OPTIMIZING FERTILIZATION AND EMBRYO CULTURE

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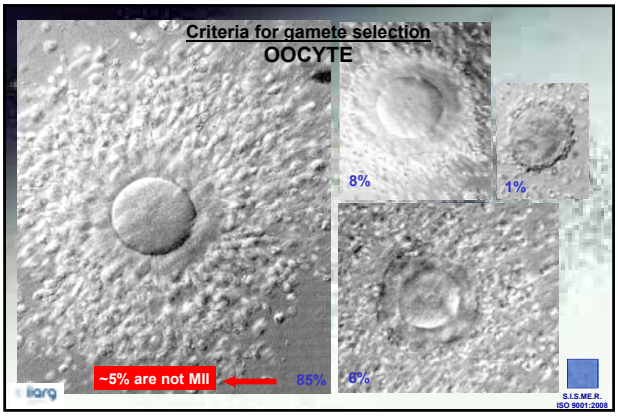
LEARNING OBJECTIVES

1) Criteria for gamete selection

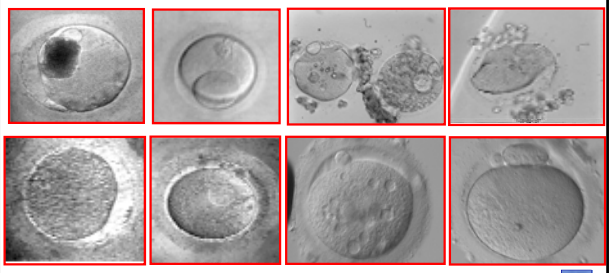
2) Insemination technique

3) Embryo culture conditions

Criteria for gamete selection
OOCYTE

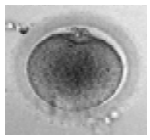


Criteria for gamete selection
OOCYTE



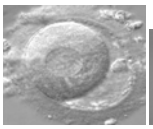
Criteria for gamete selection
OOCYTE

Enlarged perivitelline space

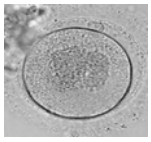


Dark cytoplasm

Decreases by 83% good quality embryos (Ten et al., 2007)



Presence of vacuoles



Granular cytoplasm

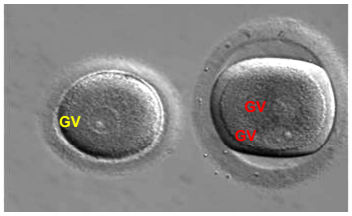
50% aneuploid (Kahraman et al., 2000)

Hairy zona

Implantation rate

Abortion rate

GIANT OOCYTES



23 univalent (chromatid) chromosomes

↓

- Cytokinetic failure
- Fusion of 2 GV

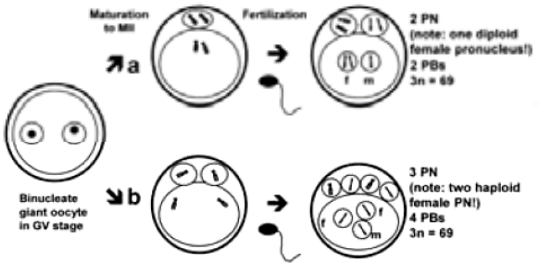
↓

Tetraploid GV

Frequency approximately 0.3%
Mean diameter 200 µm (vs. 155 µm)
Contribution to digynic triploidy

Balakier et al., 2002
Rosenbusch et al., 2002

GIANT OOCYTES



Rosenbusch et al., 2002

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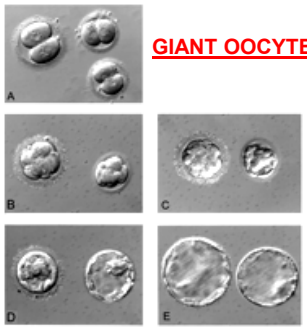
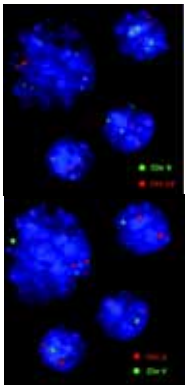
GIANT OOCYTES





Balakier et al., 2002

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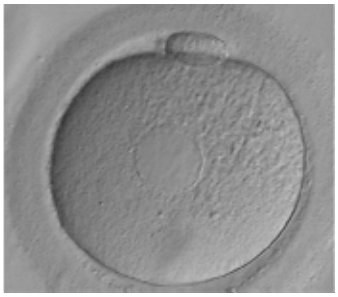
AGGREGATION OF SER



-6.2 - 9.4% of cycles affected

- < 2% of oocytes affected (25% in positive cycles)

Ebner et al., 2008; Otsuki et al., 2004

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AGGREGATION OF SER

IR, PR	no difference
Biochemical pregnancies	58% vs 22% (P<0.01)
Take-home baby rate	42% vs 78.% (P<0.001)
Increase in obstetric problems	33% vs. 5%
Lower birth weight	2500g vs. 3100g
Stillbirths	2/6

(one Beckwith-Wiedemann syndrome)

Ebner et al., 2008; Otsuki et al., 2004

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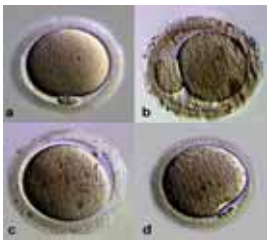
Criteria for gamete selection
OOCYTE



A. Normal MII
B. Large PB
C. Small PB
D. Fragmented PB

Courtesy: Lucinda Veeck Gosden, N.Y.

The presence of an enlarged PB was related to poorer rates of fertilization, cleavage, and top quality embryos but not fragmentation (Navarro et al., 2008)

Fragmented polar body was associated with reduced blastocyst formation rate (Ebner et al., 2006; Balaban & Urman, 2006)-

Correlation to timing of PB1 formation??

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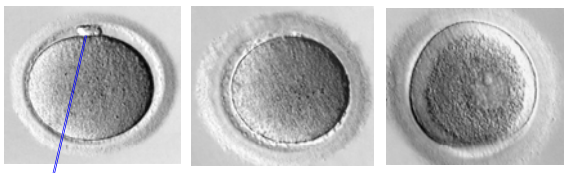
NUCLEAR MATURATION



73%

23%

MII
MI → MII
GV → MII

45%

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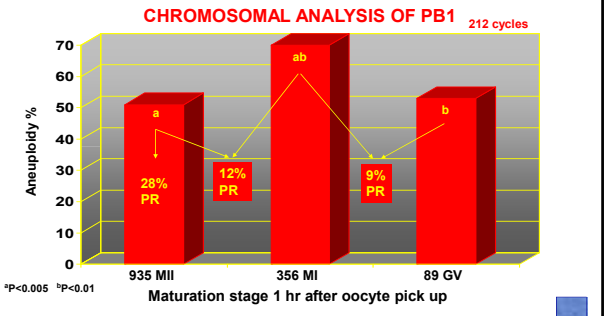
NUCLEAR MATURATION



Updated from: Magli MC, Ferraretti AP, Crippa A, Lappi M, Feliciani E, Gianaroli L (2006) First meiosis errors in immature oocytes generated by stimulated cycles. Fertil Steril 86, 629-635.

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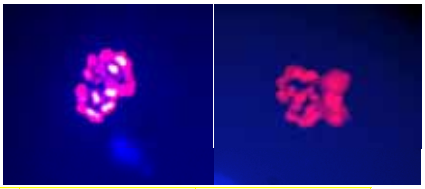
NUCLEAR MATURATION

DNA FRAGMENTATION



NUMBER OOCYTES	FRAGMENTED DNA	INTACT DNA	
20 IVM	7 (35%)	8 (53%)	P<0.01
44 MII	5 (11%)	3 (95%)	

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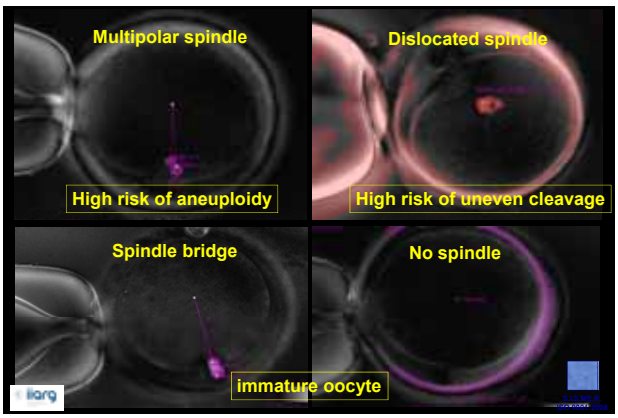
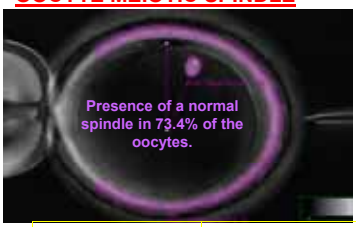
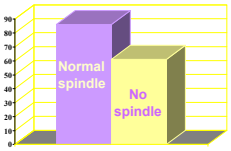


OOCYTE MEIOTIC SPINDLE



Fertilization rate
P<0.001



	ICSI PB biopsy
Analyzed	359
No spindle (%)	23 (6.4)
Abnormal spindles	14 (3.8)
Displaced spindles	59 (16.4)

Clinical pregnancy rate 37%
Implantation rate 22.2%

57 cycles, 39.1±3.9 yrs

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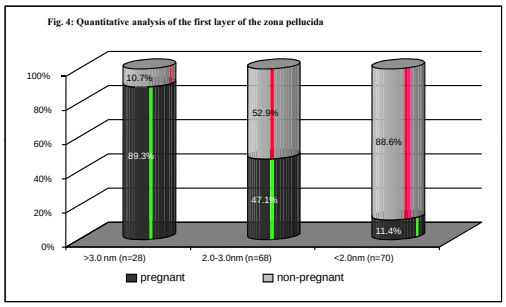
OOCYTE ZONA PELLUCIDA



(Shen et al., (2005) Hum. Reprod. 20:1596-1606)

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Criteria for gamete selection

OOCYTE

-Do not inseminate giant oocytes

-Do not inseminate oocytes with aggregation of smooth endoplasmic reticulum

-Give second priority to oocytes with cytoplasmic abnormalities

-Give second priority to oocytes with abnormal polar body

-Give second priority to in vitro matured oocytes

-Spindle view (and zona pellucida) under polarized light improves the selection of normal oocytes

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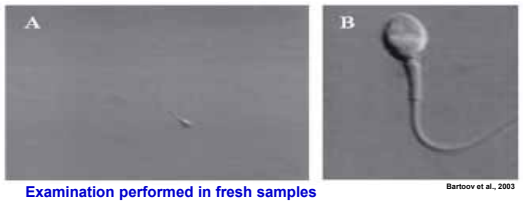
Criteria for gamete selection
SPERMATOZOA

IMSSI

INTRACYTOPLASMIC MORPHOLOGICALLY SELECTED SPERM INJECTION
MOTILE SPERM ORGANELLE MORPHOLOGY EXAMINATION

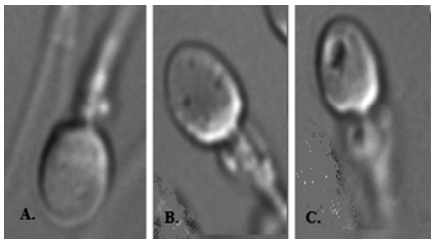


Bartoov et al., 2003

- Examination performed in fresh samples
- Inverted light microscope
- Equipped with high-power Nomarski optics
- Enhanced by digital imaging to achieve a magnification up to 6300

Criteria for gamete selection
SPERMATOZOA

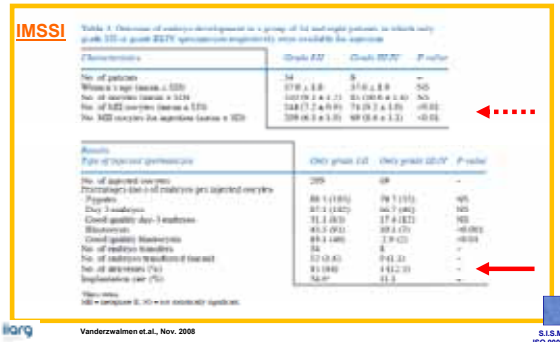
IMSSI



Peer et al. 2007 Fertil Steril 88, 1589-1594

Impact of vacuoles on pregnancy and abortion rates

IMSSI



Vanderzwalmen et.al., Nov. 2008

Criteria for gamete selection
SPERMATOZOA

Physiologic ICSI

Sperm cells have a receptor for Hyaluronic acid (HA)

Correlation between binding to hyaluran-coated surfaces and:

- sperm maturity
- normal morphology
- euploidy

Jakab et al. 2005
Huszar et al. 2006
Nasr-Esfahani et al. 2008
Parmegiani et al. 2010

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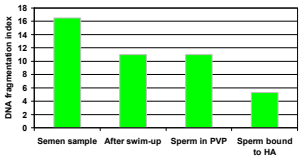
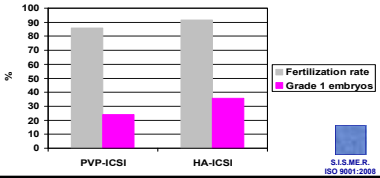
Criteria for gamete selection
SPERMATOZOA

Physiologic ICSI

HA favours the selection of spermatozoa:

- without DNA fragmentation
- with normal nucleus





Parmegiani et al. 2010

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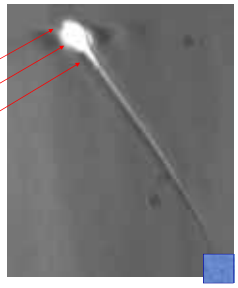
Criteria for gamete selection
SPERMATOZOA

Sperm head's birefringence

Human spermatozoa possess characteristics of birefringence due to the anisotropy of their protoplasmic texture.



protein subacrosomal filaments - longitudinally oriented

nucleoprotein filaments - arranged in rods and longitudinally oriented

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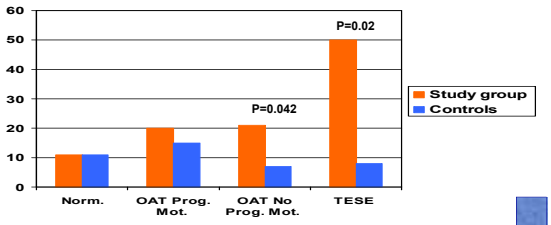
Criteria for gamete selection
SPERMATOZOA

Sperm head's birefringence:

Significantly higher ongoing-pregnancy rate after ICSI with birefringent spermatozoa

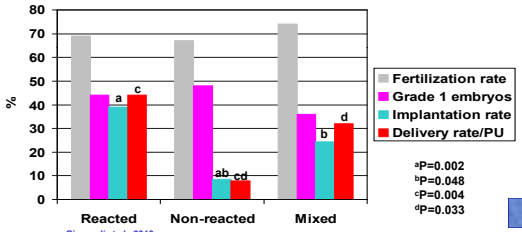


Gianaroli et al., 2008

Criteria for gamete selection
SPERMATOZOA

Sperm head's birefringence:

Significantly higher ongoing-pregnancy rate after ICSI with reacted spermatozoa

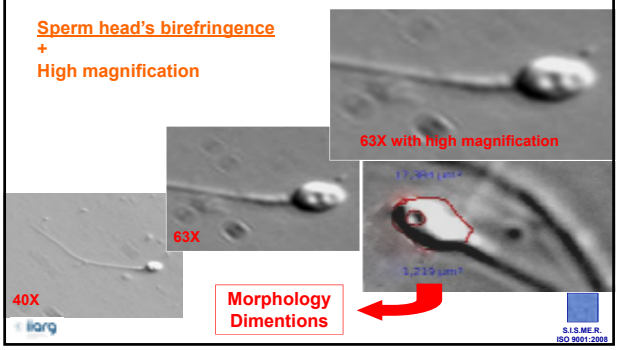


[a]P=0.002
[b]P=0.048
[c]P=0.004
[d]P=0.033

Gianaroli et al., 2010

Criteria for gamete selection
SPERMATOZOA

Sperm head's birefringence
+
High magnification



Criteria for gamete selection

SPERMATOZOA

-Select morphologically normal spermatozoa

►High magnification

►Birefringence

-Select functional spermatozoa

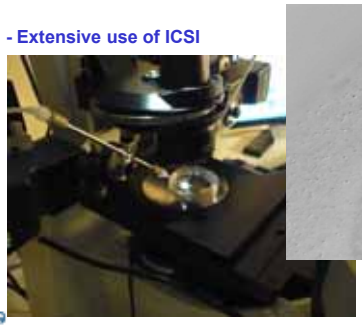
-In severe OAT samples, in TESE samples

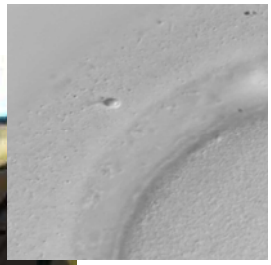
-In all sperm samples?

Insemination technique

IVF or ICSI

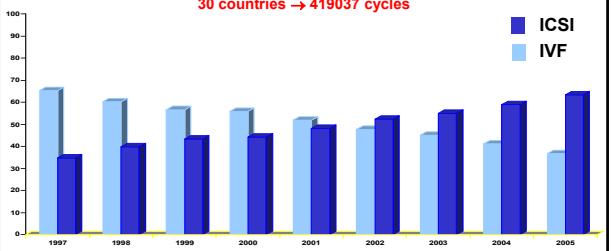
- Extensive use of ICSI



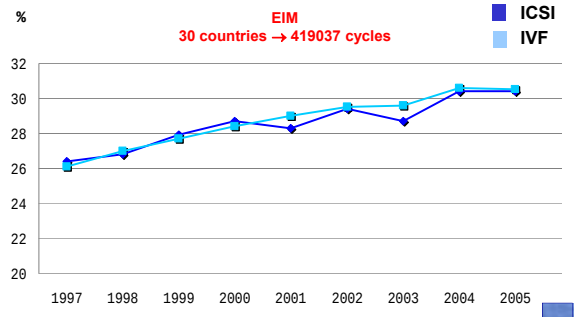


Distribution IVF / ICSI (1997-2006)

EIM
30 countries → 419037 cycles



Distribution IVF / ICSI (1997-2006)



liarg

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Insemination technique
IVF or ICSI

- Each center decides its own policy

eshre

June 2008

Good Clinical Treatment in Assisted Reproduction – *An ESHRE position paper*

INTRACYTOPLASMIC SPERM INJECTIONS (ICSI)

ICSI should be considered in the presence of severe sperm abnormalities or a history of fertilisation failure in conventional IVF attempts. It must be emphasised that ICSI does not represent the most suitable treatment for female pathologies such as poor ovarian response or previous implantation failures.

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Embryo culture conditions

- Controlled environment
  - ►Temperature
  - ►pH
  - ►air quality
  - ►Oxygen tension

liarg

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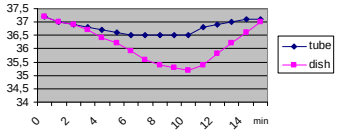
Embryo culture conditions
►Temperature

- Use calibrated thermometers
- Define set points for each block / stage to keep medium at 37°C

Temperature changes in tubes (heating block) and dishes (heating stages) set at 37°C



Courtesy of Ronny Jannsen

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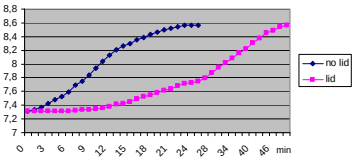
Embryo culture conditions
►pH

- Equilibration of culture dishes: ~4 hours
- Fast rise of pH in ambient air
- Oil → limited protection to pH changes

Evolution of pH in medium under oil out of the incubator



Courtesy of Ronny Jannsen

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Embryo culture conditions
►pH

- pH ↔ CO_2 concentration

pH measurement

- Difficult (measuring errors - protein deposit on probes, calibration, sampling and equilibration problems)
- Does not detect fast changes in CO_2 concentration
- Not suitable for routine control

Courtesy of Ronny Jannsen

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Embryo culture conditions
►air quality

- **Prevention** - Elimination of known/possible sources
  - Alcohol - disinfectants
  - Anesthetic gasses
- **Detection**
  - VOC meters
- **Removal**
  - Active charcoal absorption
  - Oxydation (Potassium permanganate
  - Photo-Catalytic Oxidation

CODA filters
(active charcoal – permanganate)

For more details: www.eshre.eu>Specialty Groups>Special Interest groups>SIG Embryology>Archive

Embryo culture conditions
►Oxygen tension

- Oxygen is necessary for embryo metabolism

→ consumed in oxydative phosphorylation

→ free radicals are generated from leakage of high energy e^- as they proceed down the e^- transport chain

→ reactive oxygen species (ROS) are more abundant as more oxygen is available.

Embryo culture conditions
►Oxygen tension

- ROS can have a harmful effect for embryos. If ROS escape detoxification by superoxide dismutase, they will react with and possibly harm:

→ mitochondrial DNA (increased mutations, damage to RNA transcripts)

→ proteins (conformational changes and loss of function)

→ lipids (affects on membrane stability and permeability)

Embryo culture conditions
►Oxygen tension

- Culture under reduced oxygen tension

ADVANTAGES
- Easy access
- Easy cleaning
- Low gas use
- Fast temperature recovery
- Fast pH recovery
- Space-saving

DISADVANTAGES
- Very expensive
- Gas humidification set (once a month)
- Special gas mixture (reduced O_2)

CONCLUSIONS

1) Criteria for gamete selection
   → fertilization rate
   → embryo viability

2) Insemination technique
   → customized selection of the most appropriate technique
   → high fertilization rate ≠ high embryo viability

3) Embryo culture conditions
   → important for high fertilization rate
   → crucial for embryo viability

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