PGS and prevention of recurrent miscarriage: facts and fiction

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ESHRE Campus symposium

Effects of ART and endometriosis on pregnancy outcome

SIGs Early Pregnancy and Endometriosis and Endometrial disorders

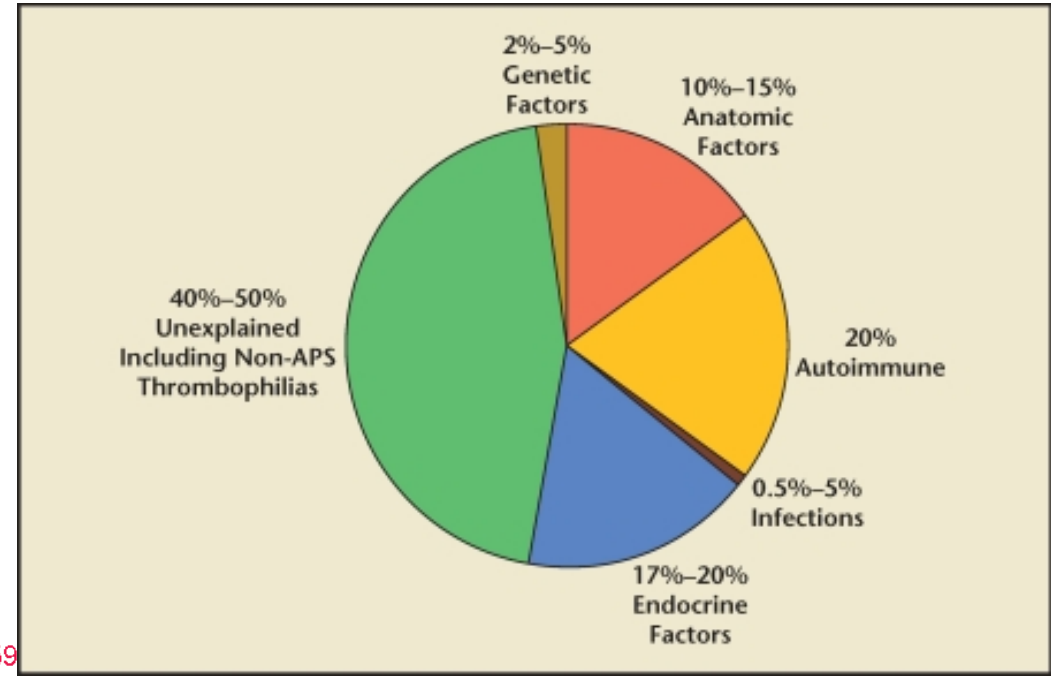
27 - 28 January 2017, Sofia, Bulgaria

Disclosure

Conflicts of interest: none

Recurrent pregnancy loss (RPL), also referred to as recurrent miscarriage or habitual abortion, is historically defined as 3 consecutive pregnancy losses prior to 20 weeks from the last menstrual period.

Ford and Shust, Rev. Obstet. and Gynecol. 2009



RPL

Second World Congress of RPL, Cannes, France 19-22 Jan, 2017

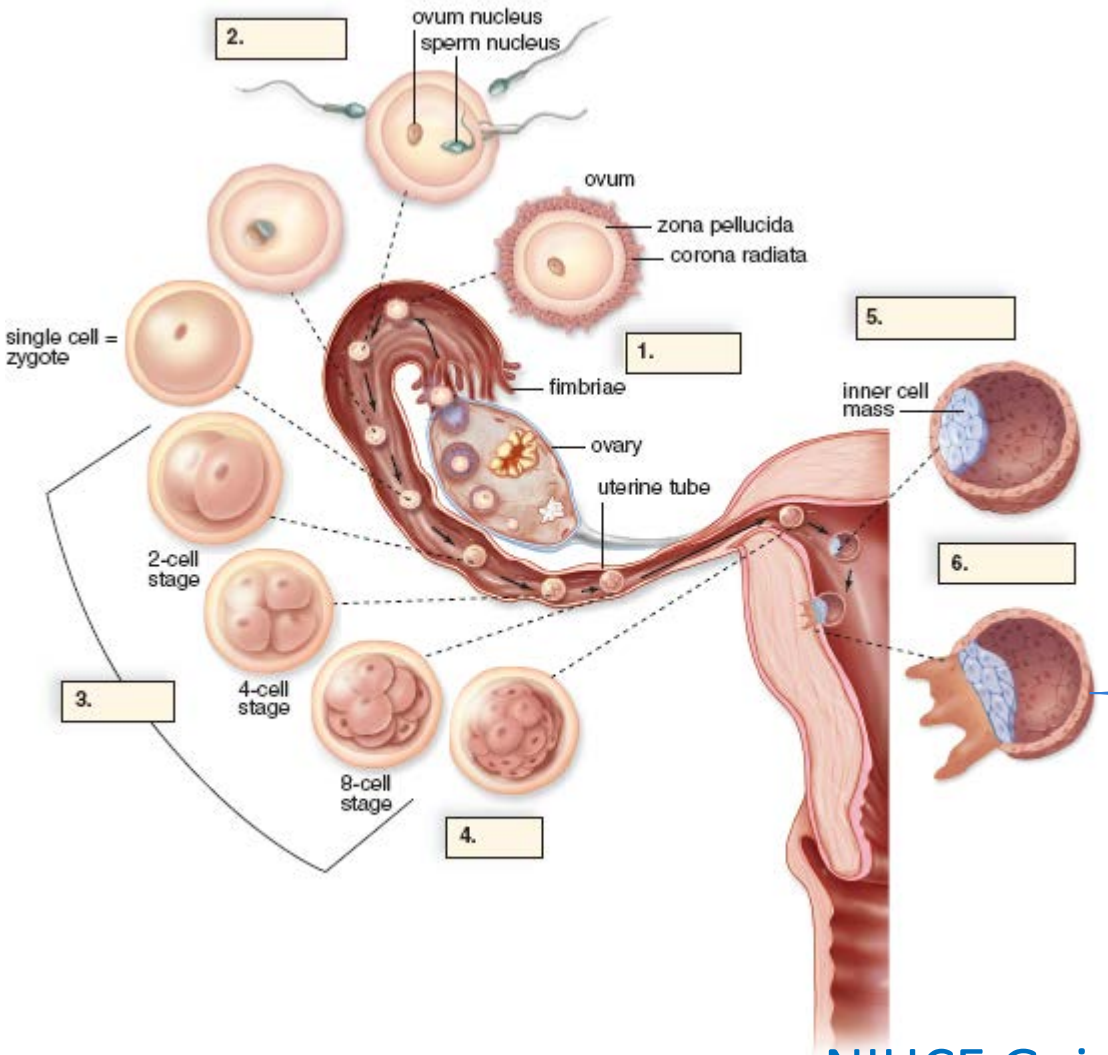
Reasons:
Endocrine aspects, BMI, Vit D3, Prevalence of Vitamin D;
Imm.processes: at Feto-maternal interphase, Decidual Th1/Th2&NK1/NK2 phenotyping, Anti-P Allo-Antibodies, APLA Syndrome, Elevated peripheral NK cells, Hereditary Thrombophilia;
Mol.and Chrom. level: Trombophilia gene mutation, Deficiency of Placental Copy Number Variations, Trophoblast Growth Pathways, RNA-Seq Analysis of Chorionic Villi, Chromosomal Abnormalities e.g. Balanced Structural Chromosomal Anomalies;
Infections: Gardnerella Vaginalis, toxoplasmosis, rubella, CMV, endometritis;
Role of Oxidative Stress, Anatomical Causes, Cervical Incompetence;

Treatment: G-CSF Treatment, Lymphocyte Immunization, Anti-Oxidants Treatment, Endoscopic Septectomy, HS, Aspirin, Medformin, Low Molecular Weight Heparin;

Methods: Time-lapse, Endometrial biopsy, Embryo morphology, Role of Sperm Selection (IMSI), PGS;

Other side effects of RPL: Depression and Anxiety

Implantation and ART

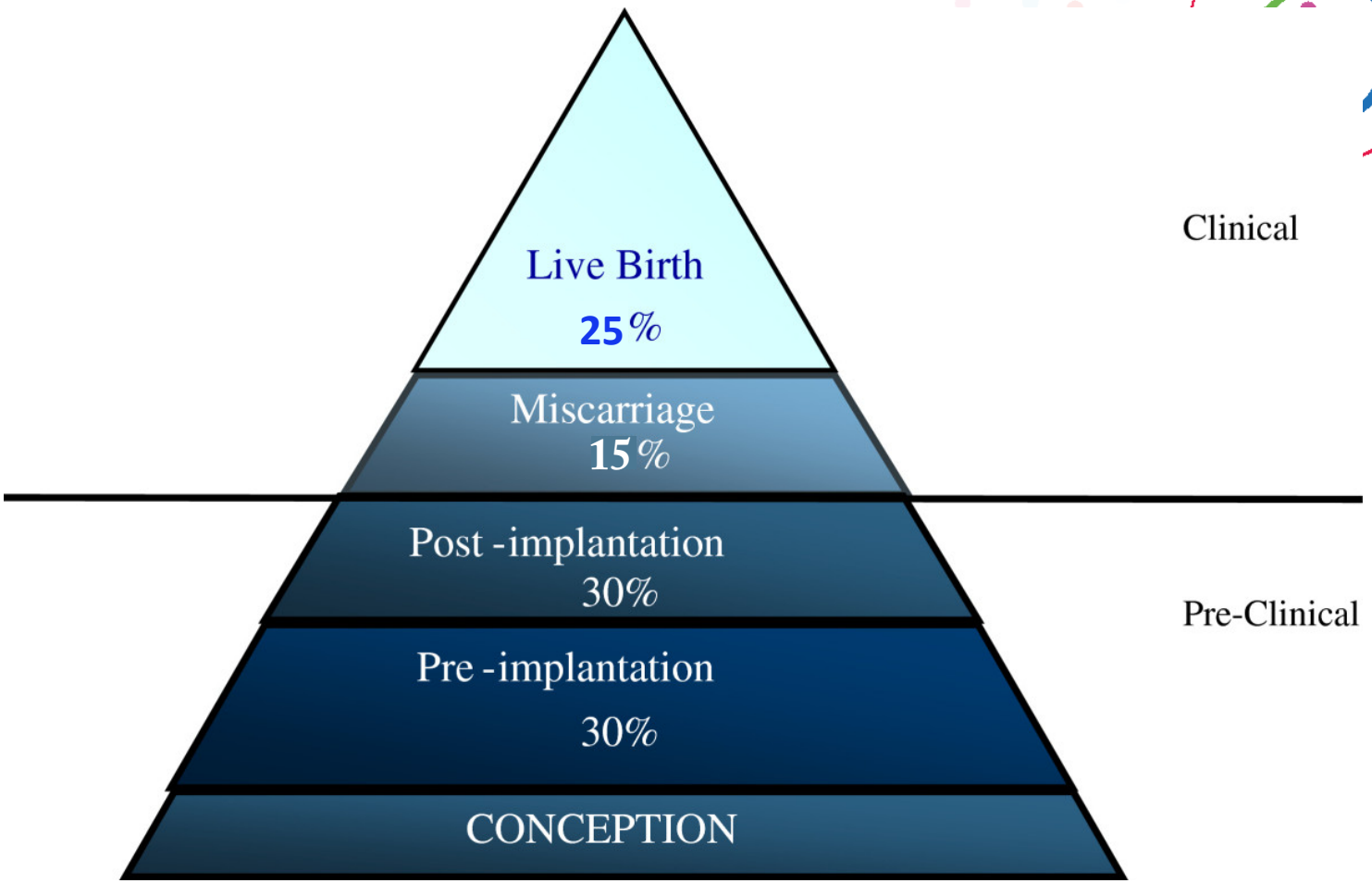


- Hydrosalpinx
- Mock ET
- Endometrium
- Uterus anomaly
- Endometriosis
- BMI
- Immunological factors
- Genetic factors
- Life style

NIHCE Guidance, 2013

The pregnancy loss iceberg

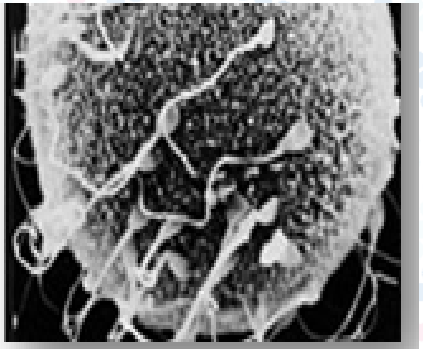
An overview of the outcome of spontaneous human conceptions



75% of embryos are lost before delivery

Most of those loses are in the period BEFORE implantation

Larsen et al. BMC Medicine 2013 11:154



- *Less than **5% of oocytes**, collected after controlled ovarian stimulation, can lead to a pregnancy and then to the birth of a child.*

Stoop D et al. Reproductive potential of a metaphase II oocyte retrieved after ovarian stimulation: an analysis of 23 354 ICSI cycles. Hum Reprod 2012

How many frozen eggs does everyone need?

How many MII are needed to achieve a pregnancy?

Outcomes compared between patients 30-36 and 37-39

TABLE 2

The outcome comparison between young age versus advanced age patients' oocytes after vitrification.

	Young age group 30–36 y (n = 11)	Advanced age group 37–39 y (n = 11)	*P* value
Patient age (mean y ± SD)[a]	32.91 ± 1.97	37.90 ± 0.83	< .0001
Mean basal FSH (mean mIU/mL ± SD)	6.20 ± 2.26	6.20 ± 0.92	NS
Survival rate (%)	80/97 (82.5)	68/89 (76.4)	> .9999 NS
Fertilization rate (%)	68/97 (70.1)	56/89 (62.9)	.3639 NS
No. of good-quality embryos on day 3 (%)[a]	54/97 (55.6)	36/89 (40.4)	.3509 < .05
No. of embryos transferred (mean ± SD)[b]	24 (2.18 ± 0.6)	29 (2.64 ± 1.0)	NS
No. of clinical pregnancies (%)	7/11 (63.6)	3/11 (27.3)	.2056 NS
No. of implantations (%)	10/24 (41.7)	6/29 (20.7)	.1984 NS
No. of take home babies (%)	6/11 (54.5)	2/11 (18.2)	.1357 NS
No. of live births	8	3	.1827 –
Percentage of oocyte to achieve a live birth (%)	8/97 (8.2)	3/89 (3.3)	NS .2173

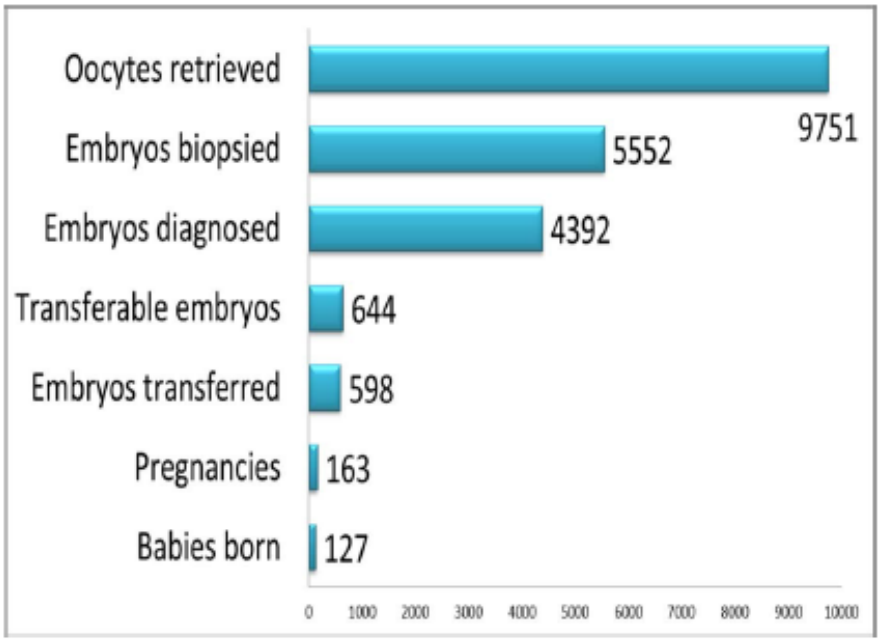
Nb of oocytes to obtain a live birth **N=12** **N=29**

How many OPU ?????

Chang et al., Fertil Steril 2013

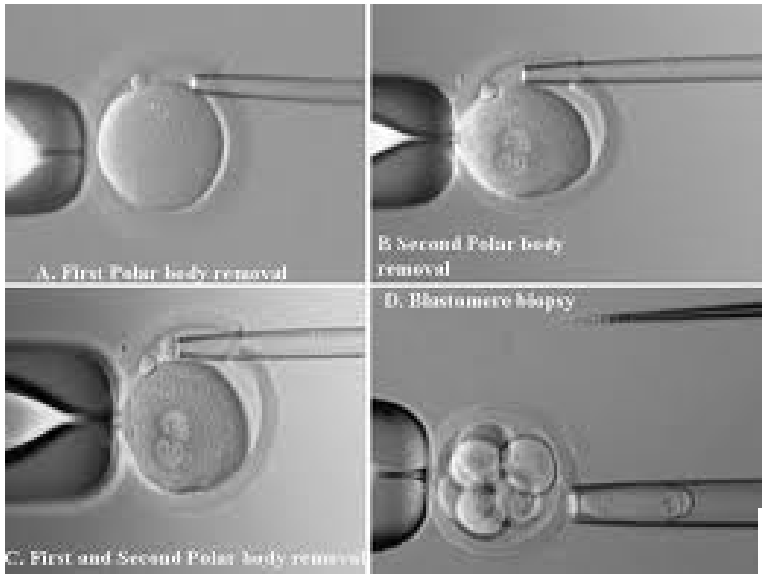
1.3% of oocytes give a baby

Example of some results



Pregnancy rate/cycle initiated	23.2%
Pregnancy rate/embryo transfer	41.1%
Fetal heart beat (FHB)/cycle initiated	22.0%
FHB/embryo transfer	39.0%
FHB/embryos transferred	25.9%
Live birth rate (LBR)/cycle initiated	18.0%
Live birth rate/embryo transfer	32.0%

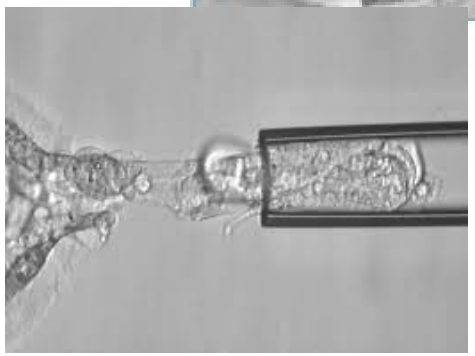
ESHRE PGD consortium meeting, Helzinki 2016



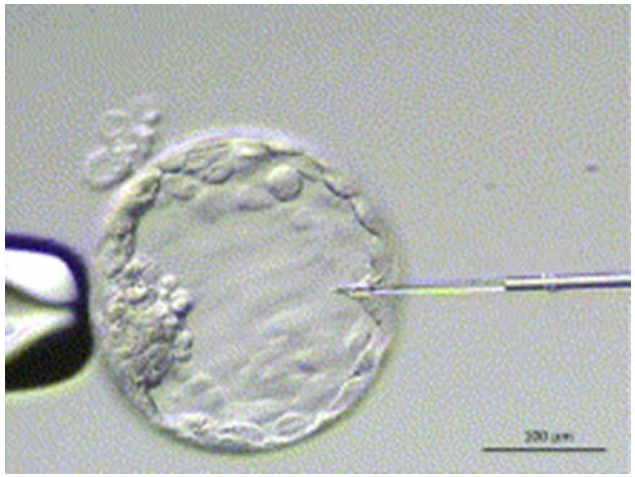
50-70%
60%-70%
60-70%
70-90% FR
80-85% M2

Blastocyst (unhatched)

Biopsy (hatched blastocyst)



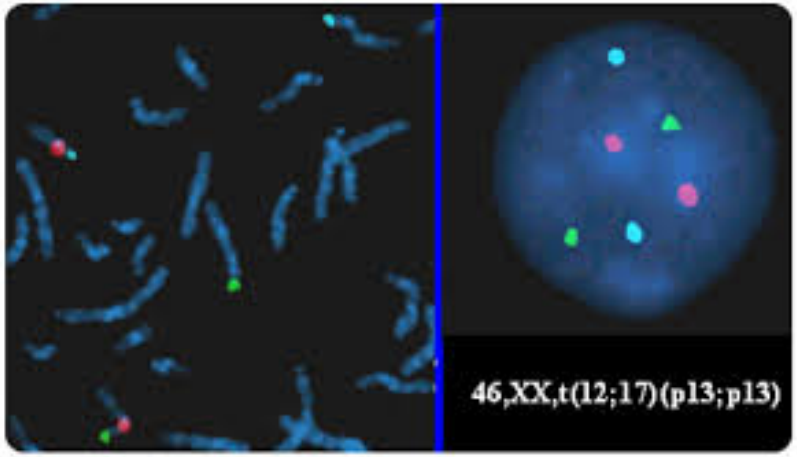
Still no consensus of which stage to biopsy

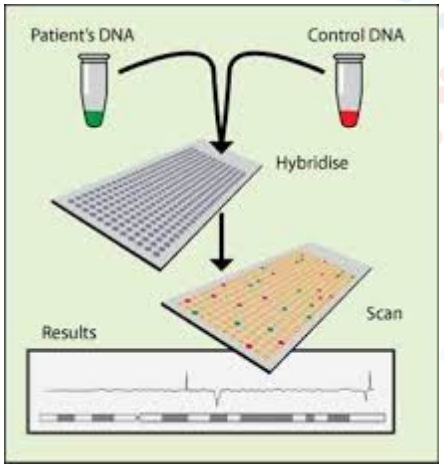


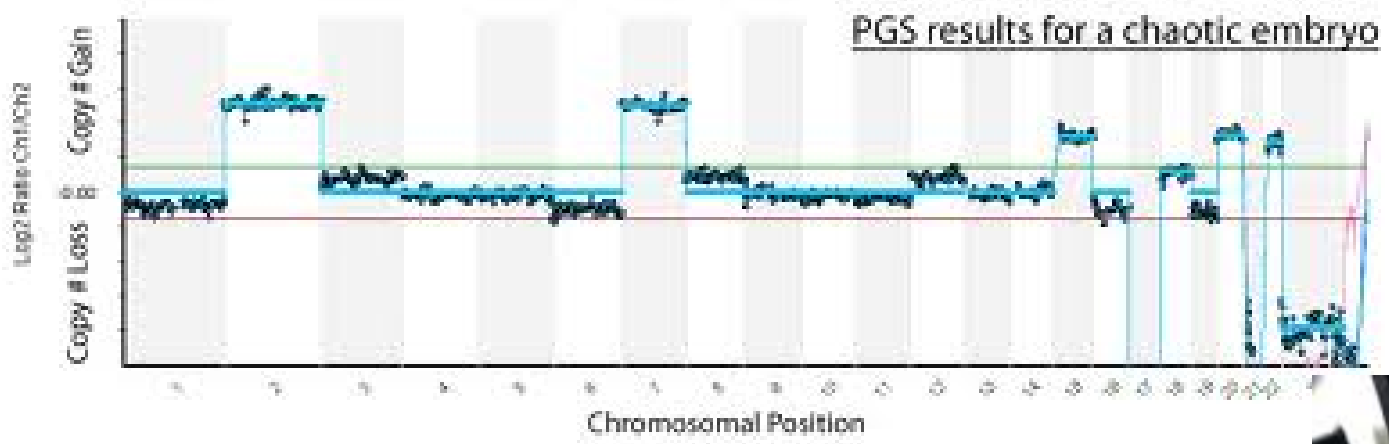
Palini et al., 2013; Gianaroli et al., 2014

Cell-free DNA in spent culture media

Galluzzi et al., 2015; Shamonki et al., 2016


46,XX,t(12;17)(p13;p13)

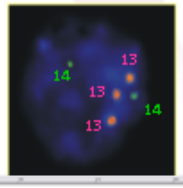

Patient's DNA
Control DNA
Hybridise
Scan
Results


PGS results for a chaotic embryo
Log2 Ratio Ch1/Ch2
Copy # Gain
Copy # Loss
Chromosomal Position

NGS performance for chromosomal translocations

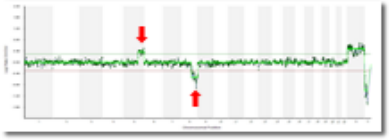
Evolution of PGD for translocations

- **Fluorescence In Situ Hybridisation (FISH)**



- **PCR**-based STR Analysis (Fiorentino et al., 2010)

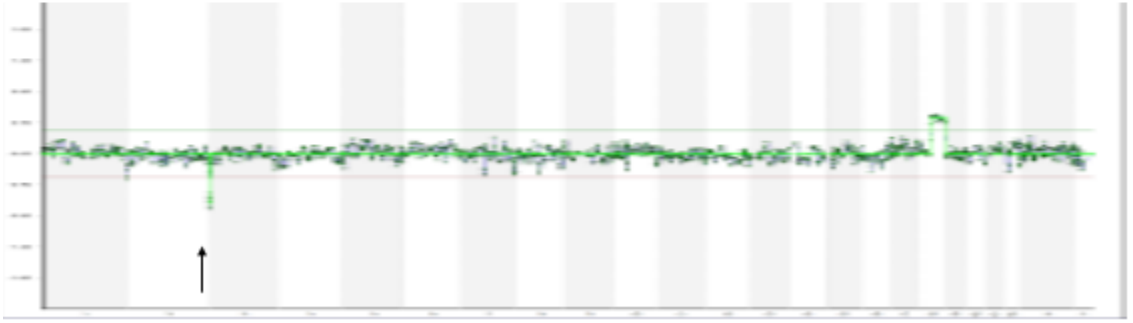
- **A**rray **C**omparative **G**enomic **H**ybridisation (**aCGH**)
(Fiorentino et al., 2011)



- **N**ext **G**eneration **S**equencing (**NGS**) (Bono et al., 2015)

NGS performance for <5Mb size fragments

TRANSLOCATION		Chromosome A[a]		Chromosome B[b]			
		Centric segment[c]	Translocated segment[c]	Centric segment[c]	Translocated segment[c]	NGS	aCGH
46,XY,t(3;10)(p13;q26)	D5	125,5	74,0	134,4	1,0	✗	✗
46,XY,t(3;18)(p26;q12)	D5	197,0	2,8	33,7	42,4	✗	✓
46,XY,t(14;15)(q32;q1)	D5	104,6	1,8	39,0	61.3	✗	✗
46, XY,t(2;11)(q37;p10)	D5	239,8	3.2	88,4	46.1	✗	✗



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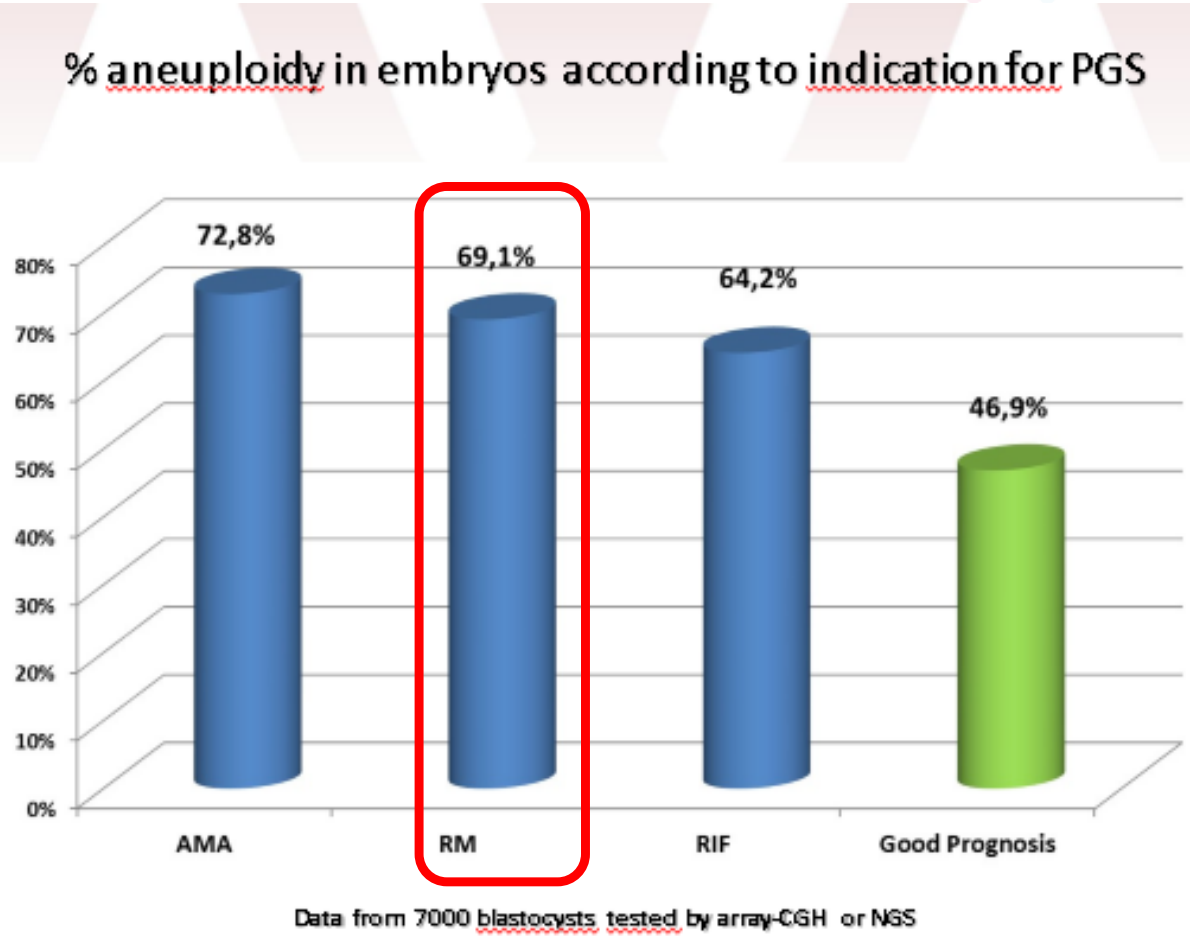
PGS and prevention of recurrent miscarriage: facts

- There is still risk of miscarriage 16-30% after PGS
- We can not completely avoid the miscarriages
- PGS improve pregnancy rate in those patients
- Combined with AMA there is a very small chance for euploid embryo and for deliver a healthy baby

De Rycke et al., 2015 - ESHRE PGD Data collection XII



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ESHRE PGD Consortium data collection XIII: cycles from January to December 2010 with pregnancy follow-up to October 2011

Table Vb
Cycles performed for PGS, data collection XIII.

Indication	AMA	AMA + misc	AMA + RIF	Rec.misc	RIF	SMF	Prev abn preg	AMA + Rec mis Pre abn Preg	Num Abnor	AMA + Num abno	No indication	Ovum donation	AMA + Ovum donation	Total
Cycles to OR	1083	265	312	415	456	278	44	2	33	1	51	37	2	2979
Number infertile	688	162	297	145	405	245	14	0	25	1	43	36	2	2063
Female age (years)	39	37	40	36	32	37	36	42	36	41	37	43	44	39
ART method														
Delivery rate (% per OR/% per ET)	10/17	15/25	5/8	22/28	16/20	22/26	34/42	0	27/32	0	29/38	16/18	50/50	14/21
Miscarriages	33	6	7	18	19	9	1	0	1	0	2	3	0	99
Miscarriage rate (% per clinical pregn – pregn lost to FU)	23	13	30	16	19	12	6	0	9	0	11	33	0	18
Clinical pregnancies lost to FU	2	3	5	5	14	8	1	0	0	0	0	1	0	39

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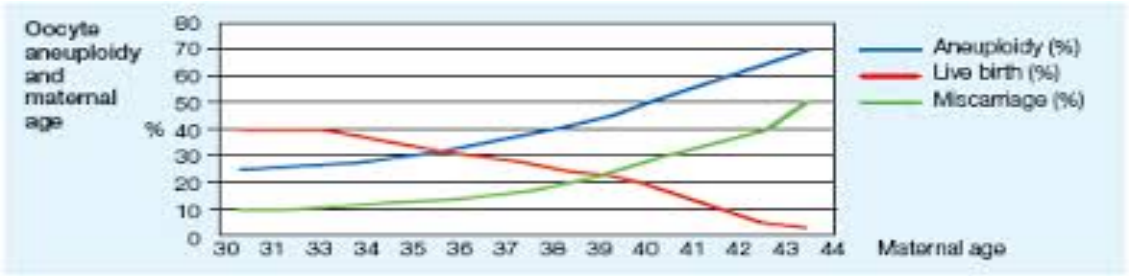
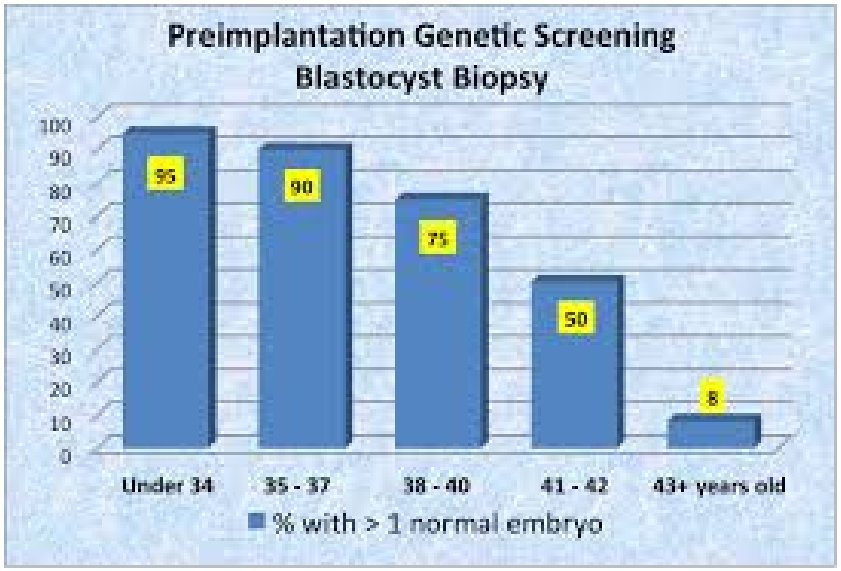
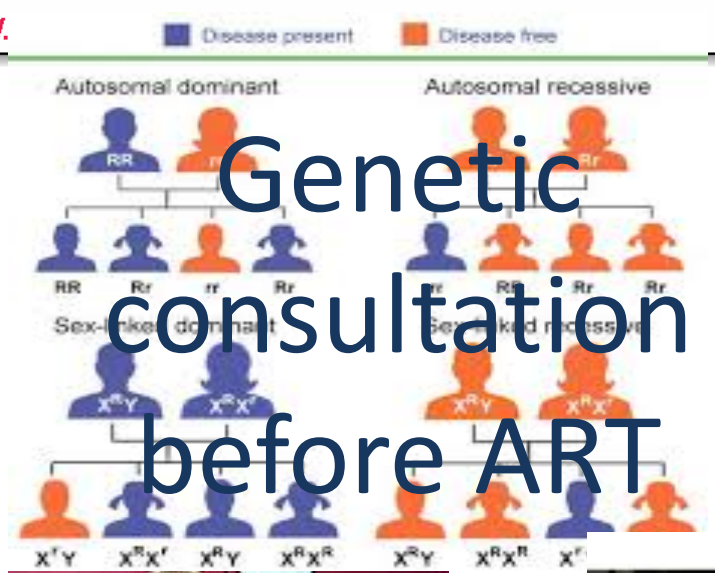




National Institute for Health and Clinical Excellence (2013). Fertility: Assessment and treatment for people with fertility problems; NICE clinical guideline 156.
Dunson DB, Baird DD, Colombo B (2005). Increased infertility with age in men and women. Obstet Gynecol.103(1):51-6.

Genetic consultation before ART



Y-microdeletions
Lachapelle
Morris syndrome
Teacher Collins syndrome

Genetic counseling / TIME

- Medical history – family members
- Evaluation of severity of the genetic problem
- Evaluation of all risks
- Give a realistic expectations
- Information about all processes, including confirmation of results
- Additional testing if needed
- Discussion of other options – pro and cons
- Inform consent

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Seven reasons to be concerned about the use of the new - PGS

1. Do not forget evidence-based medicine

The patients should not be randomized by the number of embryos. Usually, in the new-PGS there are a minimum number of viable blastocysts as a rule to initiate patient randomization.

2. An adverse past and an uncertain future

A minimum of 6 to 8 embryos available for biopsy. AMA? DOR?

3. The trophectoderm is an area of chromosomal variability

The trophectoderm blastocyst biopsy: The aneuploidy rate can be around 70% in day 3 versus approximately 20%-50% in the blastocyst with significant degree of embryo self-correction.

4. Data are missing for several indications as RIF

At the moment, there is no proper RCT was carried out with the PGS-new in populations RIF.

5. Indicating PGS-new for infrequent populations

In 2011, 3 RCTs were published about the use of PGS in patients labeled as good prognosis (Staessen et al., 2008; Jansen et al., 2008; Meyer et al., 2009). Back then, they did not find significant differences in *terms of live birth rate* among patients with or without PGS.

The original intent of this study was to improve *IVF pregnancy rates*. As this failed, their original intent was replaced by the listed secondary goal of this study: reduction of twin pregnancies at elective single transfer (Gleicher et al., 2014).

Jose Franco, JBRA, 2015

6. Concerns about **extended culture to the blastocyst stage**

- Blastocyst stage in vitro cultures risks: 1- prolonged embryo culture has been related to significant *epigenetic changes* (Lonergan et al., 2003; Calle et al., 2012); 2- blastocyst stage culture are associated with increased *risk of premature delivery* in comparison to embryos transferred on days 2 or 3 (Maheshwari et al., 2013; Dar et al., 2014).

7. **Non-maleficence**

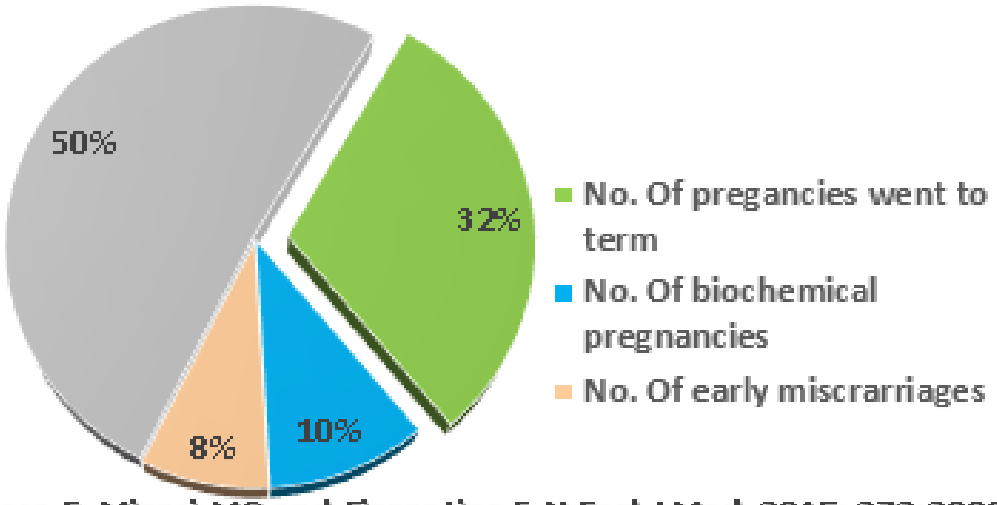
- In 2008, ASRM, ESHRE and the British Fertility Society declared that PGS (day 3 biopsy + FISH technique) is *ineffective* in improving IVF pregnancy rates and reducing miscarriage. Seven years later, these societies have not yet settled for or against PGS-new (day 5-6 biopsy + CGH or qPCR or NGS), as well as the Brazilian Society of Assisted Reproduction (SBRA).

Jose Franco, JBRA, 2015

- Mosaics embryos: 55% до 73% [Munne et al., 2006; Bielanska et al, 2002];

Mosaic embryos can develop into healthy newborns

Clinical outcome	No.
No. of ET	49
No. of embryos tranferred	50
No. of +βhCG pregnancies	24
No. of biochemical pregnancies	5
No. of early miscarriages	4
No. of ongoing clinical pregnancies per ET	15 (30.6%)
No. of pregnancies went to term	15 (30.6%)
No. of babies born	16



Updated data from: Greco E, Minasi MG and Fiorentino F. N Engl J Med 2015; 373:2089-90

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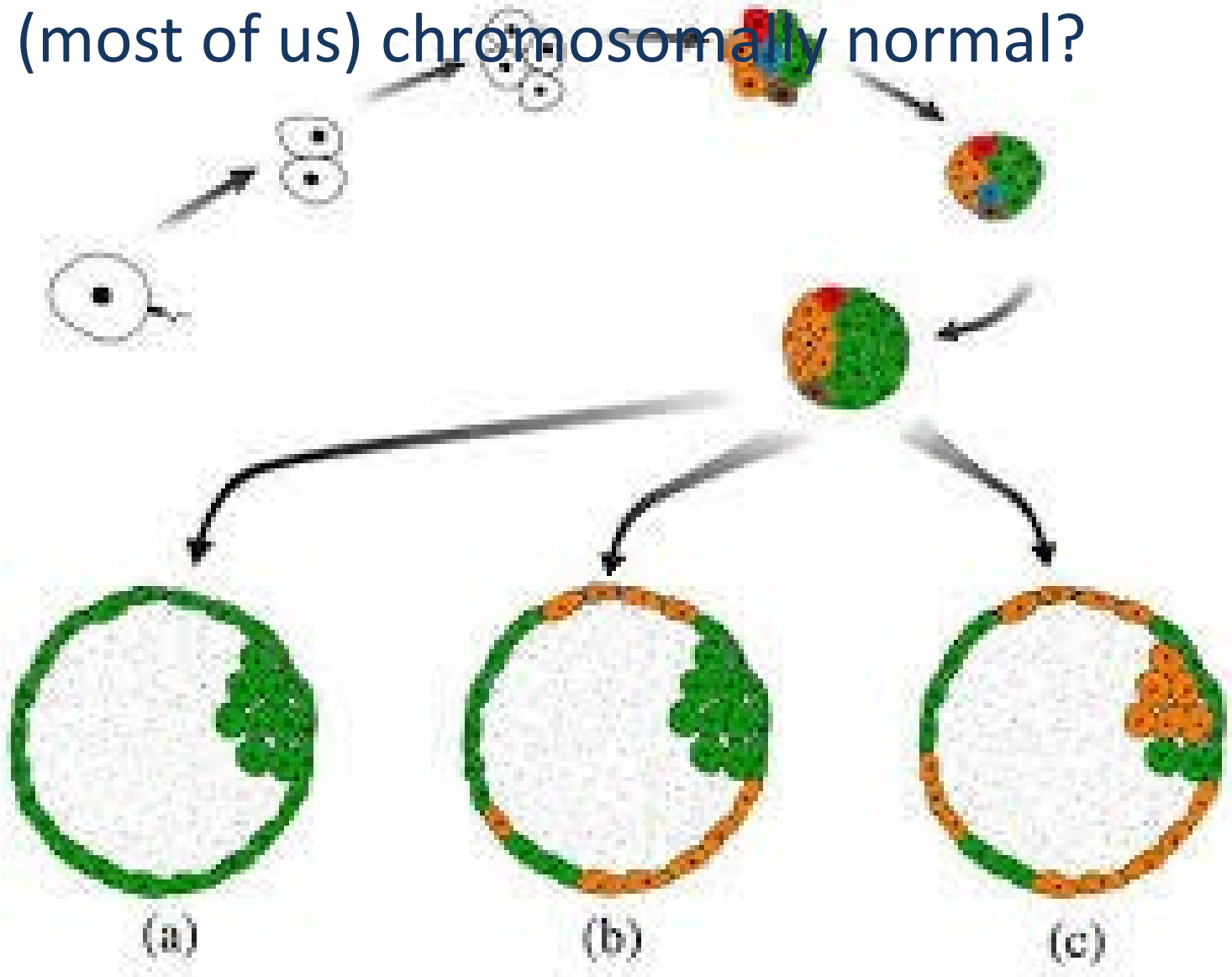
Why are (most of us) chromosomally normal?



Survival of the fittest cell (Robberecht, 2010);

20-30% of de novo unbalanced translocations: postzygotic origin;

PGS and prevention of recurrent miscarriage: facts and myths

Facts:

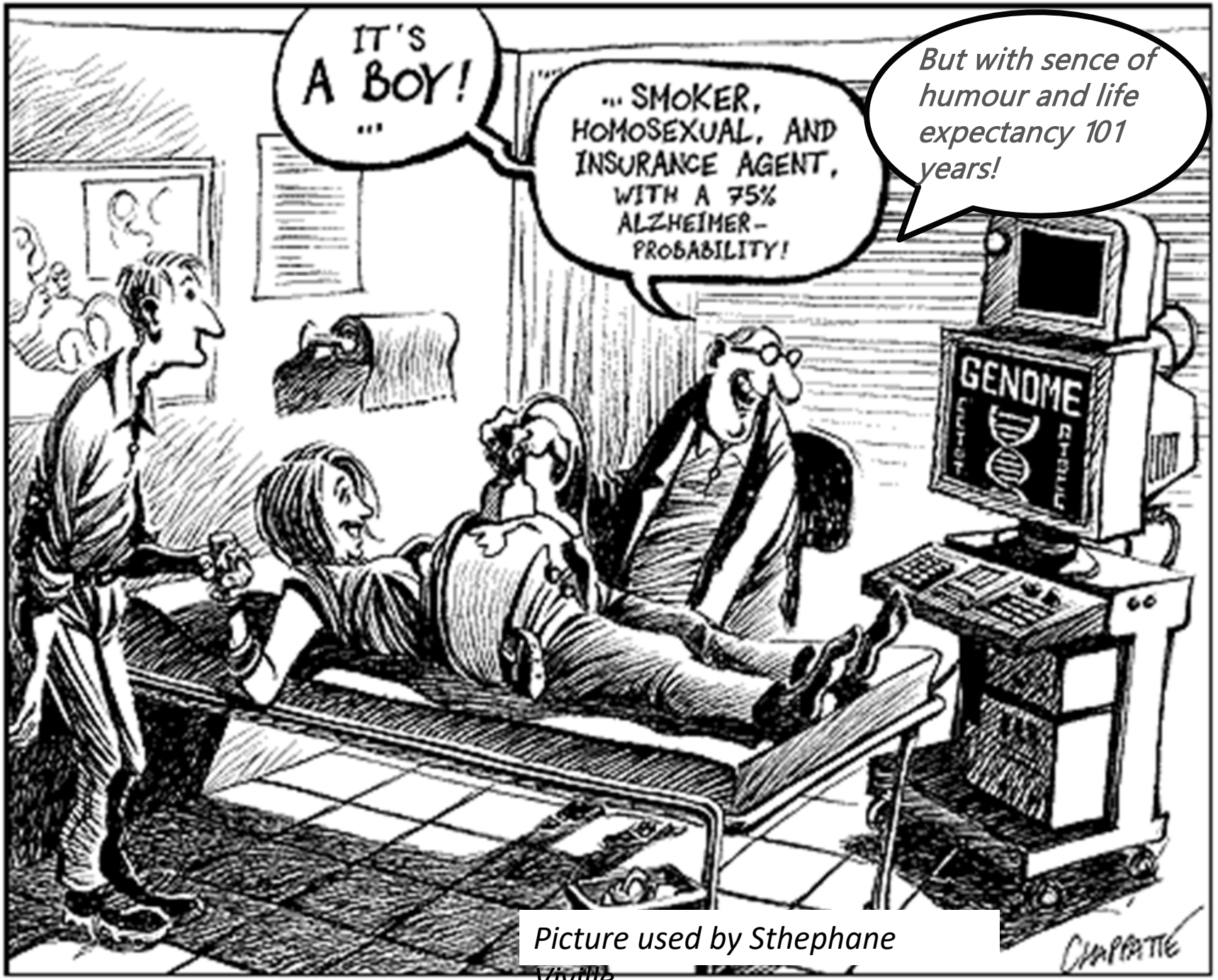
- Many embryos have *'self-correct' mechanism reaching the blastocyst stage and also after this stage (up to* 4%);
- Near 70% of embryos in patient with RM are aneuploid;
- 28% Delivery Rate per ET in young female patients;
- RM – there is 16-18% risk of miscarriage;
- 0% Delivery Rate in patients with RM, when is combined with AMA and previous abnormal pregnancy;

Myths:

- We can completely avoid the miscarriage risk by using PGS in couples with RM
- There is a high chance for euploid embryos after PGS
- There is a high chance for delivery in AMA cases

Johnson et al., 2010; Baart et al, 2007; Munne et al., 2005; De Rycke et al., 2015 - ESHRE PGD Data collection XII

Still nearly 30% of patients with RM have chance for take-home baby after PGS if there is no issue of AMA



Picture used by Sthephane Viville

Conclusions:

1. In the future: Are we be able to find the perfect embryo with the fast development of NGS?
2. How many embryos per couple we will need?
3. Is there a perfect human being at all?
4. For now we are capable to chose the less affected embryo among all others?
5. New data shows that mosaic embryos can develop into a healthy newborn in 32% of all cases.
6. Every case must be well discussed and genetically consulted prior and after PGS

The complexity and the wonder of life…..A simple friendship, a sunny day, our life

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