Vrije Universiteit Brussel

Aneuploidy screening of gametes and embryos Does it have any role in ART for male infertility?

Staessen Catherine

CENTRE FOR MEDICAL GENETICS
CENTRE FOR REPRODUCTIVE MEDICINE

Outline of the presentation

- Introduction
- Sperm aneuploidy rate in infertile males
- Sperm parameters and ART outcome
- Chromosomal abnormalities in embryos from couples with male infertility
- Benefit of PGD-AS in male infertility

Introduction

- The contribution of sperm to normal fertilization and embryogenesis include
  - The centrosome
  - Oocyte activation factors
  - Epigenetic gene modifications
  - Possibly RNA regulation mechanisms

  (Boerke et al., 2007; Carrell et al., 2007; Chatzimeletiou et al., 2007; Emery and Carrell, 2006; Haaf, 2006)
- Transmission of a haploid chromosome complement is the most fundamental and essential contribution

Introduction

- ICSI has facilitated fertilization in cases of extreme spermatogenesis defects
  - Beneficial to infertility patients
  - The cause of heightened concern about the possibility of increased genetic risk, including the potential of an elevated risk of embryo aneuploidies

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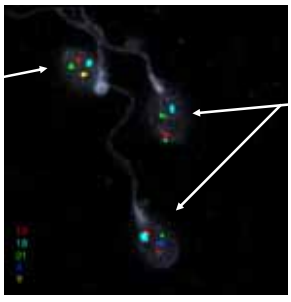
FISH on sperm



Carrell D, J. of Andrology 2007

Sperm aneuploidy in infertile men

- Infertile male patients with a normal somatic karyotype produce abnormal spermatozoa as a result of an altered intra-testicular environment that affects negatively the mechanisms controlling chromosome segregation

Mroz et al., 1998

For review: Calogero A, RBMonline, 2003

Sperm aneuploidy in infertile men

- The rate of aneuploid spermatozoa production is significantly higher in patients with abnormal sperm parameters compared with those of normozoospermic subjects or infertile patients with normal sperm parameters

Laholetie et al., 1997
Bernardini et al., 1997
McInnes et al., 1998
Pang et al., 1999

For review: Calogero A, RBMonline, 2003

Sperm aneuploidy in infertile men

- A negative correlation has been reported to exist between aneuploidy and the main sperm parameters

Vegetti et al., 2000
Ushijima et al., 2000
Calogero et al., 2001

For review: Calogero A, RBMonline, 2003

Sperm aneuploidy in infertile men

- Testicular and epididymal spermatozoa have a greater rate of aneuploidy compared with that of ejaculated spermatozoa.
- Some authors have also shown that patients with non-obstructive azoospermia have a significantly higher sperm aneuploidy rate compared with that of patients with obstructive azoospermia

Levron, et al., 2001
Palermo et al., 2002

Not confirmed by other studies

Platteau et al., 2004
Calogero et al., 2003

For review: Calogero A, RBMonline, 2003

Sperm aneuploidy in infertile men

- All chromosomes are subject to aneuploidy, although at a different rate; the sex chromosomes are more often altered than are the autosomes.

For review: Calogero A, RBMonline, 2003

Frequency of aneuploidy in sperm FISH analysis: XY,13,15,16,17,18,21,22

- 5 normospermic samples: 1117 sperm cells
  98.5% normal haploid (98%–99%)
- 27 OAT samples: 3749 sperm cells
  88% normal haploid (73%–98%)
- 11 testicular samples: 893 sperm cells
  84% normal haploid (71%–94%)

Gianaroli et al., Hum. Reprod., 20, 2140-52, 2005

Sperm aneuploidy in infertile men

Syndrome	Aneuploidy (%)	Reference
Severe Morphology Defects (Multiflagellar, macrocephalic, Tail Agenesis) 1997)	15-100	(Benzacken *et al.*, 2001) (Devillard *et al.*, 2002) (Carrell *et al.*, 2004; In't Veld *et al.*, (Carrell *et al.*, 2004)
Round Head Only Syndrome	15-60	(Carrell *et al.*, 2001) (Carrell *et al.*, 1999)
Nonobstructive Azoospermia	1-51	(Burrello *et al.*, 2005)
Unexplained Recurrent Pregnancy Loss	1-34	(Bernardini *et al.*, 2004) (Carrell *et al.*, 2003)
Repeated IVF Failure	2-7	(Petit *et al.*, 2005)

Benefit of sperm aneuploidy assay

- 1 000 to 10 000 sperm cells
- 2 – 3 rounds of varying probe mixtures
  => expensive and time-consuming analysis
- Automated testing has been described, although hardware and software expensive
- Cost/benefit of the assay will depend on the outcome of large scale studies to validate the benefit of testing

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- Benefit of PGD-AS in male infertility

Human Reproduction Vol.17, No.10 pp. 2600–2614, 2002

Prenatal testing in ICSI pregnancies: incidence of chromosomal anomalies in 1586 karyotypes and relation to sperm parameters

Maryse Bonduelle[1,3], Elvire Van Assche[1], Hubert Joris [2], Kathelijn Keymolen[1], Paul Devroey[2], André Van Steirteghem[2] and Inge Liebaers[1]

[1]Centre for Medical Genetics and [2]Centre for Reproductive Medicine, Dutch-speaking Brussels Free University (Vrije Universiteit Brussel), Brussels, Belgium.

[3]To whom correspondence should be addressed at: Centre for Medical Genetics, Academisch Ziekenhuis (AZ-VUB), Laarbeeklaan 101, B- 1090 Brussels, Belgium. E-mail: maryse.bonduelle@az.vub.ac.be

De-novo aberrations were found in 1.6% of the tested ICSI children

Bonduelle et al., Hum. Reprod.,Vol.17, No.10, 2600-14, 2002

Table V. Sperm parameters in relation to non-inherited karyotype anomalies

Karyotype result	Sperm concentration		Sperm morphology[a]		Sperm motility[a]	
	$<20\times10^6$	$\geq20\times10^6$	Abnormal	Normal	Abnormal <50%	Normal ≥50%
Normal	1120	419	1269	270	1267	272
Abnormal	24	1	4	21	25	0
Normal + abnormal	1144	420	1290	274	1292	272
%	73.15	26.85	82.48	17.52	82.61	17.39
Fisher's exact test	$P = 0.006$		$P = 1.00$ (NS)	$P = 0.014$		

2.1 % 0.24 % 1.9 %

[a]In the analysis of morphology and motility, missing values were considered as abnormal.
NS = not significant.

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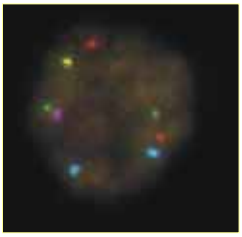
Development of PGD-AS

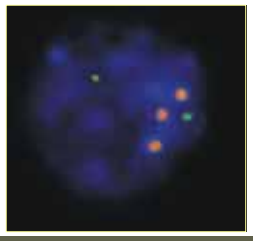
- Morning of day 3:
  ≥ 6 cells : 2 blastomeres
- A hole is made in the zona using laser technology
- Aspiration of the blastomeres

FISH



Round 1 : X p11.1-q11.1 Blue
Y p11.1-q11.1 Gold
13 q14 Red
18 p11.1-q11.1 Aqua
21 q22.13-q22.2 Green

Round 2 : 16 q11.2 Orange
22 q11.2 Green

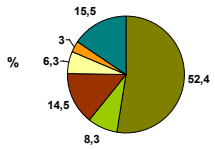


% abnormal embryos in male infertility

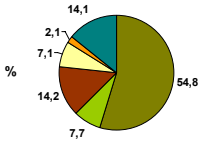
	N patients Age mean ± SD	N embryos analyzed (Chromosomes tested)	% abnormal embryos
Gianaroli et al., 2001	Mesa/TESE: 39 31.8 ± 2.4	169 (XY,13,14,15,16,18,21,22)	70.0%
Kahraman et al., 2004	Macro (23): 31.2 ± 4.6 Zero N (14): 34.1± 4.3	82 47 (XY,13,16,18,21,22)	46.4% 37.5%
Aran et al., 2004	Meiotic abn: 27 31.5 (24-39)	183 (XY,13,16,18,21,22)	42.5%
Platteau et al., 2004	NOA: 30.6 ± 4.6 OA: 33.5 ± 3.9	203 121 (XY,13,16,18,21,22)	52.5% 60.0%

Fresh ejaculated sperm – Concentration - Maternal age ≤ 35 years

Sperm conc. < 20 x 10^6 (n= 1292)



Sperm conc. ≥ 20 x 10^6 (n= 1652)



Normal Trisomy Monosomy Mosaic Hapl/tripl Comb abnor

Unpublished data, UZ Brussel

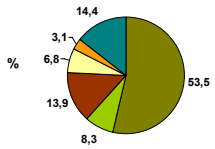
Fresh ejaculated sperm – Concentration – Maternal age ≤ 35 years

	Sperm conc. < 20 x 10^6	Sperm conc. ≥ 20 x 10^6
No. of cycles with PGD-AS	216	281
Age ± mean SD	31.2 ± 3.1	31.9 ± 2.7
Embryo/biopsy ± mean SD	6.4 ± 3.9	6.2 ± 4.3
No. of transfers	179	225
Embryo/transfer ± mean SD	1.7 ± 0.8	1.6 ± 0.7
No. of + HCG	78	101
+ HCG/transfer	43.6%	44.9%

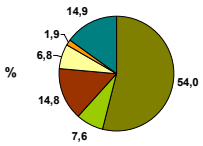
Unpublished data, UZ Brussel

Fresh ejaculated sperm – Motility - Maternal age ≤ 35 years

Motility < 50% (n= 1434)



Motility ≥ 50% (n= 1510)



Normal Trisomy Monosomy Mosaic Hapl/tripl Comb abnor

Unpublished data, UZ Brussel

Fresh ejaculated sperm – Motility - Maternal age ≤ 35 years

	Mot. < 50 %	Mot. ≥ 50 %
No. of cycles with PGD-AS	244	253
Age ± mean SD	31.5 ± 3.0	31.7 ± 2.8
Embryo/biopsy ± mean SD	6.2 ± 3.9	6.3 ± 4.4
No. of transfers	205	199
Embryo/transfer ± mean SD	1.7 ± 0.8	1.6 ± 0.7
No. of + HCG	95	84
+ HCG/transfer	46.3%	42.2%

Unpublished data, UZ Brussel

Chromosomal constitution of embryos obtained after ICSI with testicular sperm of OA and NOA men

- Azoospermic couples: 2 semen samples including a centrifugation step at high speed
- Both partners: genetic work-up
  - Karyotype analysis
  - Assessment for Yq deletion
- NOA: histological confirmation of spermatogenesis failure (maturation arrest, germ-cell aplasia, tubular sclerosis and atrophy) → n= 39 cycles
- OA: histological confirmation of normal spermatogenesis → n= 23 cycles

Platteau et al., 19, 1570-74, 2004

Chromosomal constitution of embryos obtained after ICSI with testicular sperm of OA and NOA men

- Control population of 14 couples (14 treatment cycles) who underwent PGD to determine fetal gender with regard to sex-linked disease
- Female age : 33.6 ± 5.4

Platteau et al., 19, 1570-74, 2004

Chromosomal constitution of embryos obtained after ICSI with testicular sperm of OA and NOA men

	NOA	OA	Sexing
No. of cycles	39	23	14
Age (years)	30.6	33.5	33.6
No. of COC	12.6	13.6	11.9
No. of MII	11.1	11.0	10.1
% 2PN	59.1	69.8	76.7
No. of emb. biopsied	203	121	85
Diagnosis			
% normal	42.1	34.8	56.4
% abnormal	52.5	60.0 *	40.5
% no diagnosis	5.2	2.0	2.9

*P <0.05 between obstructive azoospermic group and sexing

Platteau et al., 19, 1570-74, 2004

Gianaroli., J. Endrocrinol. Invest., 23, 711-16, 2000

Probes:XY,13,14,15,16,18,21 22

Table 3 - Chromosomal abnormalities in IVF and ICSI embryos generated by patients with a normal karyotype.

	IVF	ICSI			
		Group 1	Group 2	Group 3	Group 4
No. cycles	29	22	9	24	13
Mean age (mean±SD)	32.0±2.4	31.7±1.8	32.5±2.4	31.4±2.8	31.2±2.8
No. diagnosed embryos	155	131	45	127	53
FISH abnormal (%)	98 (63)	78 (59)	28 (62)	76 (60)	38 (72)
Monosomies and trisomies (%)	27 (27)*	21 (27)*	9 (32)	29 (38)	18 (47)*
Gonosomal aneuploidies (%)	2 (2.0)	2 (2.6)	1 (3.6)	4 (5.3)	3 (7.9)

*p<0.05. FISH: fluorescence in situ hybridization; ICSI: intracitoplasmic sperm injection; IVF: in vitro fertilization.

Group 1 : normospermic
Group 2 : oligoasthenoteratospermic with ≥ 0.5 x 10^6 total motile count
Group 3 : < 0.5 x 10^6 total motile count
Group 4 : epididymal or testicular sperm

Silber et al., Fertil. Steril., 79, 30-38, 2003

Chromosome abnormalities in ICSI and TESE embryos.

Group	ICSI–oligospermia, n (%)	ICSI–TESE, n (%)
Normal	347 (41.8)[a]	22 (22)[a]
Polyploid	39 (4.7)	5 (5)
Haploid	25 (3.0)	4 (4)
Aneuploid	199 (23.9)	16 (16)
Aneuploid and mosaic	19 (0.2)	1 (1)
Mosaic	201 (24.2)	52 (52)
Total aneuploid	218 (26.2)	17 (17)
Total mosaic	220 (26.5)[b]	53 (53)[b]
Total	830	100

[a,b]$P<.001$ between the ICSI and TESE embryo groups.

Silber. Chromosome abnormalities in TESE embryos. Fertil Steril 2003.

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Does PGD -AS change the selection of embryos in NOA and OA men?

- Study :

  to retrospectievely review all the embryology data available from azoospermic patients undergoing ICSI with PGD and to examine whether the embryo selection on day 5, based only on the developmental and morphological criteria, would have been different from the selection based on PGD-AS results

Donoso et al., Hum Reproduction, 2006

Does PGD -AS change the selection of embryos in NOA and OA men?

NOA (37 cycles)			
	Correct choice	Incorrect choice	False-Hope
SET	64.8% (n=24)	10.8% (n=4)	24.3% (n=9)
DET	72.9% (n=27)*	2.7% (n=1)	24.3% (n=9)
TET	72.9% (n=27)*	2.7% (n=1)	24.3% (n=9)
OA (22 cycles)			
	Correct choice	Incorrect choice	False-Hope
SET	54.5% (n=12)	36.3% (n=8)	9.1% (n=2)
DET	86.5% (n=19)**	4.5% (n=1)	9.1% (n=2)
TET	86.5% (n=19)**	4.5% (n=1)	9.1% (n=2)

Donoso et al., Hum Reproduction, 2006

Aim of PGD-AS

- **IVF benefit :**
  - to improve implantation
  - to improve pregnancy rate
  - to reduce spontaneous abortion
  - to prevent multiple pregnancies
- **Genetic benefit :**
  - to prevent viable trisomic offspring (XY, 13, 18, 21)

Effectiveness of PGD-AS

Randomized trials :

None

Comparative studies :

Kahraman et al., RBM Online 2004 9: 79-85

Aran et al., RBM Online 2004 8: 470-476

Kahraman et al., RBM online, Vol.9, No.1, 79-85, 2004

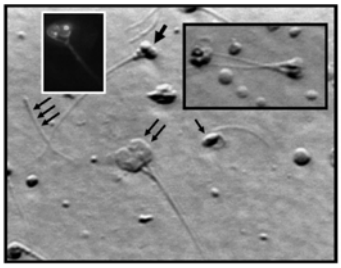


Figure 1. Macrocephalic sperm sample including slightly abnormal spermatozoon (small arrow). In the upper centre, two distinct head with two clear distinct midpiece with one tail is clearly seen (big arrow). A fluorescence in-situ hybridization image (upper left) shows a spermatozoon with two sets of chromosome Y. In the upper right box, two heads, two tails and attachment point is clearly visible. In the middle a giant flat head with clearly visible attachment line is apparent (double arrows). A pinhead spermatozoon without head (triple arrows).

Kahraman et al., RBM online, Vol.9, No.1, 79-85, 2004

Table 1. Cycle characteristics and outcome in PGD and non-PGD cycles for macrocephalic sperm samples.

Parameter	*PGD*[a]	*Non-PGD*[a]
Number of patients	21	52
Number of cycles	23	60
Female age (years)	31.2 ± 4.6	30.5 ± 5.0
Male age (years)	33.7 ± 5.1	34.0 ± 5.2
Duration of infertility (years)	7.8 ± 4.4	9.2 ± 6.0
Sperm concentration ($\times 10^6$/ml)	6.7 ± 13.8	11.9 ± 14.9
Motility (%)	14.1 ± 13.9	17.0 ± 14.6
Oestradiol on HCG day (pg/ml)	2580 ± 998	2259 ± 989
Average oocytes/patient (*n*)	16.3 ± 5.5	14.5 ± 7.7
Average MII oocytes/patient (*n*)	12.5 ± 5.0	10.8 ± 5.8
Fertilization rate (%)	43.9	46.5

[a]There were no significant differences between the two groups ($P > 0.05$).

Kahraman et al., RBM online, Vol.9, No.1, 79-85, 2004

Table 4. Embryo transfer outcome in PGD and non-PGD cases for macrocephalic sperm samples.

Parameter	*PGD*	*Non-PGD*
Embryo transfer cycles (*n*)	21	54
Embryo transfer cycles cancelled (*n*)	2[a]	6[b]
Embryos transferred (mean ± SD)	2.3 ± 1.3	3.4 ± 1.5[c]
Pregnancy rate (%)	33.3 (7/21)	27.8 (15/54)
Implantation rate (%)	25.0	12.3[c]
Abortion rate (%)	14.3 (1)	46.7 (7)

Values in parentheses are numbers.
[a]Due to lack of chromosomally normal embryos.
[b]Due to fertilization failure ($n = 2$) and cleavage-stage arrest ($n = 4$).
[c]Significant ($P \leq 0.01$).

Kahraman et al., RBM online, Vol.9, No.1, 79-85, 2004

Table 2. Cycle characteristics and outcome in PGD and non-PGD cycles for patients having spermatozoa with zero normal morphology.

Parameter	*PGD*[a]	*Non-PGD*[a]
Number of patients	14	57
Number of cycles	14	66
Female age (years)	34.1 ± 4.3	30.8 ± 5.0
Male age (years)	38.1 ± 5.4	36.1 ± 6.3
Duration of infertility (years)	10.2 ± 8.3	8.8 ± 5.1
Sperm concentration ($\times 10^6$/ml)	5.7 ± 15.8	8.8 ± 23.7
Motility (%)	22.7 ± 14.6	15.7 ± 17.4
Oestradiol on HCG day (pg/ml)	2347 ± 979	2410 ± 1439
Average oocytes/patient (*n*)	12.4 ± 7.9	14.3 ± 6.8
Average MII oocytes/patient (*n*)	9.1 ± 5.0	11.7 ± 15.7
Fertilization rate (%)	66.4	70.4

[a]There were no significant differences between the two groups ($P > 0.05$).

Kahraman et al., RBM online, Vol.9, No.1, 79-85, 2004

Table 5. Embryo transfer outcome in PGD and non-PGD cases for patients having spermatozoa with zero normal morphology.

Parameter	*PGD*	*Non-PGD*
Embryo transfer cycles (*n*)	13	63
Embryo transfer cycles cancelled (*n*)	1[a]	3[b]
Embryos transferred (mean ± SD)	2.5 ± 1.8	3.8 ± 2.1[c]
Pregnancy rate (%)	46.1 (6/13)	53.9 (34/63)[d]
Implantation rate (%)	17.5	20.5[d]
Abortions (%)	16.7 (1)	23.5 (8)

Values in parentheses are numbers.
[a]Due to lack of chromosomally normal embryos.
[b]Due to fertilization failure (*n* = 1) and cleavage-stage arrest (*n* = 2).
[c]Significant ($P \leq 0.01$).
[d]Not significant ($P \geq 0.05$).

Kahraman et al., RBM online, Vol.9, No.1, 79-85, 2004

Probes:X,Y,13,18,21

Table 3. Fluorescence in-situ hybridization results. Values relate to results from blastomeres analysed.

	Macrocephalic spermatozoa		*Zero normal morphology*	
	n	%	n	%
Analysed	82		47	
Diagnosed	69	84.1	40	85.1
Normal	37	53.6	25	62.5
Abnormal	32	46.4	15	37.5
Aneuploidy	27	84.4	14	93.3
Monosomy	2	7.4	3	21.4
Trisomy	10	37.0	10	71.4
Tetrasomy	1	3.7	–	–
Complex aneuploidy	14	51.9	1	7.4
Haploidy	–	–	–	–
Triploidy	4	12.5	1	6.6
Tetraploidy	1	3.1	–	–

Aran et al., RBM online, Vol.8, No.4, 470-476, 2004

Table 1. General characteristics and clinical outcome of couples with meiotic abnormalities after ICSI cycles with and without PGD. There were no significant differences between groups.

	Meiotic abnormalities with PGD	*Meiotic abnormalities without PGD*
No. couples	25	44
No. cycles	27	66
Mean age male (years)	34.6 (28–51)	34.9 (21–59)
Mean age female (years)	31.5 (24–39)	33.1 (20–41)
Mean oocytes recovered (no. oocytes)	19.2 (519)	13.9 (916)
Mean oocytes microinjected (no. oocytes)	14.6 (393)	11.1 (735)
Mean 2PN oocytes (no. oocytes 2PN; %)	10.9 (293; 74.5)	7.5 (496; 67.5)
No. replacements	25	66
Mean no. embryos/transfer	2.1	2.8
No. pregnancies	13	29
Pregnancy rate/cycle (%)	48.1	43.9
Pregnancy rate/transfer (%)	52.0	43.9
Implantation rate (%)	32.1	23.5
Miscarriage rate (%)	15.4	10.3

Aran et al., RBM online, Vol.8, No.4, 470-476, 2004

Table 2. Embryo analysis results.

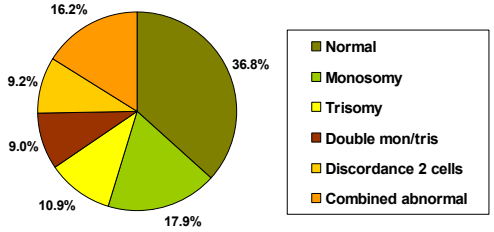
No. cycles	27
No. biopsied embryos	250
No. analysed embryos (%)	183 (73.2)
No. diagnosed embryos (%)	160 (87.4)
No. normal embryos (%)	92 (57.5)
No. abnormal embryos (%)	68 (42.5)
No. undiagnosed embryos (%)	23 (12.6)

Effectiveness of PGD-AS: Primary testicular dysfunction – comparative studies

	AMA	PGS	Control	FHB +/ embryo transferred	+ HCG /cycle	Misc/ Clin preg
Kahraman et al., 2004	Macrocephalic sperm	23	60	25.0 / 12.3 p<0.01	33.3 / 27.8 NS	14.3 / 46.7 p<0.05
	No. embryos transferred : 2.3 ± 1.3 (PGS) versus 3.4 ± 1.5 (control) p < 0.01					
Kahraman et al., 2004	Zero normal morphology	14	66	17.5 / 20.5 NS	FHB +/cycle 46.1 / 53.9 NS	16.7 / 23.5 NS
	No. embryos transferred : 2.5 ± 1.8 (PGS) versus 3.8 ± 2.1 (control) p < 0.01					
Aran et al.,2004	Meiotic abnormalities	27	66	32.1 / 23.5 NS	48.1 / 43.9 NS	15.4 / 10.3 NS
	No. embryos transferred : 2.1 (PGS) versus 2.8 (control)					

Advanced maternal age FISH : XY,13,16,18,21,22

- N of embryos with biopsy 685
- N of embryos with diagnosis 653 (95.3%)



Staessen et al., Hum Reprod 2004, 19 : 2849 - 2858

RCT: Advanced maternal age

Included Studies		
Study	Stevens et al., 2004	Staessen et al., 2004
N patients randomized	39 (21 PGS/18 con)	400 (148 PGS/141 con)
Inclusion criteria	≥ 35 with ≥ 5 good quality embryos on day 3	≥ 37 ICSI
Outcome	Pregnancy rate/ET Implantation rate Ongoing pregnancy rate/woman	Positive hCG/cycle Implantation rate Abortion rate Women reaching ET N embryos/ET

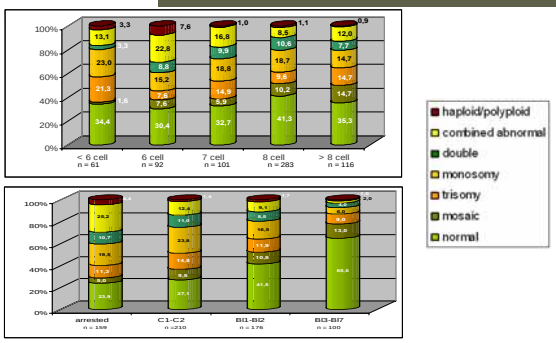
Twisk et al., 2006 The Cochrane Library Issue 1

Advanced maternal age

Outcome title	No. of studies	No. of participants	Statistical method	Effect size
01 live birth rate	1	389	Odds Ratio (Fixed) 95% CI **(11% vs. 15%)**	0.65 [0.36, 1.19]
02 ongoing pregnancy rate	2	428	Odds Ratio (Fixed) 95% CI **(15% vs. 20%)**	0.64 [0.37, 1.09]
03 proportion of women reaching embryo transfer	1	389	Odds Ratio (Fixed) 95% CI **(41% vs. 64%)**	0.39 [0.26, 0.59]
04 mean number of embryos transferred per transfer	1	202	Weighted Mean Difference (Fixed) 95% CI	-0.80 [-1.09, -0.51]
05 clinical pregnancy rate per woman randomised	2	428	Odds Ratio (Fixed) 95% CI **(15% vs. 22%)**	0.56 [0.32, 0.96] **0.42 [0.12, 1.51]**
06 multiple pregnancy rate per pregnancy	1	389	Odds Ratio (Fixed) 95% CI	0.41 [0.12, 1.36]
07 miscarriage rate per clinical pregnancy	2	428	Odds Ratio (Fixed) 95% CI	0.27 [0.04, 1.82]

Twisk et al 2006 The Cochrane Library Issue 1

Relation between FISH and developmental stage on day 3 and 5



Staessen et al Hum Reprod 2004;19;2849-2858

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In Vitro Fertilization with Preimplantation Genetic Screening

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Table 2. Outcomes in Women Who Underwent Preimplantation Genetic Screening and in Controls.

Outcome	Women Who Underwent Preimplantation Genetic Screening (N = 206)	Controls (N = 202)	Rate Ratio (95% CI)*	P Value
Women with an ongoing pregnancy — no. (%)	52 (25)	74 (37)	0.69 (0.51–0.93)	0.01
Women with ≥1 biochemical pregnancy — no. (%)	81 (39)	106 (52)	0.75 (0.60–0.93)	0.008
Total no. of biochemical pregnancies	94	118		
Women with ≥1 clinical pregnancy — no. (%)	61 (30)	88 (44)	0.68 (0.52–0.88)	0.003
Total no. of clinical pregnancies	67	92		
Women with ≥1 miscarriage — no. (%)	37 (18)	36 (18)	1.01 (0.67–1.53)	0.97
Total no. of miscarriages	43†	44‡		
Women with ≥1 live birth — no. (%)	49 (24)	71 (35)	0.68 (0.50–0.92)	0.01
Total no. of live births	59§	85¶		

* CI denotes confidence interval.
† One miscarriage occurred at 18 weeks of gestation; all other miscarriages occurred before 12 weeks of gestation.
‡ All miscarriages occurred before 12 weeks of gestation.
§ There were 29 singleton and 10 twin births; one woman underwent elective termination of pregnancy, one pregnancy ended in an intrauterine death, and one premature delivery resulted in the postpartum death of a twin.
¶ There were 57 singleton and 14 twin births; two women underwent elective termination of pregnancy, and one pregnancy ended in an intrauterine death.

Aim of PGS

- **IVF benefit :**
  - to improve implantation
  - to improve pregnancy rate
  - to reduce spontaneous abortion
  - to prevent multiple pregnancies
- **Genetic benefit :**
  - to prevent viable trisomic offspring (XY, 13, 18, 21)

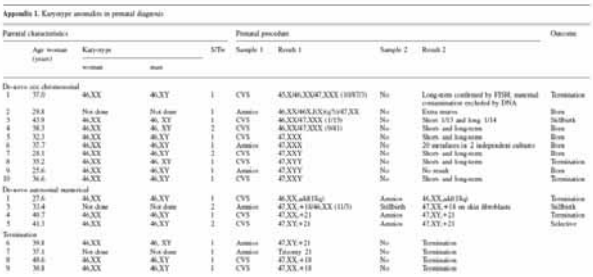
RCT : Advanced maternal age (≥ 37 y)

	PGS	Control
No. of pregnancies with FHB (%)	22 (75.9)	30 (76.9)
No. clinical miscarriage	1*	1
No. singleton births	18	23**
No. of twin births	2	6
No. of triplet births	1	0
Total live-born children	25	35
Live-born / embryo transferred	15.2 %	10.4 %

* Twin pregnancy expulsion at 20 weeks
** One child with trisomy 21

Staessen et al. (2004) Hum Reprod, 19, 2849-2858

Bonduelle et al., Hum. Reprod.,Vol.17, No.10, 2600-14, 2002



Summary

- High incidence of abnormal embryos (37 – 70%)
- PGD-AS seems to improve embryo selection in azoospermic men especially when undergoing SET
- More RCT studies are required to evaluate the value of PGD-AS in severe sperm morphological anomalies

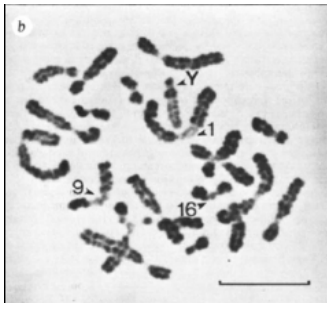
Effect of PGD-AS less than expected ?

The concept of PGD-AS is valid, although the effect is less than expected

- Screening for too few chromosomes : aneuploidy involving unscreened chromosomes
- Adverse impact of embryo biopsy
- Mosaicism complicates the diagnostic abilities
- Other causes of implantation failure

- Thanks for your attenion

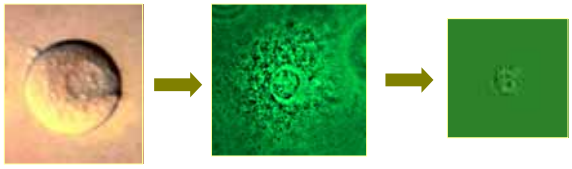
Karyotype obtained from sperm/hamster oocyte fusion technique



Rudak et al., 1978

Fixation

Spreading of the individual blastomere using 0.01N HCl/0.1% tween 20 solution on Superfrost slides *(Coonen et al., 1994)*



Chromosomal constitution of embryos obtained after ICSI with testicular sperm of OA and NOA men

	NOA	OA	Sexing
I Sex chromosomal abnormalities No. of sex chromosome abnormalities / no. of embryos analysed (%)	11/194 (5.6)	2/117 (1.7)	0/82 (0.0)
II Autosomal abnormalities No. of autosomal abnormalities / no. of embryos analysed (%)	30/194 (15.4)	33/117 (28.2)	19/82 (23.2)
III Ploidy status abnormalities No. of ploidy status abnormalities / no. of embryos analysed (%)	6/194 (3.0)	9/117 (1.2)	1/82 (7.7)
IV Combined abnormalities No. of combined abnormalities / no. of embryos analysed (%)	71/194 (36.9)	25/117 (21.3)	16/82 (19.5)

Platteau et al., 19, 1570-74, 2004