

RESEARCH INTO INFERTILITY

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VRIJE UNIVERSITEIT BRUSSEL



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Outline lecture

- **Introduction**
- Complimentarity research on gametes, embryos and hESC
- PGD-PGS clinical research
- Causes of adverse ART outcome
- Conclusions

Introduction

- HFEA regulates research on human embryos and research topics are related to a limited number of purposes
- Currently 33 research projects are licensed
 - 13 (40%) related to hESC
 - 11 related to embryology
 - 9 related to reproductive genetics including PGD/PGS
- There is a shift of research projects towards stem cell biology

Introduction

- Stem cell research can be complimentary to research on gametes or human preimplantation embryos
- Clinical research related to effectiveness of PGD/PGS
- Research into pathogeny of adverse outcome after ART

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Complimentarity research on embryos and hESC

- Embryo research in Belgium is regulated by law (May 2003)
- Liberal law with oversight
- Two prohibitions in the law
 - Reproductive cloning
 - Sex selection on gametes and embryos for non-medical reasons

CONCERTED ACTION
RESEARCH CENTRE REPRODUCTION AND GENETICS
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**GENETIC AND EPIGENETIC REGULATION IN HUMAN
GAMETES, PREIMPLANTATION EMBRYOS AND
EMBRYONIC STEM CELLS AND THE EFFECT OF
GENETIC MUTATIONS ON DEVELOPMENT AND
FERTILITY**

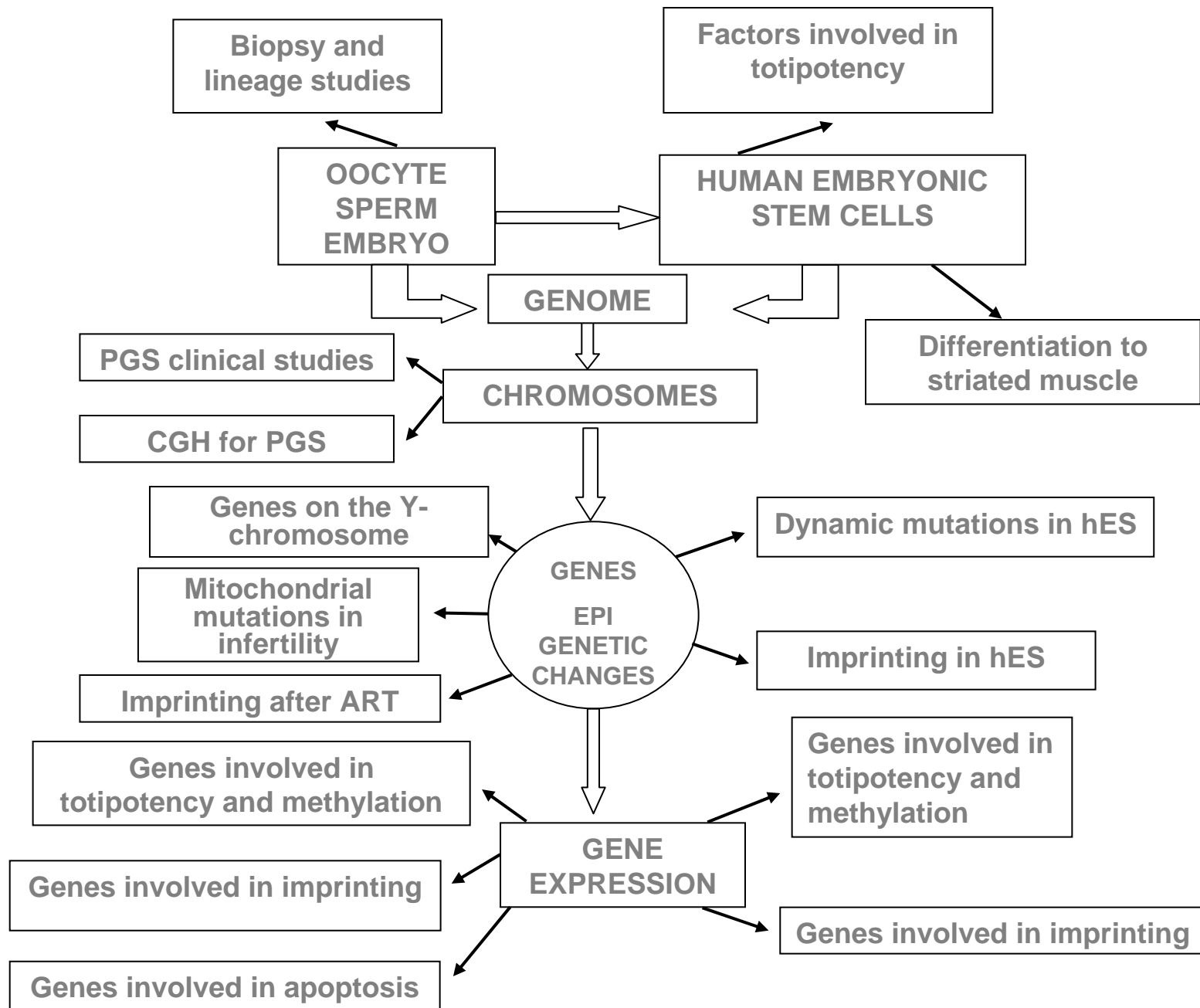


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PGD stricto sensu

- One-cell versus two-cell biopsy
 - Accuracy of diagnosis
 - Outcome: chance for couple to have a child
 - RCT needed and ongoing
- Better diagnostic procedures
 - Comparative genomic hybridization
 - Microarray PCR
 - Combination of PCR-FISH for genes and chromosomes





PREIMPLANTATION GENETIC
DIAGNOSIS BY ANEUPLOIDY
SCREENING (PGD - PGS)

What do we know about its
effectiveness?



- Pioneering work from Verlinsky et al., Munné et al., Gianaroli et al., has indicated that many oocytes and embryos are chromosomally abnormal when FISH for several chromosomes is applied on polar bodies or blastomeres → many transferred embryos with “decent” morphology will not implant
- PGS is currently widely used in ART practice to increase “success rate”

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- Most commonly used indications where PGS is carried out are 1) advanced maternal age, 2) repeated implantation failure, 3) recurrent miscarriage and ...
 - What does the current literature tell us about the effectiveness of PGS?
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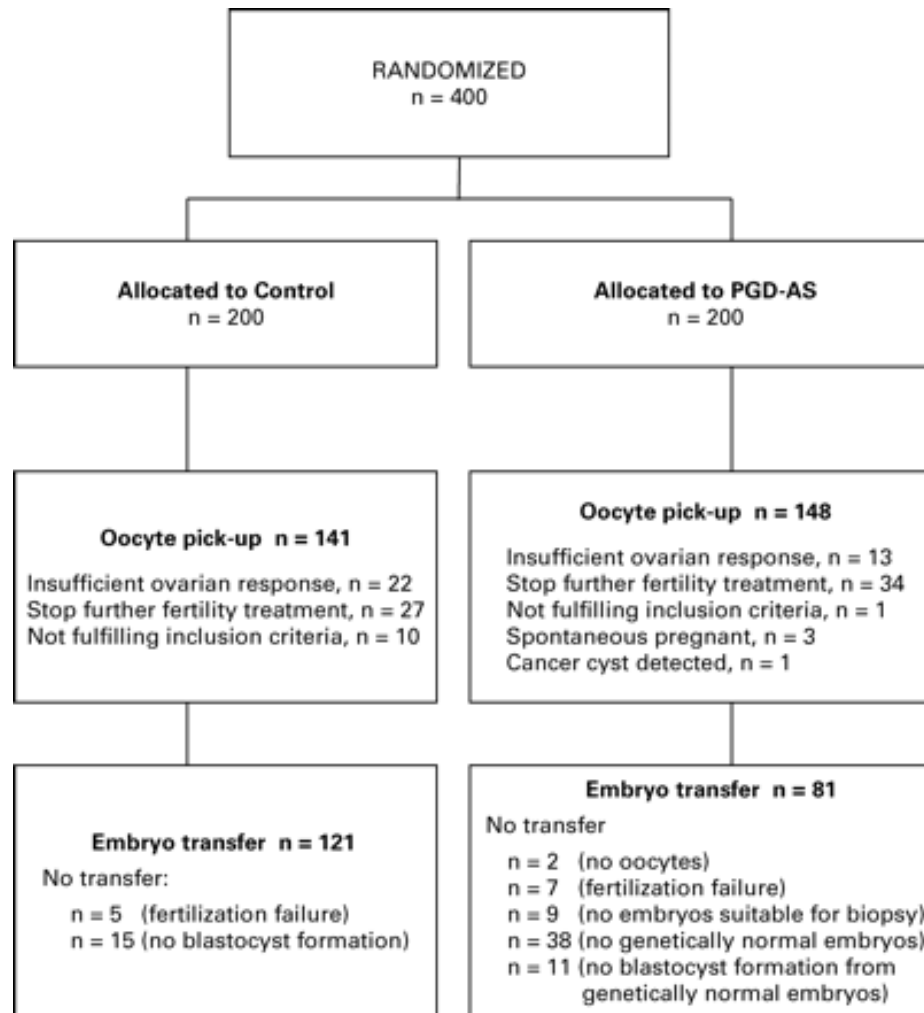
- Effectiveness of any ART treatment needs to be assessed by the number of live born (healthy, singleton) children per started treatment
- In none of the studies this outcome measure is clearly indicated
- Surrogate outcome measures in PGS literature are 1) implantation rate per embryo transferred, 2) pregnancy (HCG increased), 3) clinical pregnancy per cycle and 4) number of miscarriages per clinical pregnancy

- It is furthermore unclear whether number of cycles is 1) number of started treatments i.e. hormone stimulation or 2) number of times oocyte retrieval is carried out

PGS for advanced maternal age

- Only two randomized trials on effectiveness of PGS for advanced maternal age:
 1. Werlin et al., 2003 (Fertil Steril 80, 467, 2003): only 7 PGS and 12 control patients
 2. Staessen et al., 2004 (Hum Reprod 19, 2849, 2004): 400 couples, 148 with PGS and 141 control no difference in ongoing pregnancies between 2 groups

Patient flow through the randomized controlled trial



PGS for maternal age - randomized studies

Outcome measure	Ref	PGS	%	Control	%	OR CI 95%	RR CI 95%
Implantation (per embryo)	2	28/164	17.1	39/338	11.5	1.58 (0.90-2.76)	1.48 (0.95-2.32)
HCG + (per started cycle)	1.2	32/207	15.5	42/212	19.8	0.74 (0.43-1.26)	1.48 (0.51-1.19)
Clinical pregnancy (per started cycle)	2	22/200	11.0	30/200	15.0	0.70 (0.37-1.31)	0.73 (0.44-1.23)
Ongoing pregnancy (per started cycle)	2	22/200	11.0	29/00	14.5	0.73 (0.39-1.37)	0.76 (0.45-1.27)
Miscarriages per clinical pregnancy	2	0/22	0	1/30	3.3	-	-

Comments on Staessen et al. RCT

- Different number of embryos transferred in control and PGS group
- Less transfers in PGS group
- Statistical comments are made on implantation rate of embryos while randomisation was done per female patient → methodology is not optimal

PGS for advanced maternal age

- Analysis of six comparative studies:
 1. Gianaroli et al. Hum Reprod 12, 1762, 1997
 2. Gianaroli et al. Fertil Steril 72, 837, 1999
 3. Munné et al. Hum Reprod 14, 2191, 1999
 4. Kahraman et al. Hum Reprod 15, 2003, 2000
 5. Obasaju et al. RBM Online 2, 98, 2001
 6. Munné et al. RBM Online 7, 91, 2003

PGS for advanced maternal age

- Remarks on some papers: control group not adequate (4), only data on implantation and miscarriages (3), some of data in more than one publication (1-3), age minimum varies from 35 (3,6) to 40 years (5), different number of embryos transferred in PGS and control group

PGS for maternal age - comparative studies

Outcome measure	Ref	PGS	%	Control	%	OR CI 95%	RR CI 95%
Implantation (per embryo)	2,3, 6,5	150/781	19.2	39/338	11.5	1.58 (0.90-2.76)	1.48 (0.95-2.32)
HCG + (per cycle not started)	No data						
Clinical pregnancy (per cycle)	2 5	28/100	28.0	44/153	28.8	0.96 (0.53-1.75)	0.97 (0.65-1.45)
Ongoing pregnancy (per cycle)	No data						
Miscarriages per clinical pregnancy	2 3 5	8/70	11.4	22/79	27.8	0.33 (0.13-0.87)	0.41 (0.2-0.86)

- Notwithstanding limitations of the studies PGS at advanced maternal age increases implantation rate of transferred embryos. There is no evidence of increased clinical pregnancy rate
- PGS seems to reduce the number of miscarriages per clinical pregnancies
- So far, however, there are no data on clinical pregnancies per started cycle or number of live births per started cycle
- The effects of PGS increase with age and when more embryos are available for FISH analysis

Discussion, recommendations

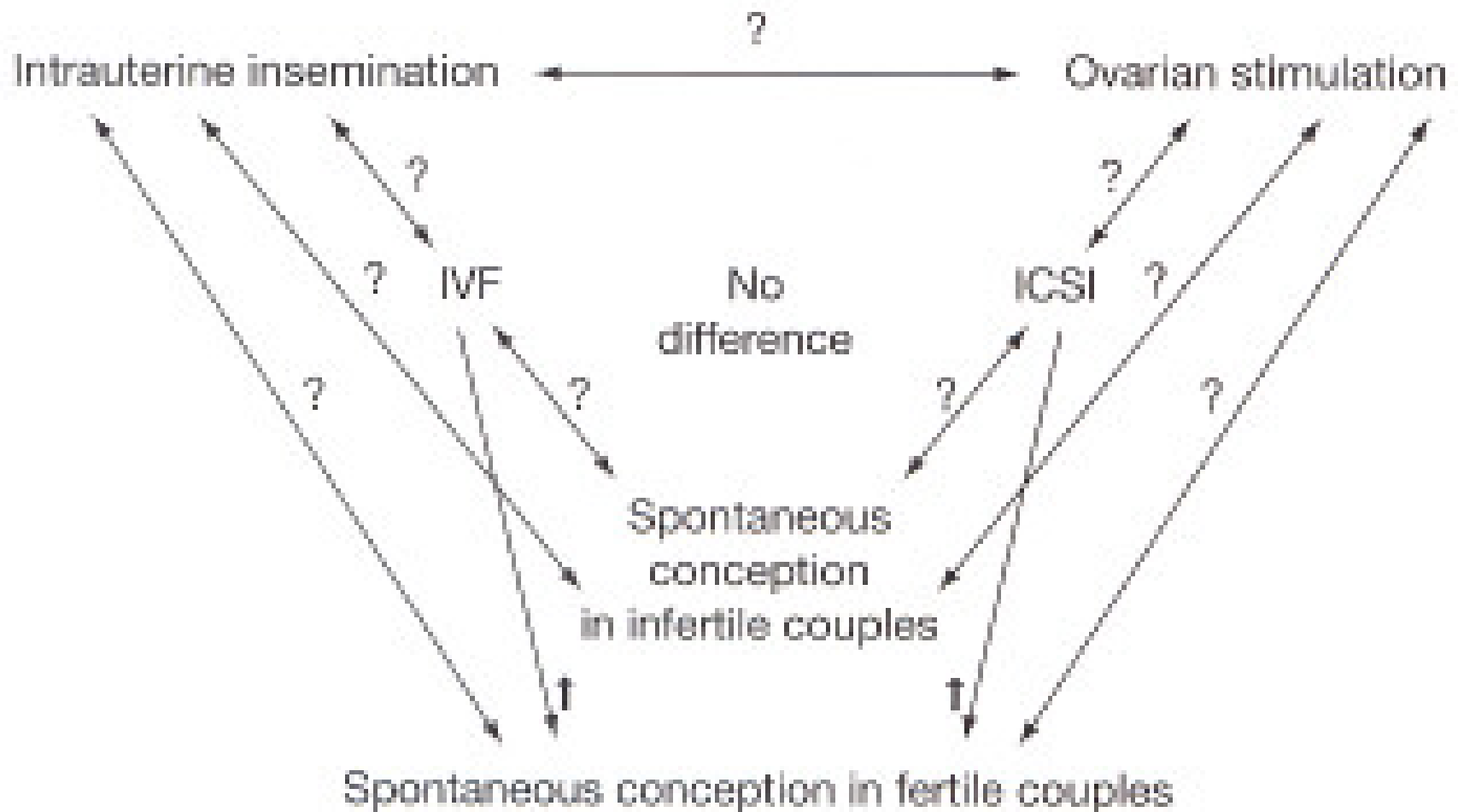
- The concept of PGS is valid
- Data to evaluate effectiveness are of suboptimal quality
 - RCT versus comparative studies
 - Primary endpoint should be number of live born (singleton) children per started cycle
- One-cell versus two-cell biopsy debate PGD versus PGS
- Pregnancy and children outcome

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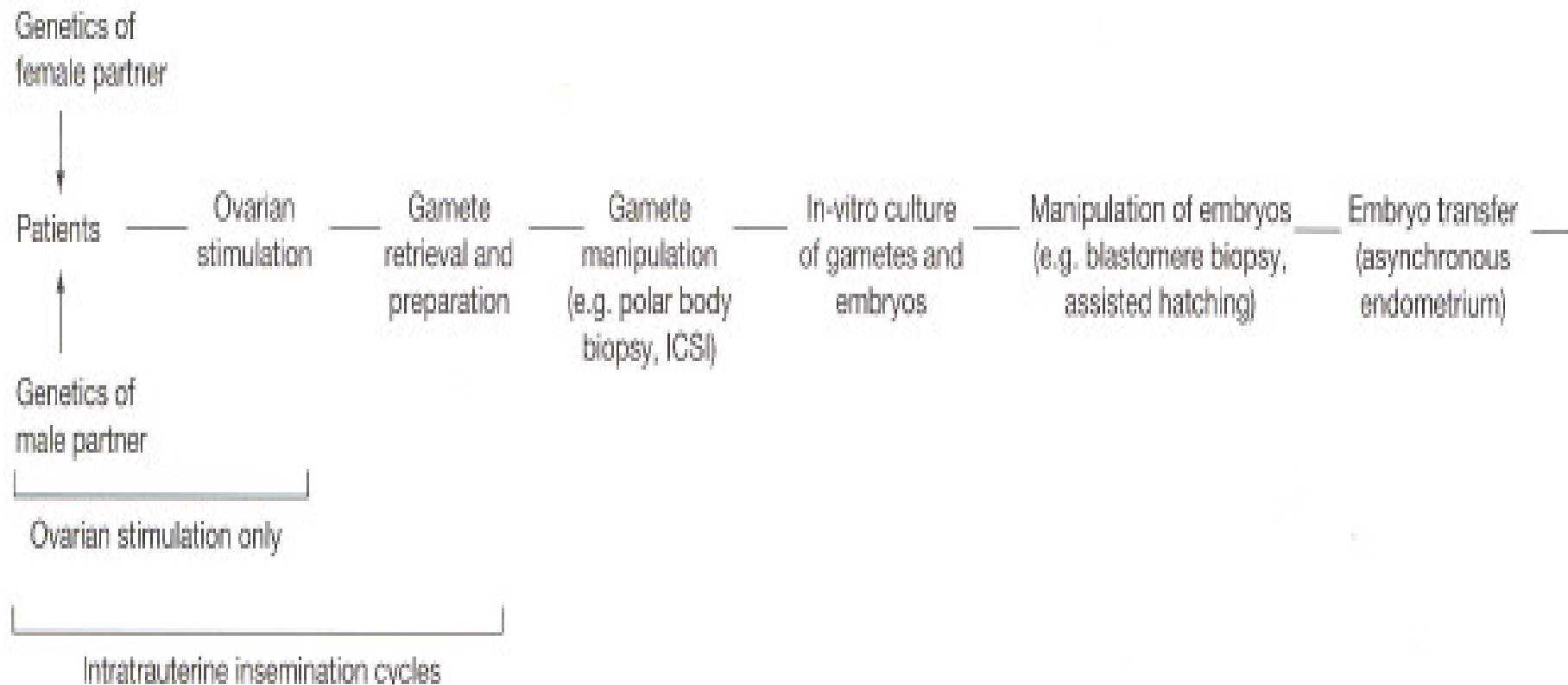
Facts



- Data only available for IVF/ICSI outcome versus spontaneous conceptions
 - More prematurity in singletons
 - Malformations at birth similar in IVF and ICSI (~3%) but slightly higher than in spontaneous conception
- Rare disorders (genomic imprinting) may be more common



Multiple pregnancies after all forms of ART

- The major problem
- Estimating that $\sim 2 \times 10^6$ children have been born after ART, at least half of these children are not from singleton deliveries !



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- Question remains why the risks are increased: in-vitro culture, infertility per se?
 - Studies are needed to assess risks of malformations after ovarian stimulation alone or after intrauterine insemination → contribution of in-vitro culture conditions
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Genomic imprinting disorders

- Case-control studies indicate increase but absolute risk remains low since disorders are very rare
- Why this increase?
 - Ovarian stimulation?
 - In-vitro culture?
 - Infertility per se?
 - Severe male factor infertility?



HOW TO COPE WITH IATROGENIC EPIDEMIC
OF MULTIPLE PREGNANCIES?



What to say to the parents?

- Multiples pregnancies remain an important risk factor
- Slightly higher risk of inherited chromosomal anomalies in relation to parental chromosomes and de novo (1-2%) anomalies related to sperm quality
- Slightly higher risk of a premature and lighter neonate

What to say to the parents

- Slightly higher risk of major malformations not excluded (3.5% instead of 2.5%) mostly in relation to maternal age, infertility and underlying parental disease
- Psycho-motor development normal, neuro-developmental outcome might be influenced by neonatal problems
- Increase of very rare disorders remains possible

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Conclusions

- Basic research shifted to include hESC
- Gamete and embryo research can be complimentary
- Clinical research topics needed
 - PGD-PGS
 - Adverse ART outcome
 - Epidemic of multiple pregnancies