IMPACT OF SPERM QUALITY ON EMBRYO VIABILITY

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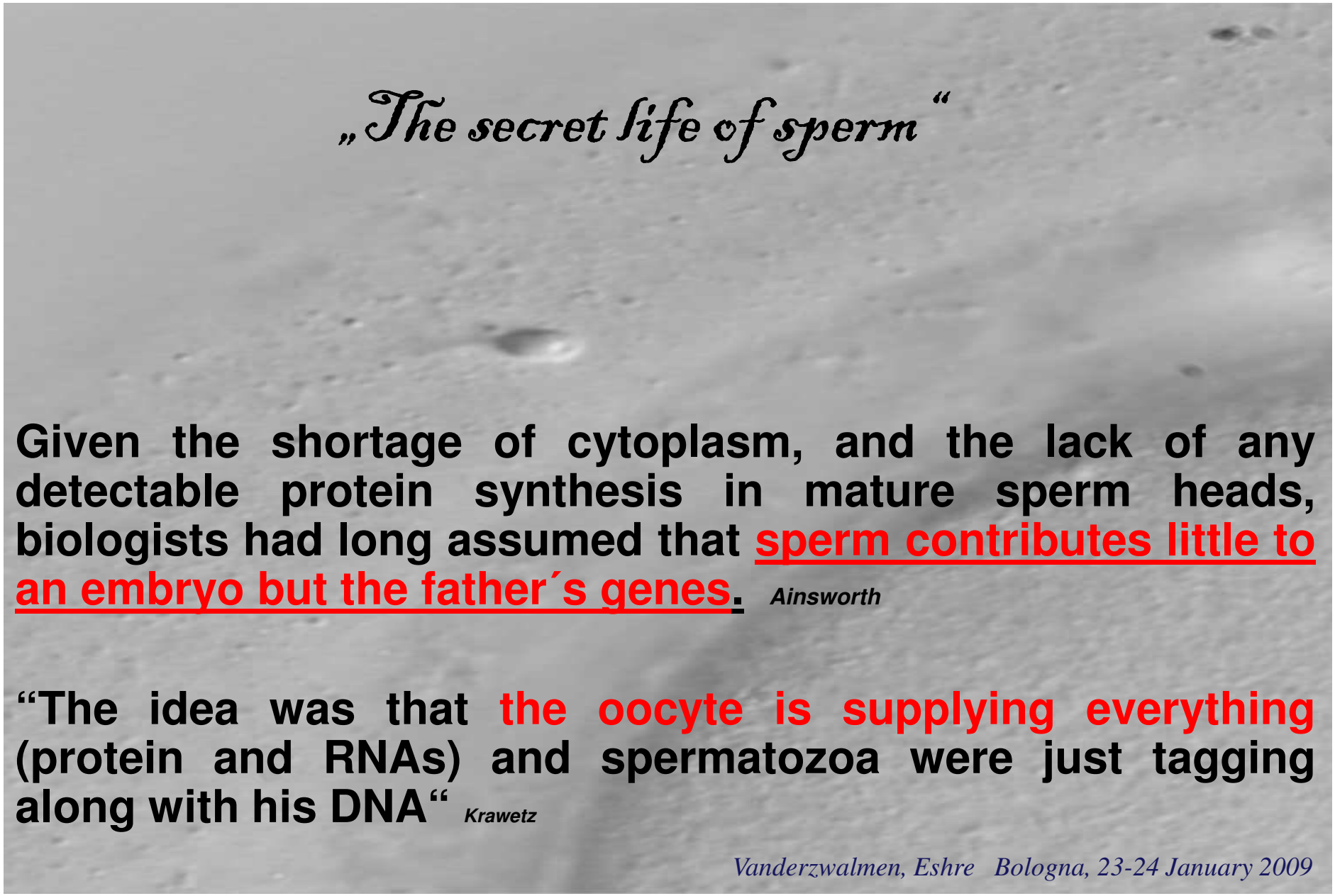
LEARNING OBJECTIVES

- To define the sperm contribution to the developing embryo
- To describe the current indicators of sperm quality
- To revise the methods for sperm selection for ICSI



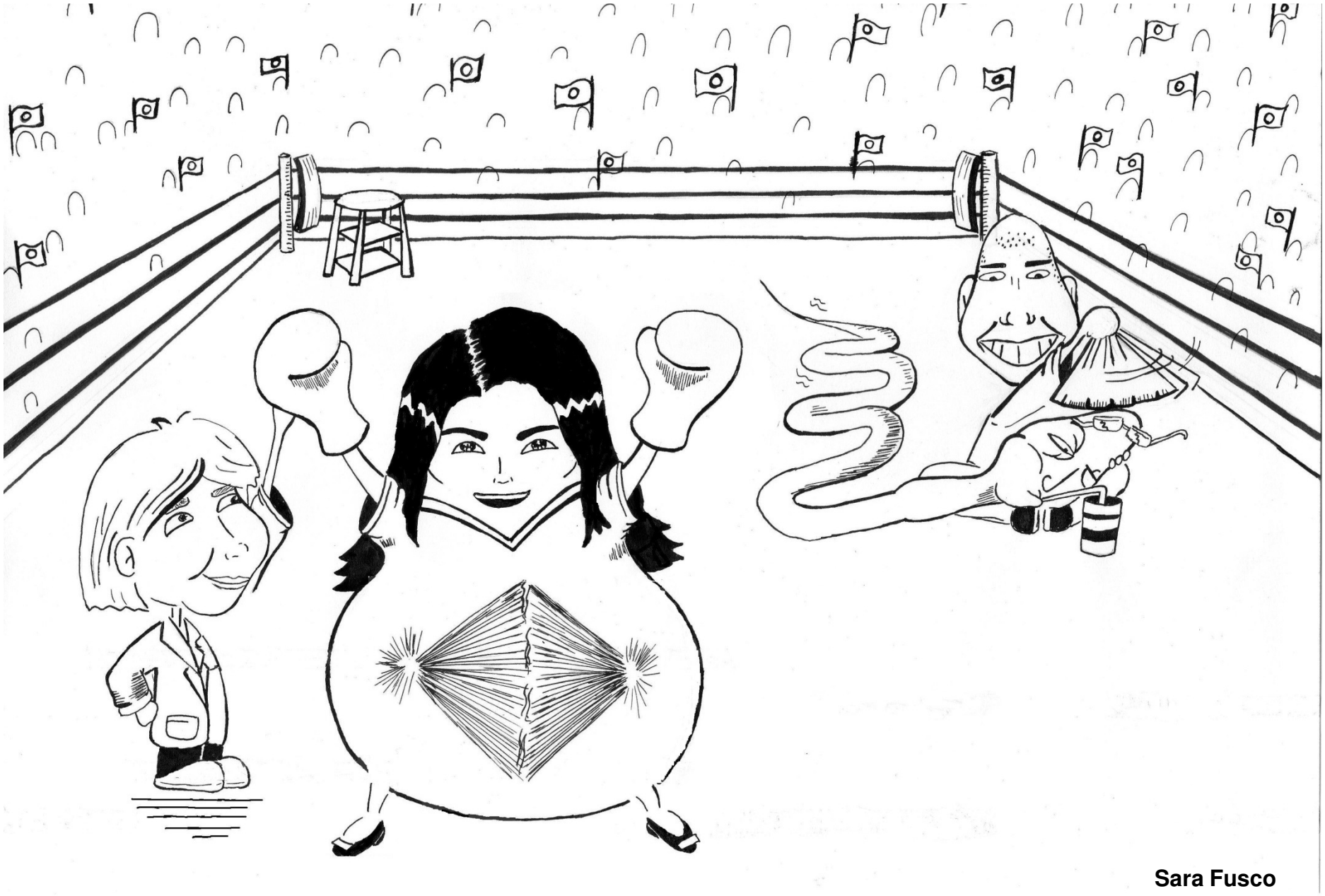
„The secret life of sperm“

- **Given the shortage of cytoplasm, and the lack of any detectable protein synthesis in mature sperm heads, biologists had long assumed that sperm contributes little to an embryo but the father´s genes.** ***Ainsworth***

- **“The idea was that the oocyte is supplying everything (protein and RNAs) and spermatozoa were just tagging along with his DNA“** ***Krawetz***

Vanderzwalmen, Eshre Bologna, 23-24 January 2009

Ainsworth C. Cell biology:the secret life of sperm. Nature 2005;436(7052):770-771.
Krawetz SA 2005 Paternal contribution: new insights and future challenges. Nature Reviews. Genetics 6, 633–642.



Sara Fusco

SPERM PARAMETERS AND NATURAL CONCEPTION

There is a correlation between the characteristics of a semen sample and the likelihood for conception *in vivo.*

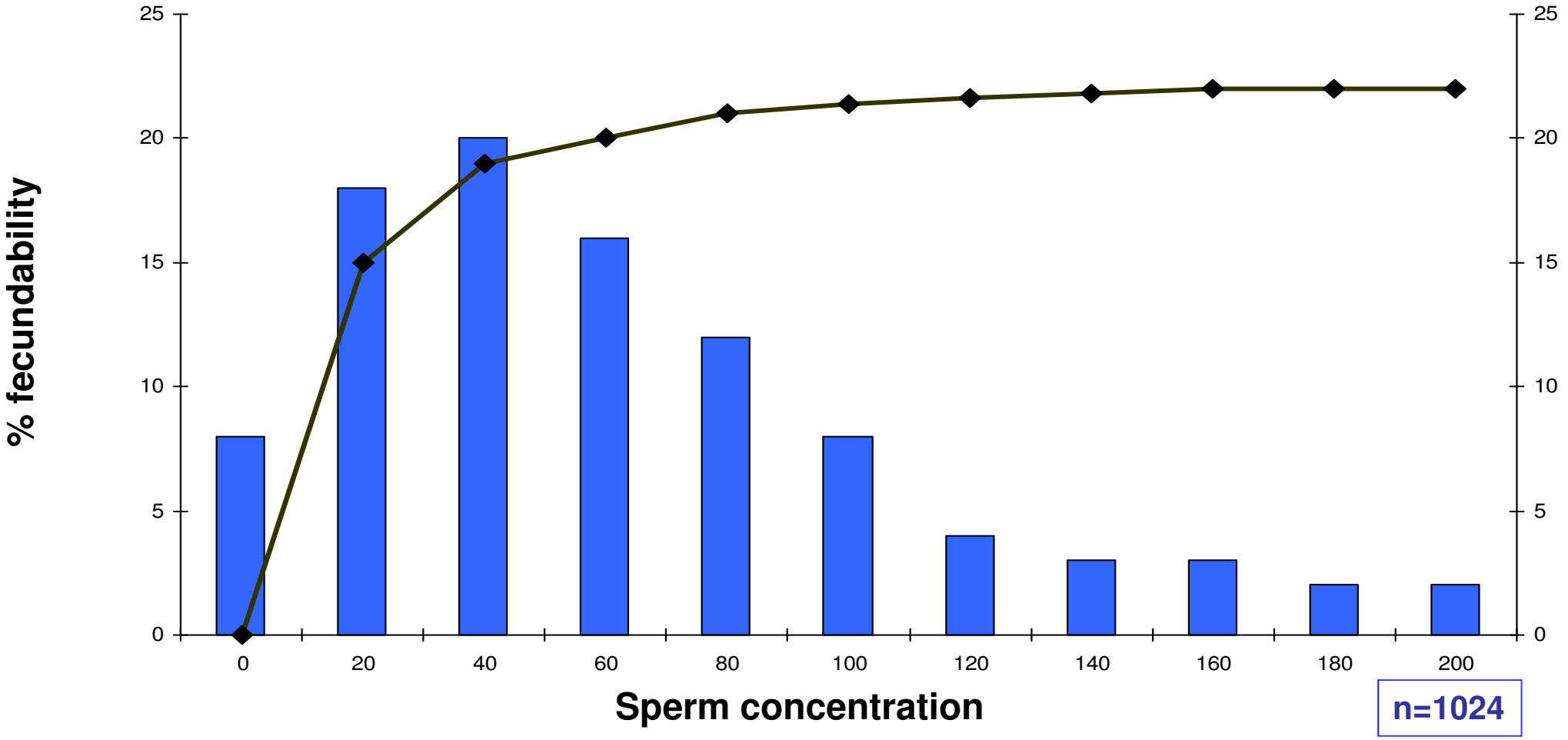
Levels where fecundity starts to decrease

	Seminal volume	< 2ml
→	Concentration	< 40 mill/ml
	Motility	<50%
→	Morphology	< 40% normal

n=430

Bonde JP, Ernst E, Jensen TK, Hjollund NH, Kolstad H, Henriksen TB, Scheike T, Giwercman A, Olsen J, Skakkebaek NE. Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. Lancet 1998;352:1172-1177.

SPERM PARAMETERS AND NATURAL CONCEPTION



Bonde JP, Hjollund NH, Kolstad HA, Abell A, Larsen SB. Environmental semen studies--is infertility increased by a decline in sperm count? Scand J Work Environ Health 1999;25 Suppl 1:12-16.

PATERNAL AGE AND MISCARRIAGE RATES

17,000 IUI

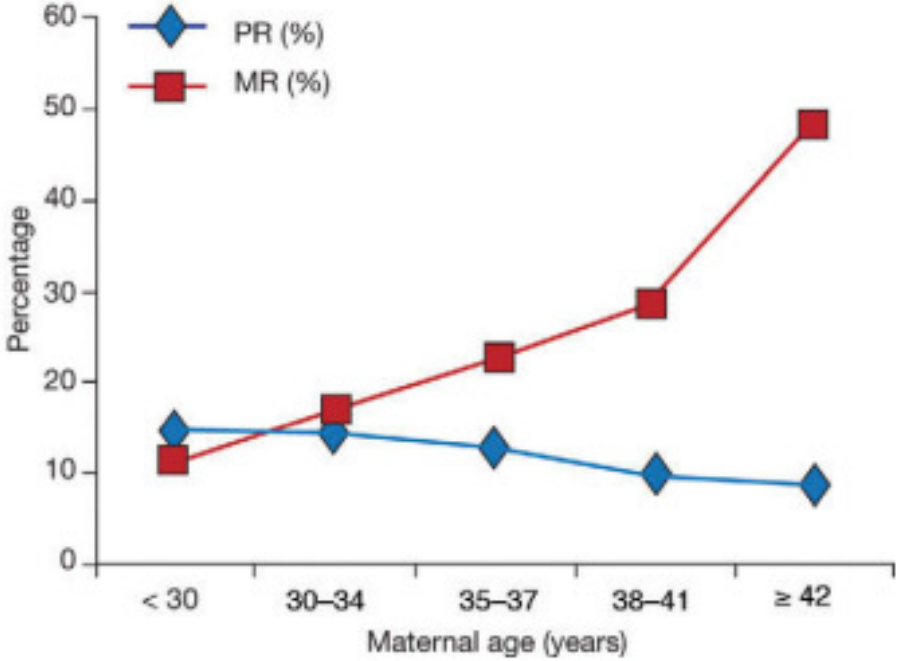


Figure 1. Clinical pregnancy rates (PR) and miscarriage rates (MR) in intrauterine insemination cycles according to maternal age.

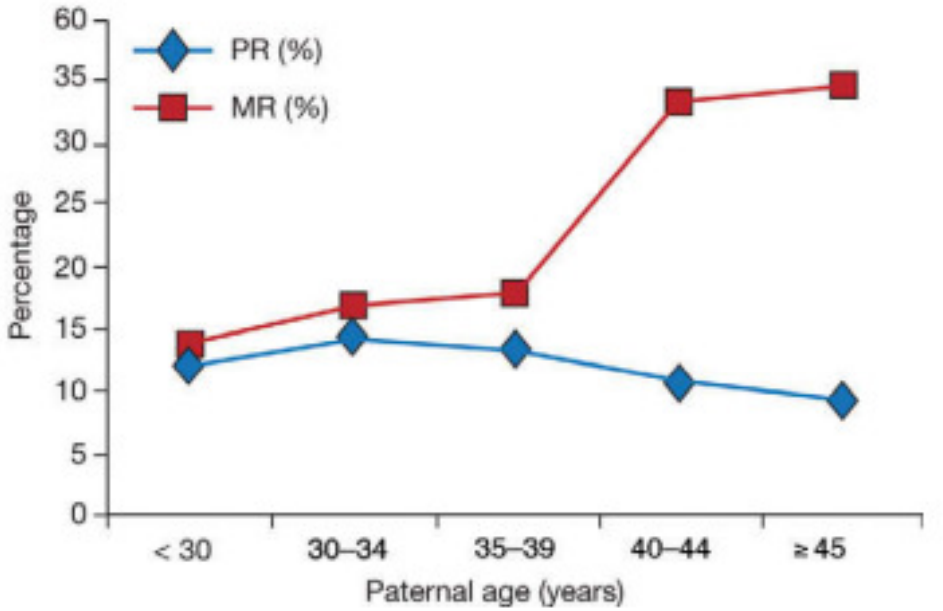
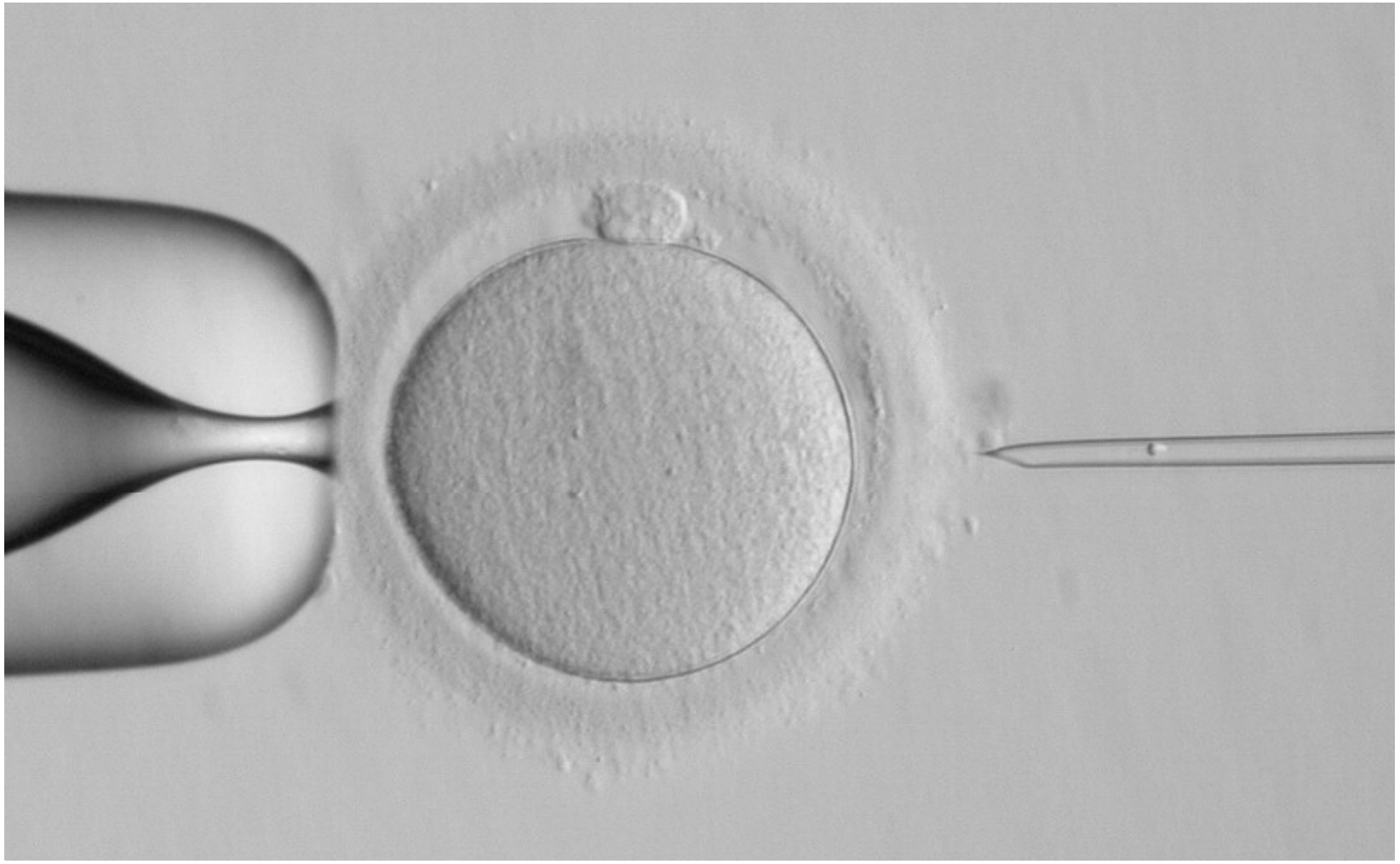


Figure 2. Clinical pregnancy rates (PR) and miscarriage rates (MR) in intrauterine insemination cycles according to paternal age.

necrospermia and sperm DNA structure ←

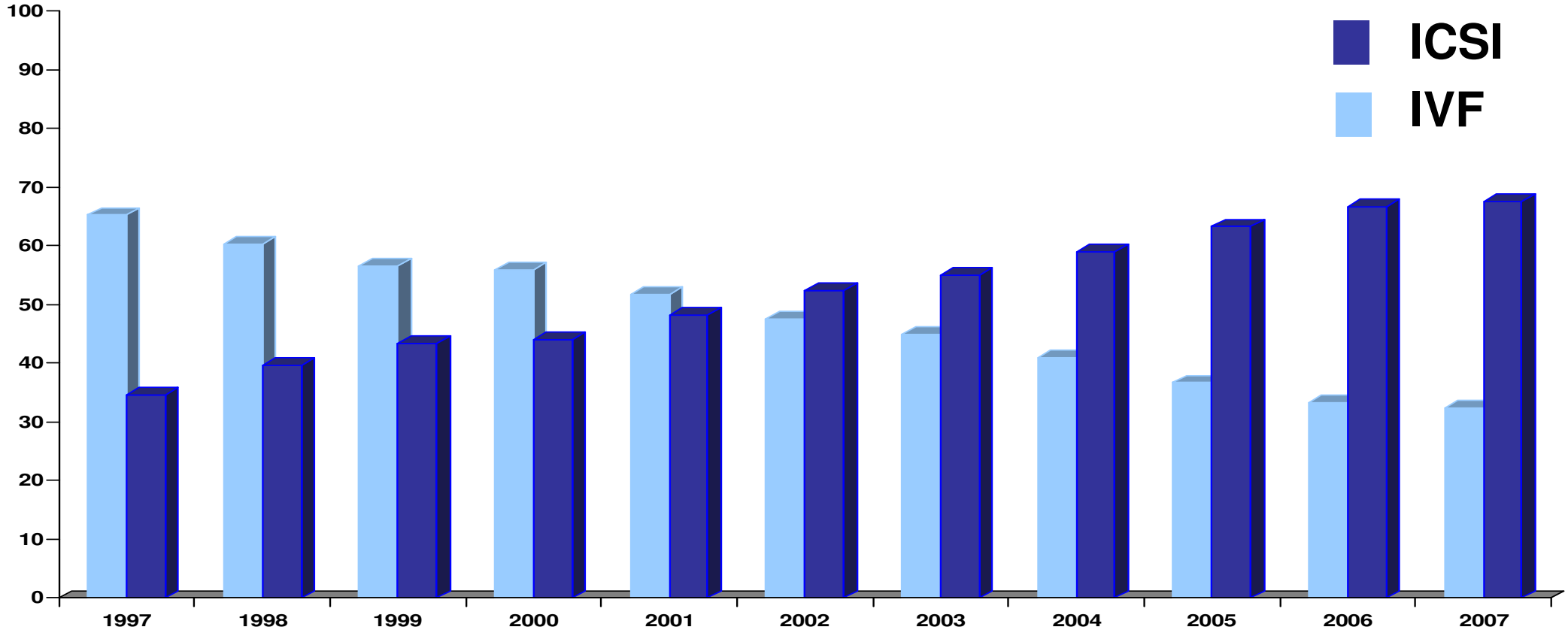
Belloc S, Cohen-Bacrie P, Benkhalifa M, Cohen-Bacrie M, De Mouzon J, Hazout A, Ménézo Y. Effect of maternal and paternal age on pregnancy and miscarriage rates after intrauterine insemination. Reprod BioMed Online 2008;17:392–397.



DISTRIBUTION IVF / ICSI CYCLES (1997-2007)

EIM

32 countries → 479288 cycles



Update to: De Mouzon J, Goossens V, Bhattacharya S, Castilla JA, Ferraretti AP, Korsak V, Kupka M, Nygren KG, Nyboe Andersen A. The European IVF-monitoring (EIM) Consortium, for the European Society of Human Reproduction and Embryology (ESHRE). Assisted reproductive technology in Europe, 2006: results generated from European registers by ESHRE Hum Reprod 2010;25:1851–1862.

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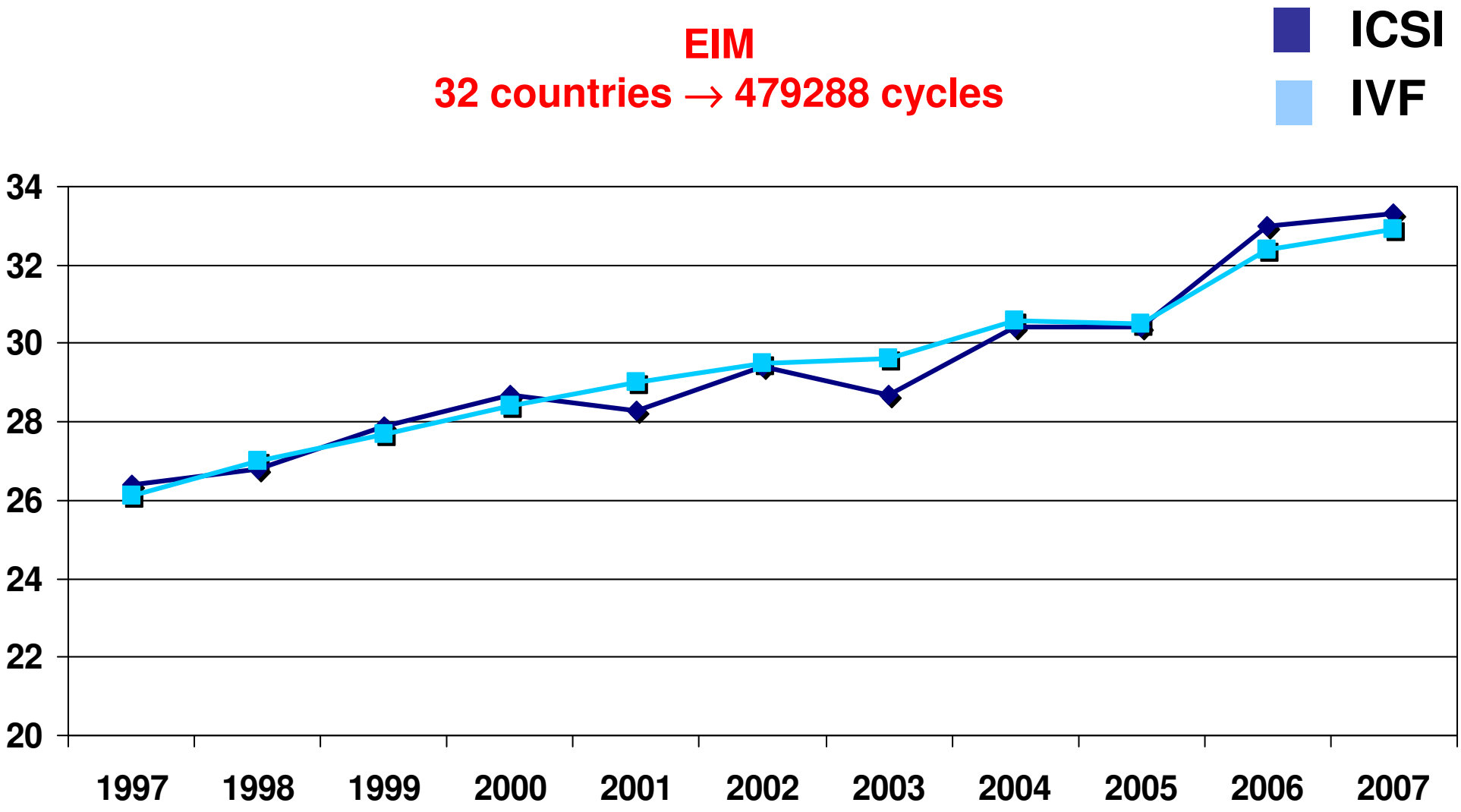

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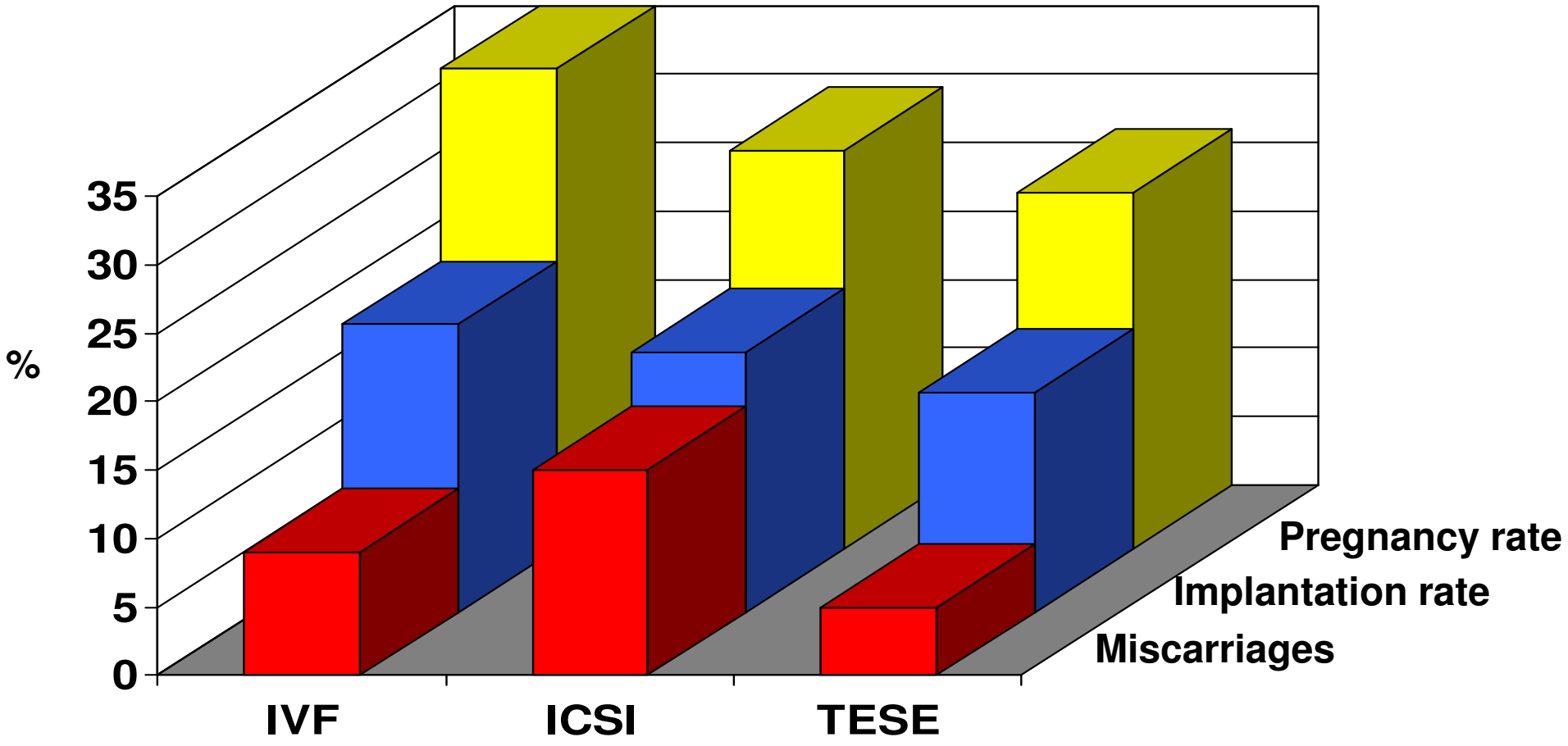
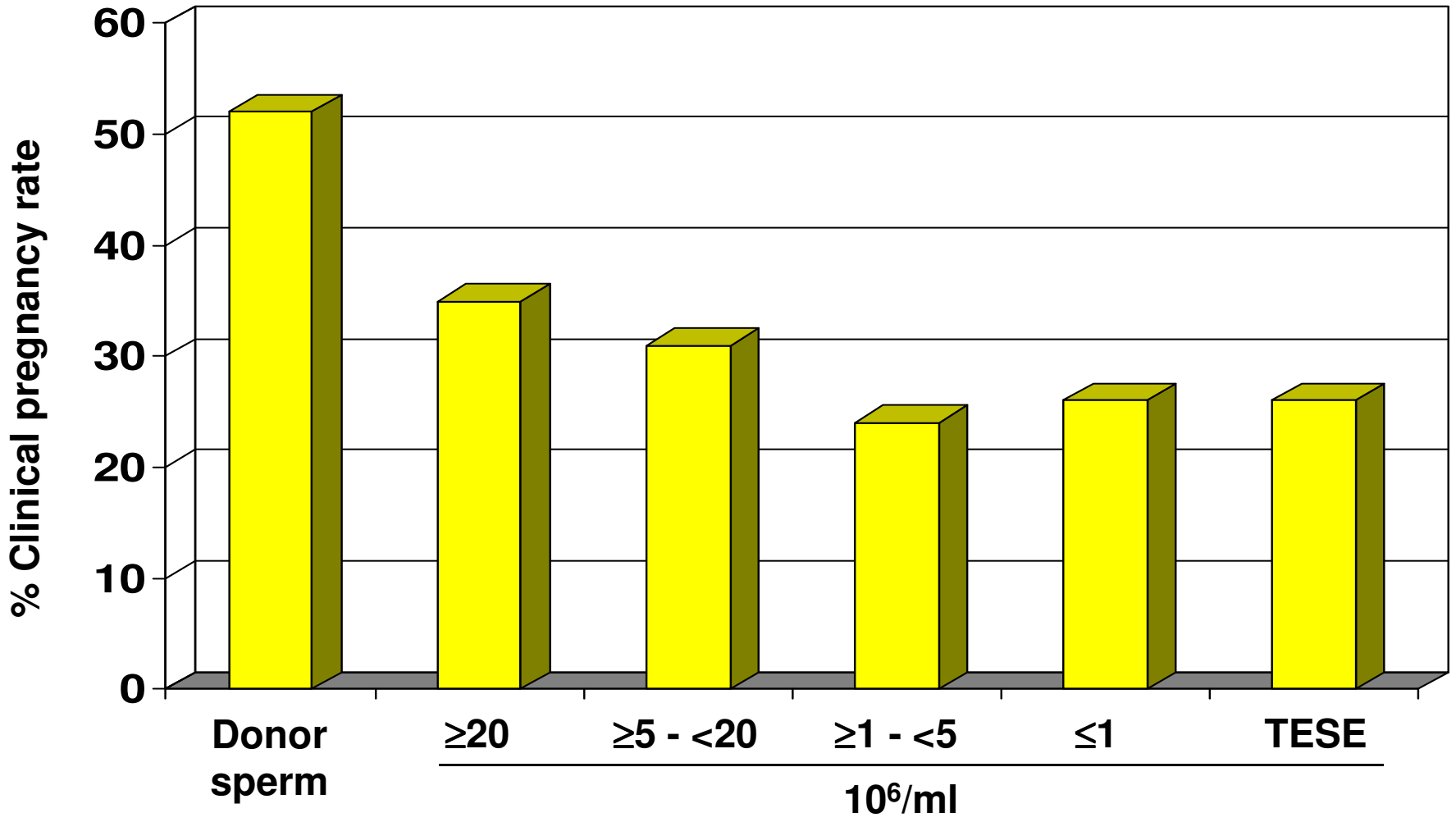
SPERM QUALITY AND CLINICAL OUTCOME

7274 oocyte retrievals



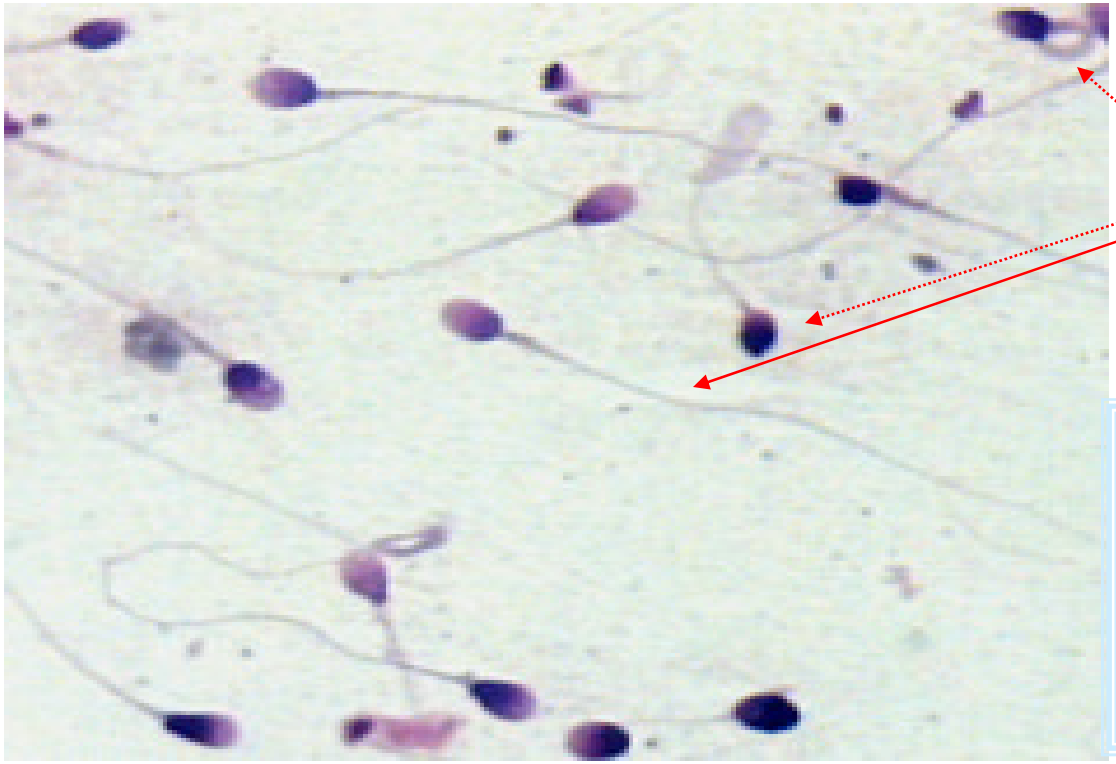
SPERM QUALITY AND CLINICAL OUTCOME

5250 ICSI cycles



WHICH SPERMATOZOON TO CHOOSE FOR ICSI?

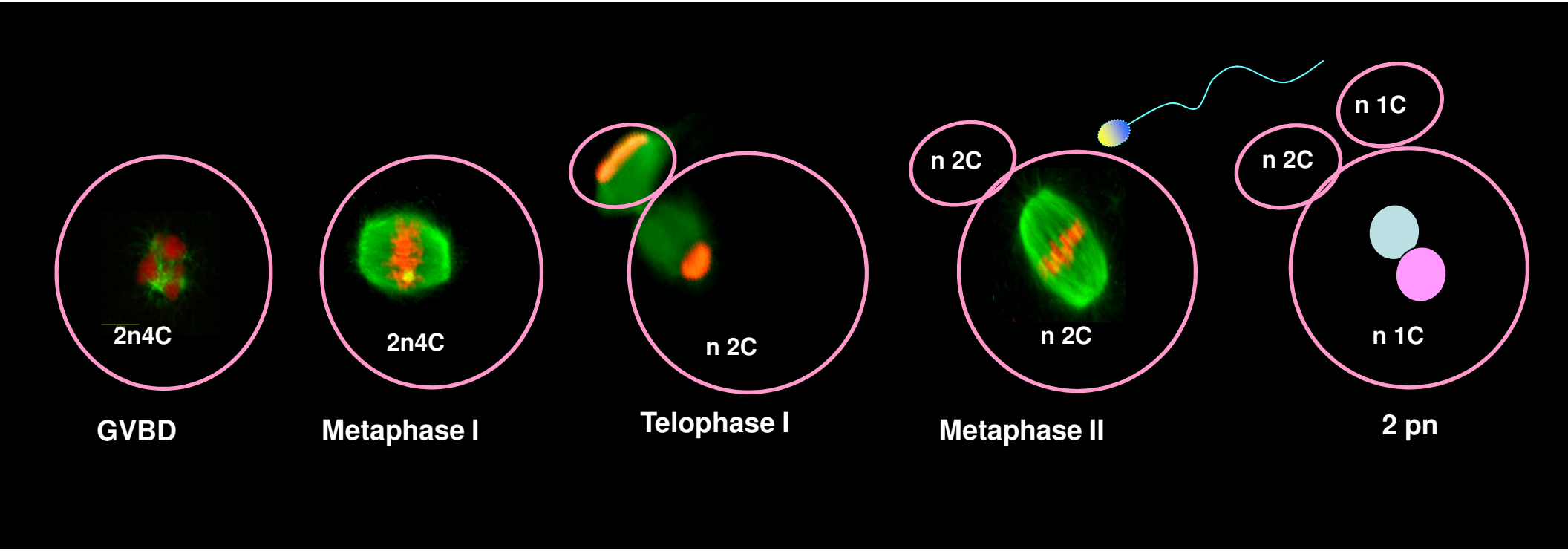
A choice is required because not all sperm cells have equal capacity to produce healthy progeny



Fertilization rate
Embryo development
Implantation
Live birth rate

Depend on the quality of the single sperm even if semen quality metrics appear to be 'normal'

SPERM CONTRIBUTION TO THE DEVELOPING EMBRYO



- Sperm chromosomes
- Cortical granule exocytosis
- Meiosis resumption and completion
- Spindle formation

SPERM QUALITY

NUCLEUS:

- **Haploidy**
- **DNA integrity**
- **DNA packaging (epigenetics)**
- **Nuclear matrix**

OTHER COMPARTMENTS:

- **Centrosome**
- **Mitochondria**
- **Membrane and cytosolic factors**

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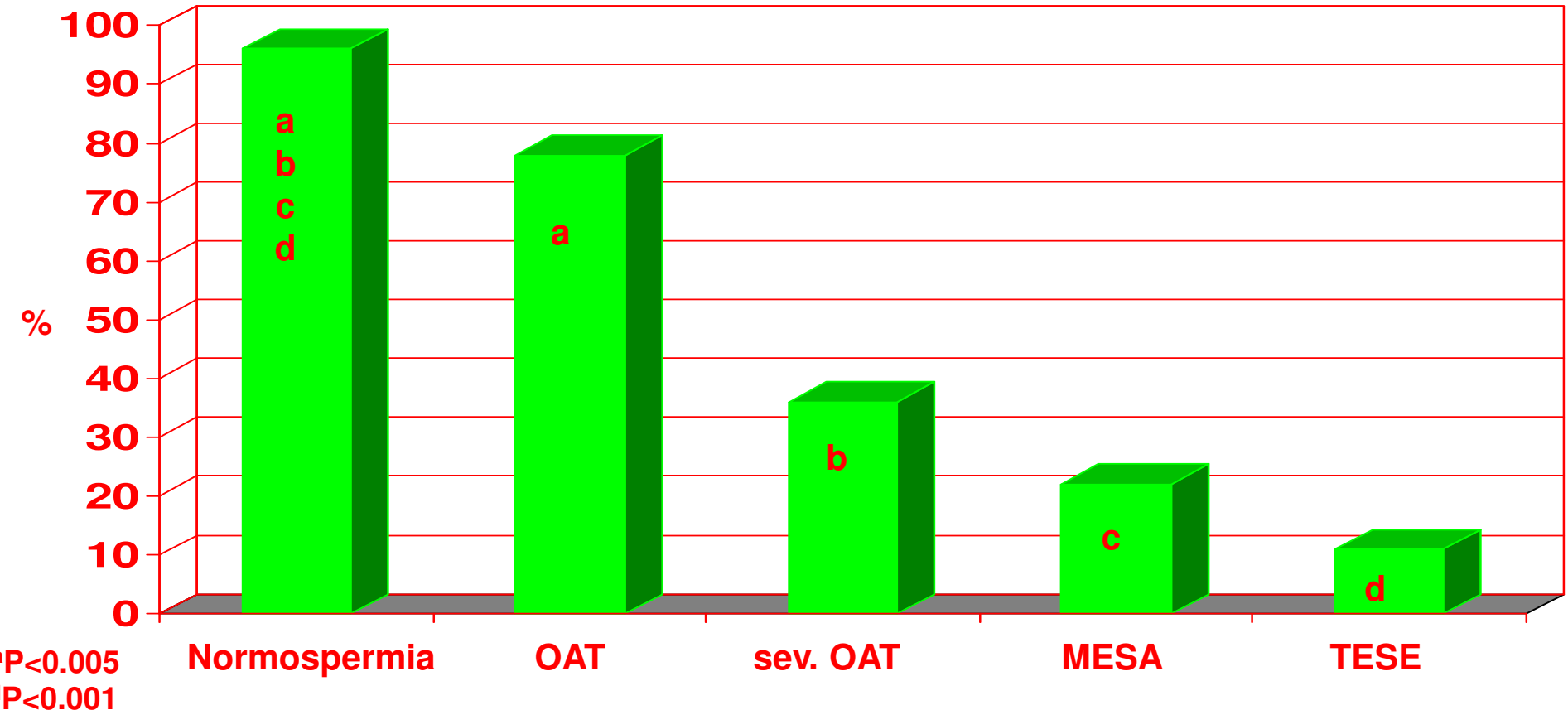
SPERM HAPLOIDY

SPERM HAPLOIDY

FISH ON SPERM - CHROMOSOMALLY NORMAL SAMPLES (n=874)



[a]P<0.005
[bcd]P<0.001

Gianaroli L, Magli MC, Cavallini G, Crippa A, Nadalini M, Bernardini L, Menchini – Fabris GF, Voliani S, Ferraretti AP. Frequency of aneuploidy in sperm from patients with extremely severe male factor infertility. Hum Reprod 2005;20:2140-2152.

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SPERM DNA INTEGRITY

The integrity of the paternal genome is essential as the spermatozoon can bring genetic damage into the oocyte at fertilization and contribute to the development of abnormal pregnancy outcome.

Exposure to physical agents or chemicals

integrity of sperm chromatin

structural, genetic and/or epigenetic abnormalities

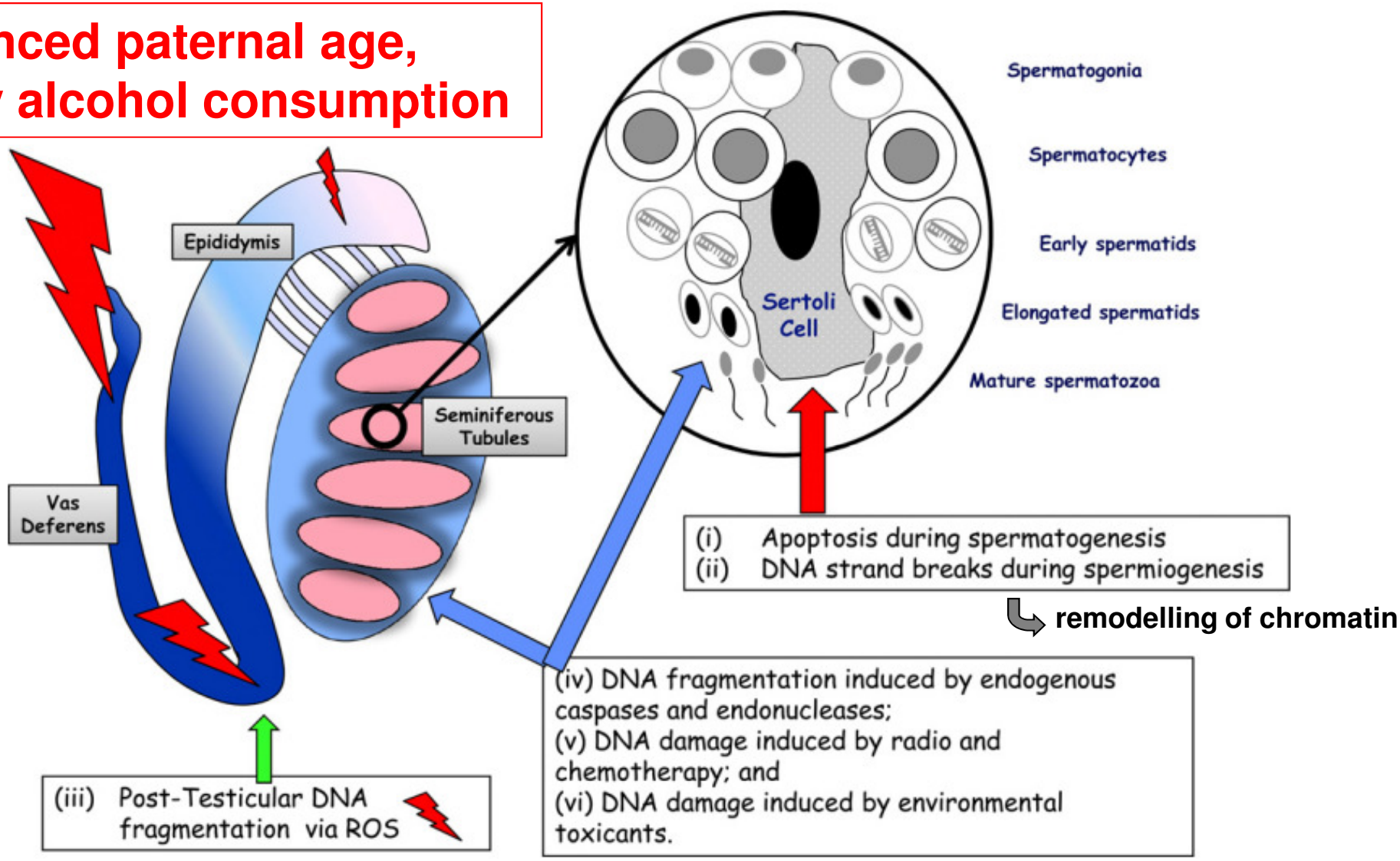
Targeting DNA
Inducing oxidative stress
Modifying epigenetic elements

Sperm chromatin non-integrity correlates well with low IVF and ICSI outcomes, and idiopathic infertility

Delbès G, Hales BF, Robaire B. Toxicants and human sperm chromatin integrity. Mol Hum Reprod 2010;16:14-22.

MECHANISMS OF DAMAGE TO SPERM DNA

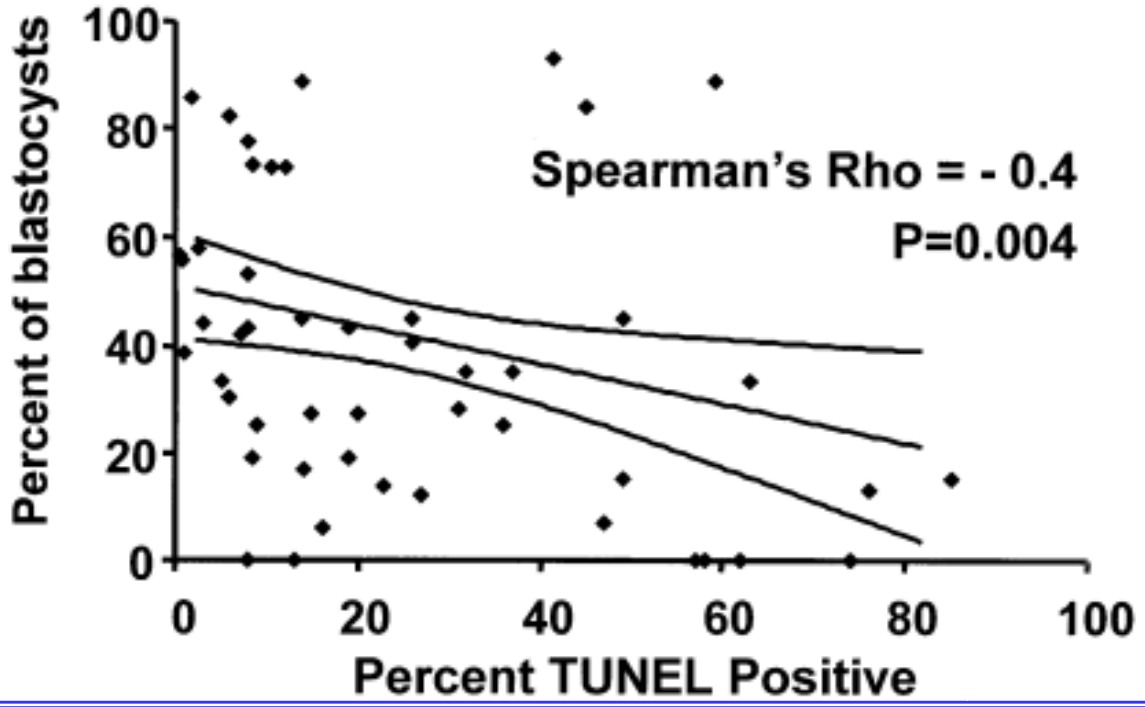
↑ advanced paternal age,
obesity alcohol consumption



Sakkas D, Alvarez JG. Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. *Fertil Steril* 2010;93:1027-1036

SPERM DNA INTEGRITY

Correlation between percentage blastocyst development and TUNEL positivity in the spermatozoa. Spearman's $\rho = -0.4$ ($P = .004$).

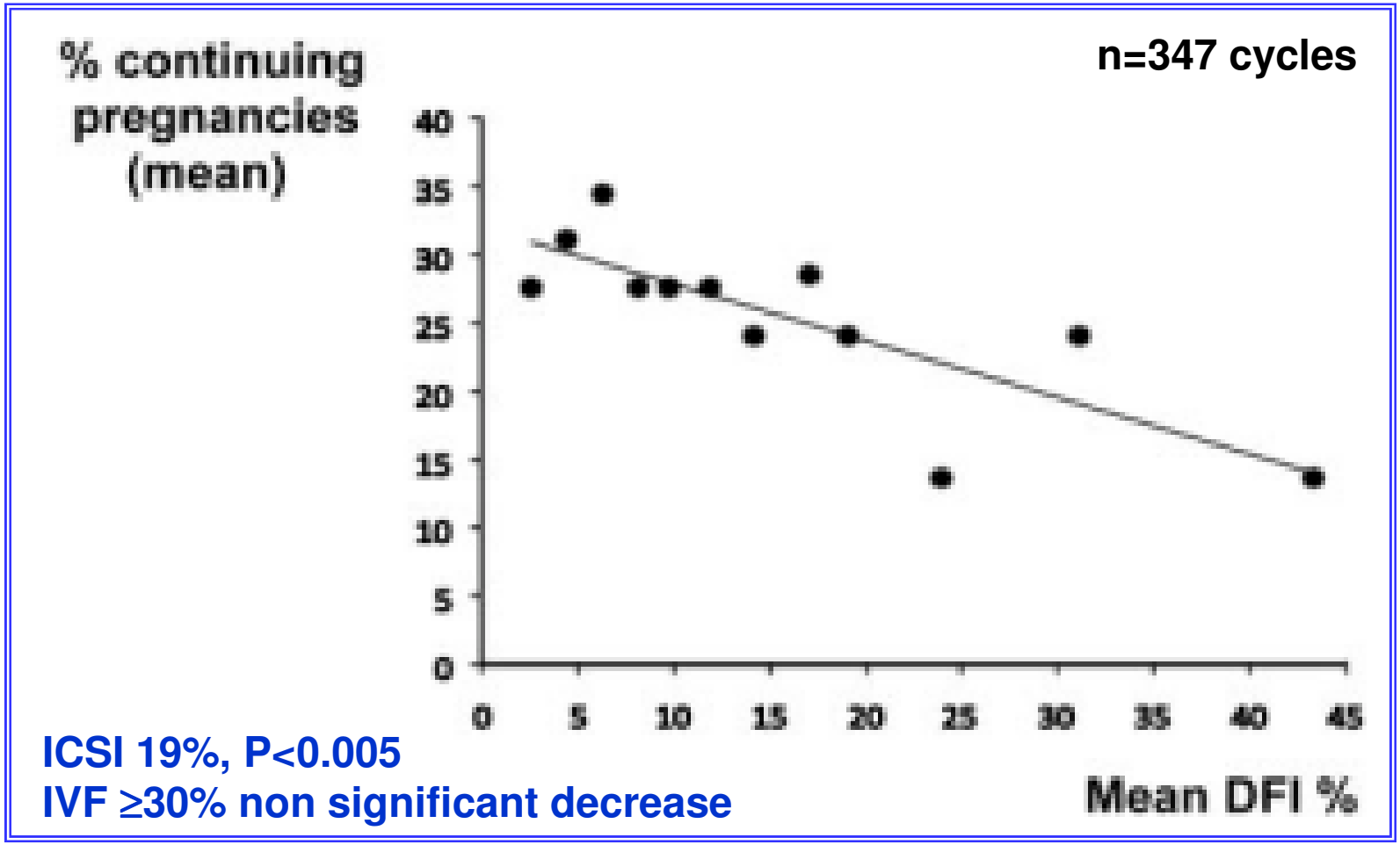


The extent of nuclear DNA fragmentation in prepared ejaculated spermatozoa for IVF negatively correlates with blastocyst development

This paternal effect on blastocyst development could also affect pregnancy outcome.

Seli E, Gardner DK, Schoolcraft WB, Moffatt O, Sakkas D. Extent of nuclear DNA damage in ejaculated spermatozoa impacts on blastocyst development after in vitro fertilization. *Fertil Steril* 2004;82:378-383.

SPERM DNA INTEGRITY



The rate of continuing pregnancies in ICSI cycles (but not in IVF cycles) showed significant negative correlation with the DFI value.

Speyer BE, Pizzey AR, Ranieri M, Joshi R, Delhanty JD, Serhal P. Fall in implantation rates following ICSI with sperm with high DNA fragmentation. Hum Reprod 2010;25:1609-1618.

SPERM DNA INTEGRITY

- **inverse relationship between DFI and sperm motility**

This may contribute to the relative lack of significant negative correlation between high DFI and continuing pregnancy in IVF cycles compared with ICSI cycles.

Spermatozoa with high DFI probably lack certain properties required to penetrate and fertilize an oocyte, the most obvious of which is motility. In IVF cycles, these sperm cells will have less chances to compete against motile spermatozoa, resulting in a normal spermatozoon fertilizing the oocyte.

Speyer BE, Pizzey AR, Ranieri M, Joshi R, Delhanty JD, Serhal P. Fall in implantation rates following ICSI with sperm with high DNA fragmentation. Hum Reprod 2010;25:1609-1618.

SPERM DNA INTEGRITY

► very strong positive correlation between DFI and sperm midpiece defects in the sperm used for ICSI cycles

The midpiece defects in these fractions are considered to result from disordered spermiogenesis leading to enzyme-containing residual cytoplasm in the midpiece.

Midpiece defects of this type have aroused recent interest because of their possible role in initiating sperm DNA fragmentation.

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SPERM RESIDENT HISTONES

Because nucleosomes are widely replaced by protamine in mature human sperm, the epigenetic contributions of sperm chromatin to embryo development have been considered highly limited.

The retained nucleosomes are significantly enriched at loci of developmental importance, including imprinted gene clusters, microRNA clusters, HOX gene clusters, and the promoters of stand-alone developmental transcription and signalling factors.

Epigenetic marking in sperm is extensive, and correlates with developmental regulators

Histones are necessary to imprinting and passing on the genetic information to the oocyte

Hammoud SS, Nix DA, Zhang H, Purwar J, Carrell DT, Cairns BR. Distinctive chromatin in human sperm packages genes for embryo development. Nature 2009;460(7254):473-478.

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SPERM RNA

Spermatozoa contain

- **Almost 3000 different kinds of mRNA**
  - **Code for proteins needed for early embryo development (signalling molecules implicated in the process of fertilization and morphogenesis, and early embryo patterning).**
  - **Others are still unknown and have no equivalent in the oocyte.**

- **Small antisense RNAs that delivered at fertilization could also participate in early post fertilization events.**

Ostermeier GC, Goodrich RJ, Moldenhauer JS Diamond MP, Krawetz SA. A suite of novel human spermatozoal RNAs. J Androl 2005;26:70–74.

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