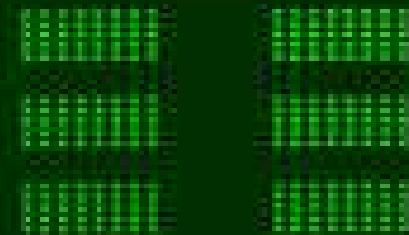
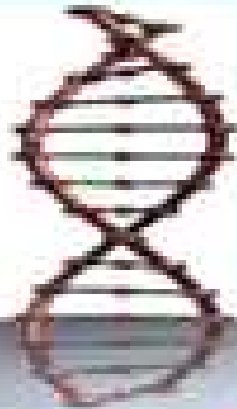
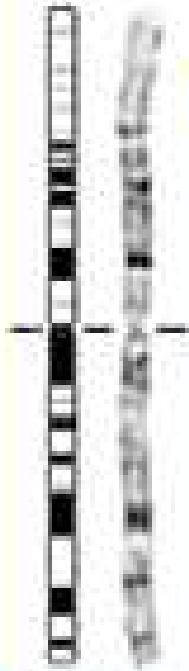


ATLR & D



Reproductive Biology & Genetics Laboratory

Natural Cycle & in vitro Maturation: Clinical & Biological Aspects

Dr Benkhalifa M, Ph.D. RBMG.

Director

ATL R&D Laboratory. Reproductive Biology & Genetics
PARIS. FRANCE. email: atl78@aol.com

Scientific Director

Chania IVF & Genetics Center . CRETA. GREECE

&

UNILABS Laboratories GENEVA

ART: Questions

- **Indications**
What is acceptable
- **ART attempts**
success rate ???
- **Causes of failures**
Genetics, Biochemical's,
clinical, others ???
- **Cost effective (taking home baby)**

ART Failure Status

Genetics: 24-26%

Clinical: 24-26%

Epigenetics Biochemical's factors : 24-26%

Endocrinology

**Physiology
status**

Others

**Culture & lab
Conditions**

**Transcription
Expression
Transductions**

**Programmed
cells death**

Others: 24-26%
???

In vitro maturation (IVM) of human oocytes is an emerging assisted reproductive technology with great promise.

From clinical management, without any ovarian stimulation we are having the risk to miss the oocyte pick up. More than this we should minimise the risk to develop a dominant follicle and have the atresia of the other antral follicles

The maturation and success rate of IVM is affected by the number of collected cumulus, the degree of atresia and the maturation rate between 24 and 48h.

Advantages of IVM

The major advantages of IVM include:

Avoidance of side effects resulting from gonadotrophin stimulation including risk of OHSS
reduced cost
simplified treatment

Social & Economic Indication

Selection Criteria for IVM patients

Patients with polycystic ovaries or polycystic ovarian syndrome (PCOS) are suitable for treatment using IVM.

Antral Follicle Count (AFC) >20 at baseline ultrasound scan.

If AFC is borderline, the treatment cycle is cancelled and patient is rescanned during the following cycle

Age of patient <35 years

Clinical preparation of Patients undergoing IVM

If the patient has irregular or no menstrual cycle, induce menstrual bleeding with progesterone (Prometrium 300 mg/day for 10 days or Provera 5 mg twice daily for 5 days). Once the medication is stopped, menstrual bleeding will occur within 3 days.

If the patient has regular cycles, she should be monitored after the onset of menses.

During the first 2 – 3 days of the menstrual cycle, a baseline transvaginal ultrasound examination is performed to ensure that no ovarian cysts are present. The number and sizes of follicles on both ovaries are recorded (AFC). Between days 6 to 9, a second ultrasound scan should be performed to confirm: follicular development (measure the sizes of the follicles, ensuring that the leading follicle does not exceed 14mm)

the lining of the endometrium should not be less than 6 mm when the patient is scheduled for human chorionic gonadotrophin (hCG)
The patient is given a 10,000 IU hCG (Profasi) subcutaneous injection 36 hours before oocyte retrieval.

Preparation of the endometrial lining

On the day of the oocyte retrieval, the patient should commence estradiol valerate tablets (Estrace) in order to prepare the endometrium of the uterus for receptivity of the embryos. Depending on the endometrial thickness on the day of oocyte collection, the patient will be given a divided dosage.

If the endometrial lining is $>7.0\text{mm}$, then a daily dose of 6 mg will be administered. If the endometrium is $<7\text{mm}$, a 8-10 mg dosage is given.

Endometrium Preparation with Progesterone/Luteal support

Preparation of the endometrial lining is done using progesterone 200mg of intra-vaginal progesterone (Prometrium) administered 3 times daily or IM progesterone (100mg/day) injections are given subcutaneously starting from the day after oocyte retrieval (or day of ICSI) and continued until the pregnancy test, along with estradiol valerate (started on day of oocyte retrieval) until 12 weeks of gestation.

Endometrial thickness at Embryo transfer

On the day of embryo transfer, endometrial thickness should be measured by transvaginal ultrasound scan. During ET, the endometrium should be at $>7.0\text{ mm}$. If the endometrial thickness is less 7.0 mm, the embryo should be cryopreserved and transferred in a subsequent cycle.

First Block

- Oocyte maturation is accompanied by a complex network of translational activation and repression of dormant maternal mRNAs
- These maternal mRNAs drive the oocyte's reentry into meiosis and control the rate of mitosis during the early cleavage divisions of the embryo.
- *The correct timing of translation of these (housekeeping) mRNAs will lead further oocyte competence i.e. further embryonic quality. It will take 2.5 days in the human embryo to start new synthesis of mRNA*

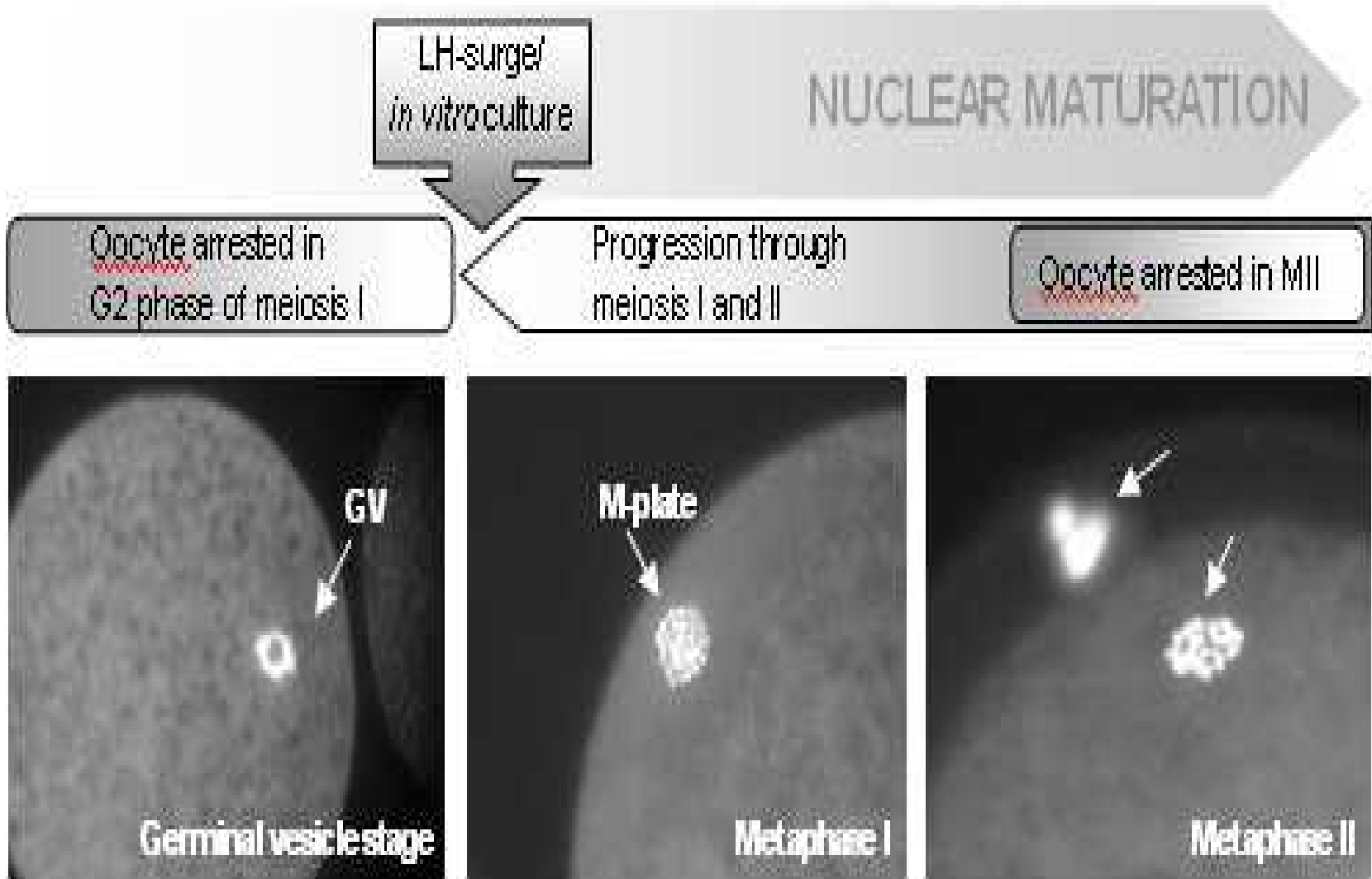
In vivo vs in vitro conditions

In vivo

Constant body temperature
Darkness
Controlled O₂/CO₂
Volume of fluid
Dynamic changes in secretions
Free radical scavengers
(continuous control)

In vitro

Thermal shocks
Variations in light
Variations
Medium volume
Static/semi Static
+/-



Cytoplasmic maturation, including:

- Migration and anchoring of cortical granules
- Migration of organelles
- Increase of intracellular glutathione synthesis
- Support of male pronucleus formation

DEVELOPMENTAL POTENTIAL

In the oocyte?

A high DNA repair activity is mandatory

Polymerase beta: up regulated by estradiol (Murdoch et van Kirk 2002)

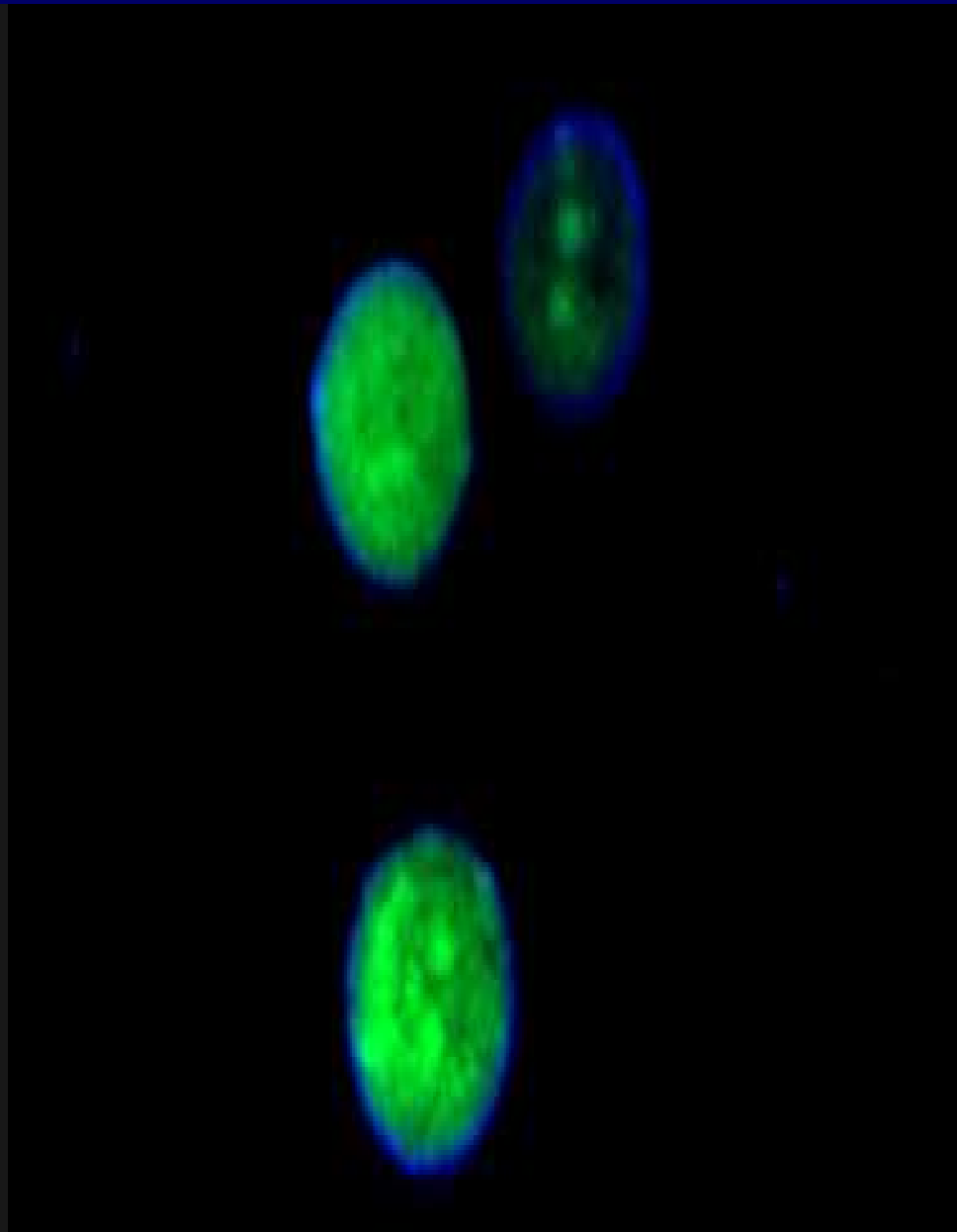
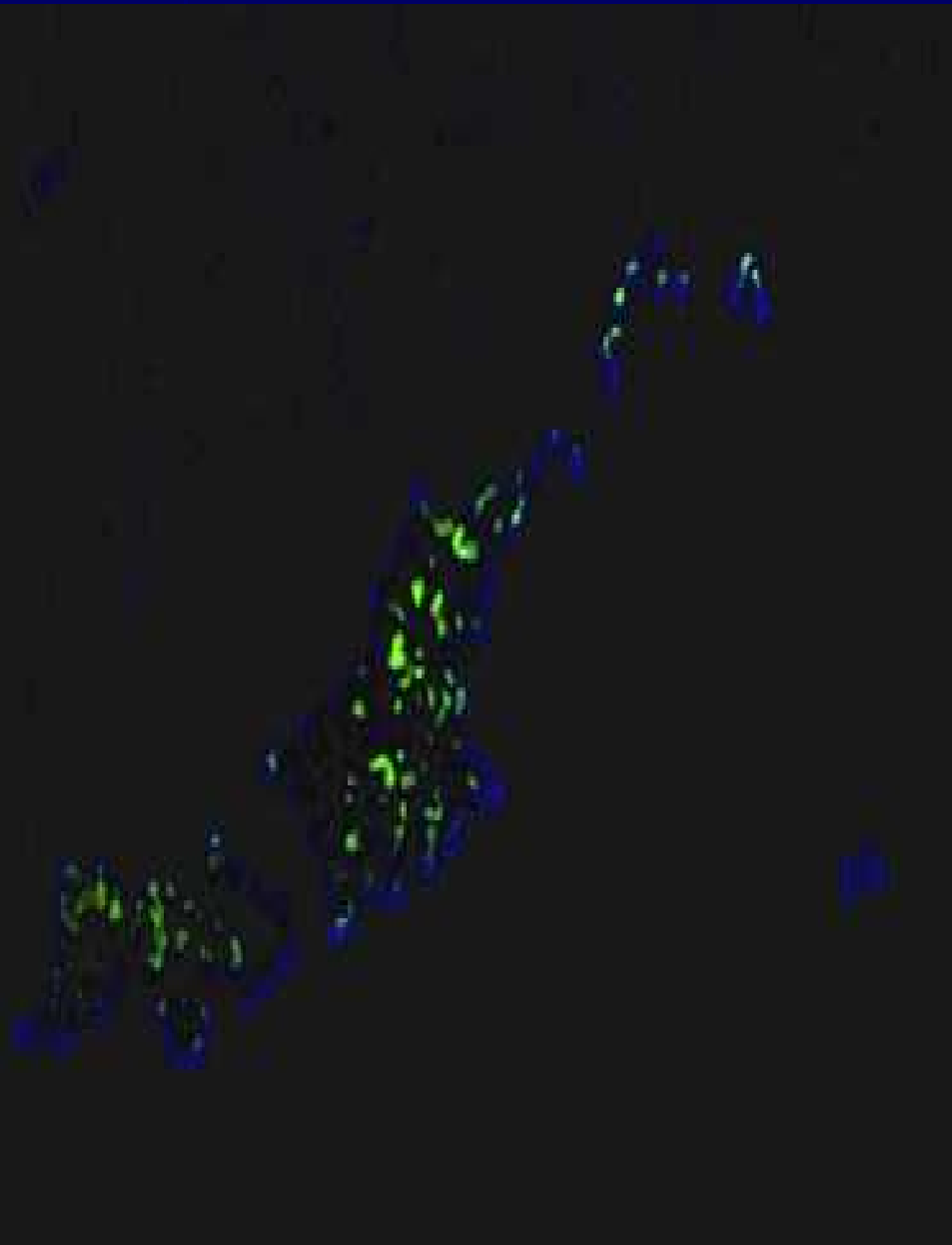
APEX/ APEN Méchanism of excision



DNA-Alkylation (overMethylation, Imprinting
One-Step Repair or Direct Reversal
of Damage



There is a 8-oxoGuanine ADN-glycosylase involved in DNA repair in human. However there is no longer any DNA repair in sperm after spermatid stage. In sperm?



Partial apoptosis at first somatic metaphase & early embryo

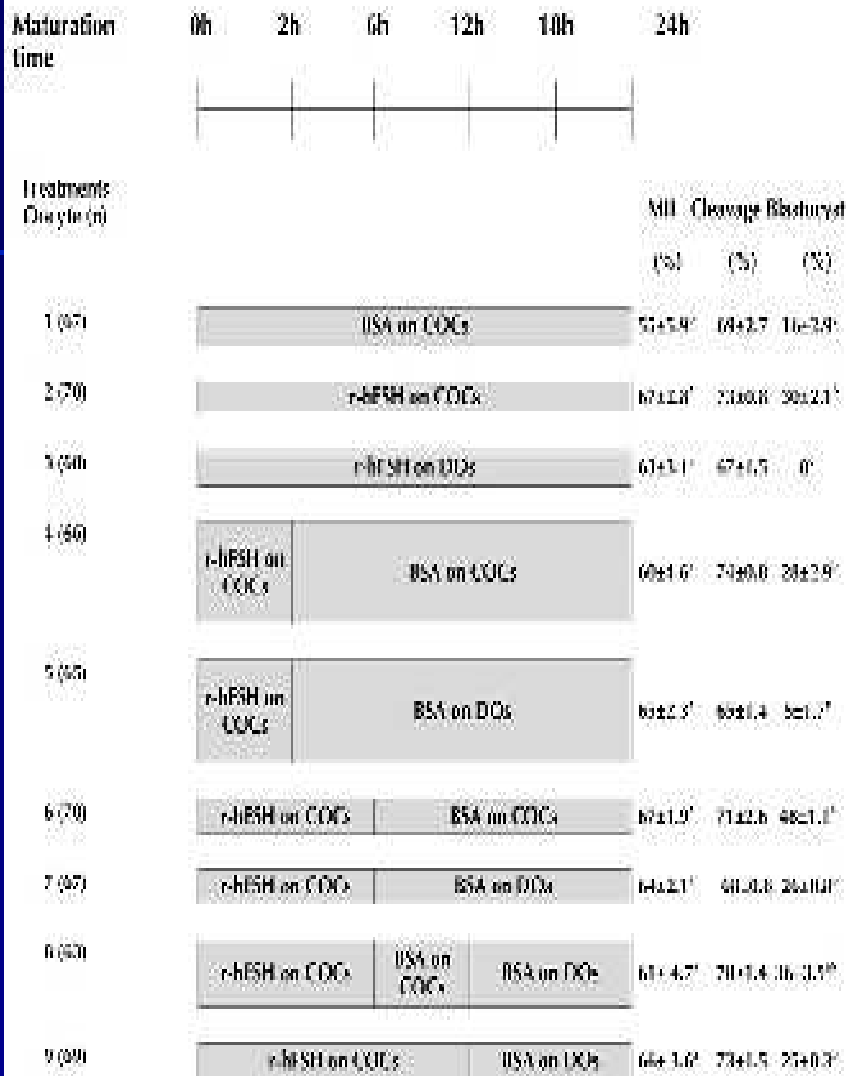


Table 1. Some factors added to in-vitro maturation media to enhance oocyte maturation and subsequent embryo development

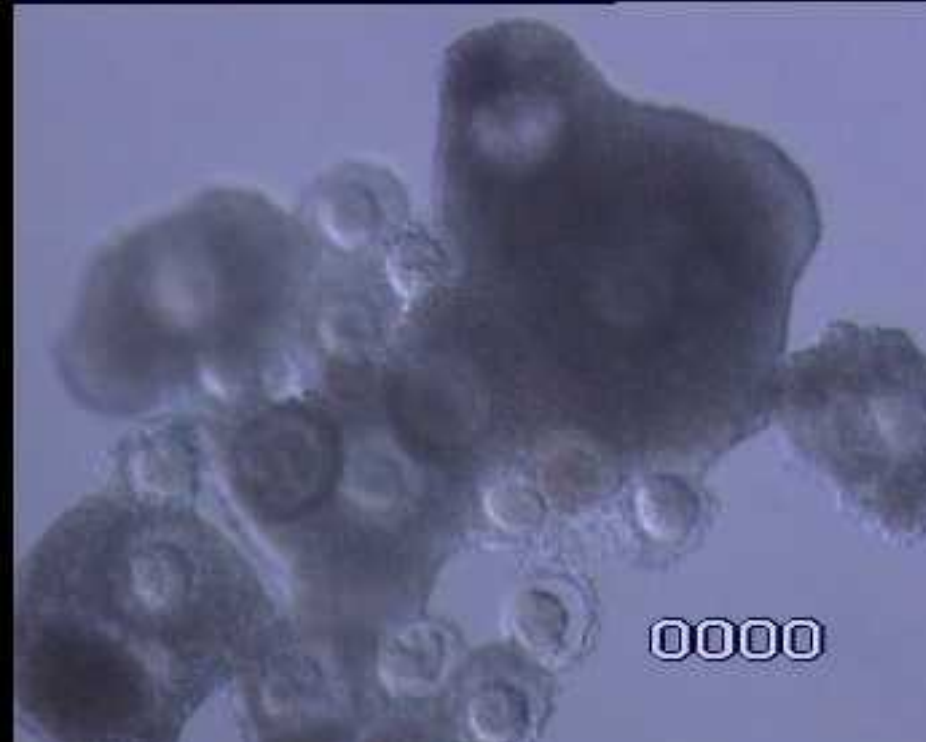
Factors	Authors	Year
FSH	Tazdyar <i>et al.</i>	1998
	Ali and Sirard	2002b, 2005
Sp-cAMPS (PKA activator)	Ali and Sirard	2005
PMA (PKC activator)	Ali and Sirard	2005
Oestradiol	Tesarik	1995
	Ali and Sirard	2002b
Growth hormone (GH)	Iga <i>et al.</i>	1998
	Tazdyar <i>et al.</i>	1998
Hyaluronic acid (HA)	Ali <i>et al.</i>	2002
Follicular fluid (FF)	Sirard <i>et al.</i>	1995
	Blondin <i>et al.</i>	2002
	Ali <i>et al.</i>	2004
Serum	Toumson <i>et al.</i>	2001
Intracellular antioxidants (cysteine, cysteamine, glutathione, β mercaptoethanol)	Jeung and Yang	2001
	Ali <i>et al.</i>	2003

Oocyte retrieval is usually performed between days 10 – 14, depending upon the thickness of the endometrial lining and the size of the follicles. Women with ovulatory cycles have their collections between days 9 – 11, to reduce the chance of observing a dominant follicle >10mm.

It is extremely important to avoid an LH surge causing the spontaneous ovulation of the dominant follicle.

When the patient arrives for the follicular scan on days 6

5–6 ml of the patient's blood is drawn and handed to the IVF laboratory. The blood will then be processed and used as a serum supplement for the culture of the immature oocytes

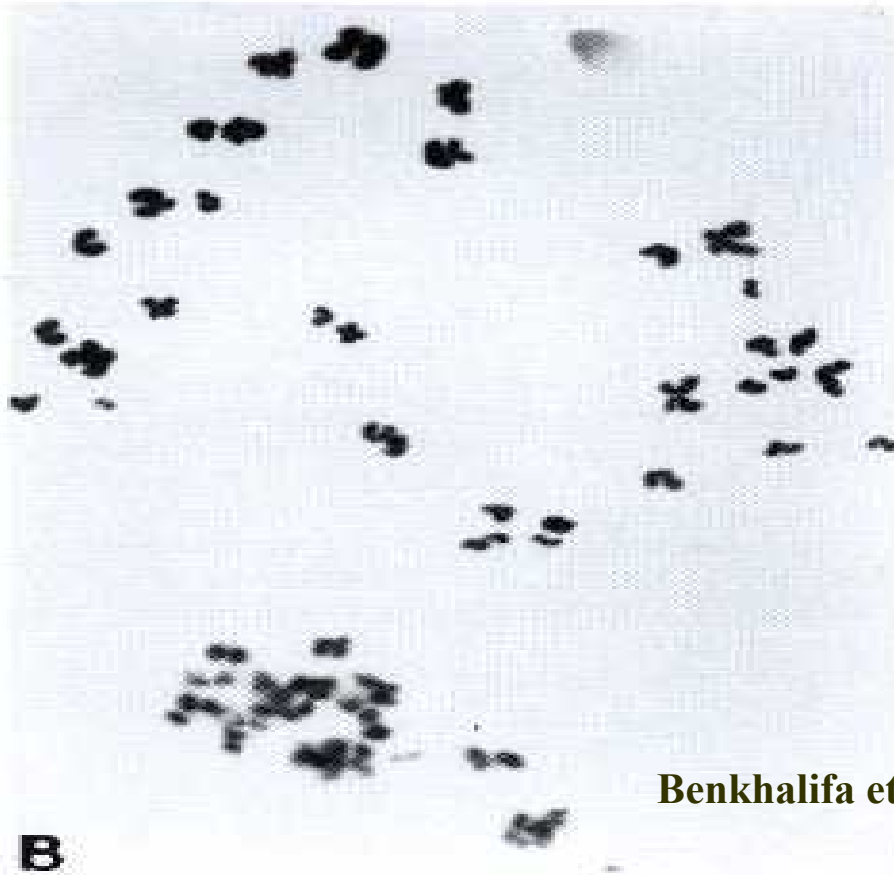
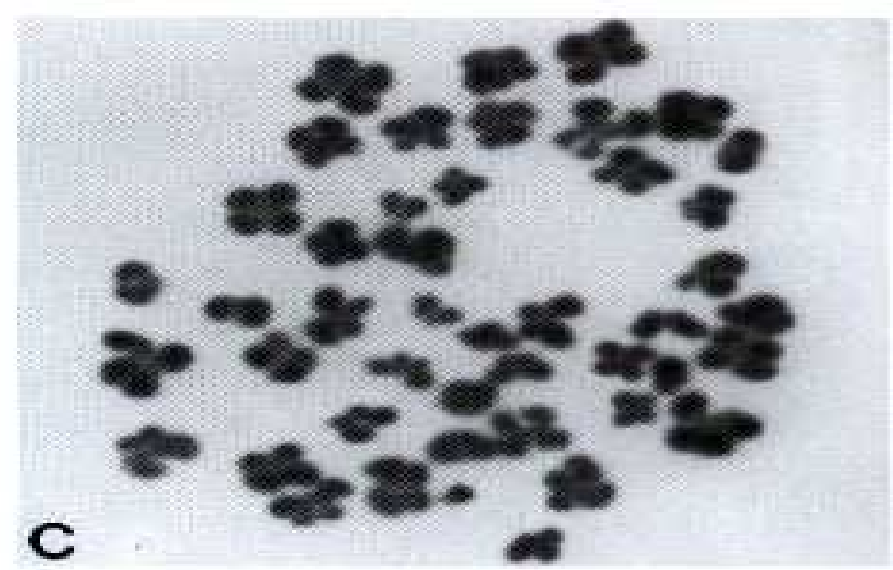
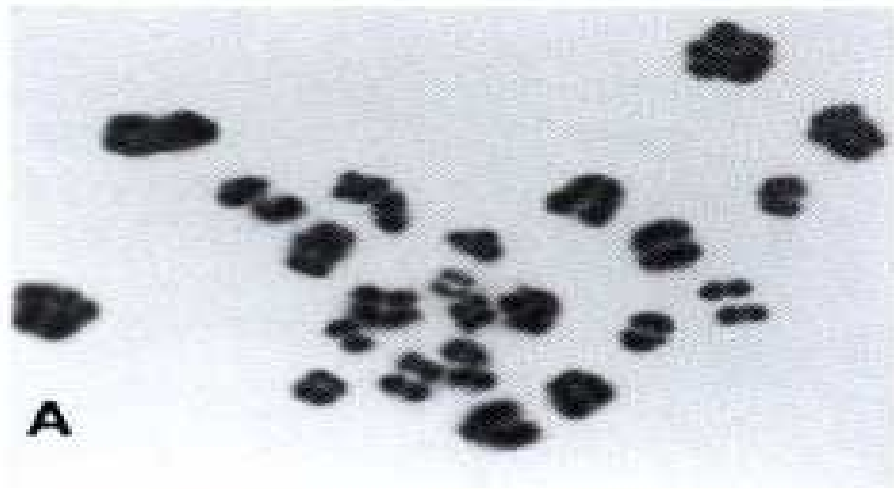


Identification of immature oocyte

Follicular aspiration is examined for Cumulus-oocyte-complexes (COCs) under the stereo-microscope. Oocytes are then transferred to the wash dish containing warm IVM washing medium at 37° C.

In order to ascertain if the oocyte is mature, a procedure call 'sliding' can be employed. The dish is tilted slightly so as to stretch the cumulus mass. It is possible to observe the germinal vesicle (GV) or if the oocyte has extruded the first polar body (Pb) in the perivitelline space. If any mature oocytes are observed, they should be inseminated by ICSI within 3 hours of collection.

Oocytes are washed several times before being transferred to the IVM oocyte medium for maturation in culture.



Sperm preparation

Semen can be collected and prepared for insemination on the day of oocyte retrieval if a mature oocyte has been retrieved from the dominant follicle. If not, semen collection and preparation should be performed the day after oocyte retrieval. Sperm preparation is as per standard IVF. ICSI is performed on all mature oocytes.

Cumulus denudation 24 hours after culture

The immature COCs are cultured in the IVM oocyte medium in the incubator for 24 to 28 hours for maturation to take place.

After 24 hours, all the COCs are stripped and maturation assessed. Cumulus denudation is performed using a drawn out pipette after one minute exposure to hyaluronidase.

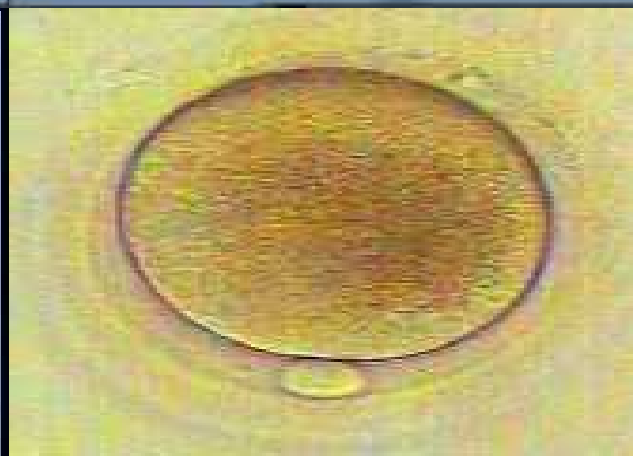
ICSI is performed on the mature oocytes and immature oocytes remain in culture for another 24 hours.

Insemination by ICSI

ICSI is performed as per standard procedure. The injection procedure may be performed on the day of oocyte retrieval, after 24 or 48 hours culture depending on the availability of mature oocytes.

ICSI dish is prepared at least 1 hour before the injection and kept at 37°C in an incubator. After ICSI, the oocytes are transferred into the 40 µl drops of IVM development medium and cultured in the incubator at 37°C in an incubator with 6% CO₂ with high humidity.

A maximum of 4 oocytes are cultured in each drop.



Identification of Fertilization

Approximately 16 to 18 hours after ICSI, oocytes are examined for fertilization under the microscope. Oocytes with 2 distinct pronuclei (2PN) and 2 polar bodies are fertilized.

Fertilized oocytes are cultured in drops; a maximum of 4 oocytes per drop.

Assessment of oocytes after 48 hours culture

The remaining oocytes are re-examined after a further 24 hours culture and mature oocytes are inseminated by ICSI.

Embryo culture

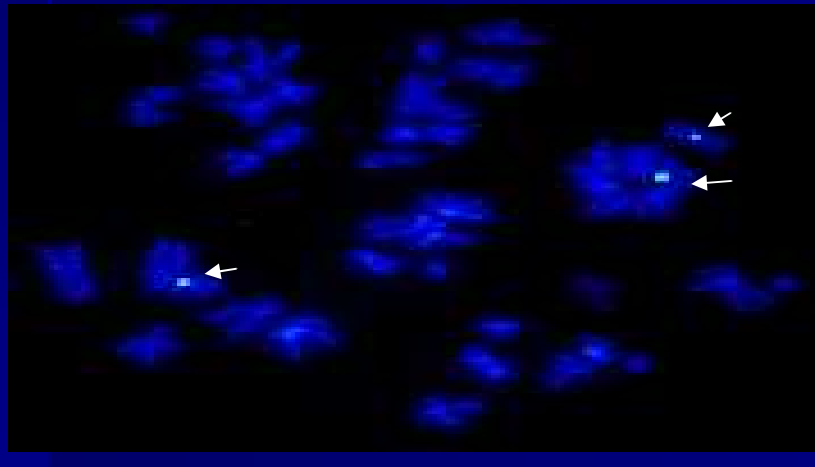
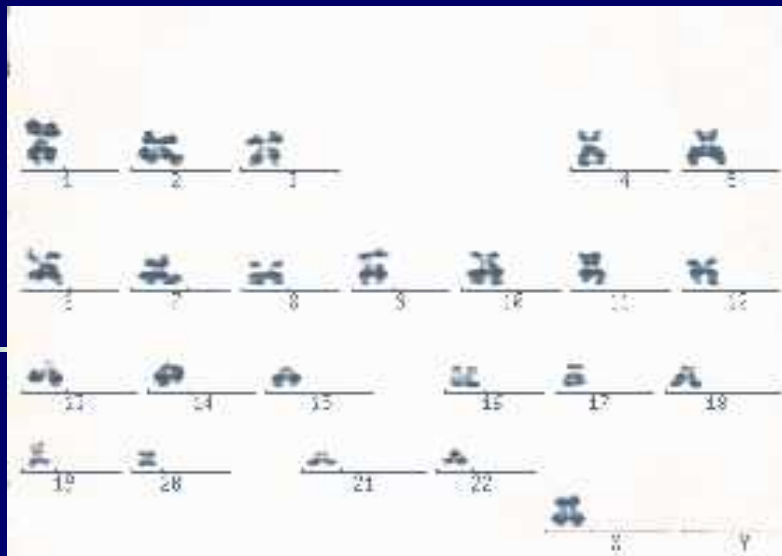
Fertilized oocytes are cultured for one or two days, depending on the number of embryos and quality of embryos available.

14. Embryo grading

Embryo grading is similar to IVF. As the oocytes may have been inseminated at different times, the development stages will be different.

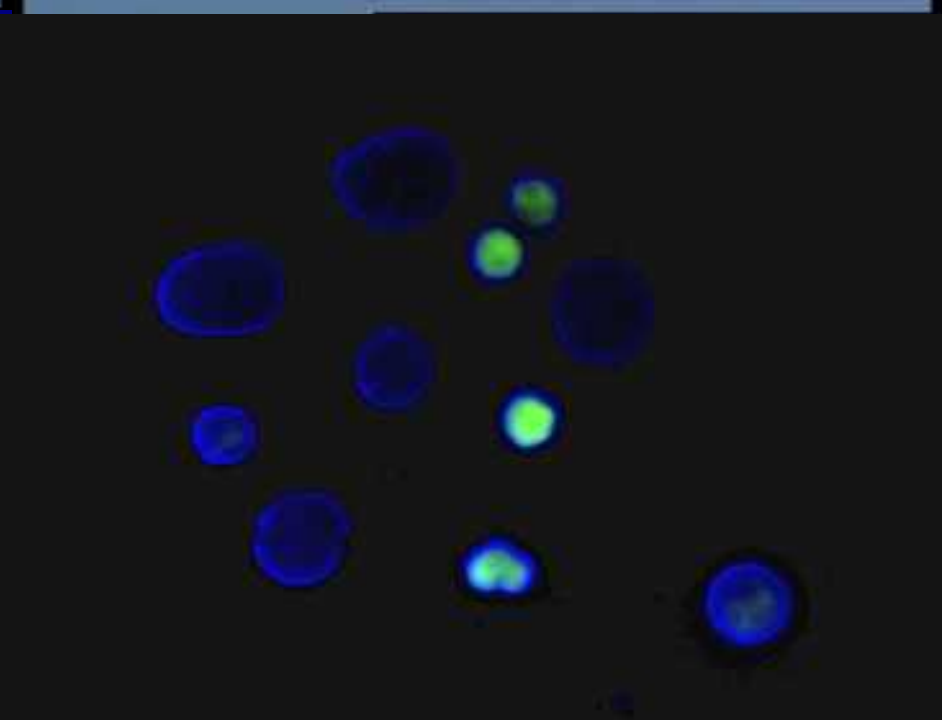
Embryos that are more advanced are preferred for transfer. It is recommended that a maximum of 3 embryos be transferred into the uterus.

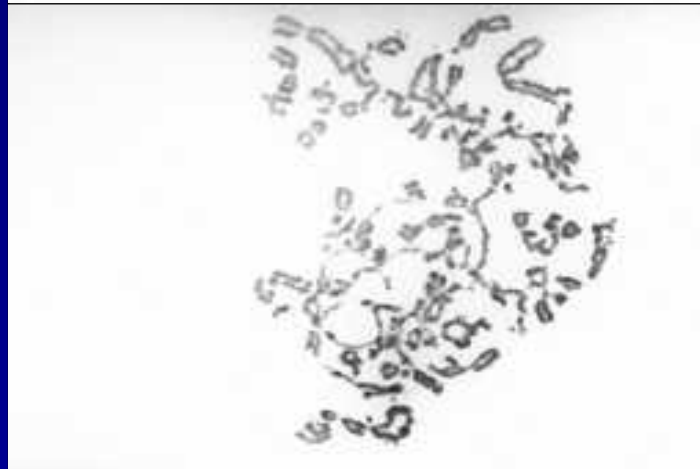
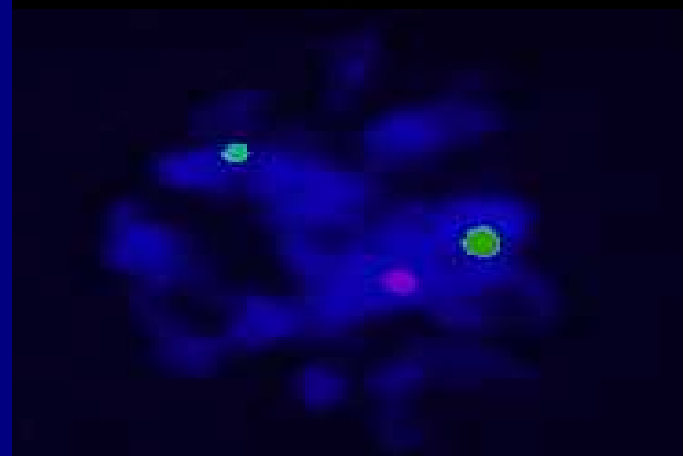
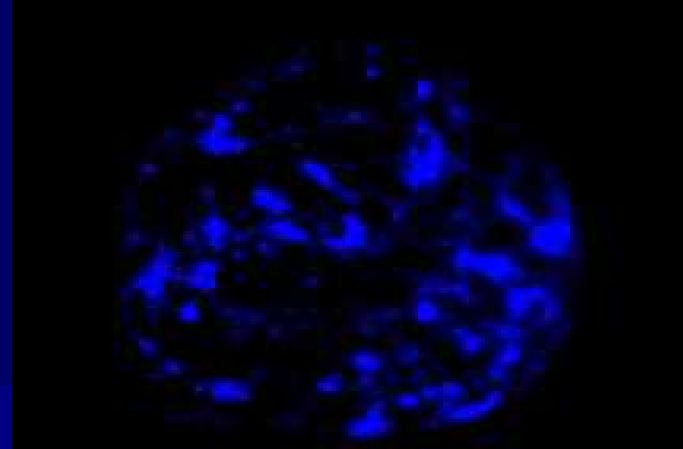
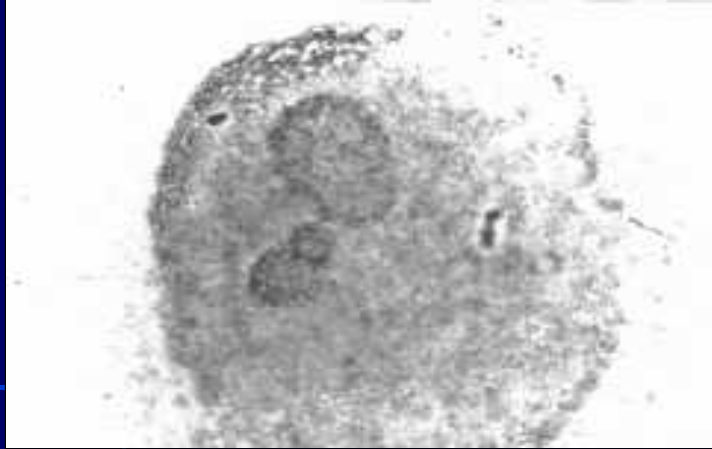




Morphological and cytogenetic analysis of intact oocytes and blocked zygotes

M. Benkhalfa^{1,2*}, S. Kahraman², D. Caserta³, E. Domez² and M. B. Qumsiyeh⁴



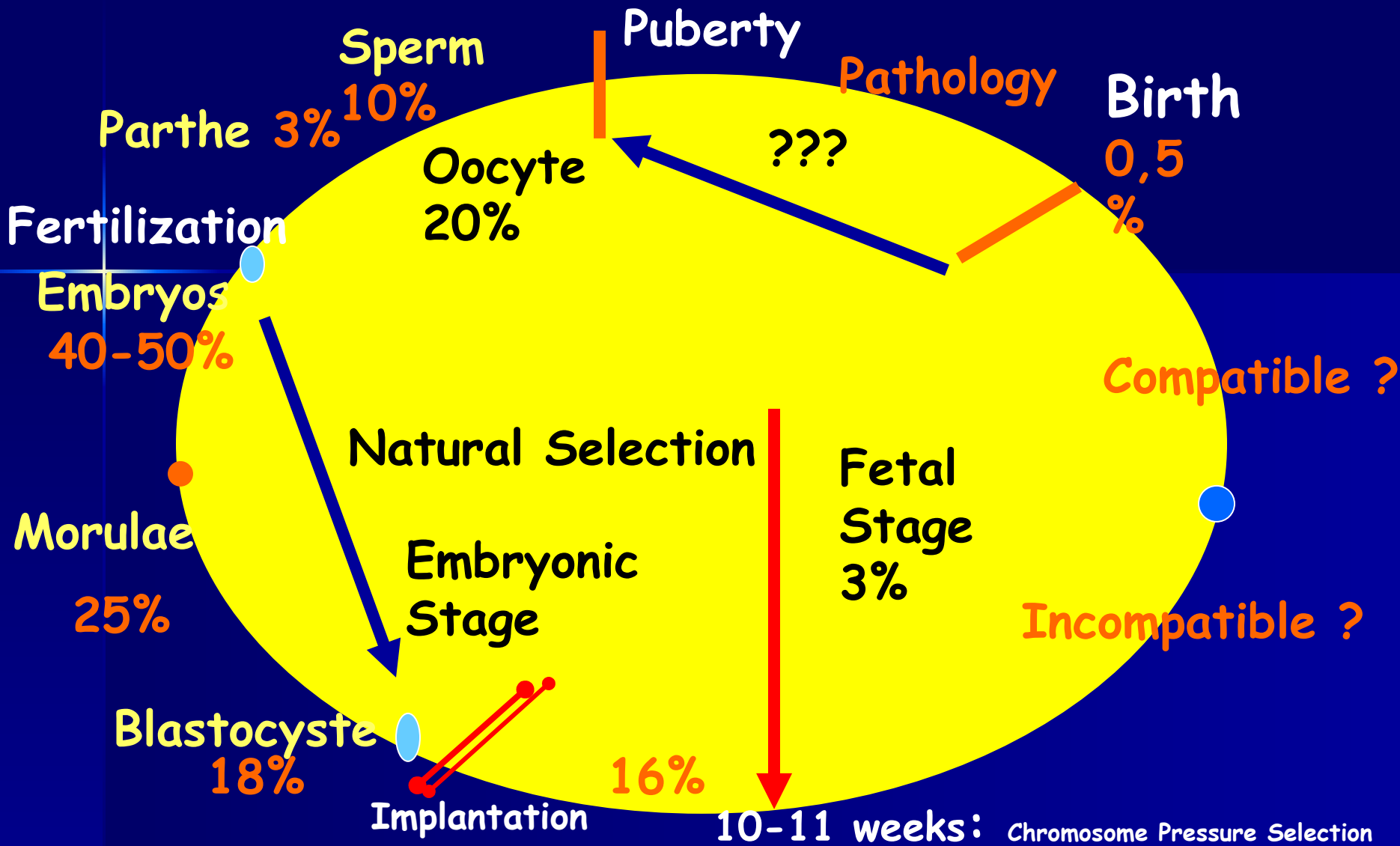


Benkhalifa et al Fertil Steril 2004

Clinical Data from pure PCO Patients

	N°	%
Patients	102	
Collected Oocytes	870	
Maturation 26-28h	415	47,7%
2PN	286	68,9%
Cleavage	185	64,7%
Embryo GI	61	33 %
GII	65	35 %
GIII	36	19,5 %
Blocked	23	12,5 %
Everage embryo Transfer		1,6 Embryo/Patient
Pregnancy rate		18,7 %

Following experience with the IVM procedure, the efficiency of producing homogeneous embryo cohort with good quality and viability is less than in classical IVF and intracytoplasmic sperm injection. When comparing oocytes matured *in vivo* versus *in vitro*, no apparent differences are seen at the level of nuclear maturation, in the rates of fertilization or cleavage, but rather in the developmental competence of the oocytes as exemplified by poor embryonic developmental competence and pregnancy rate. The clinical data have shown a biochemical pregnancy rate of approximately 18%, with a 40% early miscarriage rate (personal communication, Dr Benkhalifa). These observations indicate that the cytoplasmic competence must be different between *in-vitro* and *in-vivo* matured oocytes. Advances made in immature oocyte isolation and maturation, zygote and embryo culture conditions have increased the clinical feasibility of IVM.



From oocyte to early abortion: chromosomes abnormalities and embryo development failure

● M. Benkhalifa ^(1,2), P. Clément ⁽²⁾, F. Pellestor ⁽³⁾, G. Tachdjian ⁽⁴⁾, D. Caserta ⁽⁵⁾, A. Demiroj ⁽⁶⁾, E. Balashova ⁽⁷⁾, T. Gurgan ⁽⁸⁾

Oocyte maturation and competence

How can we explain it?

- Oocyte maturation is accompanied by a complex network of translational activation and repression of dormant maternal mRNAs
- These maternal mRNAs drive the oocyte's reentry into meiosis and control the rate of mitosis during the early cleavage divisions of the embryo.
- *The first step in translational activation of stored maternally inherited mRNAs is cytoplasmic extension of their poly(A) tails: translational activation*
- The cytoplasmic polyadenylation element (CPE) (consensus sequence UUUUUA1-2U), is necessary for cytoplasmic polyadenylation

Conclusion

- **There is room for improvement of**
 - **Gametes Quality before ICSI with new concept of clinical management**
 - **In Vitro maturation**
 - ***In vitro* embryo environment**
- **This may go through a better understanding of intermediate metabolism and molecular biology**
 - **Needs more basic research and Nanotechnology Application**

Conclusion:

Endogenous regulation of oocyte maturation is a complex sequence of events regulated by

endocrine parameters, oocyte/follicular cross-talk, and intra-oocyte kinase/phosphatase interactions

However,

in order to achieve acceptable birth rates, future studies should focus on characterization and regulation of oocyte cytoplasmic maturation, and how oocyte-derived factors influence zygotic genome activation and embryonic developmental competence

In-vitro maturation of oocytes: biological aspects

ART: Nanotechnology & molecular Biology

Perspective for Genome investigation ???

Genomics: Constitutional

Transcriptome: Development

Biomarkers & Proteomics: Implantation

WGA, molecular karyotyping
and genome profiling

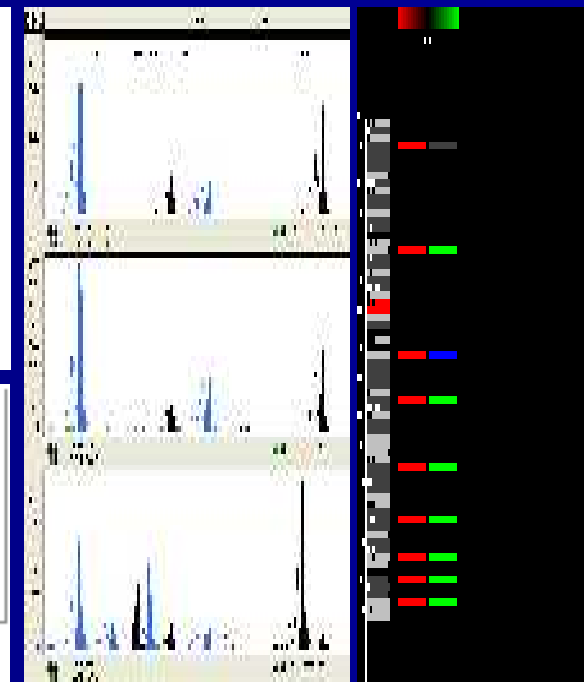
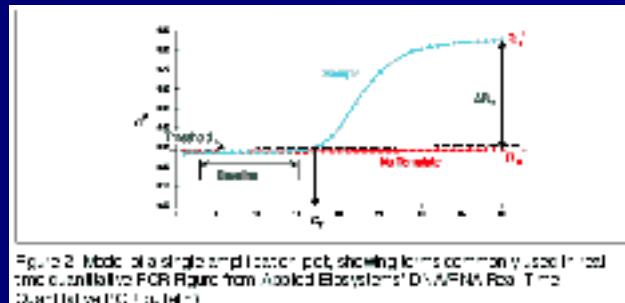
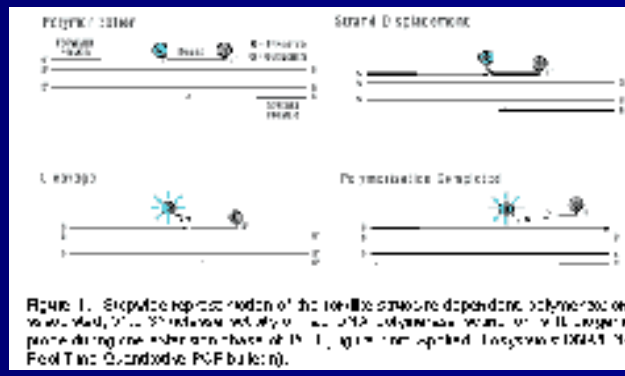
gene expression & transcription
RT PCR for qualitative and quantitative analysis

Functional proteins
embryo signature

Genes

RNAs

Proteins



**Dr Demirol A, Pr Gurgan T
&
the embryology Team**

**Women's Health Clinic.
IVF & Genetics Centre.
Ankara.
Turkey**

**Pr Y MENEZO
&
ATL R&D Laboratory Team
PARIS**

Dr Balashova E

**Planning Family and IVF Dept.
National Surgery Centre.
Moscou. Russa**

Dr Atef A, Dr Miron P

Montreal University

**Dr I Giakoumakis
M Jacoumakis**

**Mediterranean IVF & Genetics
Center. Creta. GREECE**