ASSISTED REPRODUCTIVE TECHNOLOGIES (ART)

Dr. Herve Lucas, MD, PhD,
Biologist, Andrologist

Dr. Taher Elbarbary, MD
Gynecologist-Obstetrician
Definitions of Assisted Reproductive Technologies

**Techniques involving oocyte retrieval and ejaculated sperm:**
- Gamete Intra-Fallopian transfer (GIFT), Peritoneal Oocyte Sperm Transfer (POST)
- Zygote Intra-fallopian Transfer (ZIFT).
- In Vitro Fertilization (IVF)
- Tubal Embryo Transfer (TET)

**Techniques Including sperm retrieval:**
- Testicular Sperm Aspiration (TESA)
- Per-cutaneous Epididymal Sperm Aspiration (PESA)
- Microsurgical Epididymal sperm Aspiration (MESA)
- Non-Scalpel Vasal Sperm Aspiration (NSVSA)

**For injection:**
- Intra-Cytoplastic Sperm Injection (ICSI)

Don't forget artificial Insemination...easy, low cost...!
Who are the candidates of ART?

15% of European couples who want to conceive

Indications for ART including artificial inseminations…
Steps of ART

Monitored hyperovulation

Oocyte retrieval

- Oocyte vitrification

Oocyte culture

Extracorporeal insemination

- Cryopreservation of zygotes due to OHSS risk

Fertilization control

Embryo culture

Embryo cryopreservation

Transfer to uterus after 2-5 days

AZH – biopsy

Sperm retrieval:
- Homologous sperm:
  - By ejaculation
  - By surgical retrieval (MESA-TESE-STW)
- Sperm bank
- Heterologous sperm:
  - Sperm bank

- 30-50 thousands motile spermatozoa in culture with each oocyte (IVF)
- Microassisted (ICSI)
Female Problems: PATIENT STIMULATION

PATIENT STIMULATION

- PATIENT STIMULATION
- HYPOTHALAMUS
- GnRH ANALOGUES
- HYPOTHALAMUS
- GnRH
- HYPOPHYSIS
- LH, FSH
- HYPOPHYSIS
- ESTROGENS
- LH, FSH

US image of multiple ovarian follicles

Serum estradiol level
Oocyte retrieval
Syringes with cumulus-oocyte complex in follicular fluid
Cumulus-oocyte complex
Sperm preparation (various techniques for various utilisations, AI, IVF, ICSI, GIFT…)

1- Sperm retrieval 2- Sperm washing

Adaptation of the sperm preparation:

- Ejaculate vs freezing-thawed,
- Very poor sperm vs normospermia,
- Ejaculate vs testicular or epididymal,
- Sperm with antisperm-antibodies,
- Retro-ejaculated sperm….
Artificial inseminations (indication: >1M motile spz, tubes OK.)
GAMETE INTRAFALLOPIAN TRANSFER (GIFT)
Day (0): Decoronization of mature oocytes before injection (MII)
ICSI

Day (0)
Day (0): Catch a single sperm with the injection pipette
Intra-Cytoplastic Sperm Injection
Day (0)
Fertilization control…and embryo development.

- Insemination: 0 h
- 2 PN: 16-18 h
- Early 2-cells: 25 h
- Transfer: 42-50 h
Observation of zygotes
Day (1) 16-18 h post-classical IVF

Decoronisation at day 1

Living spermatozoa (motility) at 16-18h

Sperm-ZP binding

Fertilization rate:

Fertilized (number of PN ?)

Unfertilized (1 polar body)
**Female Problems**

**Male Problems**

**Who are the candidates of ART?**

**Observation of zygote**

**Day (1) 16-18h post IVF or ICSI**

1 PN zygote: problem of oocyte activation? (spz or nuclus oocyte origin?).

3 PN after ICSI: non-extrusion of the second polar body... by lesion of meiotic spindle?

3 PN after IVF: polyspermia = too many spz inseminated or bad quality oocyte because abnormal cortical granules)

This case: ICSI

Abnormal fertilization

(1 or 3 PN and 1 or 2 PB)

Normal fertilization

(2PN and 2PB)

Scoring of zygotes...

Correlated with embryo quality (J2-J3)
• Day (1) **PM** two cell embryo: the early cleavage (25 h),

• Day (1) **AM** Zygote stage: best scoring zygote (16h),

The same indication: good timing in embryo development.
Day (2): 4-cell stage embryo quality.
Day (4): Morula stage
Embryo transfer
What are the risks of ART?

Risk due to ovarian stimulation?

Risk due to oocyte retrieval?

Risk due to embryo transfer? Multiple pregnancies

Risk due to ICSI? Increased!!!

Double in comparison with naturally obtained pregnancy (male genetic analysis in oligospermia)

Risk due to Classical IVF?

Increased in comparison with naturally obtained pregnancy (bias of observation of the children? Techniques in vitro?)
What are the success rates of ART?

**IVF**
Deliveries per retrieval .............................................................. 29.1%

**ICSI**
In women < 35 years without male factor infertility ..................... 35.7%
In women > 35 years with male factor infertility ......................... 35.1%
In women < 40 years without male factor infertility .................... 10.3%
In women > 40 years with male factor infertility ......................... 12.8%

**GIFT**
In women < 35 years without male factor infertility ..................... 34.9%
In women > 35 years with male factor infertility ......................... 22.2%
In women > 40 years without male factor infertility .................... 09.1%
In women > 40 years with male factor infertility ......................... 11.1%

**Nb of transferred embryos, indications, AI before IVF?**

Mechanical assisted hatching
Assisted hatching with acid solution… now with LASER : safer technique!
Correlation Between the Number of Embryos Transferred in the Previous Cycles and the Pregnancy Rate After the Assisted Hatching

Pregnancy Rate / No. of Transferred Fresh Embryos in The Previous Cycles

- <5
- 5-6
- 7-8
- 9-10
- 10-20

No. of fresh embryos transferred before hatching

Pregnancy rate / transfer (%)

0
10
20
30
40
Assisted Hatching indicated in:

• Failed Embryo Transfer (more than 3 ET of 2 good quality embryos).

and/or

• Thick Zona Pellucida (>15 um)- Hard ZP
But ...

ESHRE analysis doesn’t confirm the interest of Laser Assisted Hatching in any indication

Further study to conclude?
Pre-implantation genetic diagnosis (PGD)

Genetic analysis (x2 cells):

Number of Chr.: FISH
Gene alteration: PCR
Pre-implantation Genetic Diagnosis (PGD)

- The benefits of PGD in infertile couple (2 Polar body, PCGD).
  - Research of aneuploidy in older women (decrease miscarriage)
- The benefits of PGD in fertile/infertile couple.
  - Sex embryo screening (Ethically discussed…)

- Who should have PGD?
  - Fertile or infertile couples with known and genetically detectable genetic disease.

Very good genetic laboratory needed!
What is the future in ART:

- Ovarian tissue cryopreservation and graft.
- In Vitro Oocyte Maturation.
- Reducing the number of embryo transferred (blastocyst culture development).
- Pre-Implantation Genetic Diagnosis.
- In Vitro maturation of male germinal cells.
What is the future in ART:

….To have primate model for evaluation of:

NEW BIOLOGICAL TECHNOLOGIES,
NEW MALE/FEMALE TREATMENTS,

….Before use in humans.