Indications for chromosome screening

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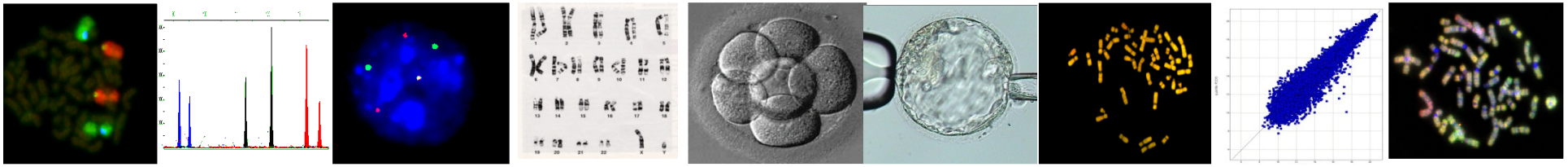
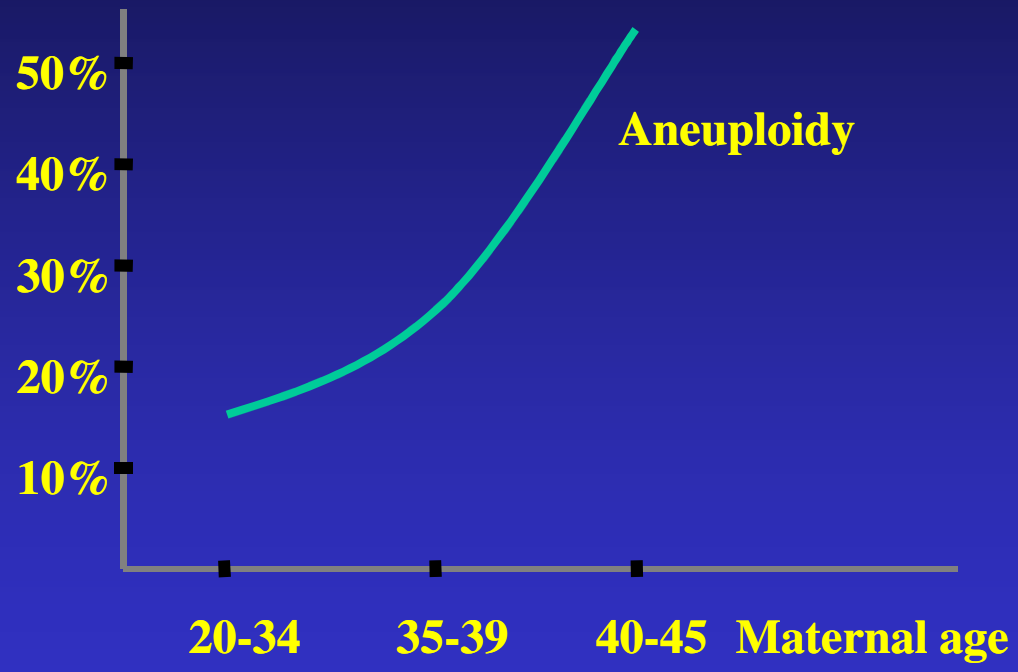


Chromosome imbalance (aneuploidy)

Uncontroversial data

The incidence of aneuploidy

Aneuploidy is extremely common in human oocytes and increases with advancing age



This trend is also reflected in the dramatic increase in Down syndrome pregnancies with maternal age

The incidence of aneuploidy

The high incidence of oocyte aneuploidy has been demonstrated using multiple techniques in laboratories worldwide

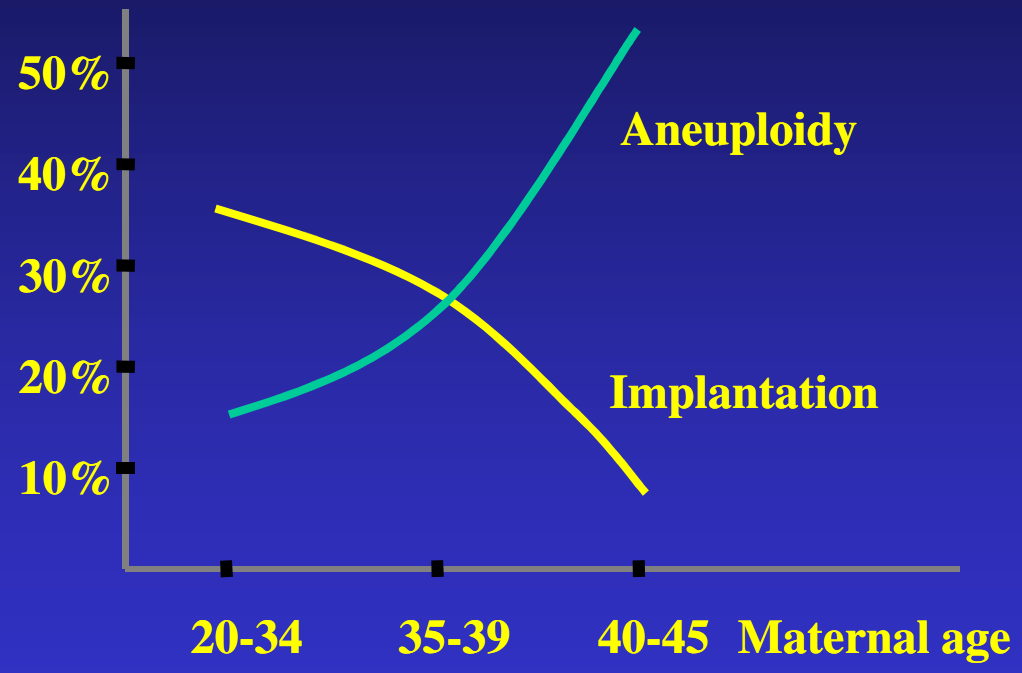
Aneuploid oocytes produce embryos abnormal in every cell

For women over 40 over 50% of cleavage stage embryos are chromosomally abnormal in every cell

What is the impact of aneuploidy?

Aneuploidy and IVF failure

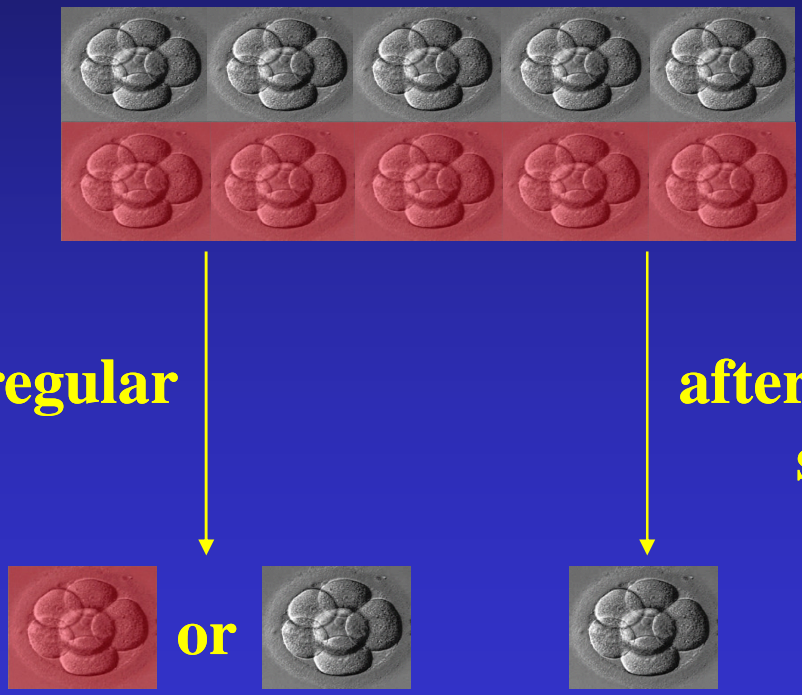
As aneuploidy increases age, so implantation rate decreases



~65% of 1st trimester miscarriages are aneuploid

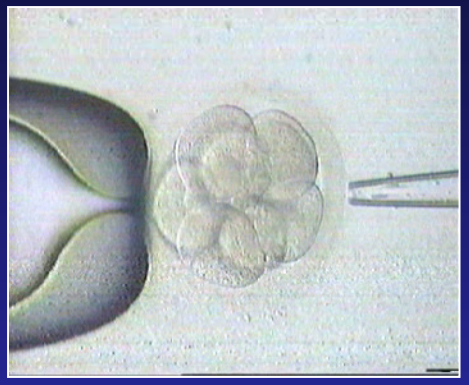
Preimplantation genetic screening (PGS)

Standard embryo evaluations do not reveal embryos with the wrong number of chromosomes

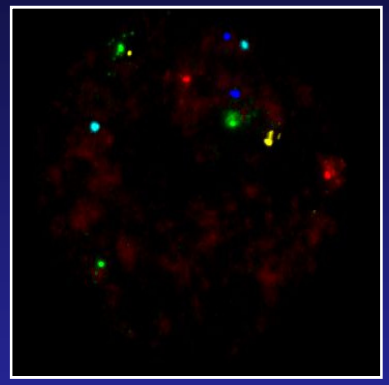


Preimplantation genetic screening

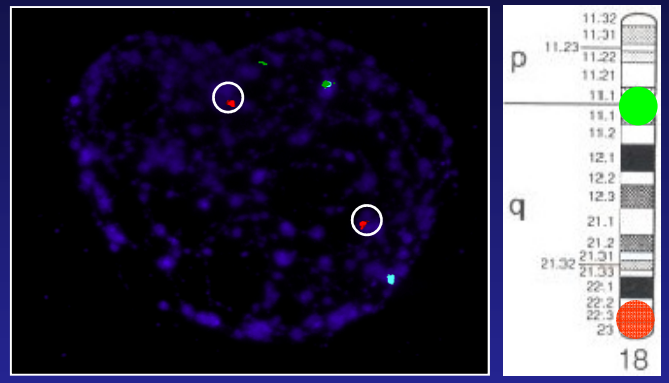
Chromosomal indications



Biopsy



1st round of FISH



2nd round of FISH

NRR

- **Theoretical benefits for patients undergoing routine IVF**
  - Increase embryo implantation/pregnancy rate
  - Reduce aneuploid syndromes
  - Reduce miscarriage

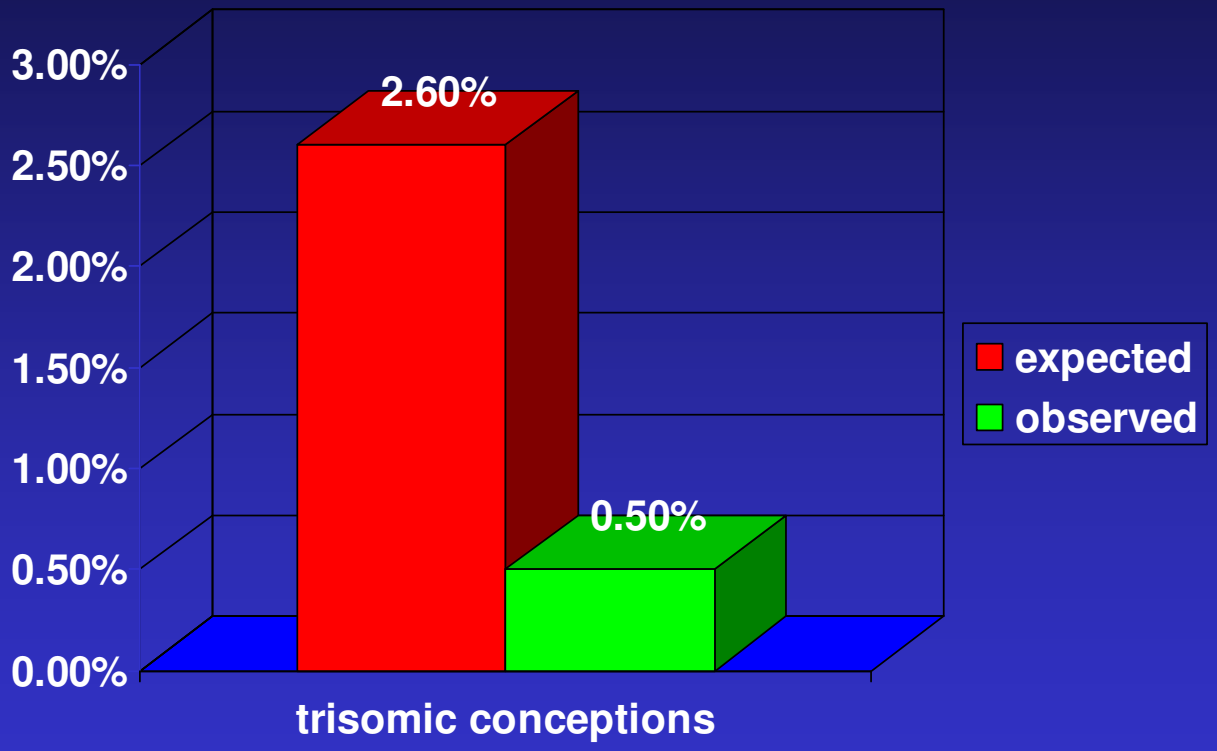
Advanced maternal age

The positive

Reduction in aneuploid pregnancies

PGS – reduction in aneuploid pregnancy

Reduction in aneuploidies 13, 18, 21, XY achieved using PGS



P<0.001

From 2,300 cases with follow-up data available, mean age 37

Munne et al 2006 and Reprogenetics data to 10/2007

PGS – reduction in aneuploid pregnancy

Are patients interested in PGS for this purpose?

Recent study of subfertile women (Twisk et al., 2007)

If PGS was assumed to have no effect on pregnancy rate

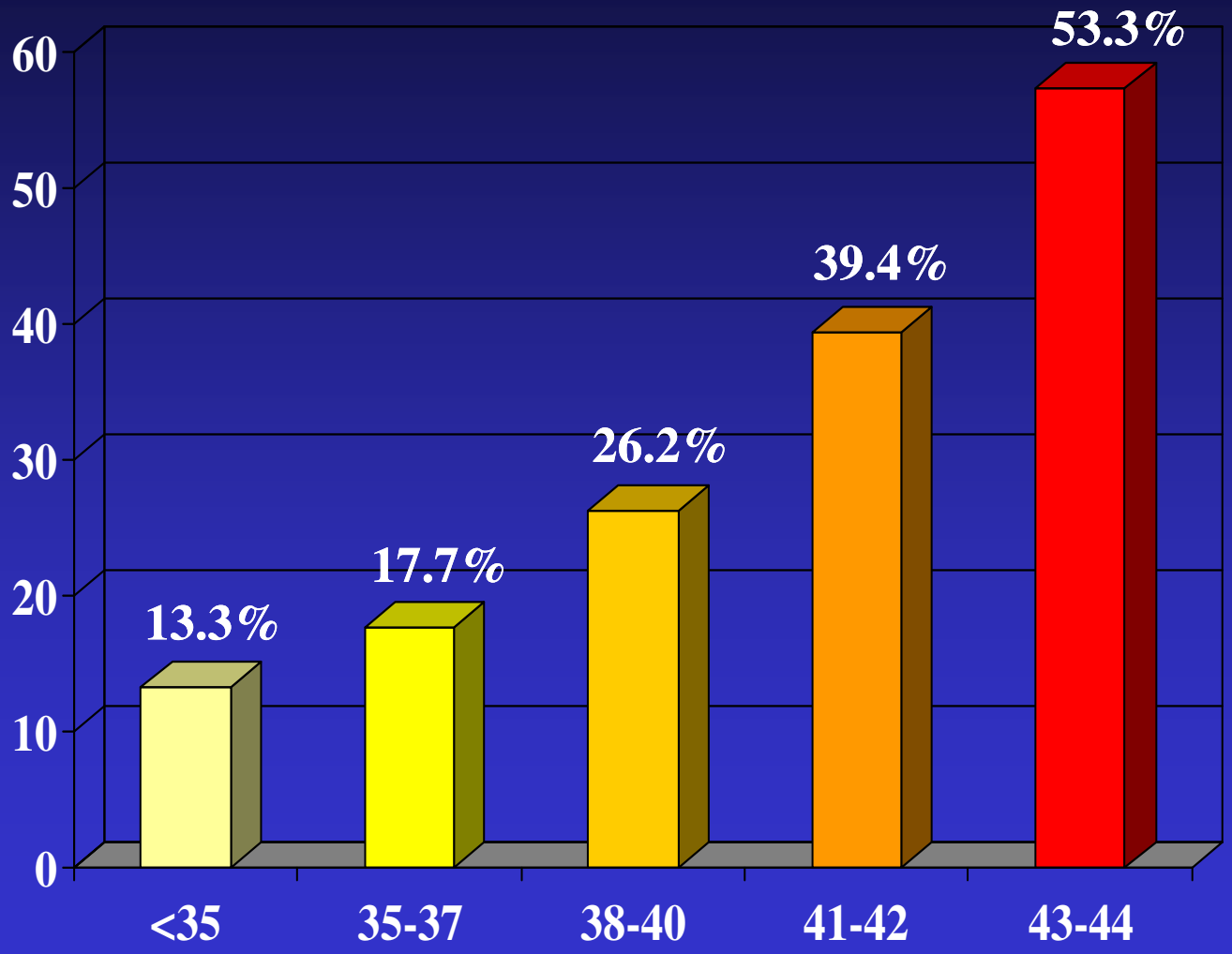
83% of patients would request PGS (75% if 80% detection)

If PGS was assumed to reduce pregnancy rate from 20% to 14%

36% of patients would still request PGS (31% if 80% detection)

Reduction in miscarriage rate

IVF pregnancy loss and maternal age



SART-ASRM (2005)

Reduction in spontaneous abortion

Pregnancy loss rates in the general IVF population and after PGD

Age:	35-40	>40
IVF population*	19%	41%
PGD**	14%	22%
	p<0.05	p<0.001

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considering pregnancies as the presence of a gestational sac, and pregnancy loss as the loss of the whole pregnancy.
Munne et al., 2006

Increase in pregnancy rates

PGS – live birth rate

Patients 38-42

Chromosomes analyzed: XY, 13, 15, 16, 17, 18, 21, 22

SART data of 5 centers with >10% PGS cases, 2003-2005

clinic	Non-PGS cycles	loss rate	live birth	PGS cycles	loss rate	live birth
1	505	27%	35%	70	22%	40%
2	210	36%	14%	72	27%	15%
3	1204	34%	12%	120	15%	23%
4	509	29%	15%	236	26%	22%
5	191	25%	17%	208	16%	25%
total	2619	30%[a]	18%[b]	706	21%[a]	24%[b]

a: p<0.01

b: p<0.001

Losses reduced by ~1/3

Live births increased by ~1/3

Munne et al 2007; Colls et al 2007

Problems with positive PGS studies

BUT….

- **Not randomized**
- **In some cases control groups questionable**

The negative

Increase in implantation/pregnancy- controversy

Implantation rate

- **Mastenbroek et al (2007), NEJM**
- **Maternal age ≥35**
- **8 chromosomes assessed, randomised**
- **No significant improvement in implantation**

Problems with negative PGS studies

BUT….

- **Many patients with <5 embryos included in study (mean 4.8)**

  Little selection possible

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  Developmental potential drastically reduced

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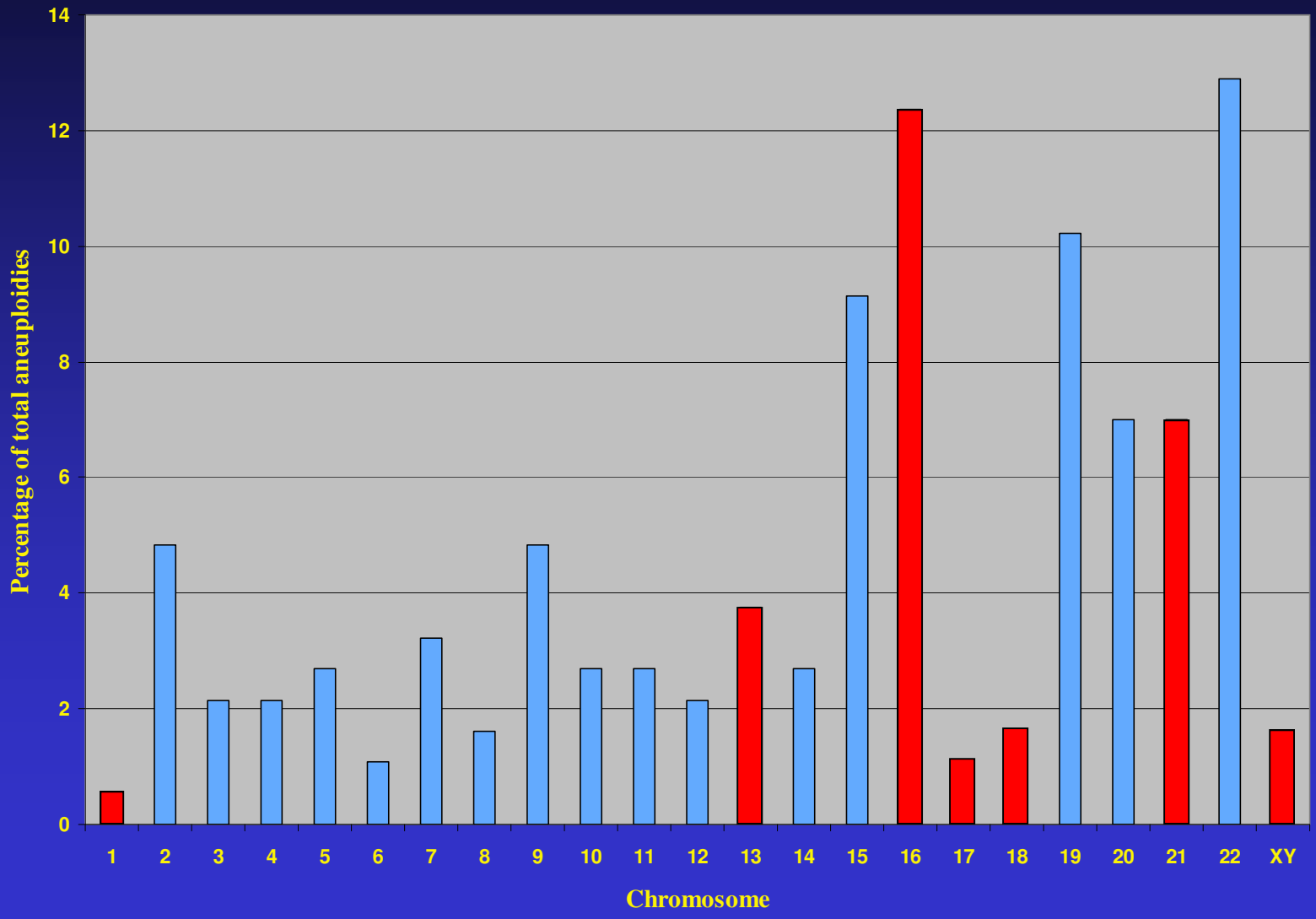
Poor selection of chromosome probes



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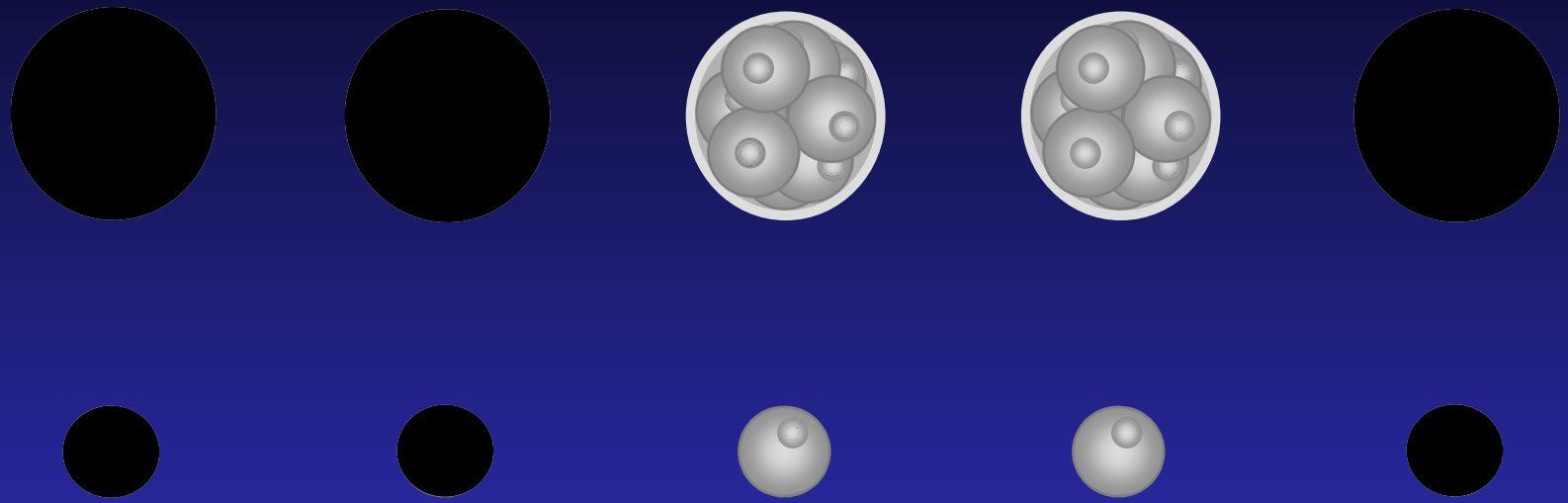
- **Did not test chromosomes 15 & 22 (only 28% of aneuploidies detected)**

  Many abnormal embryos undetected , little selection possible
- **Implantation rate for biopsied, non-diagnosed embryos= 6%**

  Developmental potential reduced. Lack of biopsy experience?

Problems with negative PGS studies



Critically damaged by biopsy

Only 28% of aneuploidies detected

No result

Pool of embryos reduced while little selective advantage has been gained

Legitimate criticisms of traditional PGS methods

- Current methodologies are not robust, limiting application
- Biopsy can have a serious impact if poorly performed
- Mosaicism will lead to the exclusion of a small number of potentially viable embryos
- No randomized study has proven that PGS is beneficial

Chromosome screening for repeated implantation failure (RIF)

Screening RIF patients

So far there is no evidence that PGS improves outcome for RIF patients (studies 1-5)

1: Gianaroli et al. 1999
2: Kahraman et al. 2000
3: Munné et al., RBO 2003
4: Pehlivan et al. 2002
5: Werlin et al. 2003

Aneuploid rate in one cycle is usually highly predictive of aneuploidy rate in the next

PGS may help patients with 100% abnormal results to consider alternative options such as gamete donation

Chromosome screening for patients with previous trisomic conception

Patients (<35 years) with previous trisomic conception

CHROMOSOME ABNORMALITIES:

	Aneuploidy rate
Patients with previous trisomy	41%
Control	19%
	P<0.001

IMPLANTATION RATE:

	% pregnancy	implantation
Patients with PGS	57%	50%
Controls	43%	22%
	P<0.025	

Munne et al. 2004b

Chromosome screening for recurrent pregnancy loss (RPL)

Patients with recurrent pregnancy loss

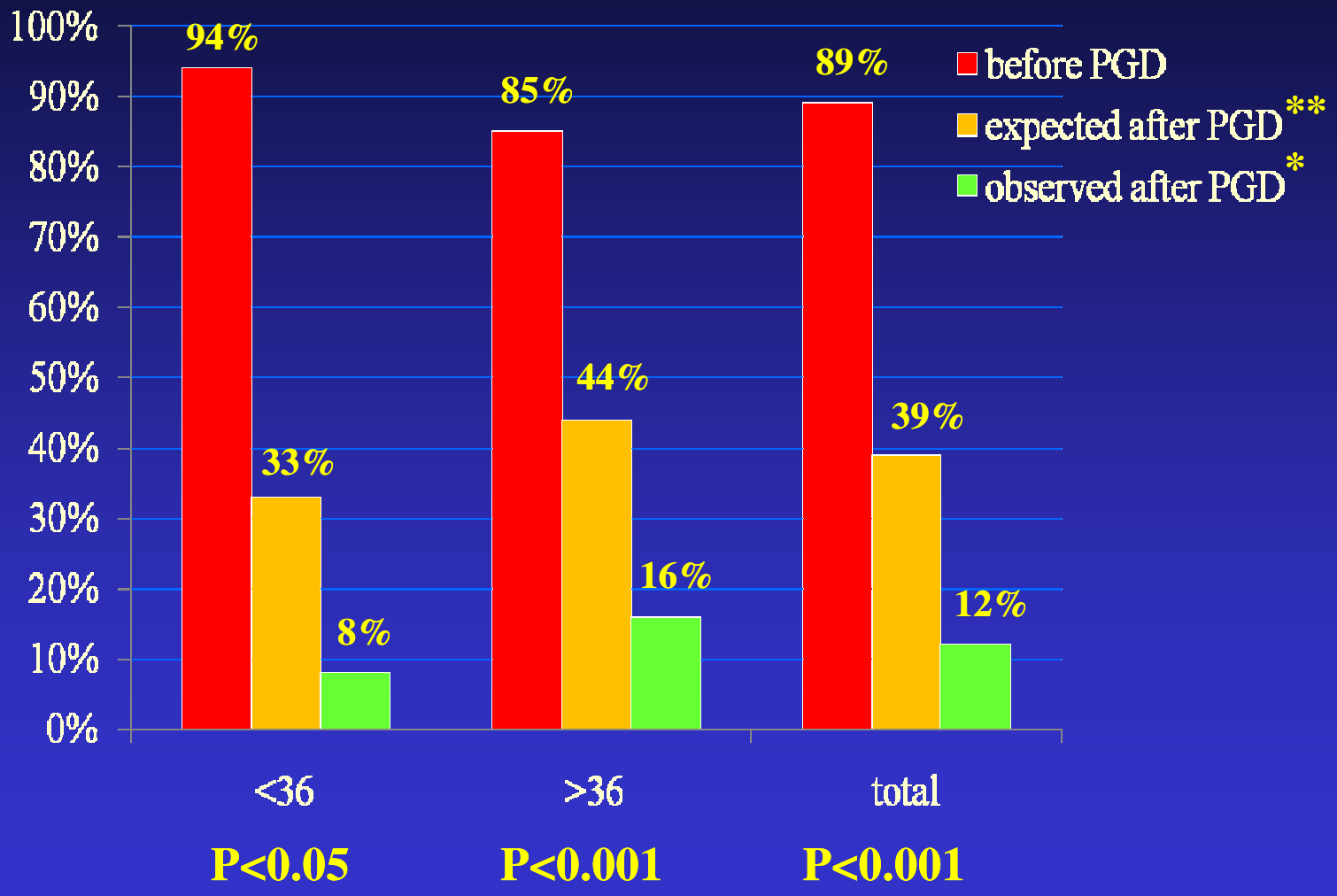
Controlled studies on idiopathic RPL :

- Werlin L, et al. (2003) Preimplantation genetic diagnosis (PGD) as both a therapeutic and diagnostic tool in assisted reproductive technology. Fertil Steril, 80:467
- Munné et al. (2005) Preimplantation genetic diagnosis reduces pregnancy loss in women 35 and older with a history of recurrent miscarriages. Fertil Steril 84:331
- Munné et al. (2006) PGD for recurrent pregnancy loss can be effective in all age groups. Abstract PGDIS
- Garrisi et al. (2008) Preimplantation genetic diagnosis (PGD) effectively reduces idiopathic recurrent pregnancy loss (RPL) among patients with up to 5 previous consecutive miscarriages after natural conceptions. Fertil. Steril in press
- Rubio et al. (in press) Prognosis factors for Preimplantation Genetic Screening in repeated pregnancy loss. Reprod Biomed Online, in press

All show a decrease in miscarriage rate

Patients with recurrent pregnancy loss

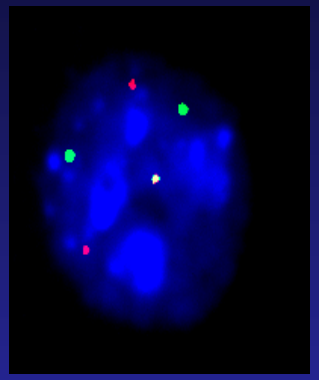
N=122
With ≥3 previous losses



*Munné et al. 2005 and unpublished data, **Brigham et al. 1999

Future developments

Limitations of conventional embryo screening techniques

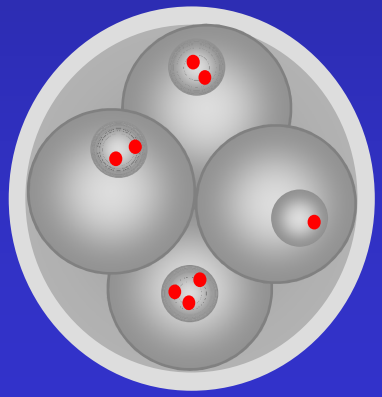


Cells are in interphase - use FISH

Limited range of fluorochromes

Less than half the chromosomes tested

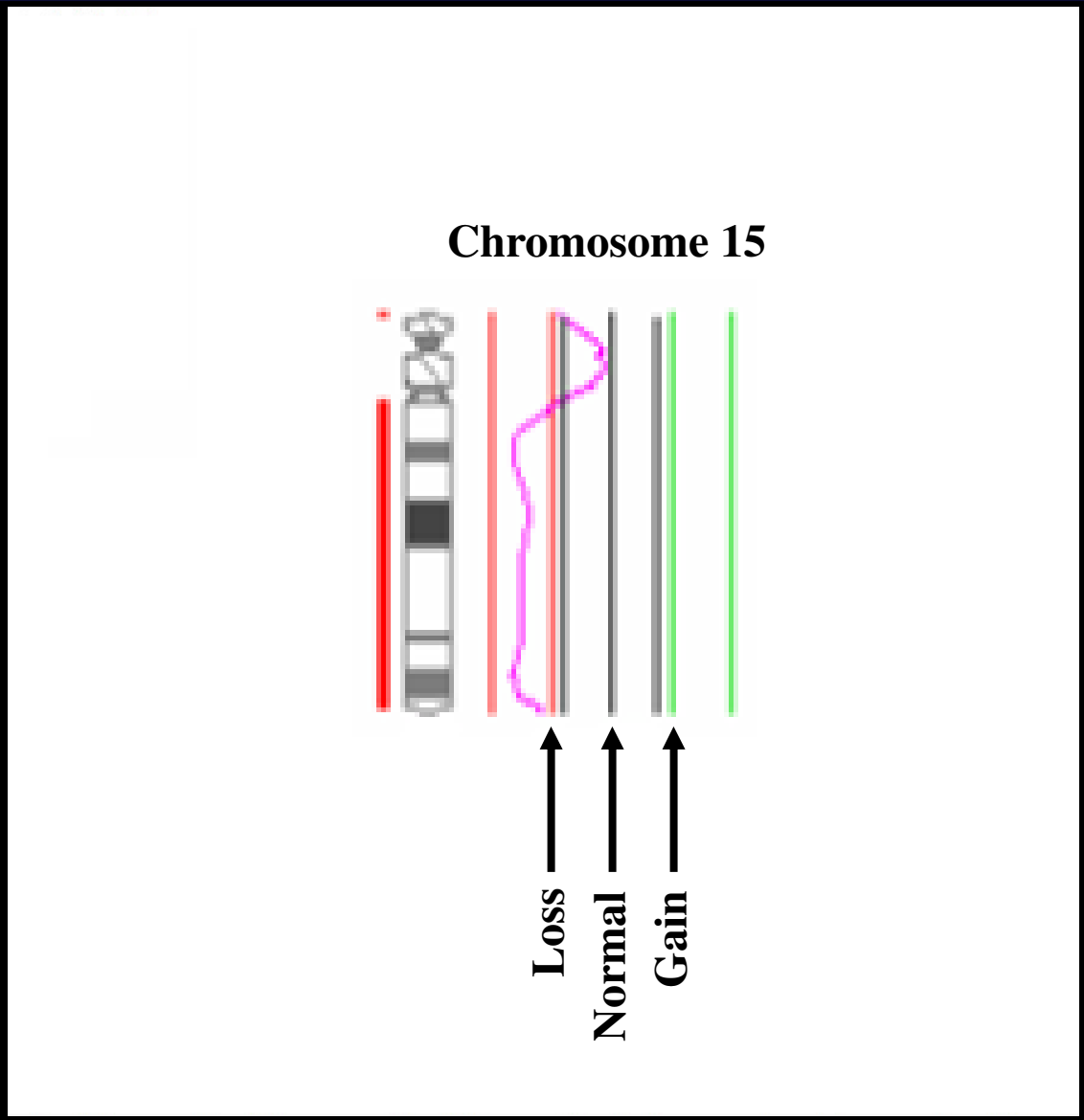
Spreading requires skill and can be inconsistent



Mosaicism Poses a significant problem for diagnosis. However, most mosaic cleavage stage embryos are aneuploid in every cell.

Cleavage stage biopsy may represent a cost to the embryo

Comparative genomic hybridization- CGH



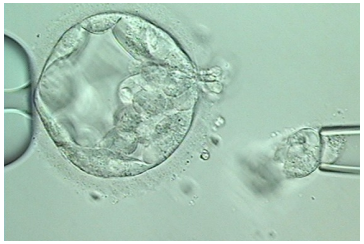
Embryo screening using CGH

Benefits

- **All chromosomes tested**
- **No spreading of cells on slides**

But what about mosaicism and the impact of biopsy?

Comprehensive chromosome screening of blastocysts



Analysis of blastocyst stage

- **Biopsy of several cells is possible**
  - **Diagnosis more robust and accurate**
  - **Less risk of misdiagnosis due to mosaicism**
  - **Reduced impact of embryo biopsy**
- **Blastocyst cryopreservation (vitrification) necessary**
- **Can overcoming the principal challenges to accurate screening allow PGS to fulfill the potential predicted by theory?**

Blastocyst CGH- clinical results

- 170 patients, mean age 38 years, 1-6 previous failed IVF cycles (mean 2)
- Near 100% survival after biopsy, freeze and thaw

• Pregnancy rate per cycle with transfer	87%	72%
• Birth rate per cycle with transfer	79%	60%
• Implantation rate per embryo	67%	28% *

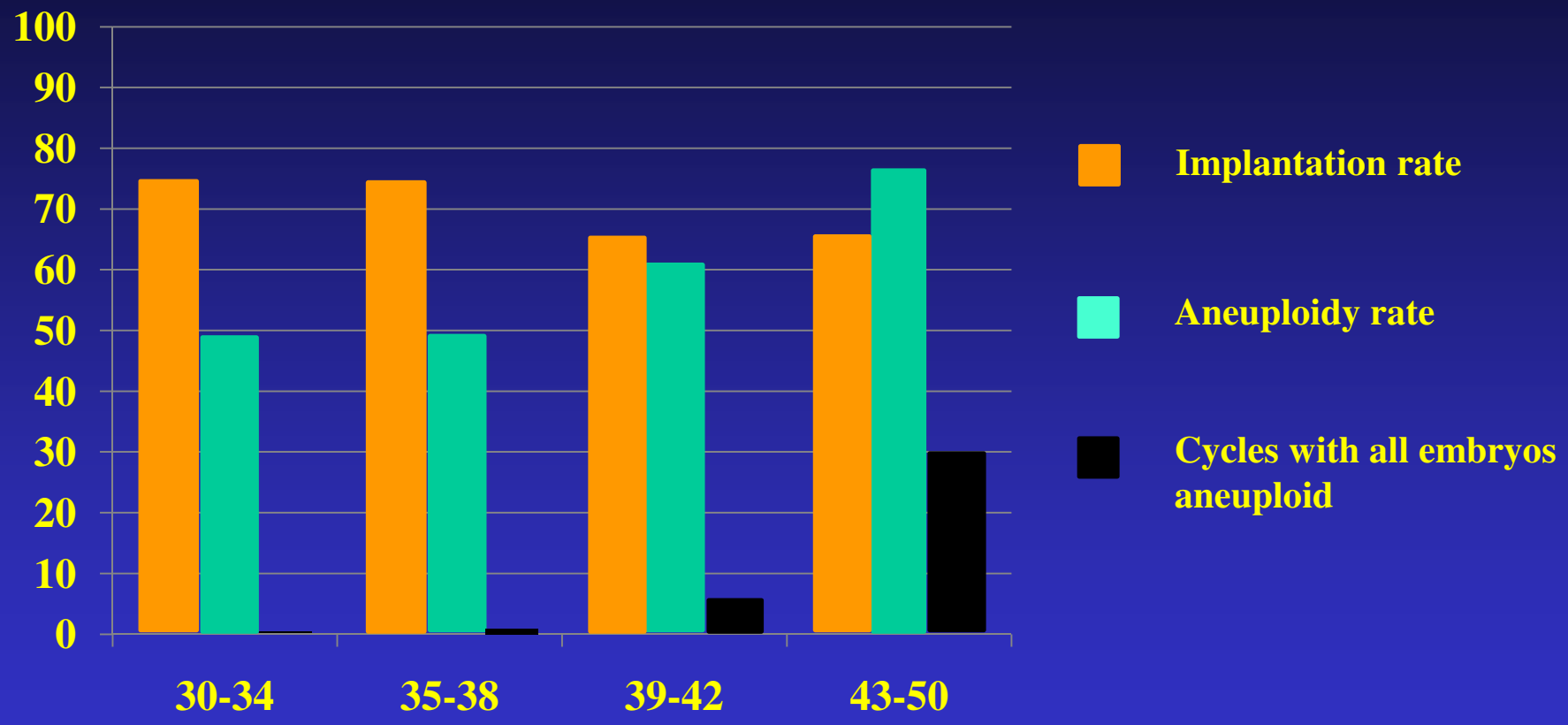
Control group matched for: maternal age, day-3 FSH, day of transfer, # oocytes retrieved, # of failed cycles

*p<0.0003 - Extremely promising for single embryo transfer

Blastocyst CGH- rates of pregnancy loss

- **Embryo loss rates are low**
- **91% of embryos that produced a fetal sac resulted in an ongoing third trimester pregnancy or live birth**
- **97% of embryos that produced a fetal heart beat resulted in an ongoing third trimester pregnancy or live birth**
- **Expected pregnancy loss rate for IVF patients in this age range is ~25%**

Blastocyst CGH- clinical results



Questions

- **Can the results obtained in the current study be replicated in a randomized controlled trial?**
- **How much of the observed benefit is due to transfer in a subsequent cycle?**
- **Aneuploidy explains most of the decline in IVF success with advancing maternal age. What explains the remainder?**
- **What patient groups will benefit the most from this type of screening?**

Reprogenetics

United Kingdom (Oxford)
Elpida Fragouli
Samer Alfarawati

United States (Livingston, NJ)
Pere Colls
Tomas Escudero
N-neka Esprit-Ngachou
Jill Fischer
Cristina Gutierrez-Mateo
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