASSISTED REPRODUCTION

In vitro fertilization with preimplantation genetic diagnosis for aneuploidies in advanced maternal age: a randomized, controlled study

Carmen Rubio, Ph.D.,[a] José Bellver, M.D.,[b,c] Lorena Rodrigo, Ph.D.,[a] Gema Castillón, M.D.,[d] Alfredo Guillén, M.D.,[e] Carmina Vidal, M.D.,[b] Juan Giles, M.D.,[b] Marcos Ferrando, M.D.,[f] Sergio Cabanillas, M.D.,[b] José Remohí, M.D., Ph.D.,[b,c] Antonio Pellicer, M.D., Ph.D.,[b,c,g] and Carlos Simón, M.D., Ph.D.[a,b,c]

[a] Igenomix Valencia/INCLIVA, Valencia; [b] Instituto Valenciano de Infertilidad, Valencia University, Valencia; [c] Department of Pediatrics, Obstetrics and Gynecology, School of Medicine, Valencia University, Valencia; [d] Instituto Valenciano de Infertilidad, Barcelona; [e] Instituto Valenciano de Infertilidad, Madrid, Universidad Juan Carlos I, Madrid; [f] Instituto Valenciano de Infertilidad, Bilbao; and [g] Instituto de Investigación Sanitaria La Fe, Valencia, Spain

Objective: To determine the clinical value of preimplantation genetic diagnosis for aneuploidy screening (PGD-A) in women of advanced maternal age (AMA; between 38 and 41 years).
Design: This was a multicenter, randomized trial with two arms: a PGD-A group with blastocyst transfer, and a control group with blastocyst transfer without PGD-A.
Setting: Private reproductive centers.
Patient(s): A total of 326 recruited patients fit the inclusion criteria, and 205 completed the study (100 in the PGD-A group and 105 in the control group).
Intervention(s): Day-3 embryo biopsy, array comparative genomic hybridization, blastocyst transfer, and vitrification.
Main Outcome Measure(s): Primary outcomes were delivery and live birth rates in the first transfer and cumulative outcome rates.
Result(s): The PGD-A group exhibited significantly fewer ETs (68.0% vs. 90.5% for control) and lower miscarriage rates (2.7% vs. 39.0% for control). Delivery rate after the first transfer attempt was significantly higher in the PGD-A group per transfer (52.9% vs 24.2%) and per patient (36.0% vs. 21.9%). No significant differences were observed in the cumulative delivery rates per patient 6 months after closing the study. However, the mean number of ETs needed per live birth was lower in the PGD-A group compared with the control group (1.8 vs. 3.7), as was the time to pregnancy (7.7 vs. 14.9 weeks).
Conclusion(s): Preimplantation genetic diagnosis for aneuploidy screening is superior compared with controls not only in clinical outcome at the first ET but also in dramatically decreasing miscarriage rates and shortening the time to pregnancy. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)
Key Words: Aneuploidy, array-CGH, embryo biopsy, maternal age, PGD-A

Advanced maternal age (AMA) is one of the most significant clinical bottlenecks in assisted reproduction. Fertility declines as women age, owing to both a diminished ovarian reserve and an impaired oocyte quality that leads to an increase in embryo aneuploidy (1). Aneuploidy is the most common genetic abnormality in humans. Large data sets from comprehensive aneuploidy screenings of preimplantation embryos demonstrate

Received December 14, 2016; revised January 27, 2017; accepted March 3, 2017.
C.R. has nothing to disclose. J.B. has nothing to disclose. L.R. has nothing to disclose. G.C. has nothing to disclose. A.G. has nothing to disclose. C.V. has nothing to disclose. J.G. has nothing to disclose. M.F. has nothing to disclose. S.C. has nothing to disclose. J.R. has nothing to disclose. A.P. has nothing to disclose. C.S. has nothing to disclose.
Partially supported by Igenomix, which covered the cost of the array comparative genomic hybridization (CGH) analysis. Illumina provided the arrays of CGH, and Instituto Valenciano de Infertilidad clinics covered the cost of embryo biopsies, so that patients in the preimplantation genetic diagnosis for aneuploidy screening (PGD-A) arm were not responsible for the cost of the PGD-A procedure.
Reprint requests: Carmen Rubio, Ph.D., Igenomix, Calle Narcís Monturiol Estarriol nº11 Parcela B, Edificio Europark, Parque Tecnológico de Paterna, 46980, Paterna, Spain (E-mail: carmen.rubio@igenomix.com).

Fertility and Sterility® Vol. ■, No. ■, ■ 2017 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2017.03.011

REFLECTIONS

Advanced maternal age patients benefit from preimplantation genetic diagnosis of aneuploidy

Chromosome abnormalities in human embryos may result in implantation failure or miscarriage. These abnormalities are common, and their incidence increases with advancing maternal age, from approximately 40% in fertile egg donors to 80% in patients 41 to 42 years old (1). Preimplantation genetic diagnosis of aneuploidy (PGD-A) is used as a selection tool for euploid embryos with potential to implant and reach term. That chromosome abnormalities are a major cause of embryo loss with advancing maternal age is demonstrated by the observation that once a euploid embryo is transferred to the uterus, it seems to have the same chance of implanting irrespective of maternal age (2). Preimplantation genetic diagnosis of aneuploidy has evolved from its first iteration using day-3 biopsy and testing for a limited number of chromosomes by fluorescence in situ hybridization, to blastocyst biopsy and comprehensive 24-chromosome screening (CCS) techniques including array comparative genomic hybridization, quantitative polymerase chain reaction, single-nucleotide polymorphism array, or next-generation sequencing.

Three previous randomized clinical trials (RCT) using the latter technologies have focused on young or good-prognosis patients and have found improvements in ongoing pregnancy rates, but the trials were underpowered. The study by Rubio et al. (3) in this issue of *Fertility and Sterility* is the first RCT involving day-3 blastomere biopsy, and PGD-A by CCS, targeting solely patients of advanced maternal age. Array comparative genomic hybridization in this multicenter study produced an acceptable no-call rate of 2.8%. On average, the study patients had five day-3 embryos, of which 62% developed to blastocyst. Compared with previous RCTs, the present study patients would be placed in the category of poor prognosis, with 78% chromosomally abnormal embryos (4). Thus, on average they produced a single euploid day-3 embryo or 0.6 euploid blastocysts per patient.

In this challenging group, with limited choice of embryos for transfer, the study shows that PGD-A significantly improved implantation, reduced miscarriage, and improved delivery rates both per transfer and per intention to treat cycle. These results offset a sharp decrease in the incidence of embryo transfer after PGD-A compared with the control arm. The design of this study offers a clear advantage over other PGD-A trials because all embryos were biopsied on the same day (day 3), whereas in blastocyst biopsy studies, data evaluation is complicated owing to biopsy being performed on both day 5 and day 6. The authors also offered a cost-effectiveness analysis, showing that in their system the costs for a single live birth were higher with PGS than without. However, they also argued that incorporating blastocyst biopsy and next-generation sequencing as standard of care could reduce these costs by 10% both in Europe and in the United States.

The cumulative delivery rate per patient was not significantly different, but an advantage of PGD-A would not be expected if all euploid embryos were to be available for transfer. Indeed, the opposite would occur, that is, the control group would show higher cumulative delivery rates if the euploid embryo pool were to be reduced either by biopsy/vitrification damage or misdiagnosis as aneuploid. From a "cumulative delivery rate" point of view PGD-A is applied to reduce the risk of miscarriage and its associated psychological and physical trauma, as well as to reduce time to pregnancy. In the present study these objectives were achieved, that is, pregnancy loss rates were reduced dramatically, and time to achieve an ongoing pregnancy was reduced by half.

The development of PGD-A over the past 10 years was predicated on two premises, [1] that 24-chromosome testing would lead to decreased error and reduced no-call rates (≤2%), and [2] that the potentially negative effects of the biopsy procedure itself would be avoided if blastocysts rather than cleavage stage embryos were to be biopsied. The study of Rubio et al. brings the second premise into question because they performed the biopsy on day 3 of development.

Although current literature suggests that blastocyst biopsy may be safer than blastomere biopsy, it is likely that any cell biopsy has an effect on the developing embryo. There is conflicting evidence from day-3 biopsy studies. Most PGD-A RCTs involving day-3 biopsy and fluorescence in situ hybridization were performed by laboratories with limited experience in day-3 biopsy, whereas centers reporting improved results had extensive experience in biopsy but failed to produce level 1 evidence. Rubio et al.'s study was performed at IVI, which is a network of centers with more than 15 years of experience in day-3 biopsy. Thus these investigators show that in skilled hands, pregnancy results can be improved even with day-3 biopsy (5). This is not to say that clinics should offer day-3 biopsy and CCS in lieu of trophectoderm biopsy. The combined evidence still suggests that blastocyst biopsy may be relatively easier to master, whereas day-3 biopsy requires a higher skill level, including years of experience performing the procedure. In our opinion, as suggested by the authors, blastocyst biopsy should be the method of choice for most fertility centers.

Like previous randomized PGD-A studies, there are limitations in the study design. Clinical and laboratory staff monitoring the RCT cycles were not blinded to the allocation of patients, and neither was allocation concealment applied to the patients. The study is underpowered, and although two culture media systems were used, data were not evaluated according to the culture system. Most exclusions after randomization were due to low number of mature oocytes (metaphase II), especially in the control group, which could potentially bias the final analysis. Some patients with previous miscarriages were excluded, but it is unclear whether those miscarriages were due to chromosomal abnormalities, as the exclusion criteria require. Similarly, recurrent implantation failure patients were excluded, but that was not an exclusion criterion.

It is important to note that the control group in this study had pregnancy outcomes within the expected range, at least

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RUBIO, 2017

Description of the embryologic outcome of the patients.

Parameter	PGD-A	Non–PGD-A
No. of cycles performed	100	105
Mean no. MII oocytes (SD)	10.2 (5.3)	10.0 (4.6)
Mean no. 2-pronuclei zygotes (SD)	7.6 (4.2)	7.1 (3.1)
Mean no. day-3 good-quality embryos[a]	5.4 (2.9)	5.8 (3.2)
Mean no. day-3 blastomeres (SD)	7.9 (1.5)	8.0 (1.5)
Mean day-3 fragmentation degree (SD)	6.4 (5.4)	6.7 (5.4)
No. of arrested embryos/day-3 embryos (%)	76/538 (14.1)	111/581 (19.1)
No. of morula/day-3 embryos (%)	127/538 (23.6)	115/581 (19.8)
No. of blastocyst/day-3 embryos (%)	335/538 (62.3)	355/581 (61.1)

Note: For all data, nonsignificant differences between groups by Fisher's exact test and Student's test for noncategorical variables.
[a] Day-3 embryos with six or more blastomere and with fragmentation degree <25%.

Rubio. PGD-A in advanced maternal age. Fertil Steril 2017.

Clinical outcome at the first attempt (per transfer and per patient).

Parameter	PGD-A	Non–PGD-A	*P* value	OR (95% CI)
No. of cycles performed	100	105	–	–
No. of cycles with transfer (%)	68 (68.0)	95 (90.5)	.0001	0.22 (0.10–0.48)
Mean no. embryos/transfer (SD)	1.3 (0.5)	1.8 (0.4)	<.0001	CI: 0.35–0.65
Implantation rate (IR), n (%)	47/89 (52.8)	48/174 (27.6)	<.0001	2.94 (1.72–5.0)
Clinical pregnancy rate/transfer (%)	37/68 (54.4)	41/105 (43.1)	NS	NS
Clinical pregnancy rate/patient (%)	37/100 (37.0)	41/105 (39.0)	NS	NS
No. of miscarriages (%)	1 (2.7)	16 (39.0)[a]	.0007	0.06 (0.008–0.48)
No. of ectopic pregnancies (%)	0	2 (4.9)	NS	NS
No. of missed sacs (%)	3/47 (6.4)[b]	22/48 (45.8)[c]	<.0001	5.57 (3.09–10.03)
Ongoing IR	44/89 (49.4)	26/174 (14.9)	<.0001	5.57 (3.09–10.03)
Delivery rate/transfer	52.9	24.2	.0002	3.52 (1.80–6.87)
Delivery rate/patient	36.0	21.9	.0309	2.00 (1.08–3.71)
No. of live births/transfer (%)	44/68 (64.7)	26 (27.4)	<.0001	4.86 (2.49–9.52)
No. of live births/patient (%)	44/100 (44)	26 (24.8)	.0050	2.39 (1.32–4.32)

[a] One fetal loss (Down syndrome).
[b] One miscarriage + two vanishing twins.
[c] Sixteen miscarriages + six vanishing twins.

Rubio. PGD-A in advanced maternal age. Fertil Steril 2017.

RUBIO 2017

conclusions:

- day 3 biopsy and CCS (aCGH; micro-array)
  - higher live birth rate per transfer (52.9 v 24.2%)
  - lower miscarriage rate (2.7% PGS v 39.0%)
  - shorter time to pregnancy (7.7 v 14.9 weeks)
  - **cumulative after 6 months: no difference**
  - cost-benefit analysis: PGS more expensive

BEWIJS VOOR PGS

trophectoderm PGS

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ACGH TROPHECTODERM

Scott *et al.*, 2013	aCGH + fresh SET	fresh SET
couples	72	83
mean age	32.2	32.4
mean number of oocytes retrieved	17.2	17.1
blastocysts	8.0	7.9
aneuploidy rate	28.6%	-
embryos transferred	1.86	2.0
sustained implantation rate	66.4%	47.9%
delivery rate per cycle	84.7%	67.5%

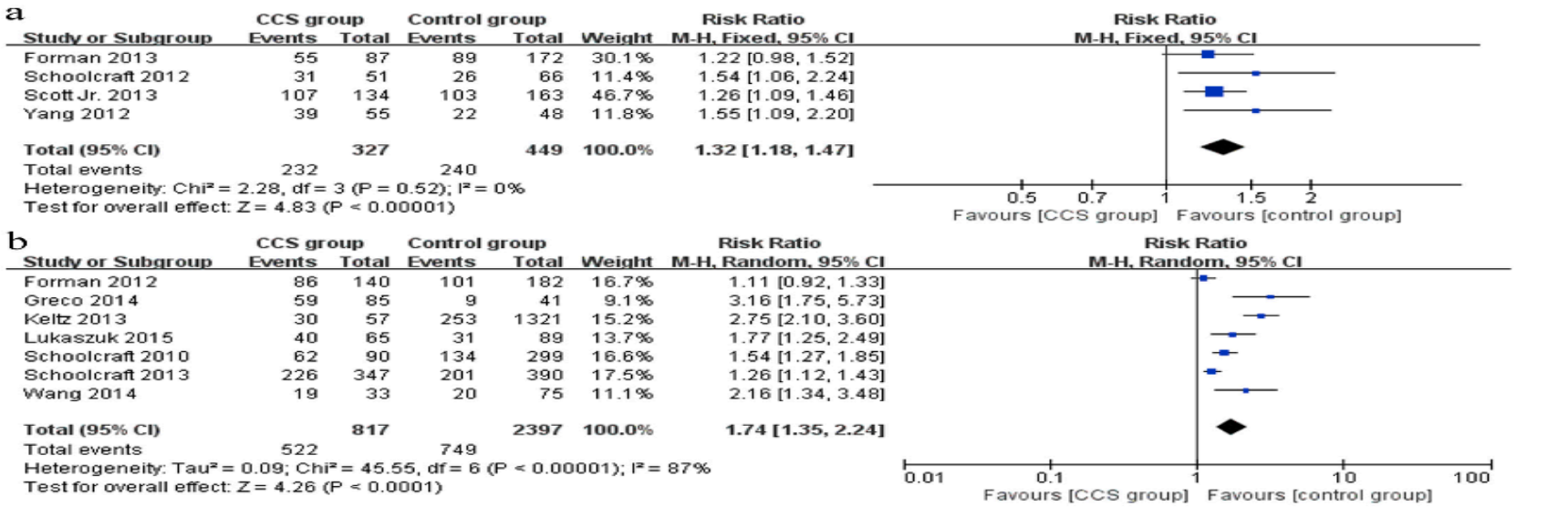
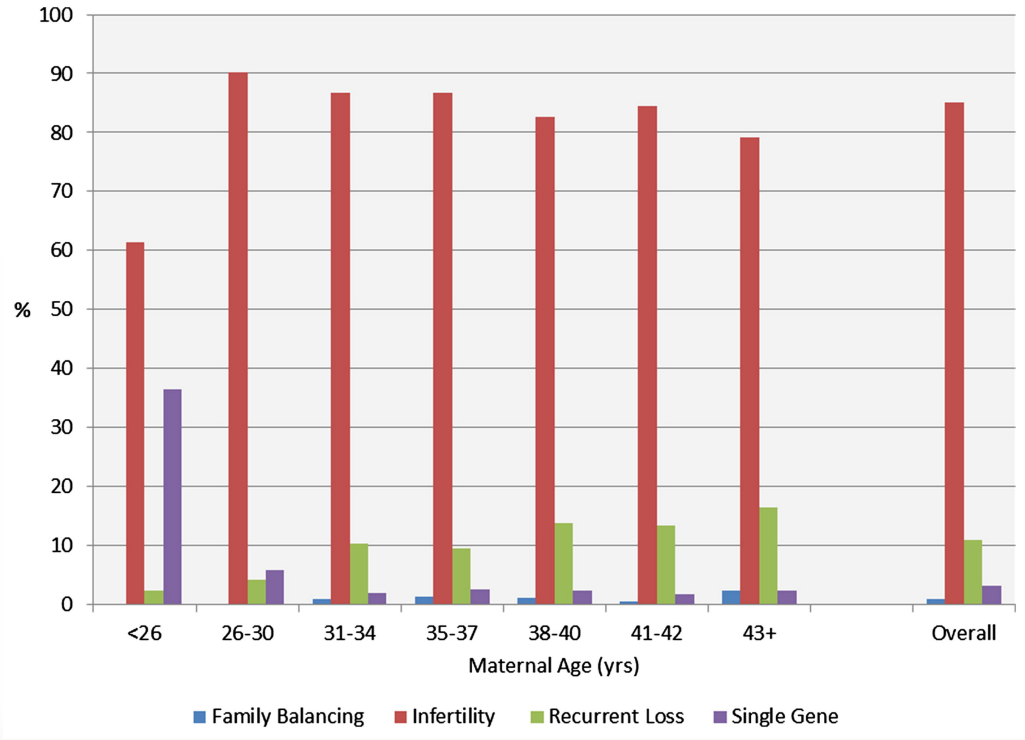


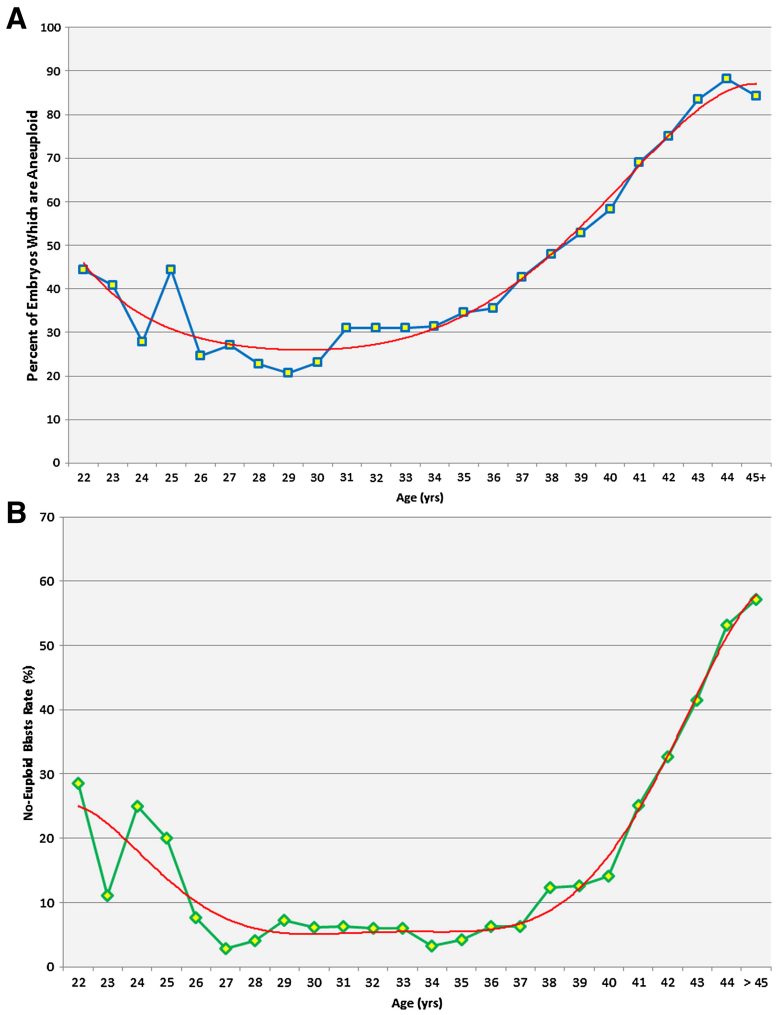
Fig 2. Forest plots showing the results of meta-analysis on implantation comparing the effect of CCS-based PGS and traditional morphological method after IVF/ICSI. (a) Forest plot of pooled RR on implantation of RCTs; (b) Forest plot of pooled RR on implantation of cohort studies.

The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening

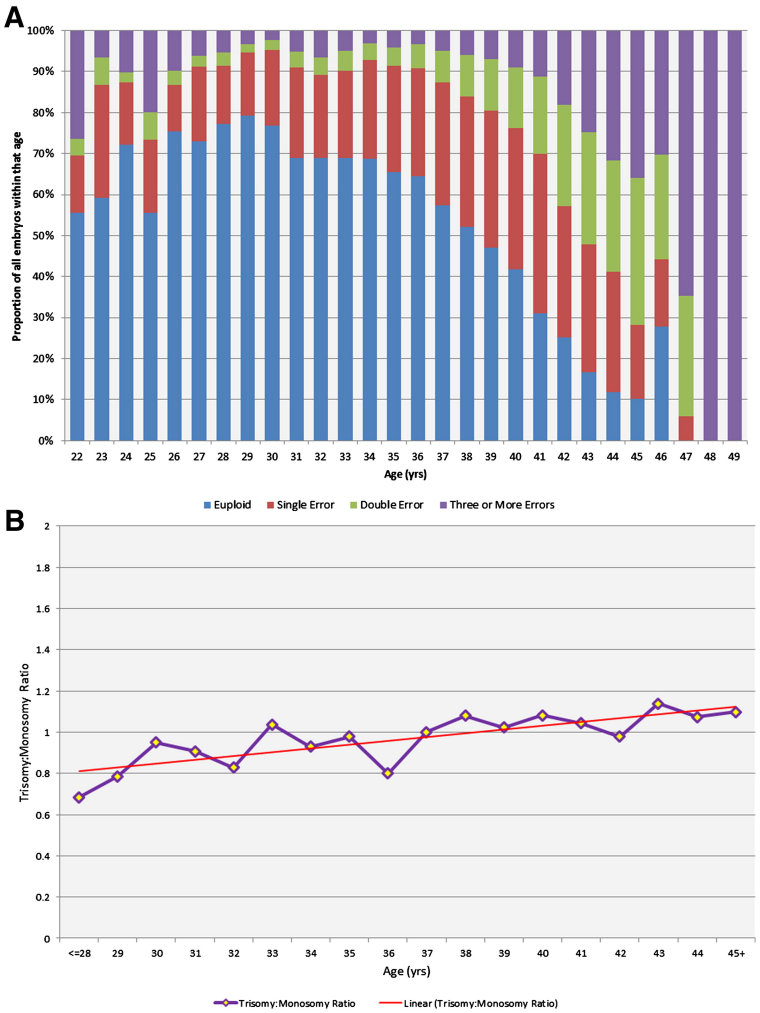
Jason M. Franasiak, M.D., Eric J. Forman, M.D., Kathleen H. Hong, M.D., Marie D. Werner, M.D., Kathleen M. Upham, B.S., Nathan R. Treff, Ph.D. and Richard T. Scott, M.D.

Volume 101, Issue 3, Pages 656-663.e1

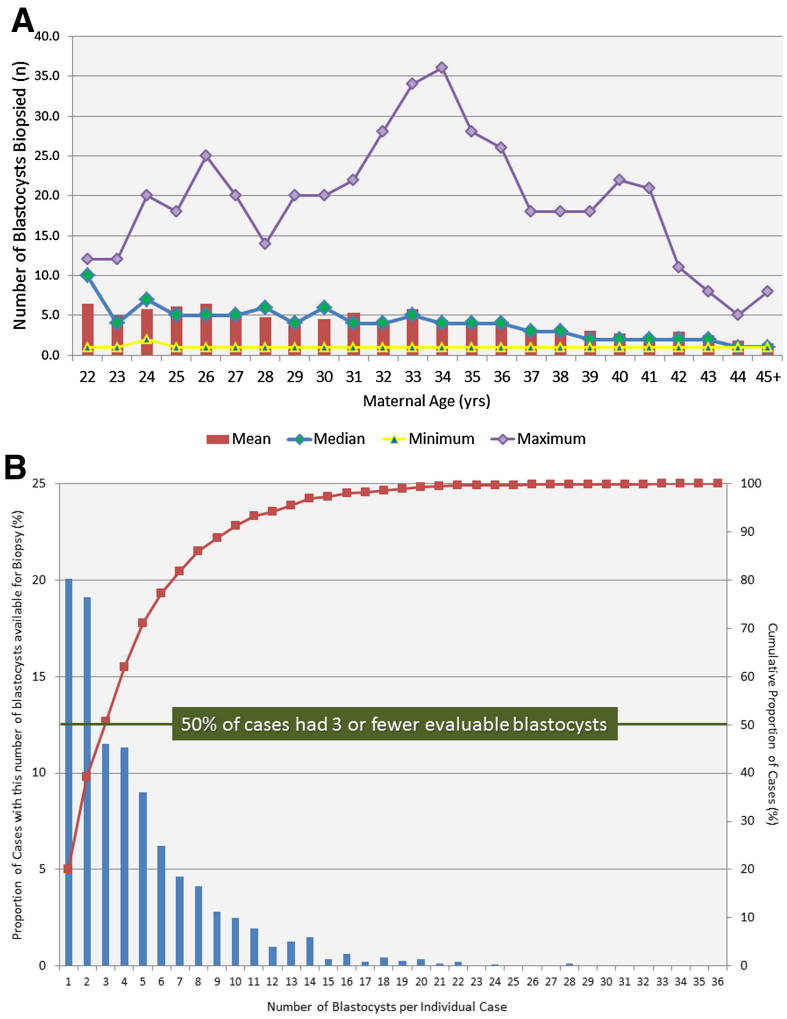




Source: Fertility and Sterility 2014; 101:656-663.e1



Source: Fertility and Sterility 2014; 101:656-663.e1



Source: Fertility and Sterility 2014; 101:656-663.e1

BESLUIT

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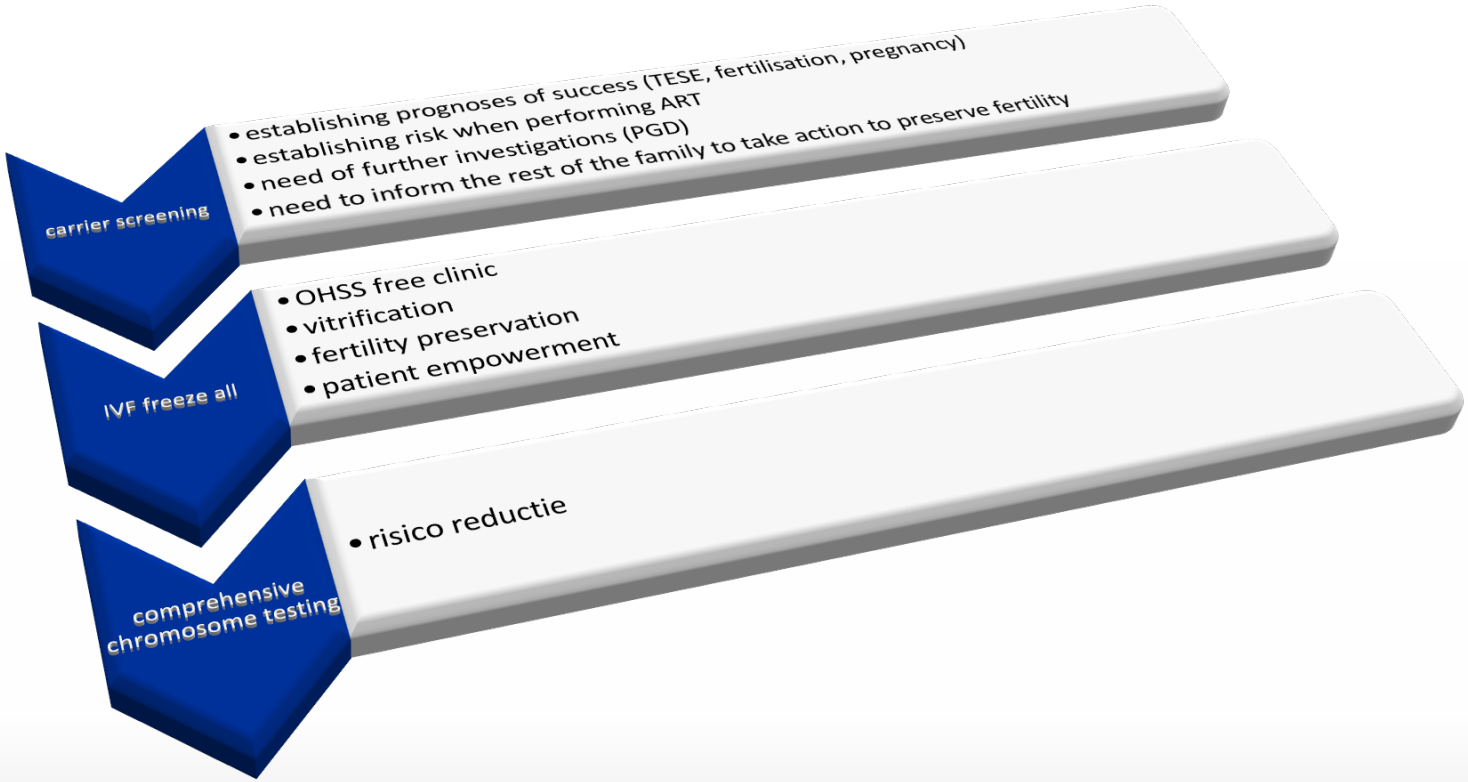
Universitair Ziekenhuis Brussel

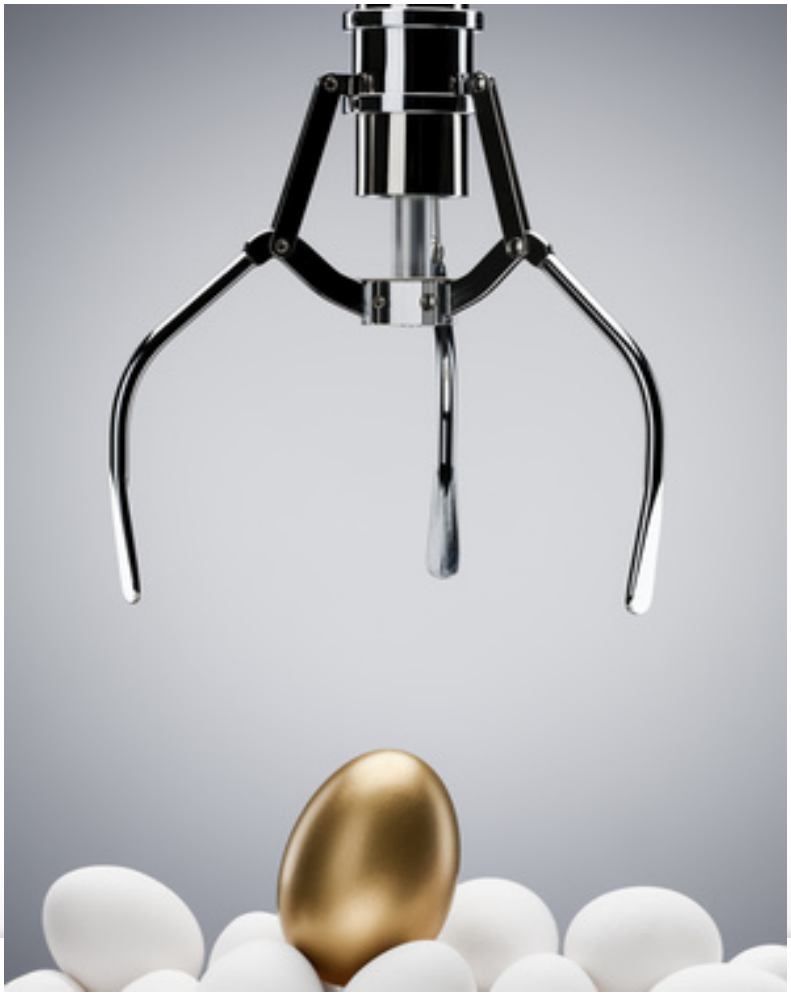

karyotype
IVF
prenatale testen

NIEUW PARADIGMA



NIEUWE PARADIGMA

- carrier screening
- eicellen en zaadcellen invriezen
- volledige chromosoomanalyse met NGS
- familieplanning



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DANK U

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