Preimplantation Genetic Diagnosis International Society (PGDIS)

15th INTERNATIONAL CONFERENCE ON PREIMPLANTATION GENETIC DIAGNOSIS (PGD)

PCC 1: "Clinical aspects of PGD/PGS"

Present and future role of PGS according to different clinical indications

Anna Pia Ferraretti, Cristina Magli , Luca Gianaroli

SISMER- Bologna - Italy

SISMER

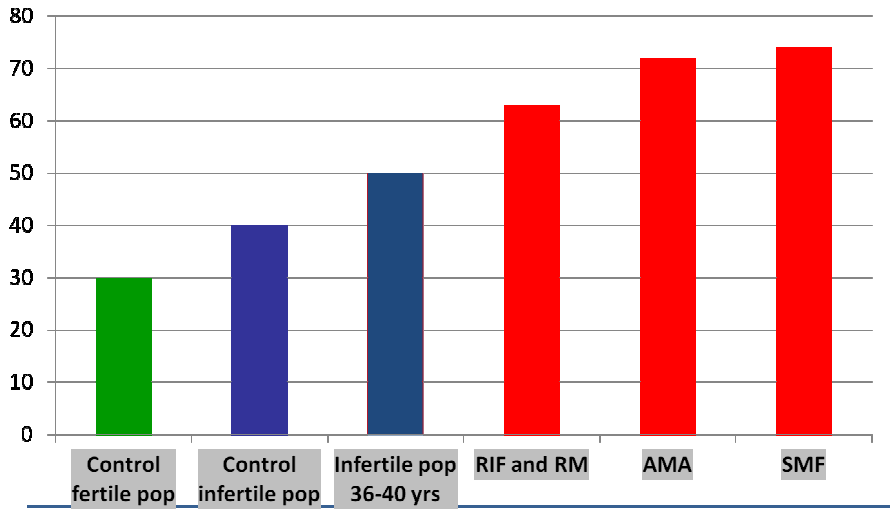
Classical indications for PGS

IVF population at higher risk to produce aneuploid embryos *("poor rognoosis patients"):*

- *advanced maternal age (AMA)*
- *recurrent idiopathic miscarriage (RM)*
- *recurrent implantation failure (RIF)*
- *association of two factors*
- *(severe male factor - SMF)*
- *(POR)*

SISMER

Aneuploidy rates



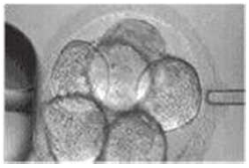
Fragouli et al,2009, Kilani et al, 2014,Verpoest et al 2008, Kuliev et al 2008, Gianaroli et al 2011

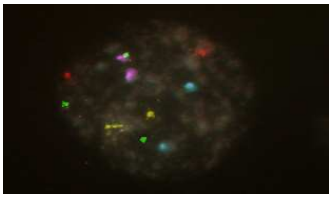
PGS in ART

- ***Since the high incidence of aneuploidies in these population, the biological rationale to select euploid embryos (PGS) does sound very logical to increase the performance of ART***

Clinical impact of PGS

First generation PGS (FISH on day 3 embryos)

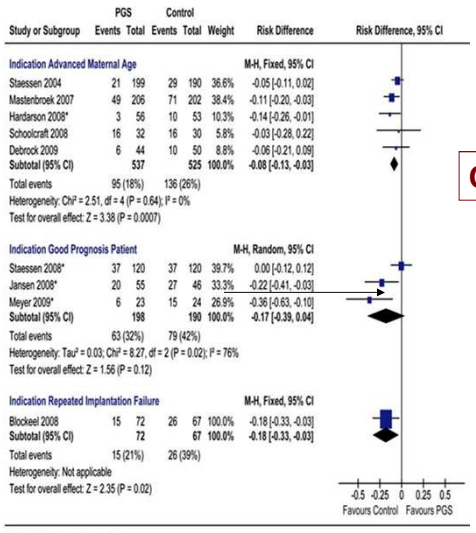




Very controversial issue

Preimplantation genetic screening: a systematic review and meta-analysis of RCTs on the effect of PGS on LBR

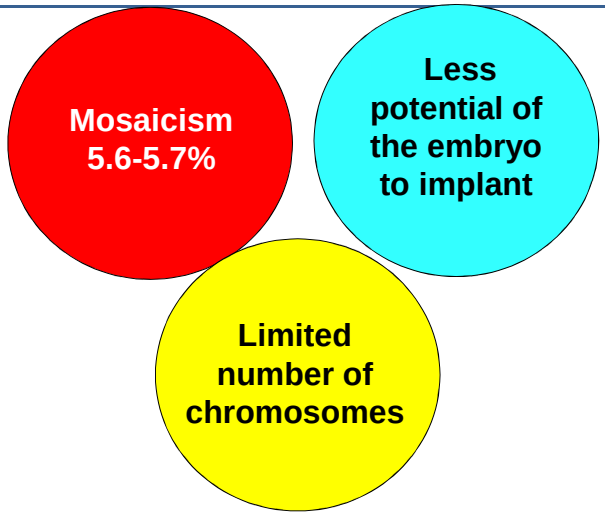
. Mastenbroek et a lHum Reprod Update 2011;17:454–466.



FISH

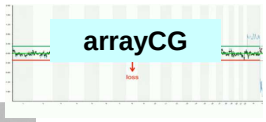
Cleavage stage

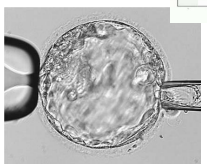
Factors affecting results in PGS first generation



PGS new generations

The present

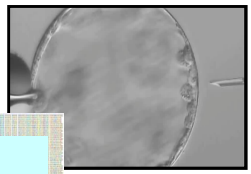


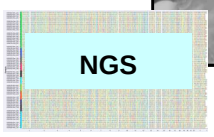


More DNA: less no results
Less mosaicism
Reduced (no) impact on embryo implantation

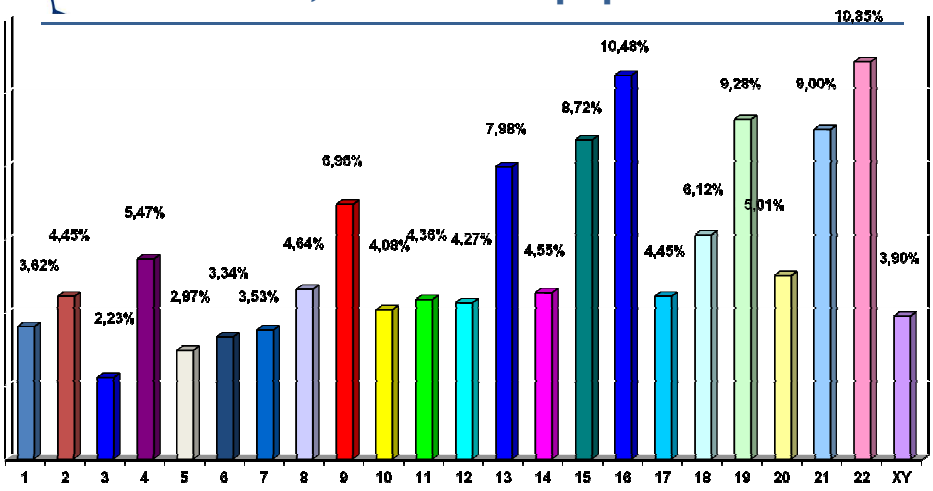
The future

Blastocentesis





CGH on polar bodies (SISMER) in AMA, RIF and RM populations



Aneuploidy rate per cromosome (CGHa)

Gianaroli et al 2013

Clinical impact of PGS

First generation PGS (FISH on day 3 embryos)

Very controversial issue

New generations PGS

Higher potential effect but the clinical value has yet to be determined

PGS clinical aspects
How to produce evidence ?

- *Gold standard : Large, Prospective Randomized Trials (very few studies)*
- *Retrospective, observational studies*
- *Meta-analysis*

PGS clinical aspects in classical indications
How to compare PGS vs no-PGS?

End-points	PGS	No-PGS	Comments pro and cons
Starting point to compare	Egg retrieval	Egg retrieval	
Transferred cycles (%)	50-60%	80-90%	Avoid (further) unsuccessful transfers (mosaicism??)
Clinical pregnancy and implantation rates	Very controversial issue with FISH on day 3 embryos		Higher with PGS by CGH on blastocysts ?
Miscarriages rate	Lower	Higher	
LBR / transfer	Higher	Lower	It is a correct indicator?
Cumulative (fresh and frozen) LBR/ egg retrieval	Similar		Shorter time to delivery with PGS Reduced costs ??

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Clinical pregnancy and implantation rates	Reduced by day 3 embryo biopsy (?)		Similar /Higher with PGS on blastocysts ?
Miscarriages rate	Lower	Higher	
LBR / transfer	Higher	Lower	It is a correct indicator?
Cumulative (fresh and frozen) LBR/ egg retrieval	Similar		Shorter time to delivery with PGS Reduced costs ??

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- *association of two factors*
- *(POR)*

The ESHRE PGD Consortium: 10 years of data collection

J.C. Harper[1,2,*], L. Wilton[3], J. Traeger-Synodinos[4], V. Goossens[5], C. Moutou[6], S.B. SenGupta[1], T. Pehlivan Budak[7], P. Renwick[8], M. De Rycke[9], J.P.M. Geraedts[10], and G. Harton[11]

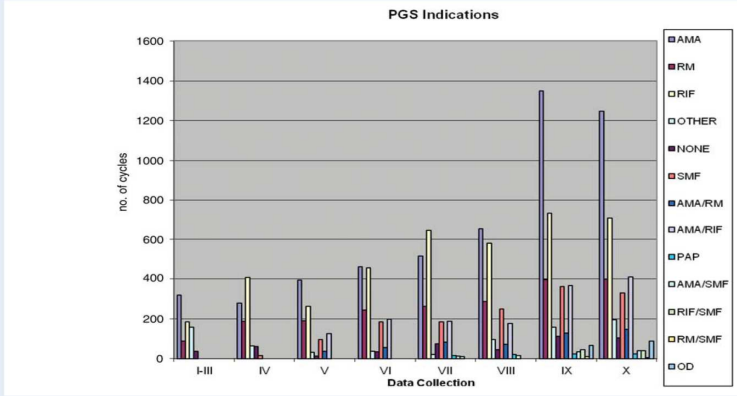
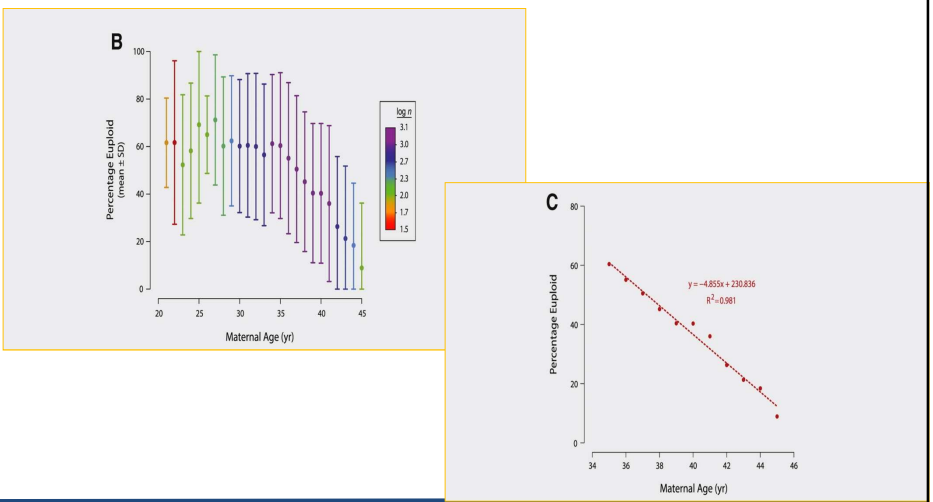


Figure 1 The evolution of PGS indications over the 10 sets of data. AMA, advanced maternal age; RM, repeated miscarriage; RIF, repeated implantation failure; SMF, severe male factor; PAP, previous abnormal pregnancy; OD, oocyte donation.

Human Reproduction Update, Vol.18, No.3 pp. 234–247, 2012

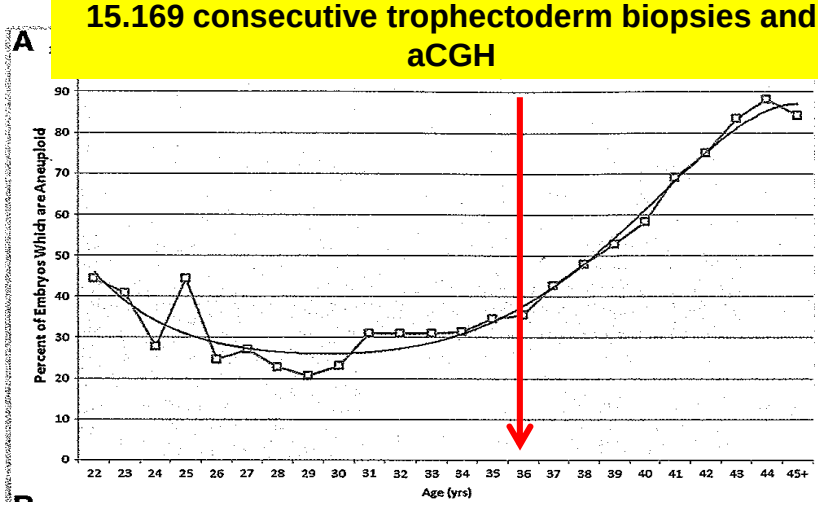
Effect of maternal age on euploidy rates (46,439 embryos analyzed by aCGH)



Demko et al, Fertil Steril 2016

PGS for AMA : from which age ?

15.169 consecutive trophectoderm biopsies and aCGH



Franasiak et al, Fertil Steril 2013

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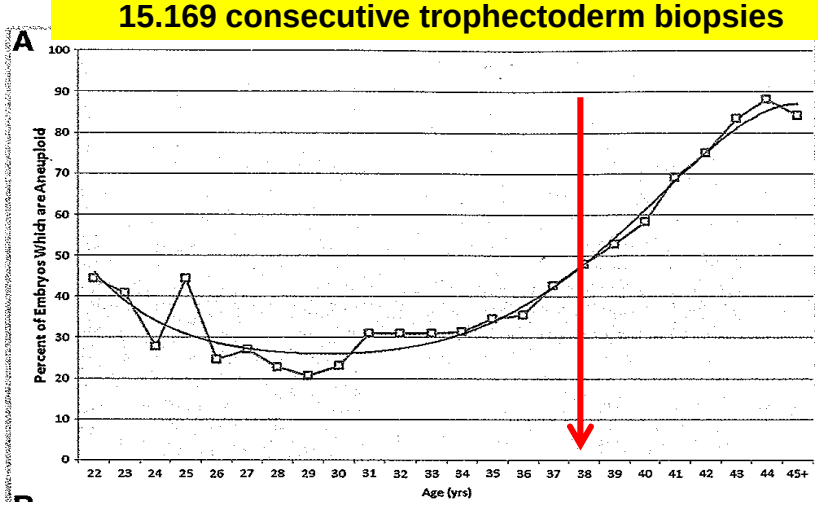

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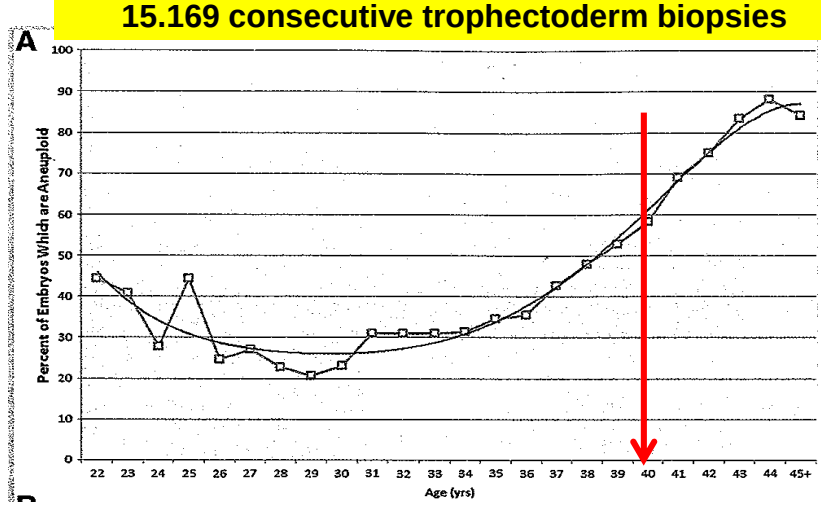
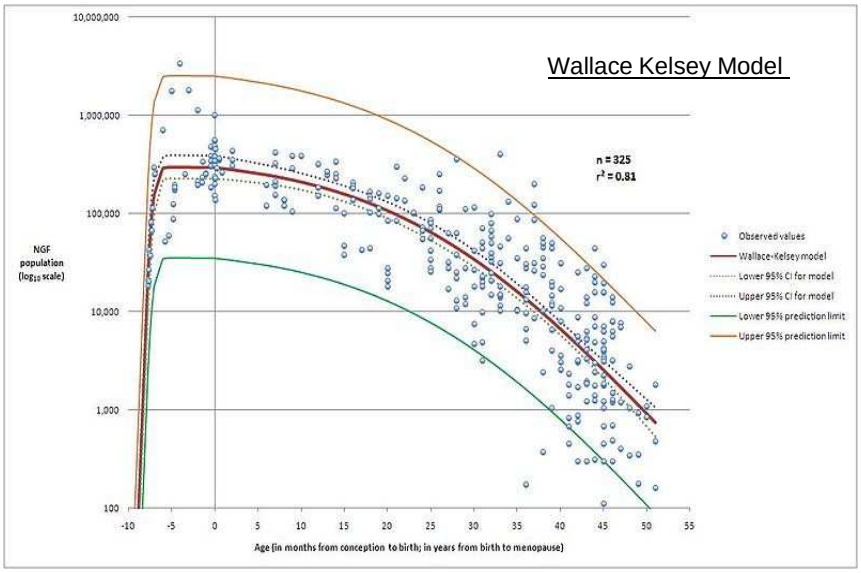


Franasiak et al, Fertil Steril 2013

REDUCED OVARIAN RESERVE



Wallace and Kelsey. Human Ovarian Reserve from Conception to the Menopause PLoS One. 2010; 5(1): e8772.

Cumulative number of blastocyst needed to produce at least one euploid blastocyst

% of patients with normal blastocysts

# of embryos	egg donors	<35 years	35-37 years	38-40 years	41-42 years	>42 years
1-3	83%	80%	71%	57%	36%	22%
4-6	97%	95%	92%	82%	59%	43%
7-10	99%	98%	96%	89%	74%	50%
10-17	100%	99%	99%	97%	88% banked	64% banked
>17	100%	100%	100%	99%	97% banked	87% banked

Conclusions:

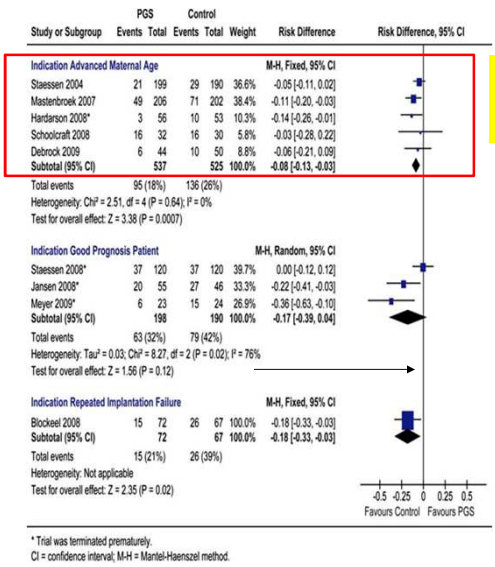
- In women 35 and older more than 50% of embryos are chromosomally abnormal

- Women 41 and older need 18 or more embryos to secure one euploid one

- Of those with no euploid embryos in the first cycle, 38% (41-42 years old) and 25% (> 42 yeas old), those that produced 17 embryos produced euploid embryos in successive cycles.

Munné et al., ASRM 201

FISH for PGS: Randomized clinical trials



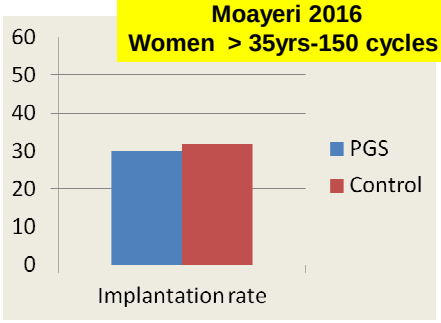
Lower LBR/women with PGS

Cleavage stage

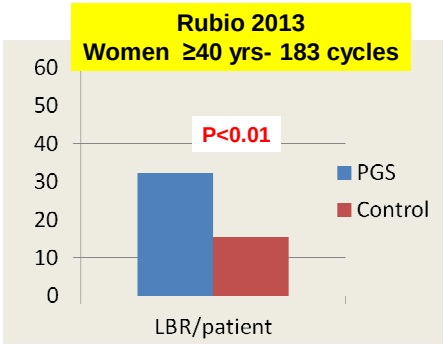
Mastenbroek et al., Preimplantation genetic screening: a systematic review and meta-analysis of RCTs. Hum Reprod Update 2011;17:454–466.

PGS in AMA
recent RCT by FISH on day 3

Moayeri 2016
Women > 35yrs-150 cycles

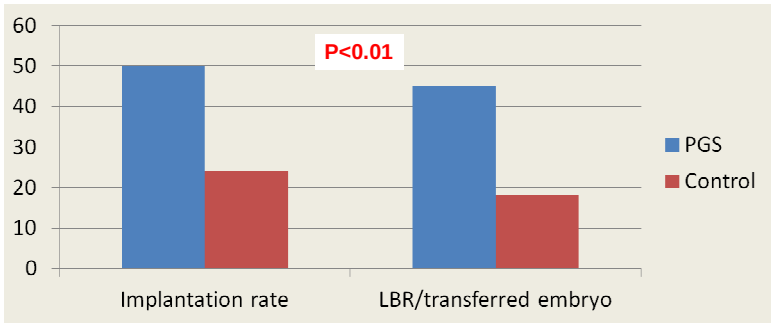


Rubio 2013
Women ≥40 yrs- 183 cycles



PGS in AMA
Retrospective studies by CGH on blastocysts

Lee HI et al, 2015
Women 40-43 yrs – 620 cycles



Outcomes of IVF with PGS : an analysis of the US ART 2011-2012 *(FS 2015)*

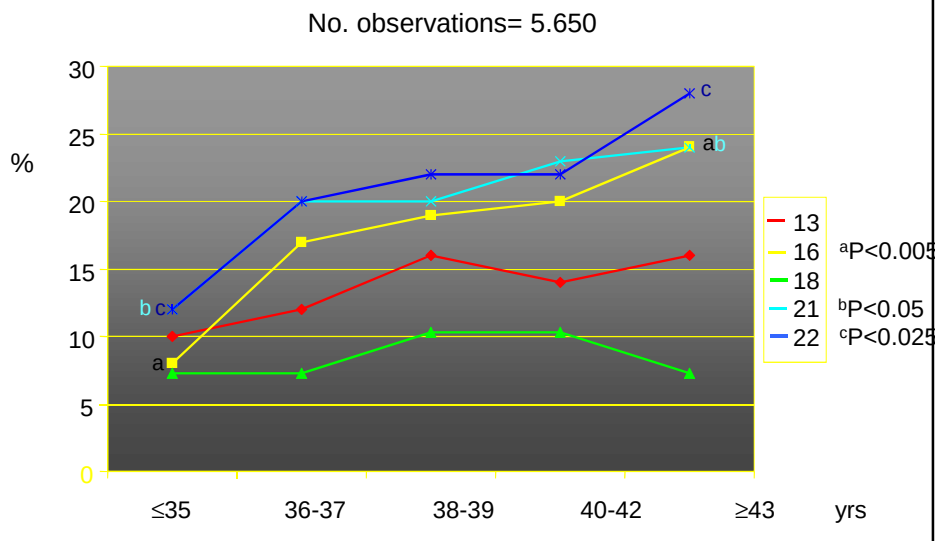
- 5741 cycles - mostly done by aCGH on day 5
- Age < 35 yrs : PGS was not associated to improved clinical pregnancy or Live birth rates
- Age ≥ 35 yrs : PGS was associated with lower odds of miscarriage per pregnancy
- Age > 37 yrs : higher likelihood of having a live birth delivery per transfer

PGS in AMA

- Can full karyotyping increase the efficacy compared to FISH ?

CHROMOSOMAL ANALYSIS ON OOCYTES

FREQUENCY OF ANEUPLOIDY IN OOCYTES ACCORDING TO AGE



SiSMer

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SiSMer

PGS in idiopathic RM (age < 40 yrs)

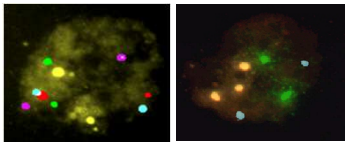
- Couples with RM have an expected miscarriage rate of 33.5%
- The % of euploid embryos by PGS is 35%
- PGS reduces the miscarriage rate to < 10% (observational studies)
- Very poor data comparing IVF with and without PGS

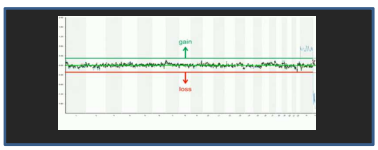
	IVF PGS	Expectant management
LBR	40%	55%
Miscarriage rate	7%	25%
Time to delivery		Higher
Emotional distress of RM	Lower	
Cost per Live Birth	43.300	418

Murugappan et al, Fertil Steril 2015

PGS in idiopathic RM (age < 40 yrs)

- Can full karyotyping increase the results compared to FISH ?
- The chromosomes chosen for FISH analyses (13,15,16,18,21,22) were those more involved in spontaneous miscarriages





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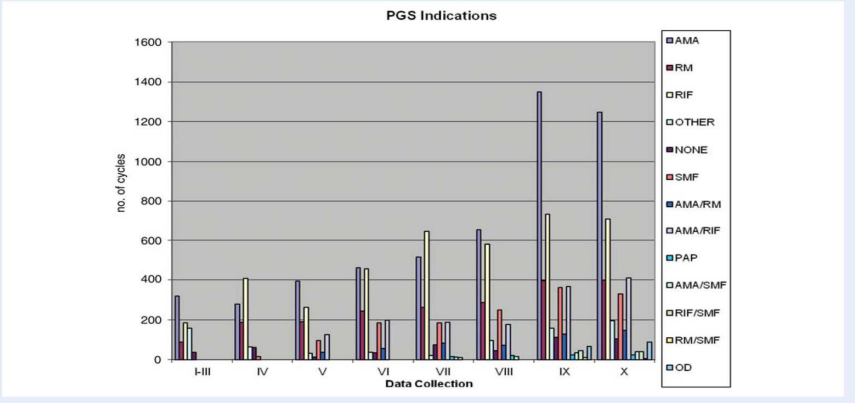

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ESHRE PGD Consortium
Definition of RIF - 2011

Failure of implantation after :

≥ 3 embryo transfers with high-quality embryos

or

the transfer of ≥ 10 embryos in multiple transfers *(exact number to be determined by each centre)*

Harton et al, Hum Reprod 2011, 26;14-24

Female age < 40 yrs, normal ovarian response, ± male factor

RECURRENT IMPLANTATION FAILURE PATIENTS

Heterogeneous population

Immunological reactions

Coagulation defects

Altered Karyotype

Endometrial anomalies

Numerous factors involved in RIF

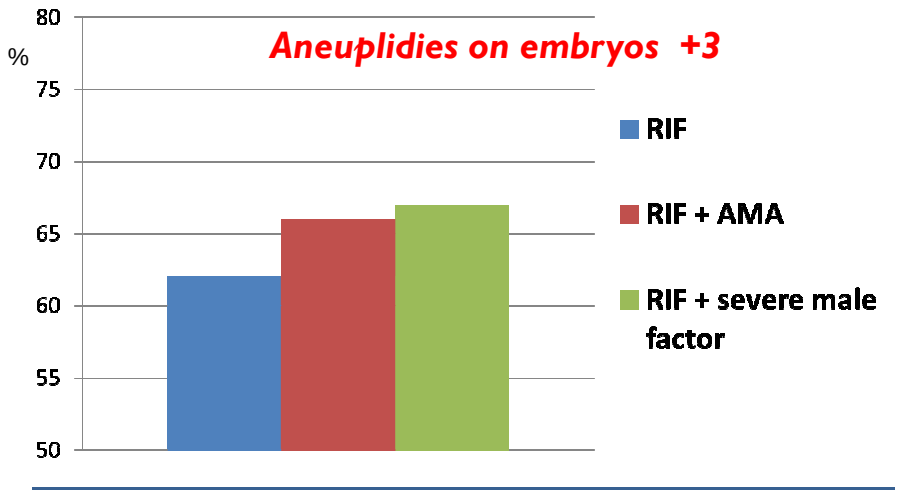
Uterine anomalies

Advanced Maternal Age

Sperm contribution

Genetic factor

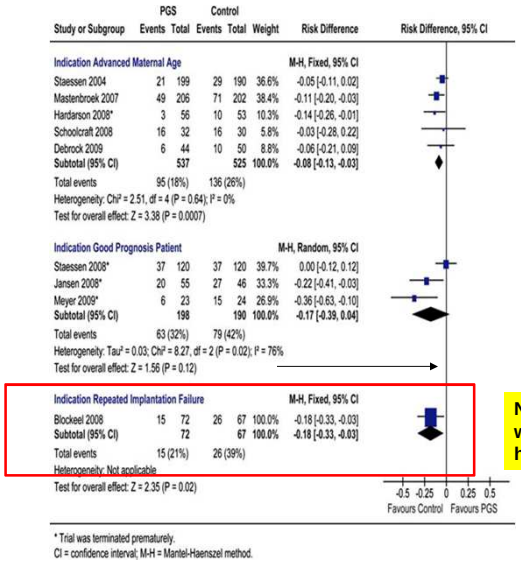
RIF ± Advanced maternal age (AMA) or severe male factor



DATA FROM THE LITERATURE

	PGS in RIF	Technique applied	Patients with RIF enrolled	normal embryos (%)	Imp. Rate (PGS vs control)	Pregn. rate (PGS vs control)	Live birth rate (PGS vs control)
Gianaroli et al., 1997	blastomere	FISH (X,Y,13,18,21)	20	18/40 (45%)	11.1% vs 4.1%	28.6% vs 16.7%	not reported
Gianaroli et al., 1999	blastomere	FISH(X,Y,13,14,15, 16,18, 21,22)	54	64/138 (46%)	17.3% vs 9.5%	25% vs 22%	not reported
Kahraman et al., 2000	blastomere	FISH (X,Y,13,18,21)	23	54%	?	30.4%	not reported
Gianaroli et al., 2002	blastomere	FISH(X,Y,13,14,15, 16,18,21,22)	66	143/356 (40%)	20.5%	28.8%	27%
Werlin et al., 2003	blastomere	FISH(X,Y,13,15,16, 17,18,21,22)	19	9/28 (32.1%)	?	20% vs 0%	not reported
Pehlivan, 2003	blastomere	FISH (X,Y,13, 16,18,21,22)	36	91/263 (34.6%)	24.6% vs 24.1%	40.7% vs 33.3%	not reported
El Toukhy, 2005	polar bodies	FISH (13,16, 18,21,22)	116	not reported	24% vs 12%	43% vs 25%	not reported
Yakin et al., 2008	blastomere	FISH (X,Y,13, 16,18,21,22)	140	not reported	11.9% vs 18.4%	14.8% vs 26.8%	14.8% vs 24.4%
Blockeel et al., 2008	blastomere	FISH (X,Y,13, 16,18,21,22)	200	not reported	21.4% vs 25.3%	32.7% vs 42.9%	20.8% vs 40.2%
Rubio et al., 2012	blastomere	FISH (X,Y,13,15,16,17,18 ,21,22)	91	not reported	not reported	37.3% vs 20.2%	47.9% vs 27.9%

FISH for PGD: Randomized clinical trials

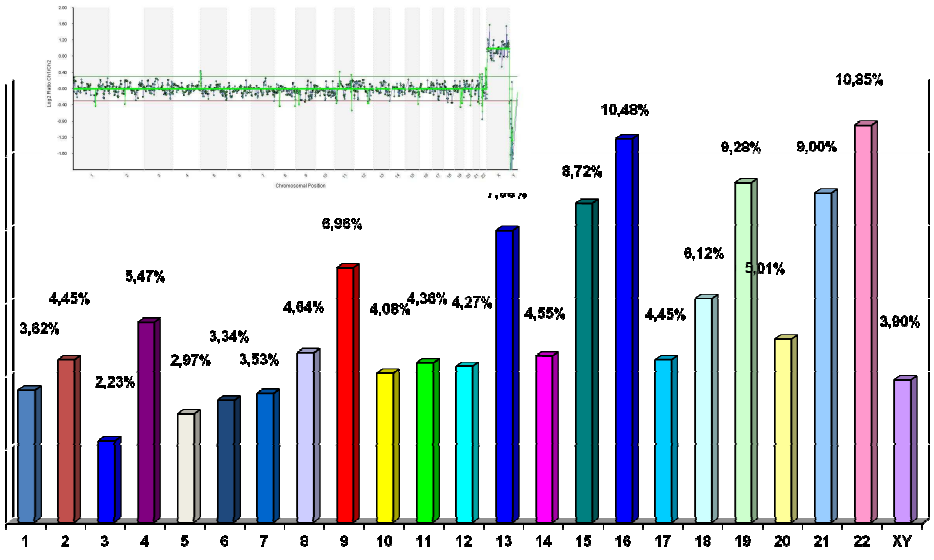


* Trial was terminated prematurely.
CI = confidence interval; M-H = Mantel-Haenszel method.

Cleavage stage

No difference in live birth rate when correcting for statistical heterogeneity.

Mastenbroek et al., Preimplantation genetic screening: a systematic review and meta-analysis of RCTs. Hum Reprod Update 2011;17:454–466.

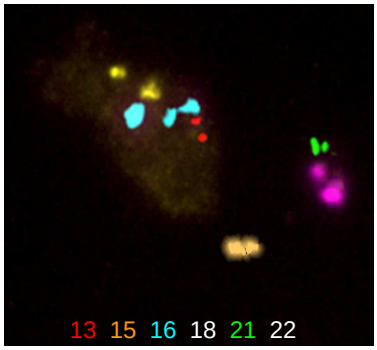


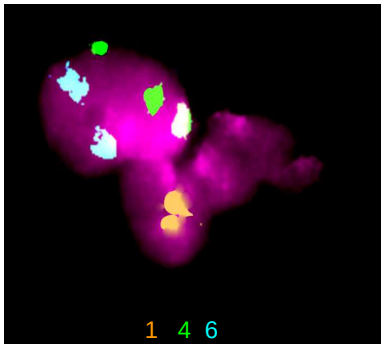
Aneuploidy rate per cromosome (CGHa)

Aneuploidy of chromosomes 1, 4 and 6
Retrospective observational study

After completion of the treatment cycle by conventional FISH, PB1s of the transferred embryos were reanalyzed for chromosomes 1, 4 and (second panel)

1st panel





2nd panel

Magli et al., Fertil Steril 2010. In press.

Aneuploidies of chromosomes 1, 4, and 6 are not compatible with human embryos' implantation

M. Cristina Magli, M.Sc.,[a] Luca Gianaroli, M.D.,[a] Andor Crippa, Ph.D.,[a] Santiago Munné, Ph.D.,[b] Francesca Robles, B.Sc.,[a] and Anna P. Ferraretti, M.D.[a]

[a] Società Italiana Studi Medicina della Riproduzione (S.I.S.Me.R.), Reproductive Medicine Unit, Bologna, Italy; and [b] Reprogenetics, Livingston, New Jersey

Abnormal conditions of these large chromosomes can allow normal early embryo development but no implantation or, should if happens, very early miscarriage occurs (before pregnancy detection)

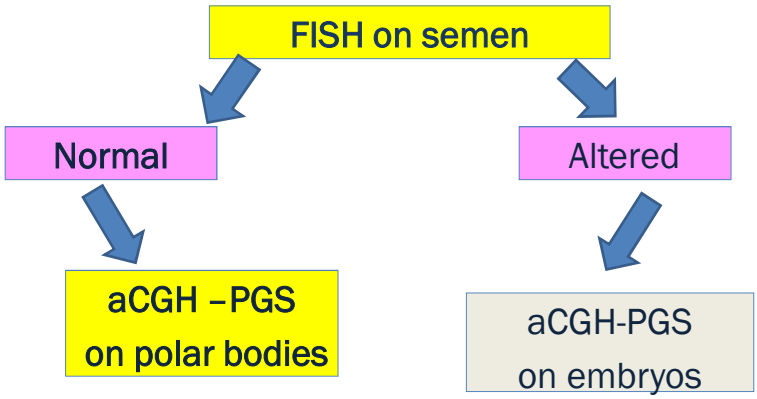
Fertility and Sterility 2010

GCH selection for RIF
(Greco et al, 2014)

- **Study design :Prospective, self randomization**
- **76 Women < 36 yrs with an history of 3-9 (mean4.9) previous IVF attempts**

	PGS	No PGS	
Embryo policy	eSET	DET	
Euploidy rate	46%	---	
Clinical PR/women	68%	22%	P < 0.001
Miscarriages	0	0	

SISMER policy in RIF
(2013-2014)



aCGH in RIF
Material

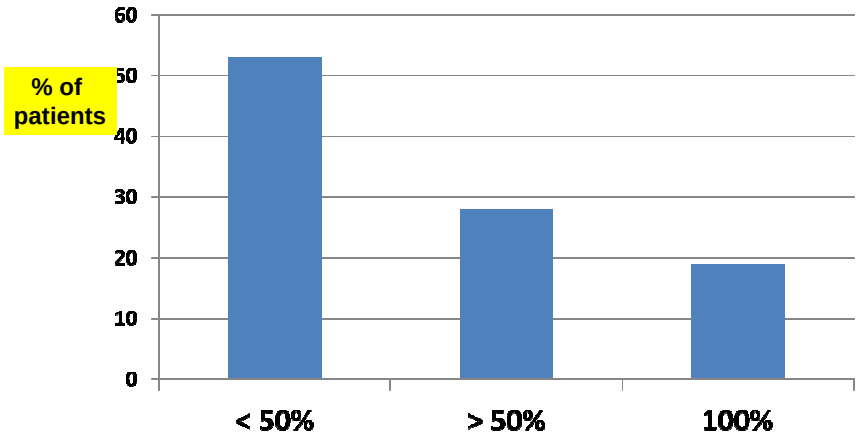
	PGS on oocytes (no male genetic factor)
Patients	*47*
Age (yrs)	*37.8*
n. of MII collecetd oocytes	*397*
n. of 2PN biopsied	*301*
n. of day 3 viable embryos	*198*
n.of oocytes analyzed	*198*
n. euploid	*65 (34%)*

PGS on oocytes in RIF *(no male genetic factor)*
Outcome

Patients	*47*
Number of transfers	*26 (55%)*
Mean embryos transferred	*1.4*
Clinical pregnancies	*13*
Implantation rate	*48% (17/35)*
Miscarriages	*1*
LBR/patient	*25.5%*
LBR/ET	*46%*

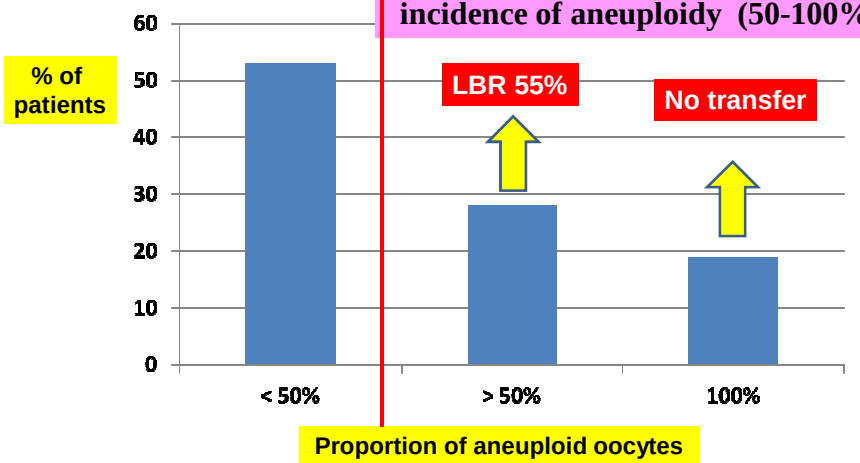
Proportion of aneuploidy

Different sub-populations of patients

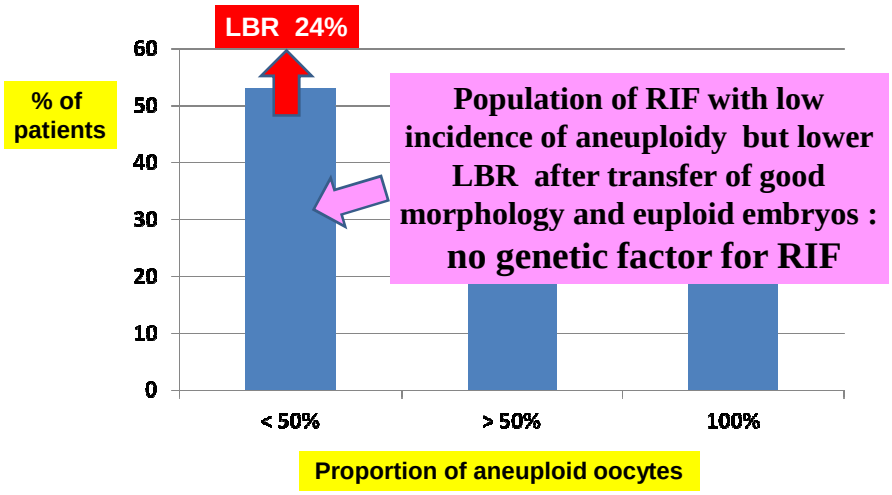


LBR/patient in relation to the proportion of aneuploidy

Population of RIF with an high incidence of aneuploidy (50-100%)



LBR/patient in relation to the proportion of aneuploidy



PGS by CGH on embryos in RIF + severe male factor

Patients	20
Euploid embryos	23 (24%)
Number of transfers	8 (40%)
Mean embryos transferred	1.2
Clinical pregnancies	2
Miscarriages	1
LBR/patient	5%
LBR/ET	12.5%

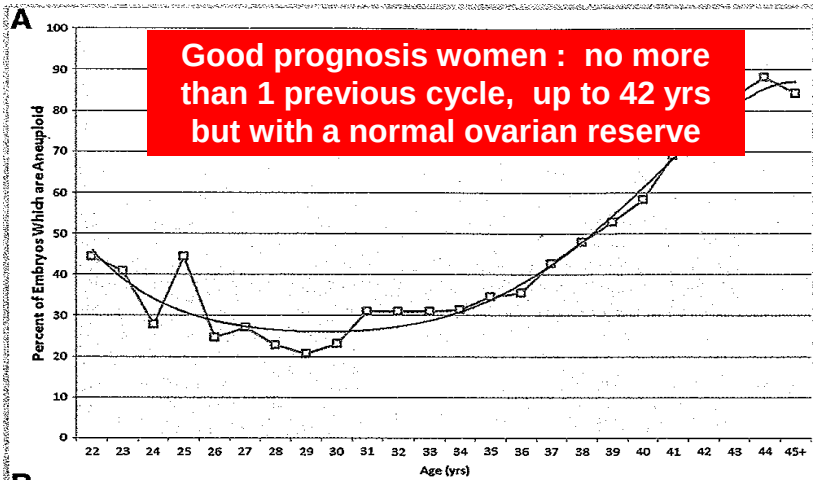
Classical indications for PGS

IVF population at higher risk to produce aneuploid embryos ***("poor prognosis patients") to improve outcomes***

- *advanced maternal age (AMA)*
- *recurrent idiopathic miscarriage (RM)*
- *recurrent implantation failure (RIF)*
- *association of two factors*
- *(severe male factor - SMF)*
- *(POR)*

New indications ?

PGS : new indication ?

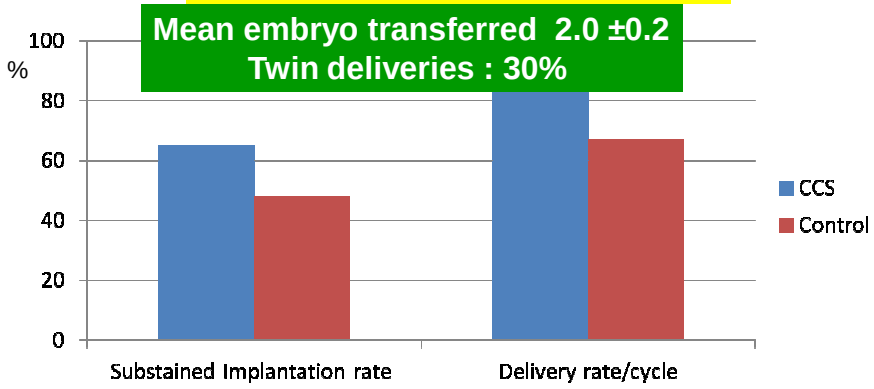


Franasiak et al, Fertil Steril 2013

Blastocyst biopsy with CCS significantly increases IVF implantation and delivery rate : a randomized controlled trial

Women 21-42 yrs (including oocyte donors) with a normal ovarian reserve

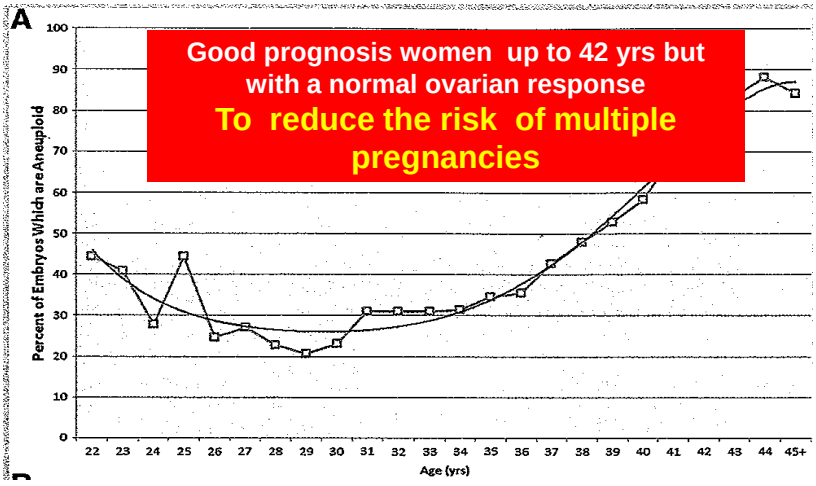
Mean embryo transferred 2.0 ±0.2
Twin deliveries : 30%



Scott et , Fertil Steril 2013

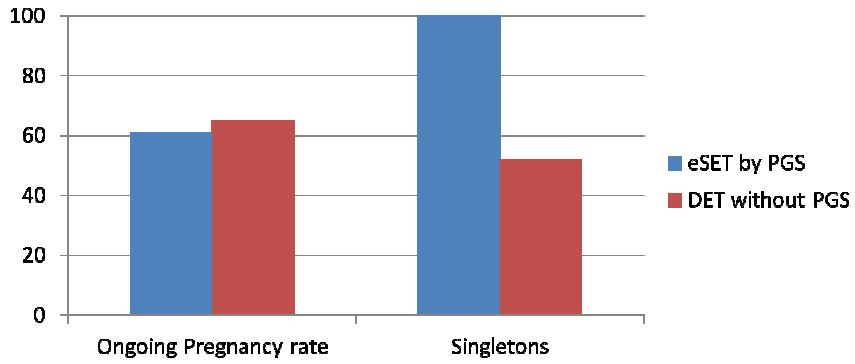
PGS : new and point ?

Good prognosis women up to 42 yrs but with a normal ovarian response
To reduce the risk of multiple pregnancies



IVF with single euploid blastocyst transfer : a randomized controlled trial

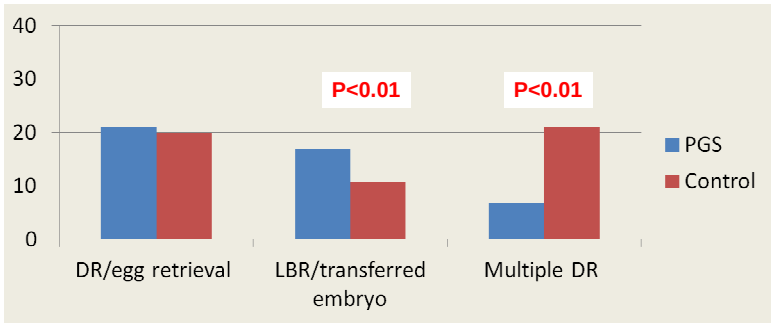
Women <43 yrs with a normal ovarian reserve
eSET after PGS (CCS) vs DET without PGS



Forman et al, Fertil Steril 2013

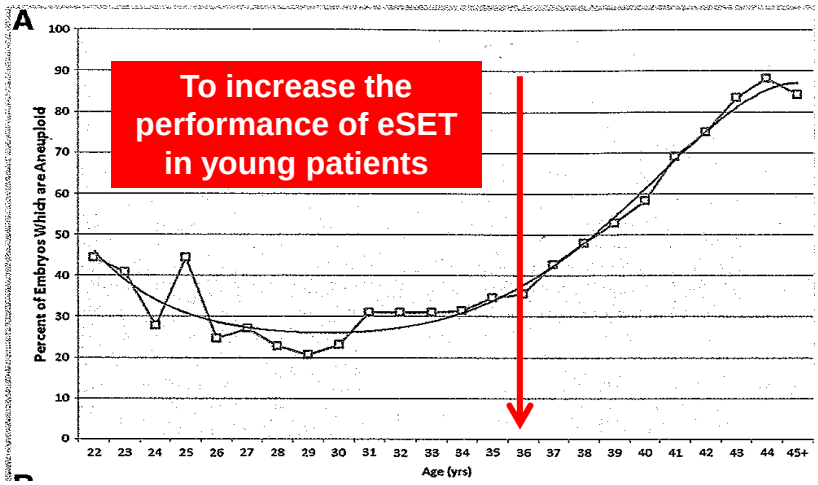
PGS in AMA
Retrospective studies by CGH on blastocysts

Ubaldi et al 2015
Women > 35 yrs
eSET after PGS vs conventional transfer (2.9±1 blastocysts)



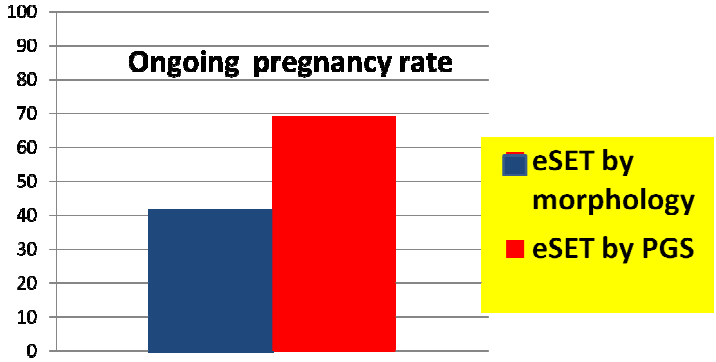
Reduction of the multiple pragnancy rate with eSET by PGS in advanced maternal age population

PGS : new end point



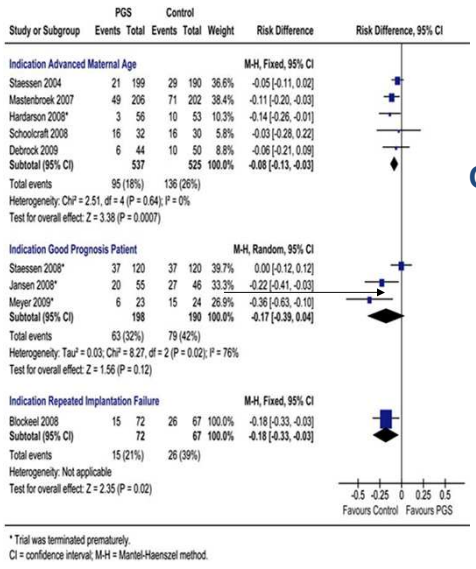
Selection of single blastocysts for fresh transfer via standard morphology or with aCGH for good prognosis patients : a randomized pilot study

103 women - Age < 35



Young et al, Mol Cytogenetics 2012

Preimplantation genetic screening: a systematic review and meta-analysis of RCTs on the effect of PGS on LBR
. Mastenbroek et a lHum Reprod Update 2011;17:454–466.



FISH

Cleavage stage

Comprehensive chromosome screening improves embryo selection: a meta-analysis

Elias M. Dahdouh, M.D., M.Sc.,[a,b,c] Jacques Balayla, M.D.,[c] and Juan Antonio García-Velasco, M.D., Ph.D.[d]

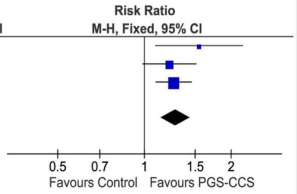
[a] Assisted Reproduction Center, CHU Sainte-Justine, University of Montreal, Montreal, Quebec, Canada; [b] PROCREA Clinics, Montreal, Canada; [c] Department of Obstetrics and Gynecology, University of Montreal, Montreal, Canada; and [d] Instituto Valenciano de Infertilidad (IVI) Madrid and Rey Juan Carlos University, Madrid, Spain

Fertility and Sterility, December 2015

Clinical implantation rate

Study or Subgroup	PGS-CCS Events	PGS-CCS Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Yang et al. 2012	39	55	22	48	13.3%	1.55 [1.09, 2.20]
Forman et al. 2013	55	87	89	172	33.9%	1.22 [0.98, 1.52]
Scott et al. 2013	107	134	103	163	52.7%	1.26 [1.09, 1.46]
Total (95% CI)		**276**		**383**	**100.0%**	**1.29 [1.15, 1.45]**
Total events	201		214			

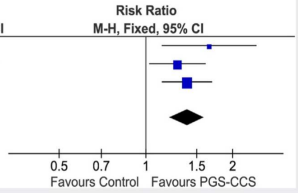
Heterogeneity: Chi² = 1.34, df = 2 (P = 0.51); I² = 0%
Test for overall effect: Z = 4.27 (P < 0.0001)



Sustained implantation rate (> 20 weeks gestation)

Study or Subgroup	PGS-CCS Events	PGS-CCS Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Yang et al. 2012	38	55	20	48	14.5%	1.66 [1.14, 2.42]
Forman et al. 2013	54	87	83	172	37.8%	1.29 [1.03, 1.61]
Scott et al. 2013	89	134	78	163	47.7%	1.39 [1.14, 1.70]
Total (95% CI)		**276**		**383**	**100.0%**	**1.39 [1.21, 1.60]**
Total events	181		181			

Heterogeneity: Chi² = 1.29, df = 2 (P = 0.53); I² = 0%
Test for overall effect: Z = 4.61 (P < 0.00001)



Meta-analysis of RCTs on PGS-CCS vs. routine care.

Dahdouh. CCS and embryo selection. Fertil Steril 2015.

iiarg SiSMer

PGS : the future

To increase safety
To increase accuracy (mosaicism?)
To reduce costs

Good prognosis patients

Euploid eSET (?)

The highest probability of the birth of a single (healthy) baby

SiSMer

"Acceptable costs for the patient and society"

Chambers et al, Fertil Steril 2013,100.319

"Under some circumstances, ART represents good values of money from a social perspective : the monetary values of providing ART treatments can be far covered by the revenue of ART children"

"These clinical circumstances are if babies born as singleton"

PGS : the future

To increase safety
To increase accuracy (mosaicism?)
To reduce costs

Good prognosis patients

Euploid eSET

The highest probability of the birth of a single (healthy) baby

Poor prognosis patients

To avoid unsuccessful transfers
To reduce the rate of miscarriages
To shorten the time to delivery
To reduce the risk of multiple pregnancies