Τεχνικές και εφαρμογές της εξωσωματικής γονιμοποίησης

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ΘΕΜΑΤΑ ΜΑΙΕΥΤΙΚΗΣ-ΓΥΝΑΙΚΟΛΟΓΙΑΣ
Υποβοηθούμενη Αναπαραγωγή
Editorial

«It is a girl congratulations» φώναξε ο Patrick Steptoe,κατά την διάρκεια της καισαρικής τομής, στο χειρουργείο του Oldham General Hospital(UK) . «It is indeed» συμπλήρωσεο Bob Edwards. «The birth of the world's first IVF baby became a reality». Το ημερολόγιο έδειχνε 25/07/1978

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EDITOR-IN CHIEF

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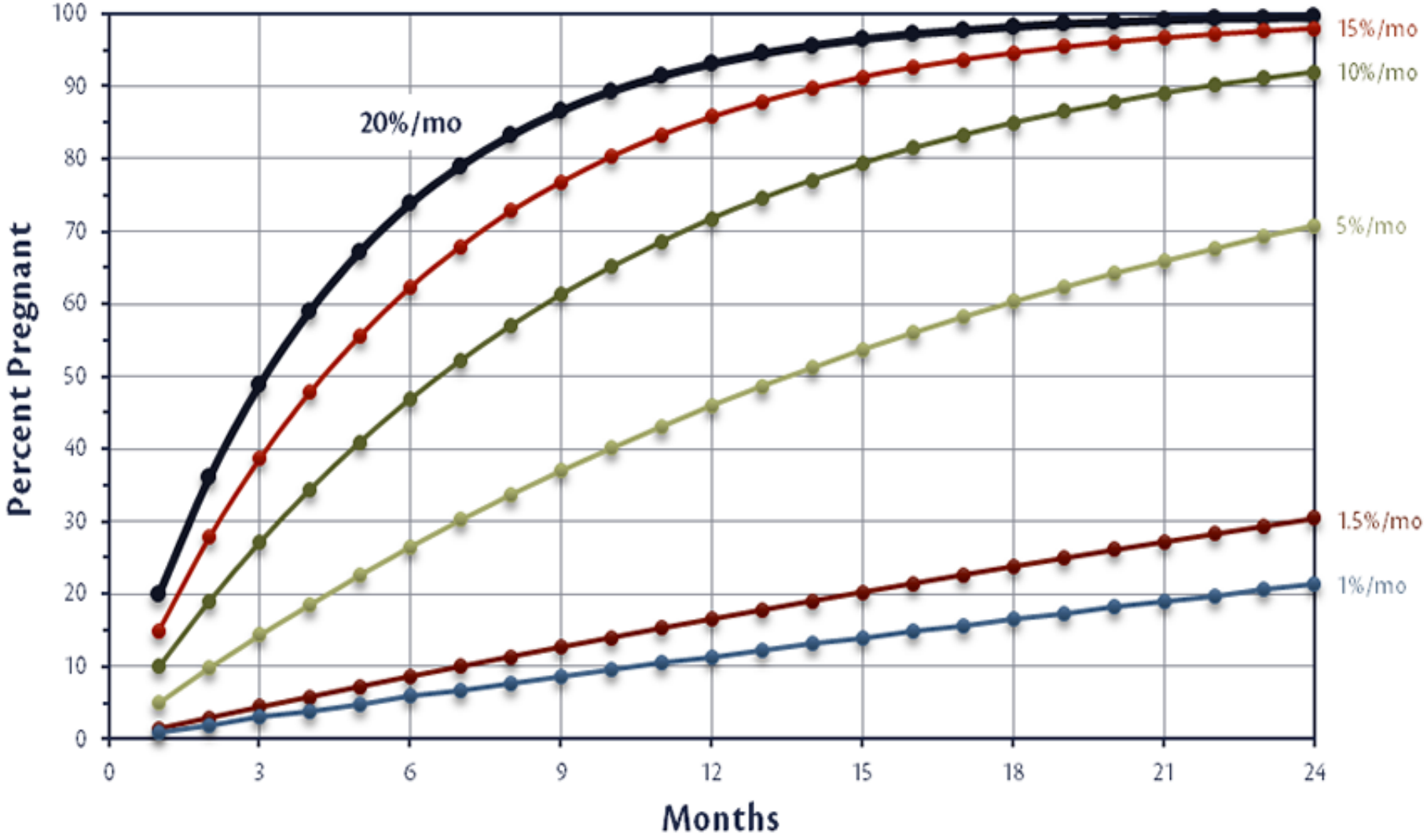
- ΥΠΟΓΟΝΙΜΟΤΗΤΑ-ΒΑΣΙΚΕΣ ΑΡΧΕΣ
  Ορισμος-Αιτια-Διερευνησεις-Θεραπειες
- ΕΛΕΓΧΟΣ ΣΠΕΡΜΑΤΟΣ – WHO CRITERIA 2010
- ΕΞΩΣΩΜΑΤΙΚΗ ΓΟΝΙΜΟΠΟΙΗΣΗ (IVF AND ICSI)
  Ενδειξεις-Πρωτοκολλα-Τεχνικες
- ΠΡΟΕΜΦΥΤΕΥΤΙΚΗ ΔΙΑΓΝΩΣΗ (PGD)
  Ποτε? Και Πως?
- ΚΑΤΑΨΥΞΗ ΓΕΝΕΤΙΚΟΥ ΥΛΙΚΟΥ
  ΕΜΒΡΥΩΝ (Embryo Freezing)
  ΩΑΡΙΩΝ(Oocyte Freezing)
  ΣΠΕΡΜΑΤΟΣ (Sperm Freezing)

- ΚΛΩΝΟΠΟΙΗΣΗ (CLONING)

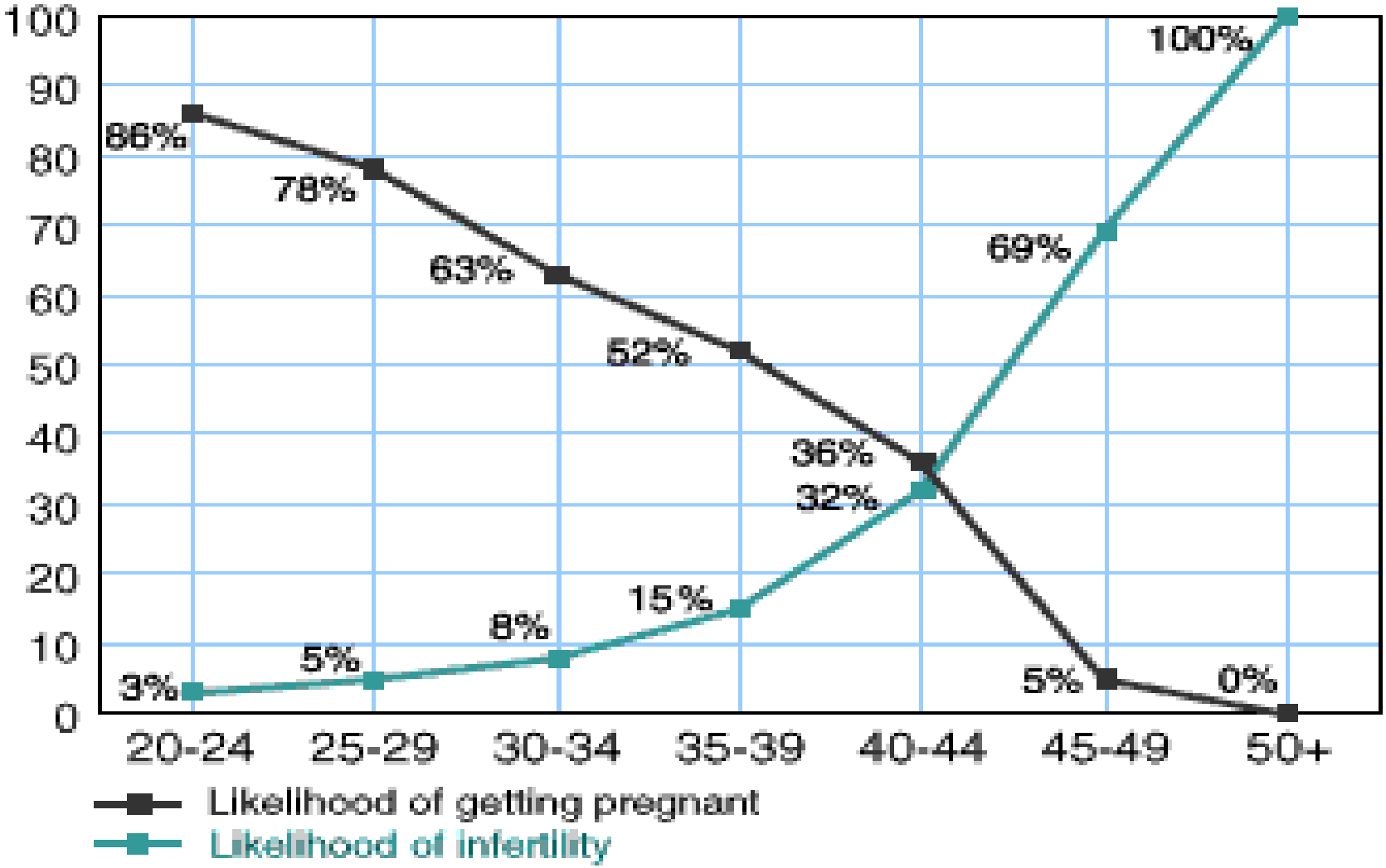
Definition of Infertility

- Infertility is "a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse."… (WHO)

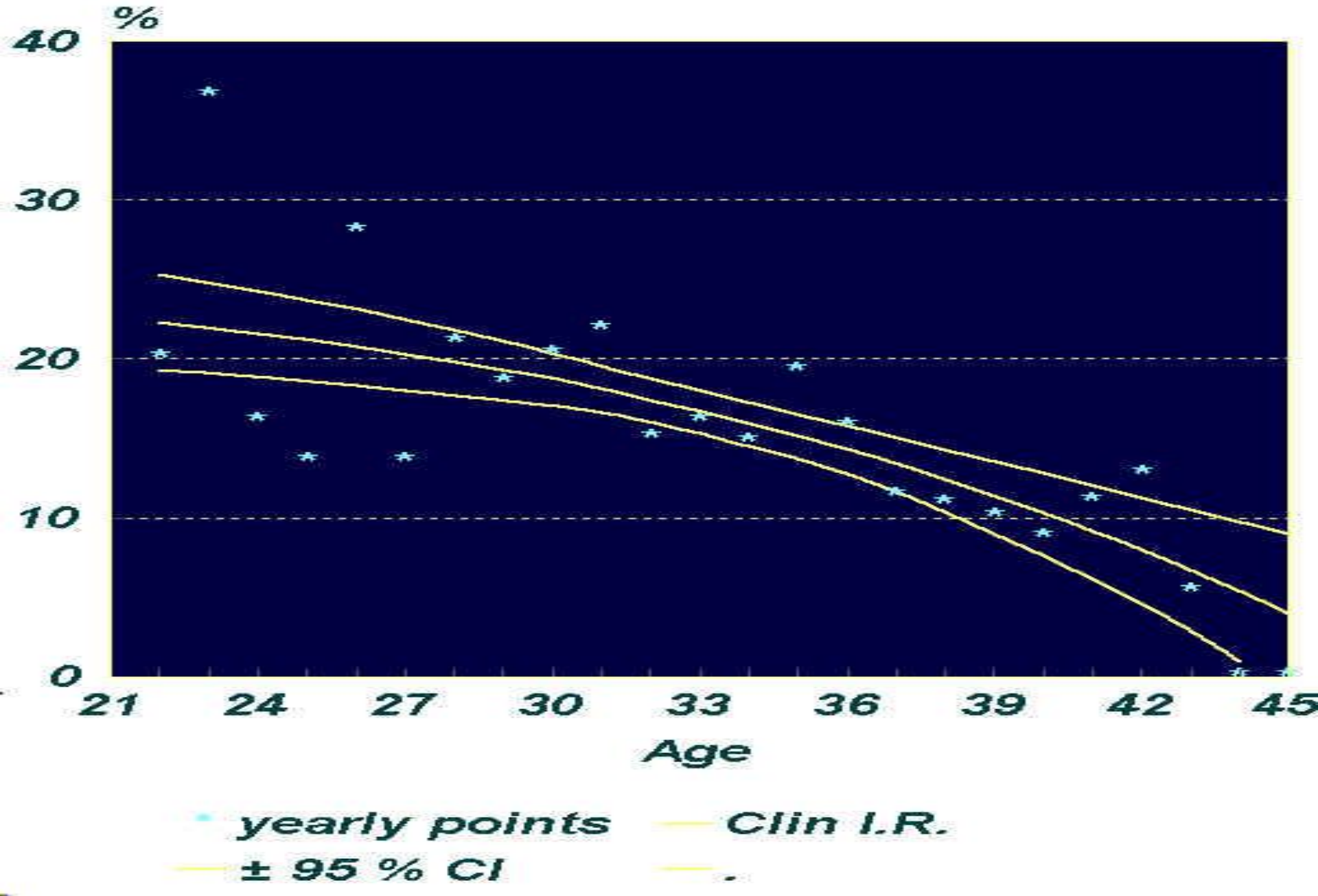
MONTHLY PROBABILITY OF CONCEPTION

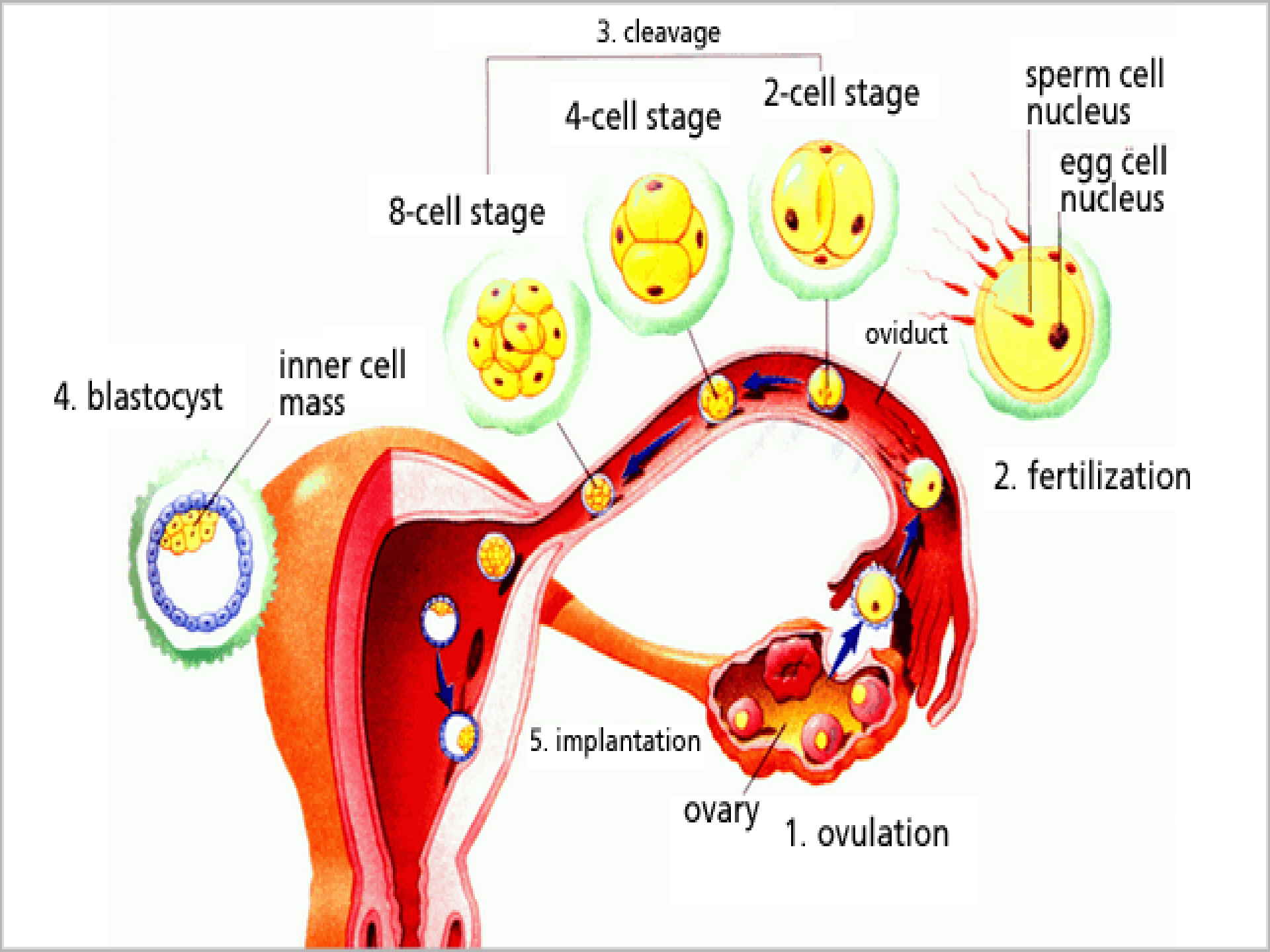


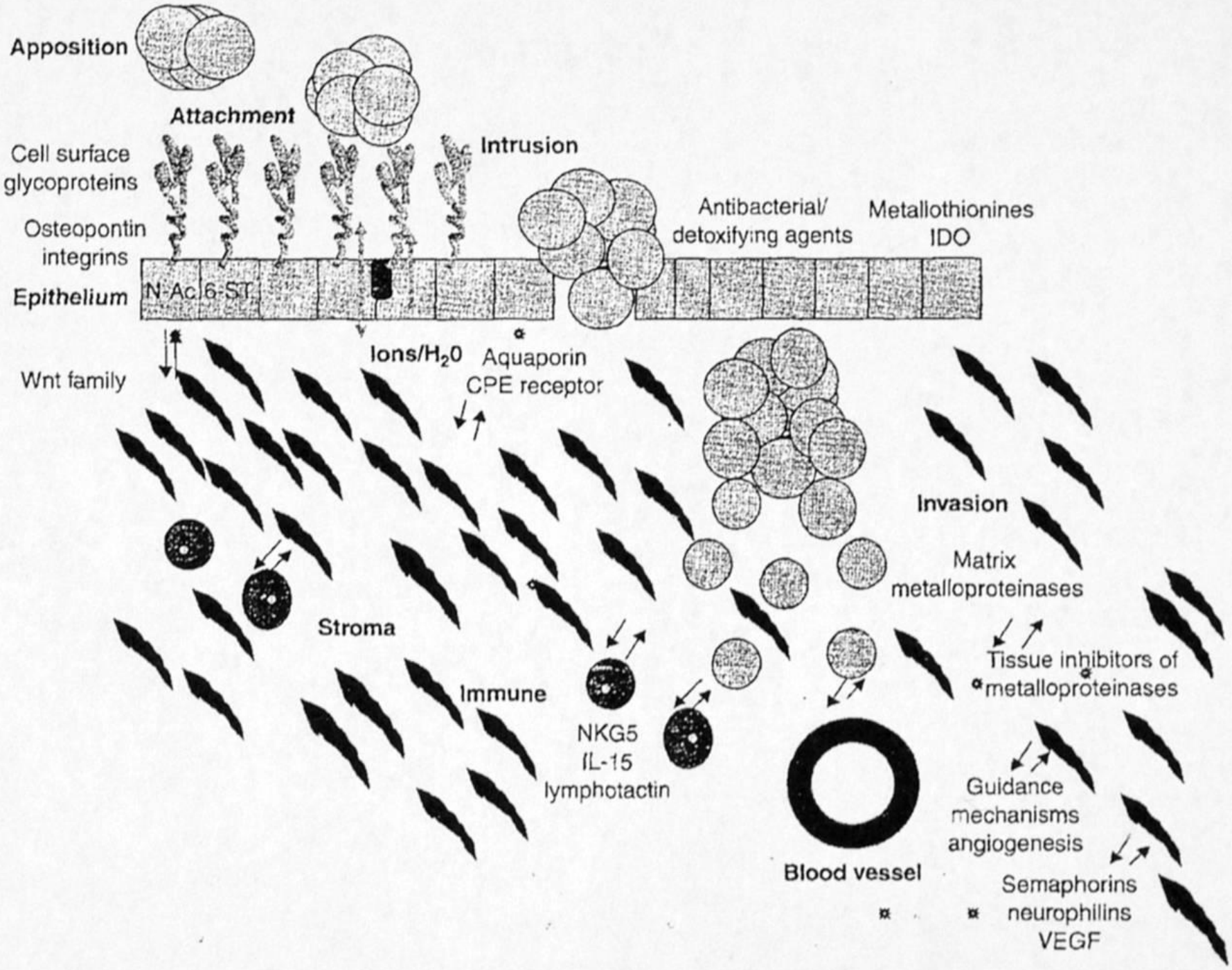
Γονιμοτης-Υπογονιμοτης σε σχεση με την ηλικια



IMPLANTATION RATE WITH AGE




3. cleavage
2-cell stage
4-cell stage
8-cell stage
sperm cell nucleus
egg cell nucleus
oviduct
2. fertilization
4. blastocyst
inner cell mass
5. implantation
ovary
1. ovulation


Apposition
Attachment
Intrusion
Cell surface glycoproteins
Osteopontin integrins
Epithelium
N-Ac 6-ST
Antibacterial/ detoxifying agents
Metallothionines IDO
Wnt family
Ions/H_2O
Aquaporin
CPE receptor
Invasion
Matrix metalloproteinases
Stroma
Immune
NKG5
IL-15
lymphotactin
Tissue inhibitors of metalloproteinases
Blood vessel
Guidance mechanisms angiogenesis
Semaphorins
neurophilins
VEGF

CAUSES OF SUBFERTILITY

MALE
35%

OVULATORY
25%

TUBAL
15%

UNEXPAINED
15%

OTHERS
10%

Basic Investigations for the infertile couple

MALE FACTOR

Semen Analysis

OVULATORY FACTOR

Progesterone D21

FSH/LH

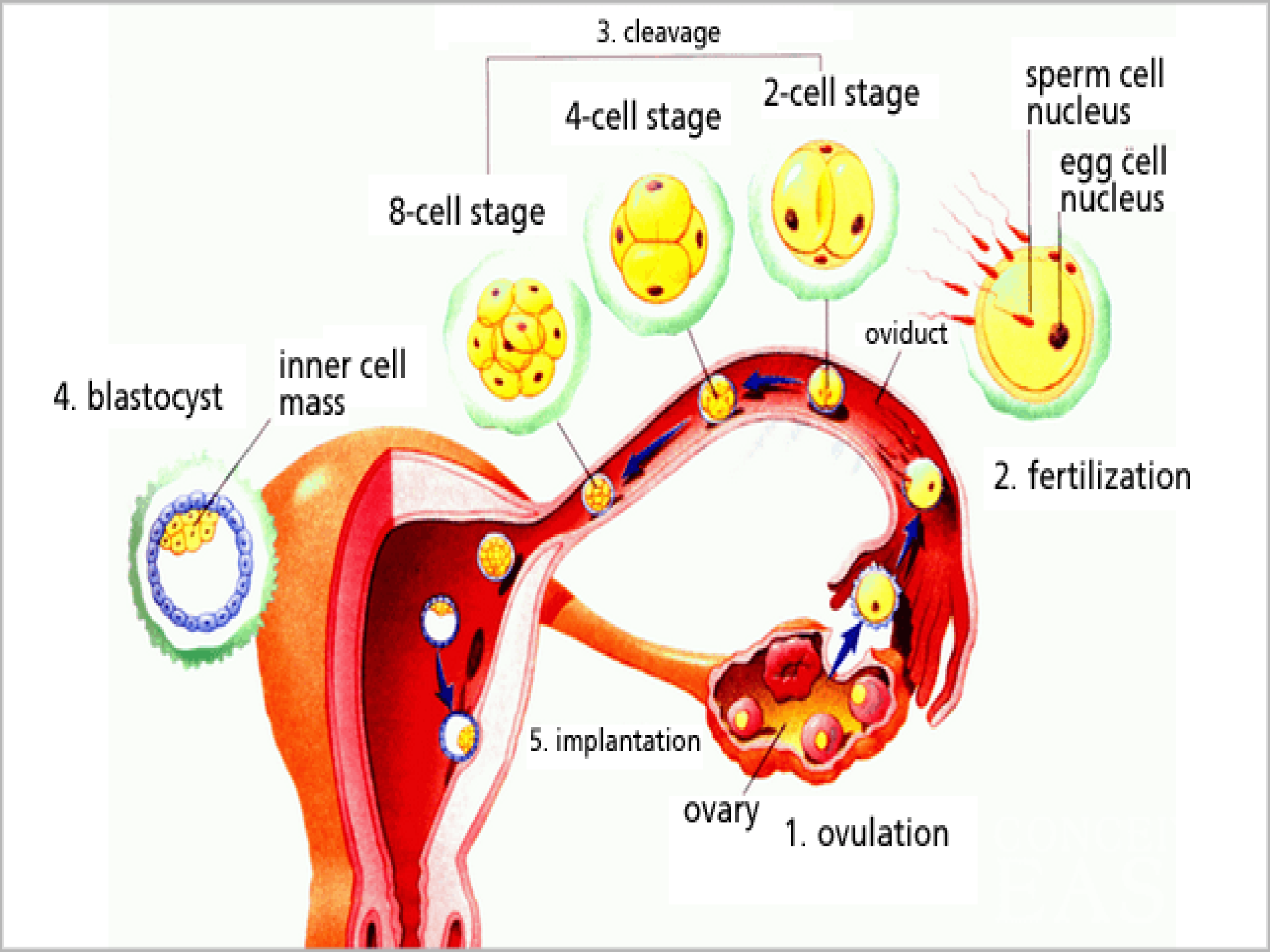
Ultrasound scan

TUBAL & UTERINE FACTOR

HSG/Hycosy

Laparoscopy

Hysteroscopy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sperm cell nucleus
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inner cell mass
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Overview

1. Contents of seminal fluid
2. indications of semen analysis
3. collection of sample
4. Examination of seminal fluid
    a. in infertility
        i. physical examination
            1. visual appearance
            2. viscosity
            3. volume
            4. pH
        ii. microscopic examination
            1. motility
            2. viability
            3. count
            4. morphology
        iii. immunologic analysis
            1. sperm MAR test – direct, indirect
            2. immunobead test
        iv. biochemical analysis
            1. fructose – seminal vesicle marker
            2. total zinc
            3. total acid phosphatase — prostate marker
            4. total citric acid
            5. alpha glucosidase — epididymis
            6. carnitine
        v. sperm function tests
            1. post coital (Sims-Huhner test)
            2. Cervical mucous penetration test
            3. Hamster egg penetration test
            4. Hypoosmotic swelling of flagella

Semen Analysis

WHO Reference Values	Reference Limit
Semen volume (ml)	1.5
Sperm concentration (10^6/ml)	15
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Progressive motility (PR, %)	32
Total motility (PR +NP, %)	40
Vitality (live sperms, %)	= / > 58
Sperm morphology (NF, %)	= / > 4
pH*	= / > 7.2
Leucocyte* (10^6/ml)	<1
MAR/Immunobead test* (%)	<50

1. WHO 2010 laboratory manual for the Examination and processing of human semen

Categories of sperm motility:

- Progressive (PR)

  Active movement regardless of speed

  - Linear
  - In large circle

- Non-progressive (NP)

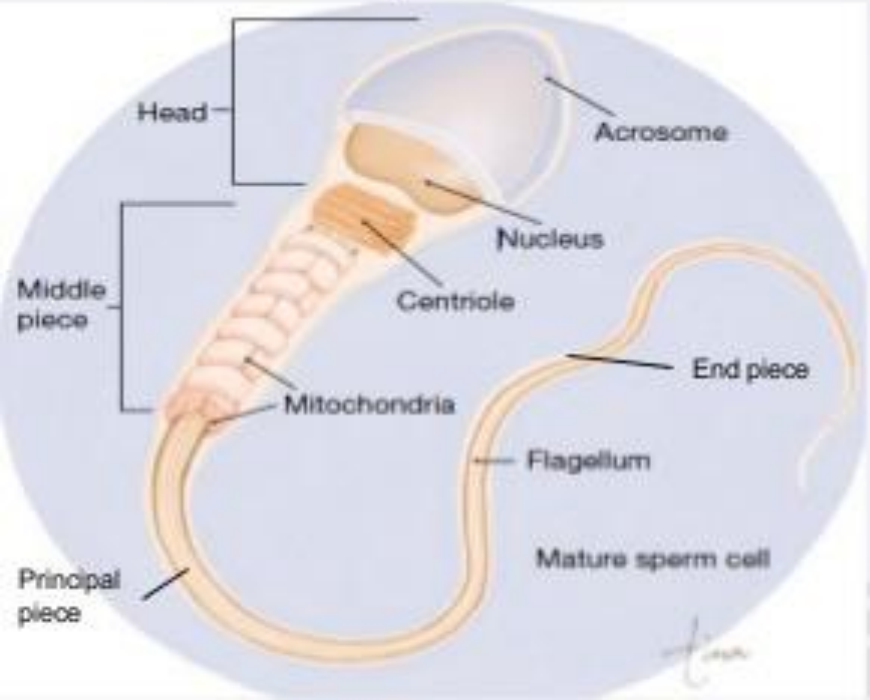
  Motility without progression.

- Immotility (IM)

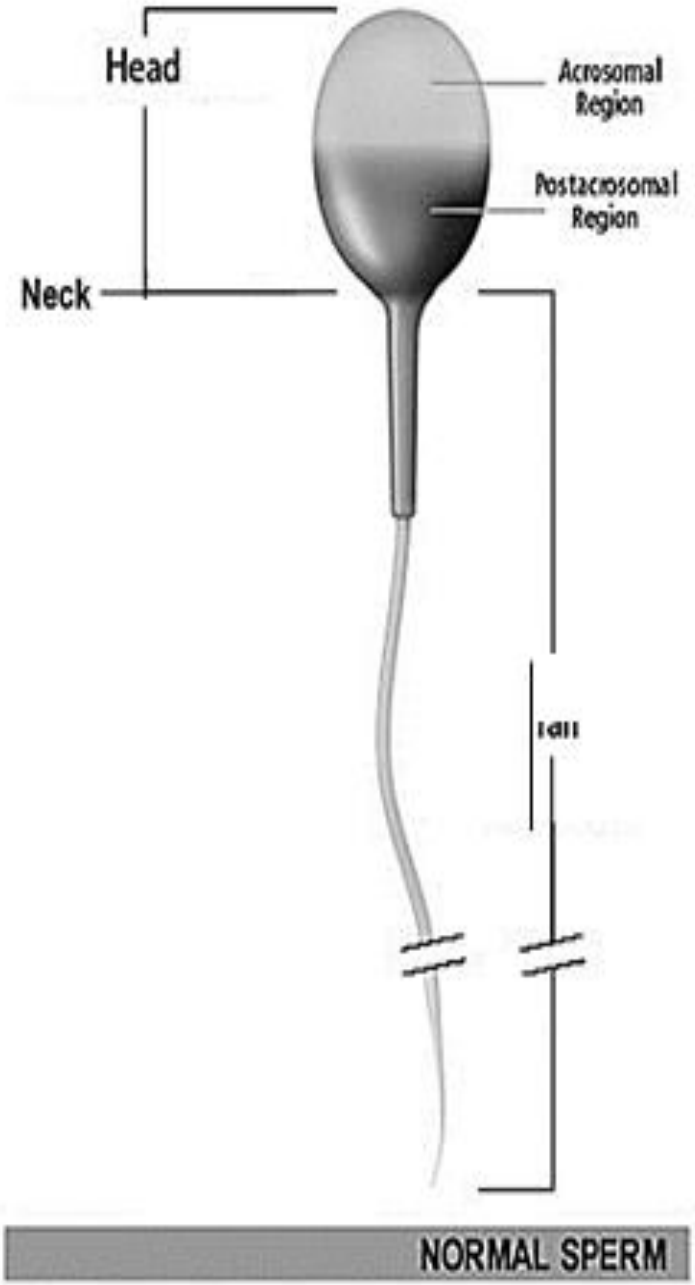
  No movement

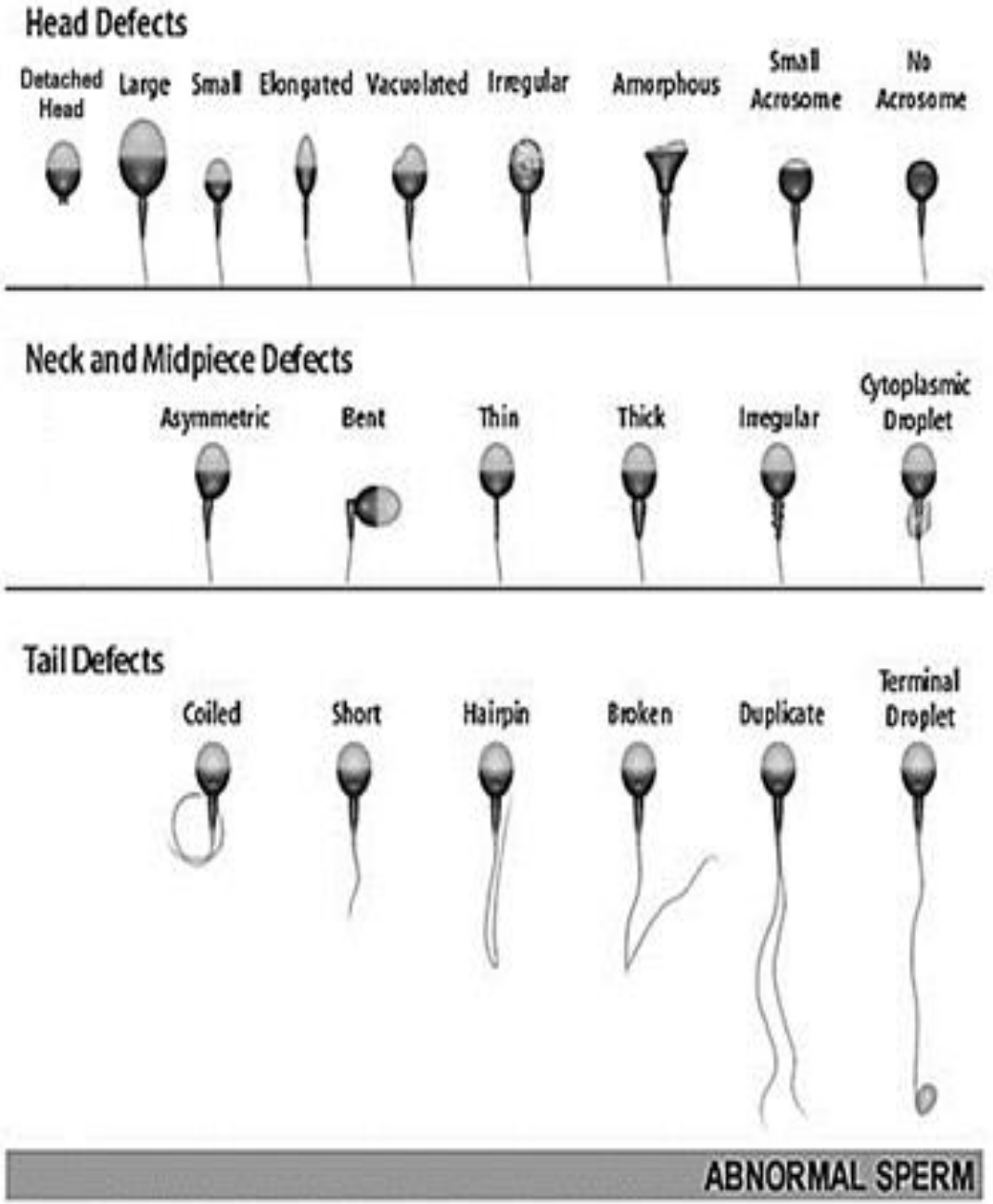
SPERM MORPHOLOGY

- A smear is prepared by spreading a drop of seminal fluid on a glass slide,stained and percentage of normal & abnormal spermatozoa are counted.
- 200 spermatozoa should be counted under oil immersion.
- Normal spermatozoa consists of : head,neck & tail.



- A normal spermatozoa has a flattened oval head and an elongated tailpiece


Head
Acrosomal Region
Postacrosomal Region
Neck
Tail
NORMAL SPERM


Head Defects
Detached Head
Large
Small
Elongated
Vacuolated
Irregular
Amorphous
Small Acrosome
No Acrosome
Neck and Midpiece Defects
Asymmetric
Bent
Thin
Thick
Irregular
Cytoplasmic Droplet
Tail Defects
Coiled
Short
Hairpin
Broken
Duplicate
Terminal Droplet
ABNORMAL SPERM

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            6. carnitine
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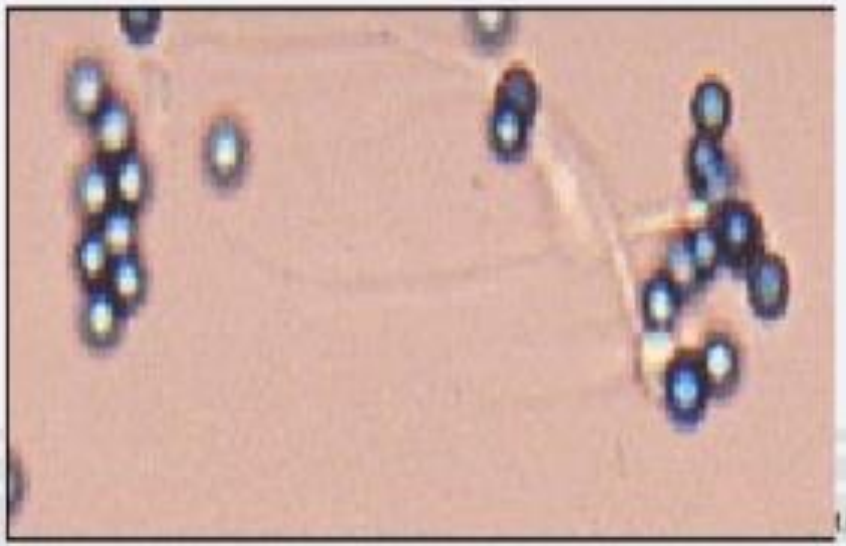
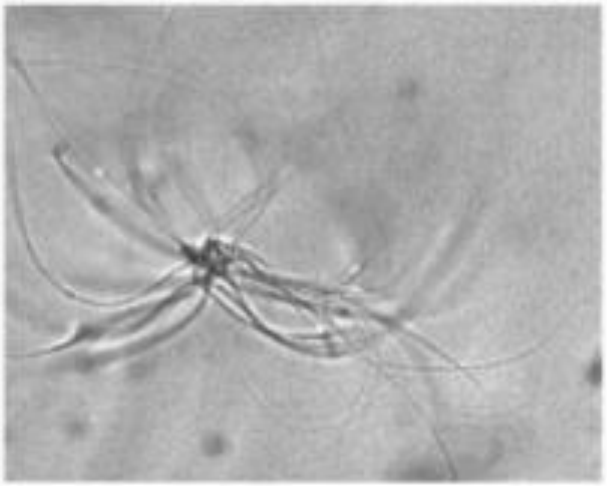
Mixed agglutination reaction(MAR) test

- The MAR test is a screening procedure used primarily to detect the presence of IgG antibodies.
- The semen sample containing motile sperm is incubated with IgG antihuman globulin (**AHG)** and a suspension of IgG coated-latex particles.
- The bivalent AHG will bind simultaneously to both the antibody on the sperm and the antibody on the latex particles, forming microscopically visible **clumps of sperm and particles.**
- Less than 10 percent of the motile sperm attached to the particles is considered normal.

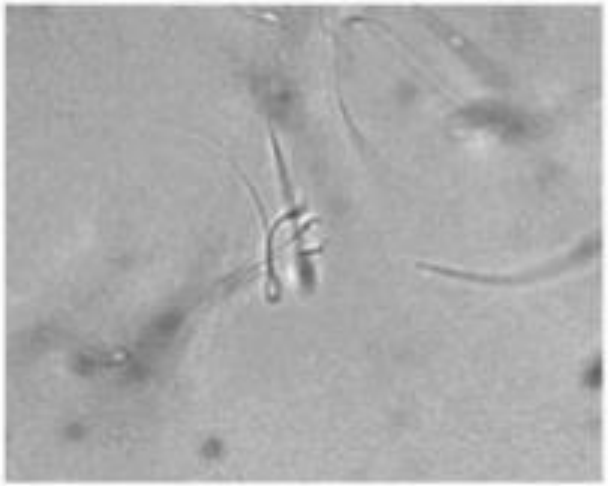


Sperm Agglutination

- Motile sperms sticking to each other by their heads, tails or mid-pieces.



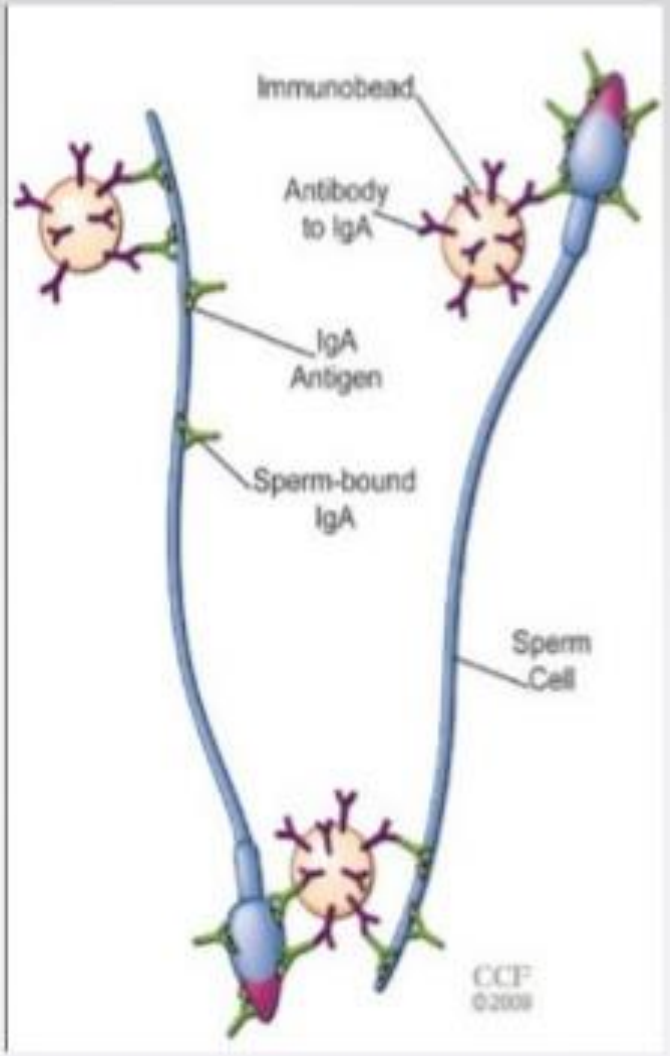
control



anti-BSp66

The immunobead test

- It is a more specific procedure
- It can be used to detect the presence of IgG, IgM, and IgA antibodies and will demonstrate what area of the sperm (head, neck, or tail) the autoantibodies are affecting.
- Sperm are mixed with polyacrylamide beads known to be coated with either anti-IgG, anti-IgM, or anti-IgA.
- Microscopic examination of the sperm will show the beads attached to sperm at particular areas.
- Depending on the type of beads used, the test could be reported as “IgM tail antibodies,” “IgG head antibodies,” and so forth.
- The presence of beads on less than 20 percent of the sperm is considered normal.



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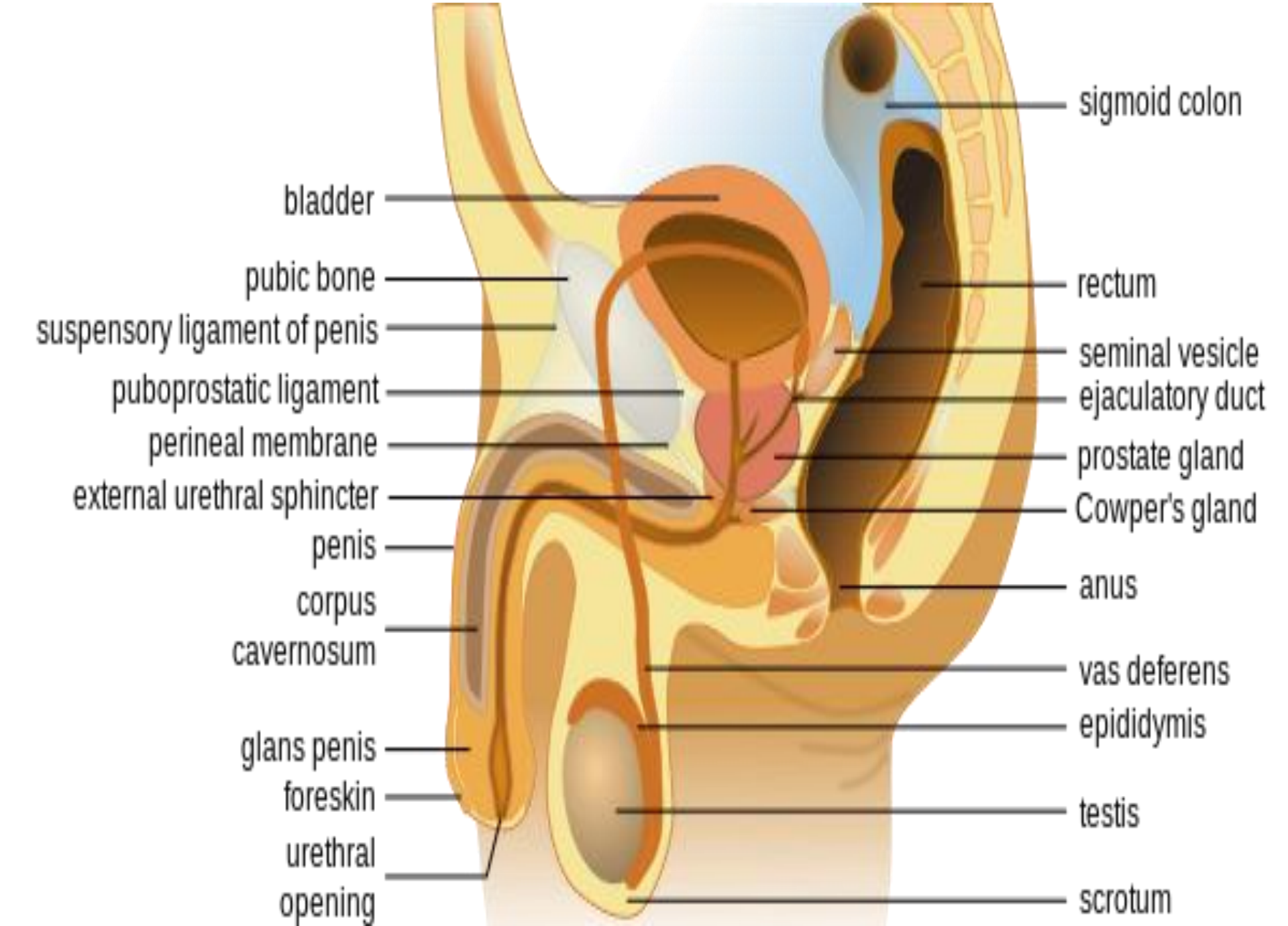
MALE REPRODUCTIVE TRACT



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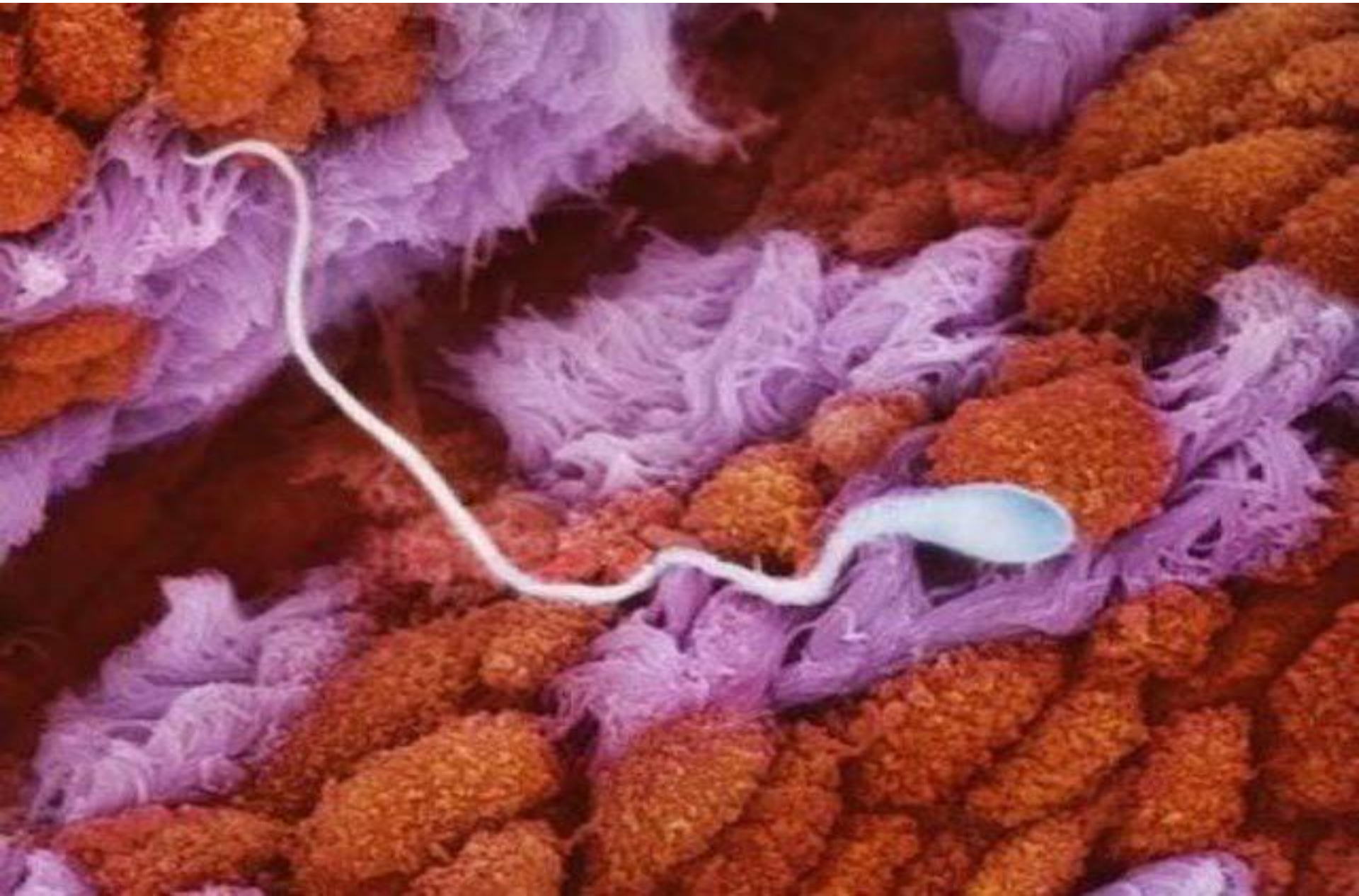
pH*	= / > 7.2
Leucocyte* (10^6/ml)	<1
MAR/Immunobead test* (%)	<50

1. WHO 2010 laboratory manual for the Examination and processing of human semen

SPERM IN THE FALLOPIAN TUBE



THE SPERM IS APPROACHING THE OOCYTE

ΛΙΓΟ ΠΡΙΝ ΤΗΝ ΣΥΛΛΗΨΗ....!!!!

ΕΠΙΤΕΛΟΥΣ ΕΦΘΑΣΑ!!! ....ΓΟΝΙΜΟΠΟΙΗΣΗ

Therapeutic options

- **Tubal factor infertility**

1. IVF
2. Laparoscopic Tubal surgery- depending on the age and the degree of tubal damage

- **Unexplained Infertility**

1. COS + IUI X 3 cycles (if female <40)
2. IVF

ΔΙΑΤΑΡΑΧΕΣ ΤΗΣ ΩΟΡΡΗΞΙΑΣ

Hypo-Hypo WHO Group I	PCO WHO Group II	Hyper-Hypo Premature Ovarian Failure
HMG with LH	Clomiphene HMG+/- IUI Ovarian drilling	No Hormonal Rx
Pulsatile GnRH	IVF	Oocyte Donation

AZOOSPERMIA
THERAPEUTIC OPTIONS

NORMO GONADOTROPHIC	HYPER GONADOTROPHIC	HYPO GONADOTROPHIC
PESA/TESE+ ICSI	TESE+ICSI	HMG
DONOR SPERM	DONOR SPERM	DONOR SPERM

Oligo-Astheno-Teratozoospermia Therapeutic options

MILD

15M>SD>10M

50%>SM>25 %

14%>NF>4%

COS+IUIX3

SEVERE

5M>SD>0

25%>SM>0%

>10%NF>0%

ICSI

Woman over 40 years and mild OAT Poor Success with….IUI!!

THE PREFFERED OPTION IS IVF

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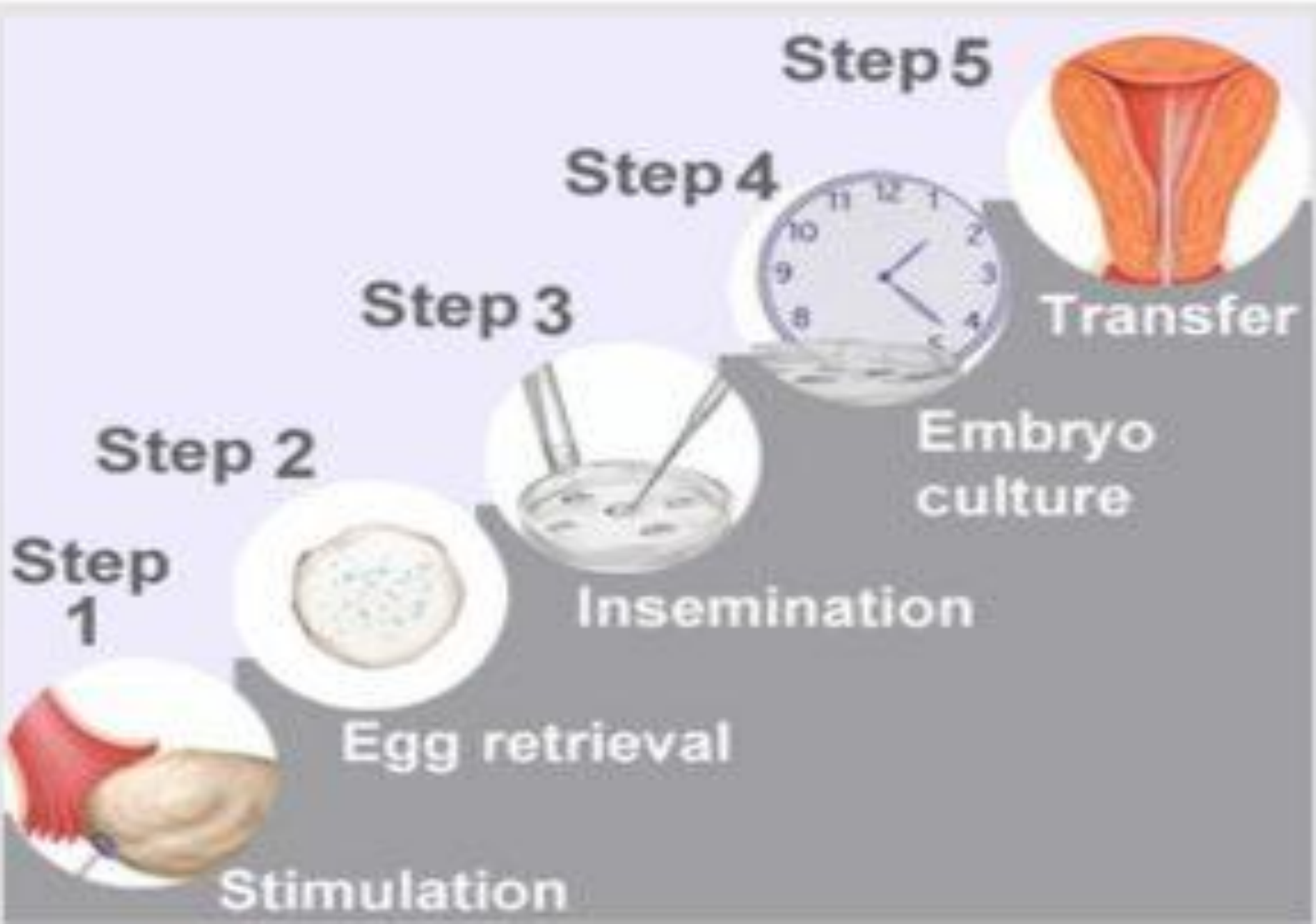
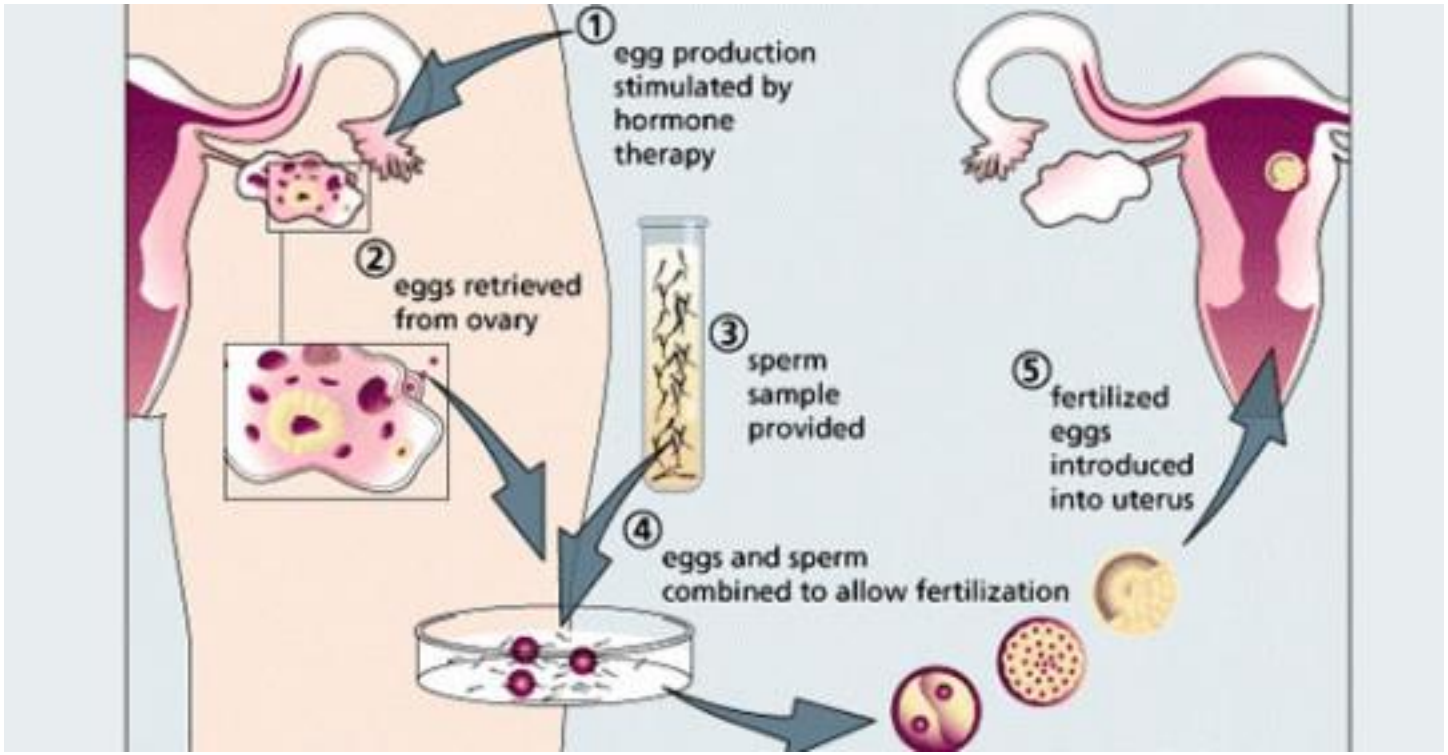
  Σπερματος (Sperm Freezing)
  Ωαριων(Oocyte Freezing)
  Εμβρυων (Embryo Freezing)

- ΚΛΩΝΟΠΟΙΗΣΗ (CLONING)

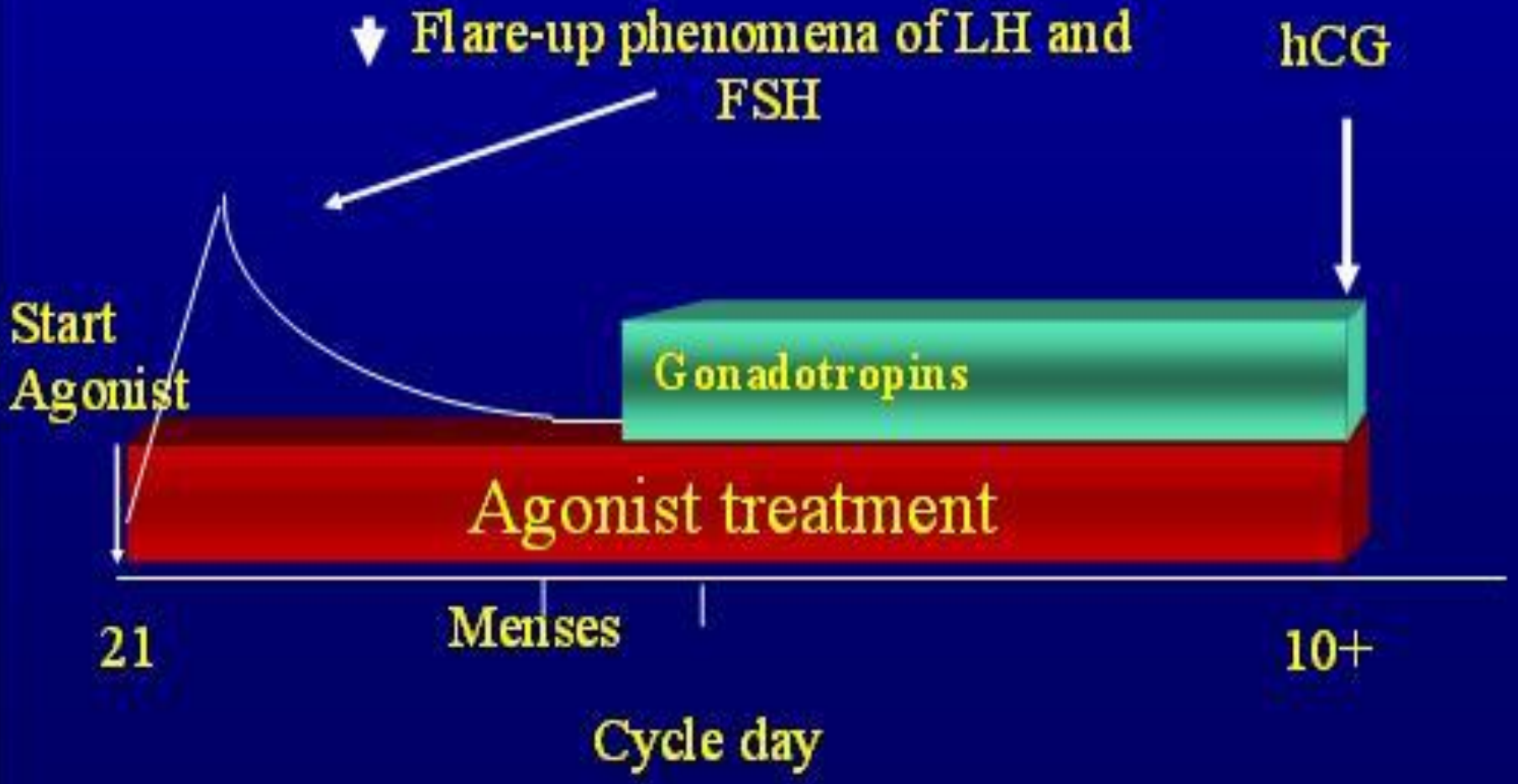
IVF is 5 Steps Procedure



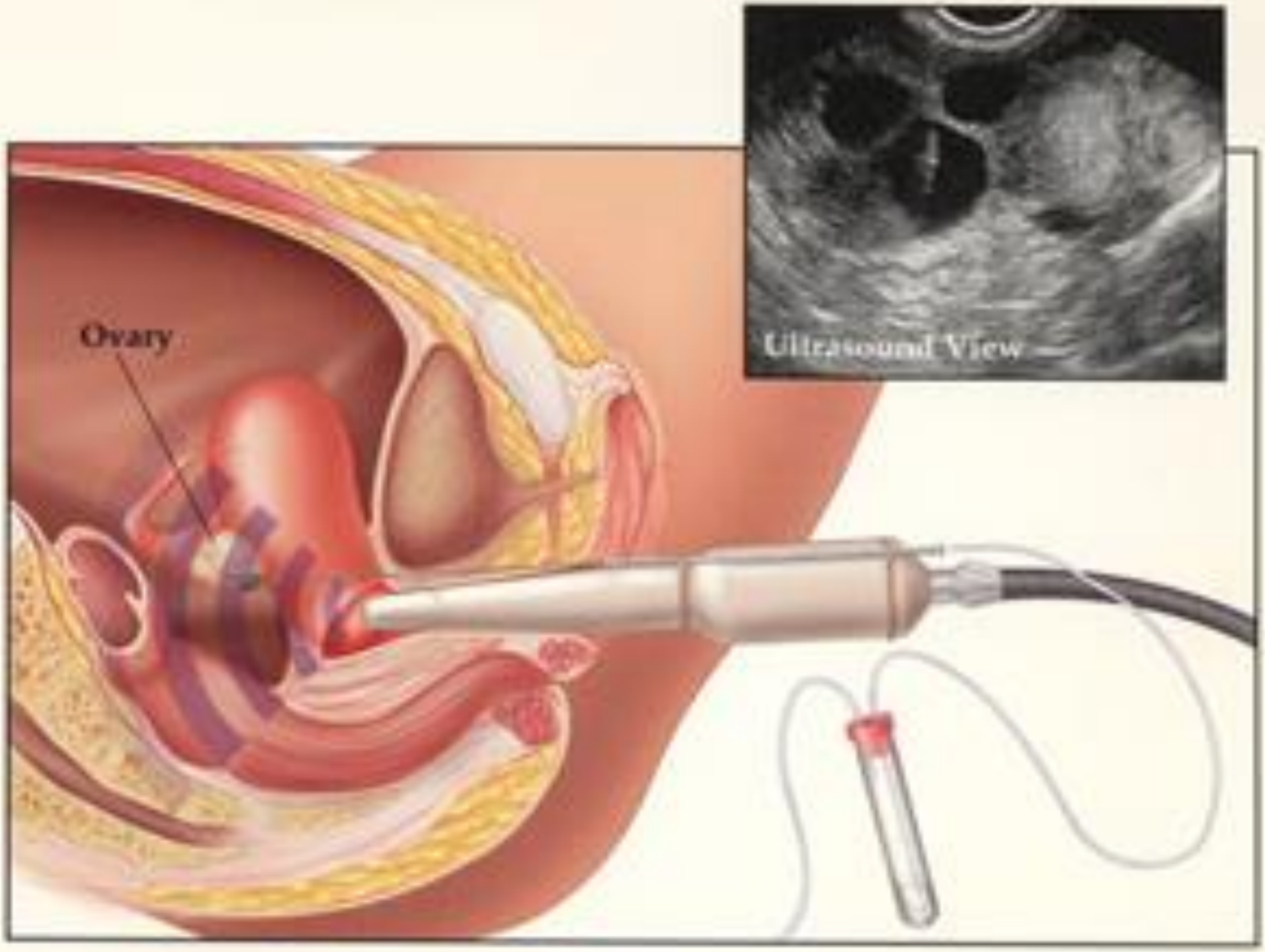

1
egg production stimulated by hormone therapy
2
eggs retrieved from ovary
3
sperm sample provided
4
eggs and sperm combined to allow fertilization
5
fertilized eggs introduced into uterus

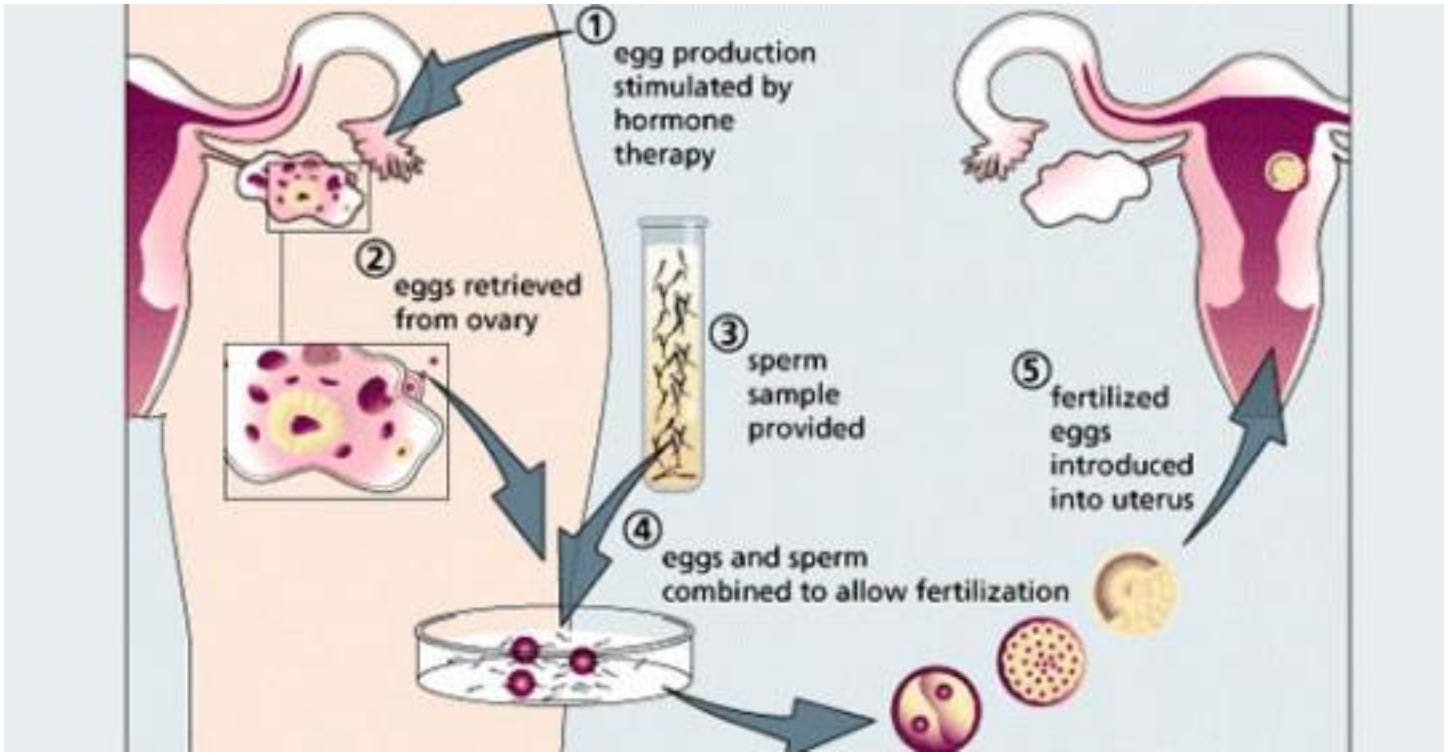
GnRH agonist (long protocol)

Starting day 21 (luteal phase)



Treatment protocol with the GnRH antagonist


Ovary
Ultrasound View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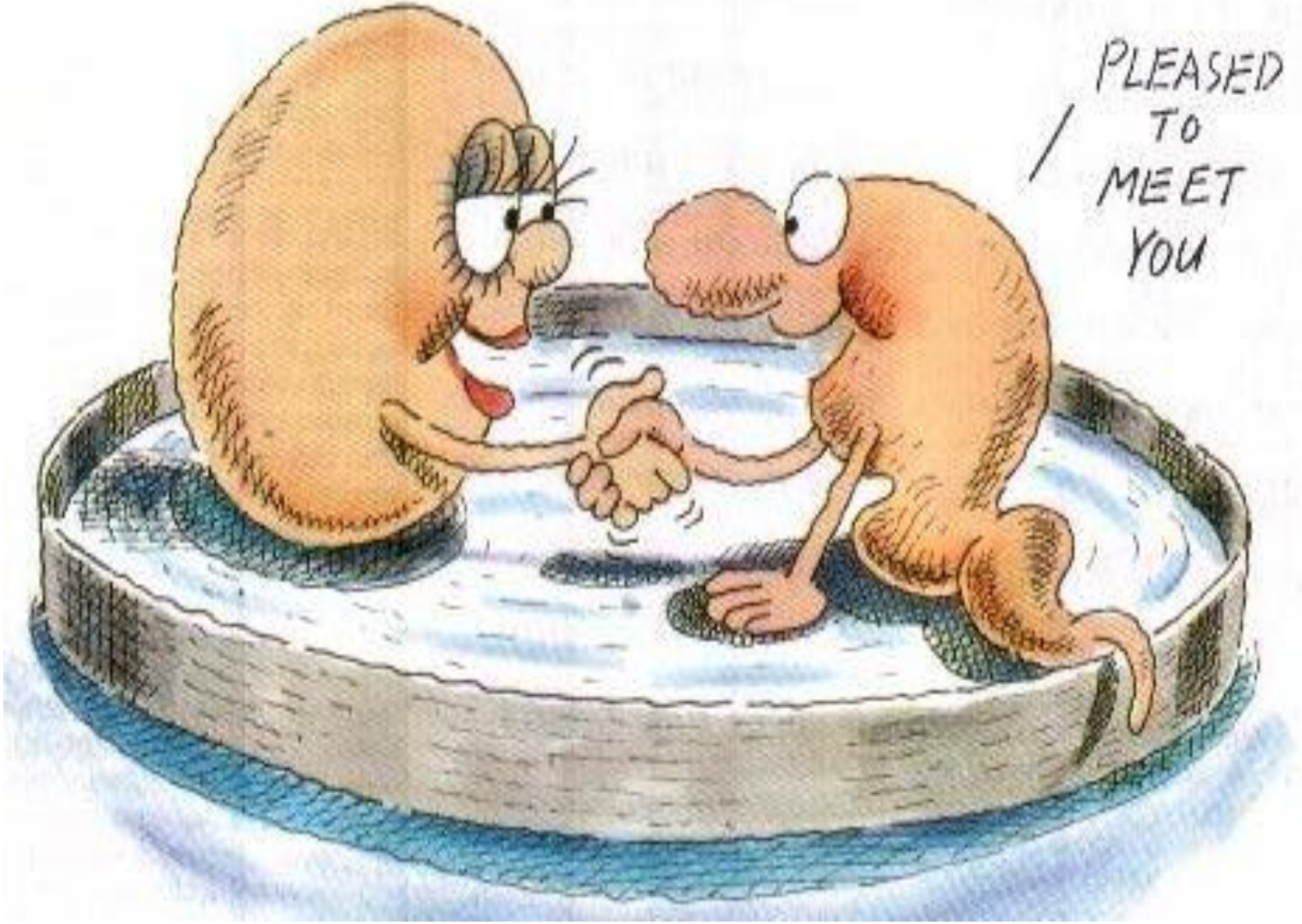
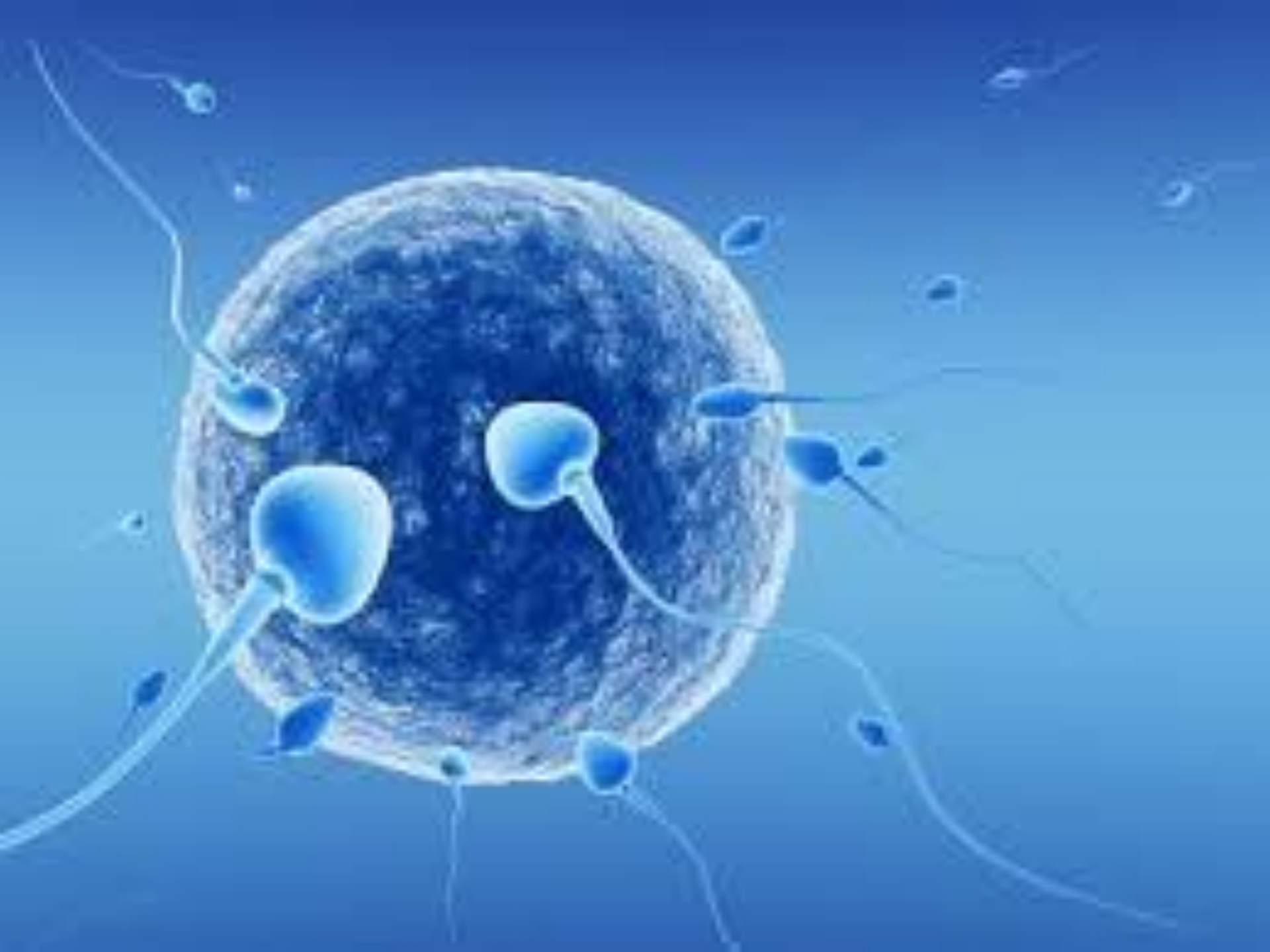
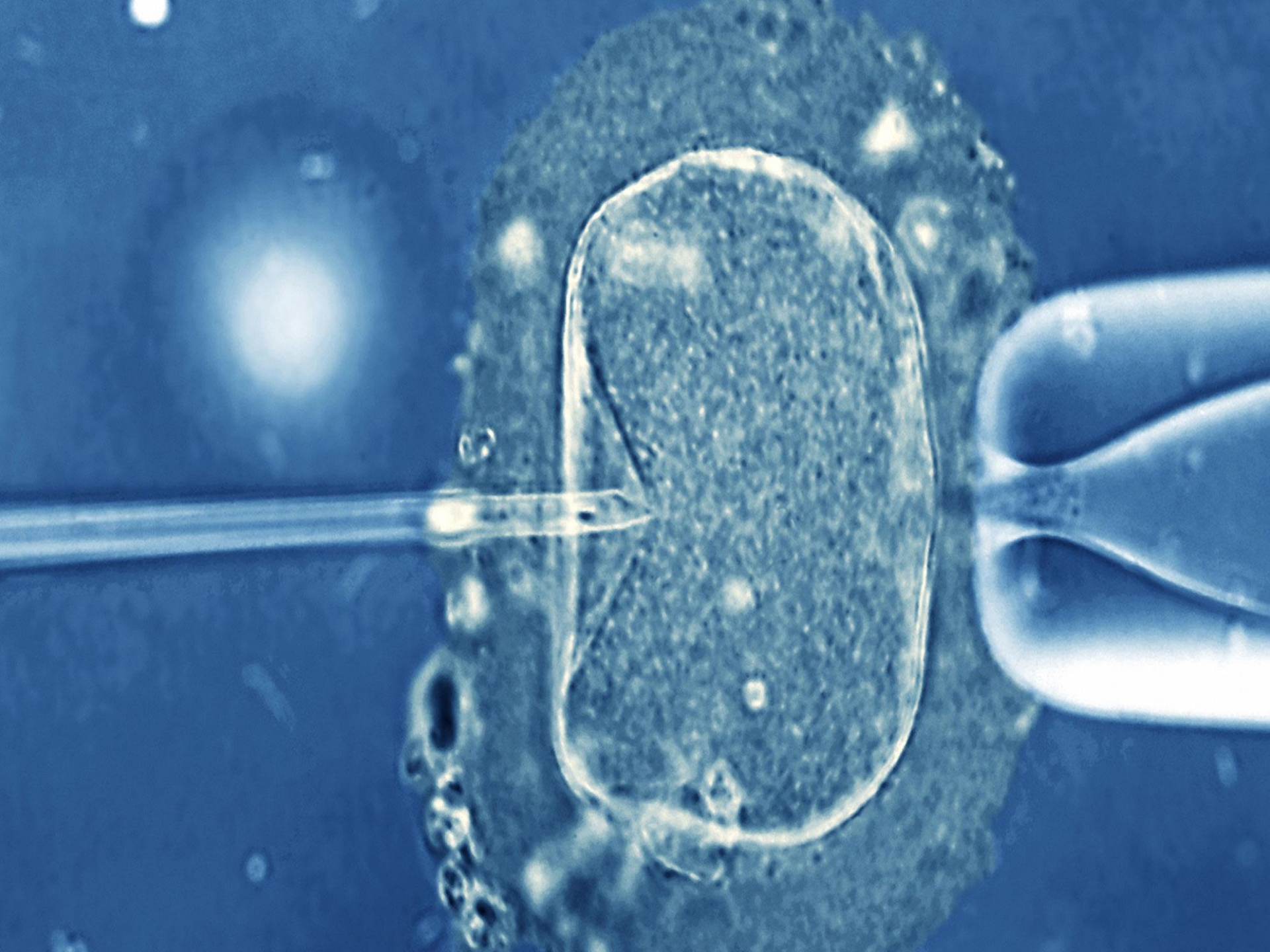
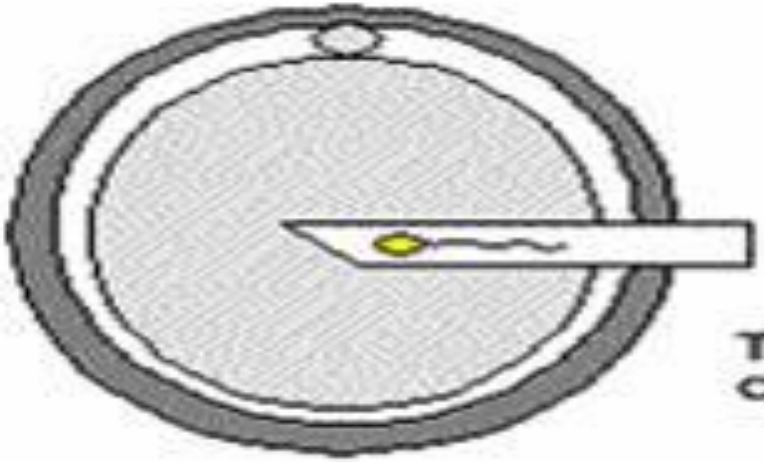

PLEASED TO MEET YOU

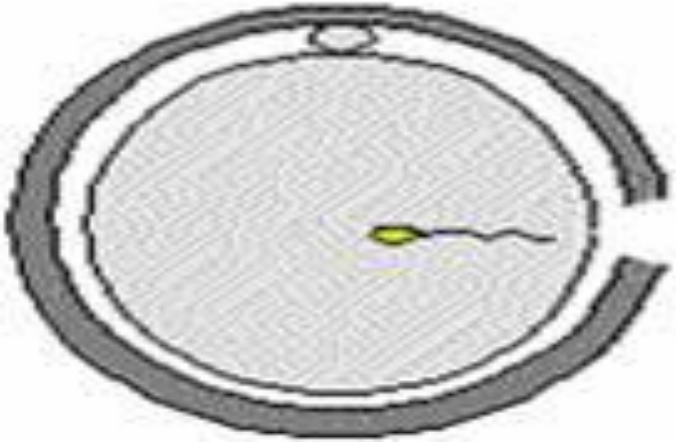




The sperm is immobilsed and loaded into the injection pipette

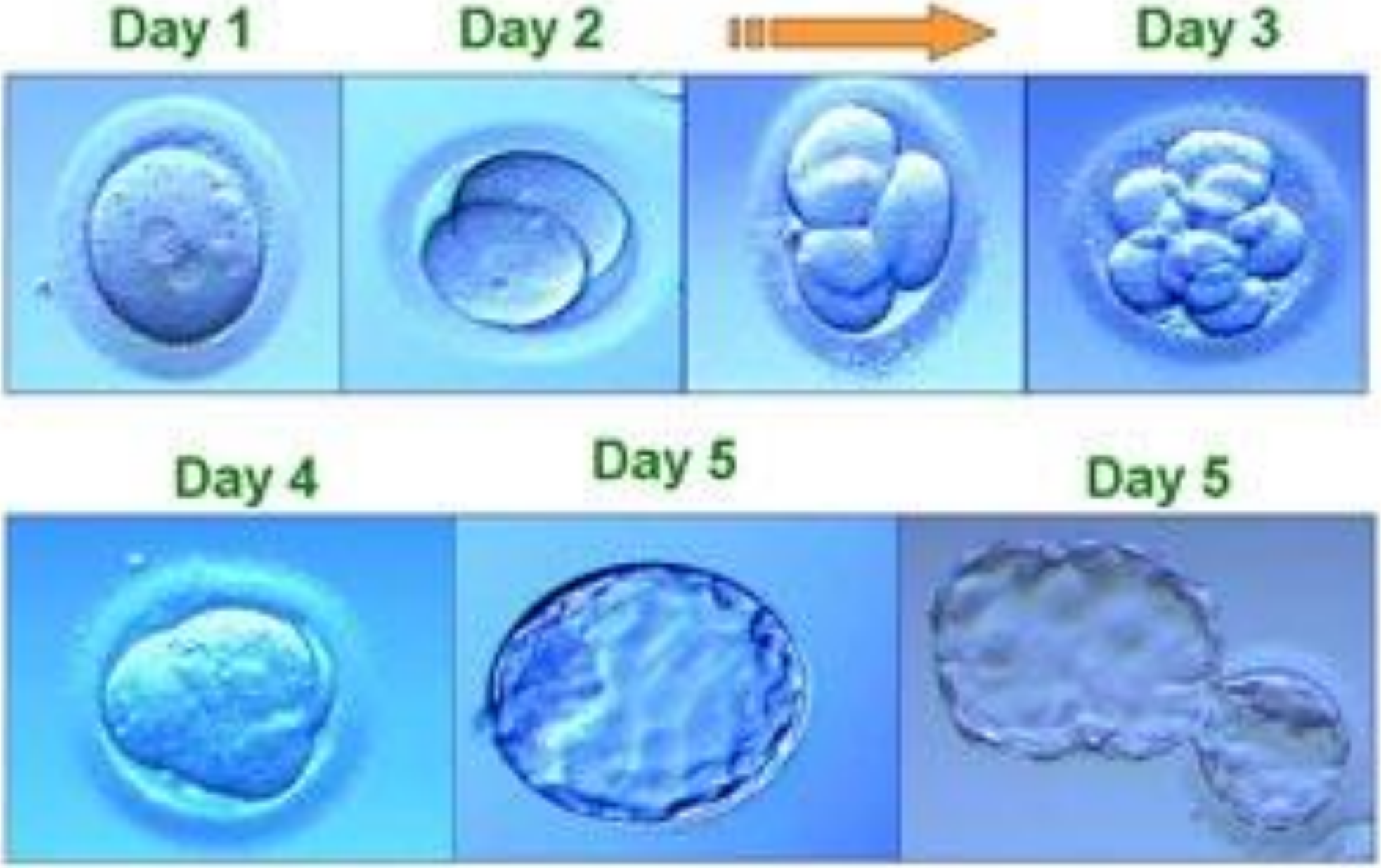


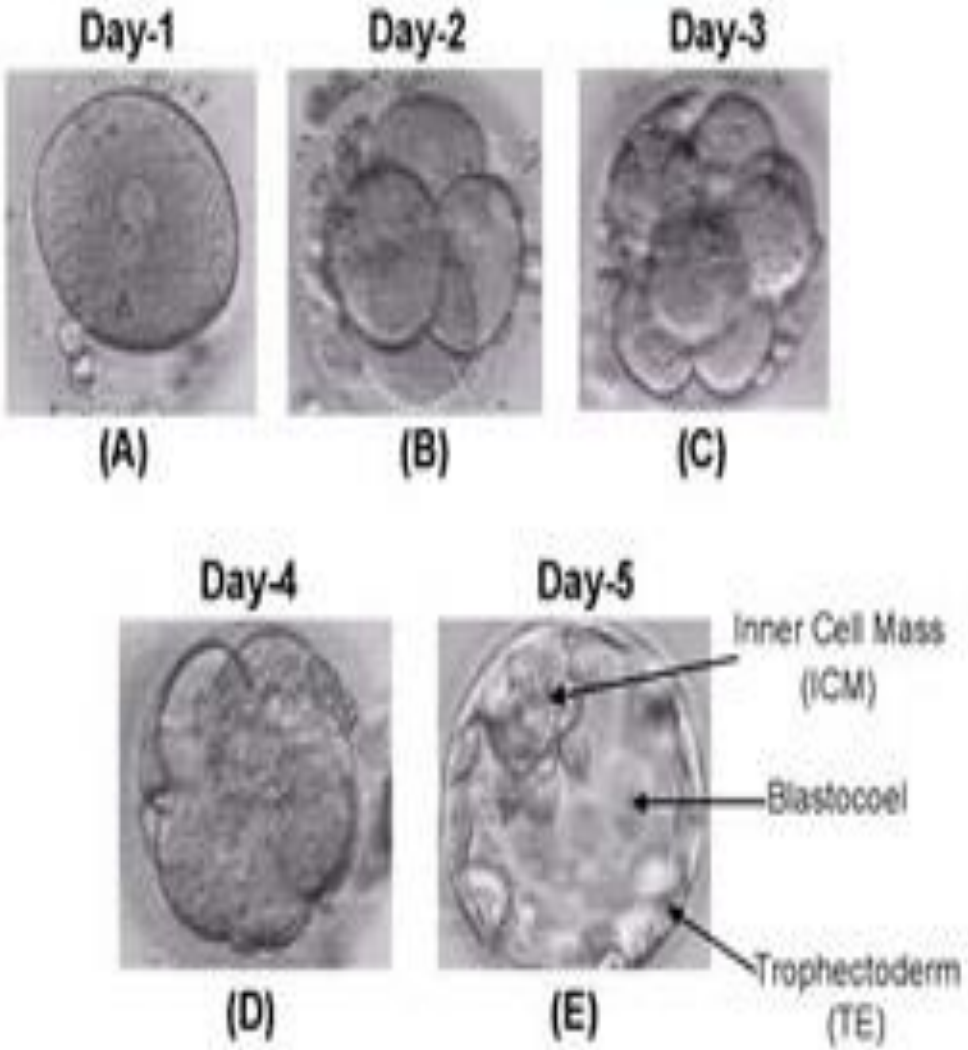
The pipette is inserted into the centre of the egg



The sperm is expelled and the injection pipette withdrawn

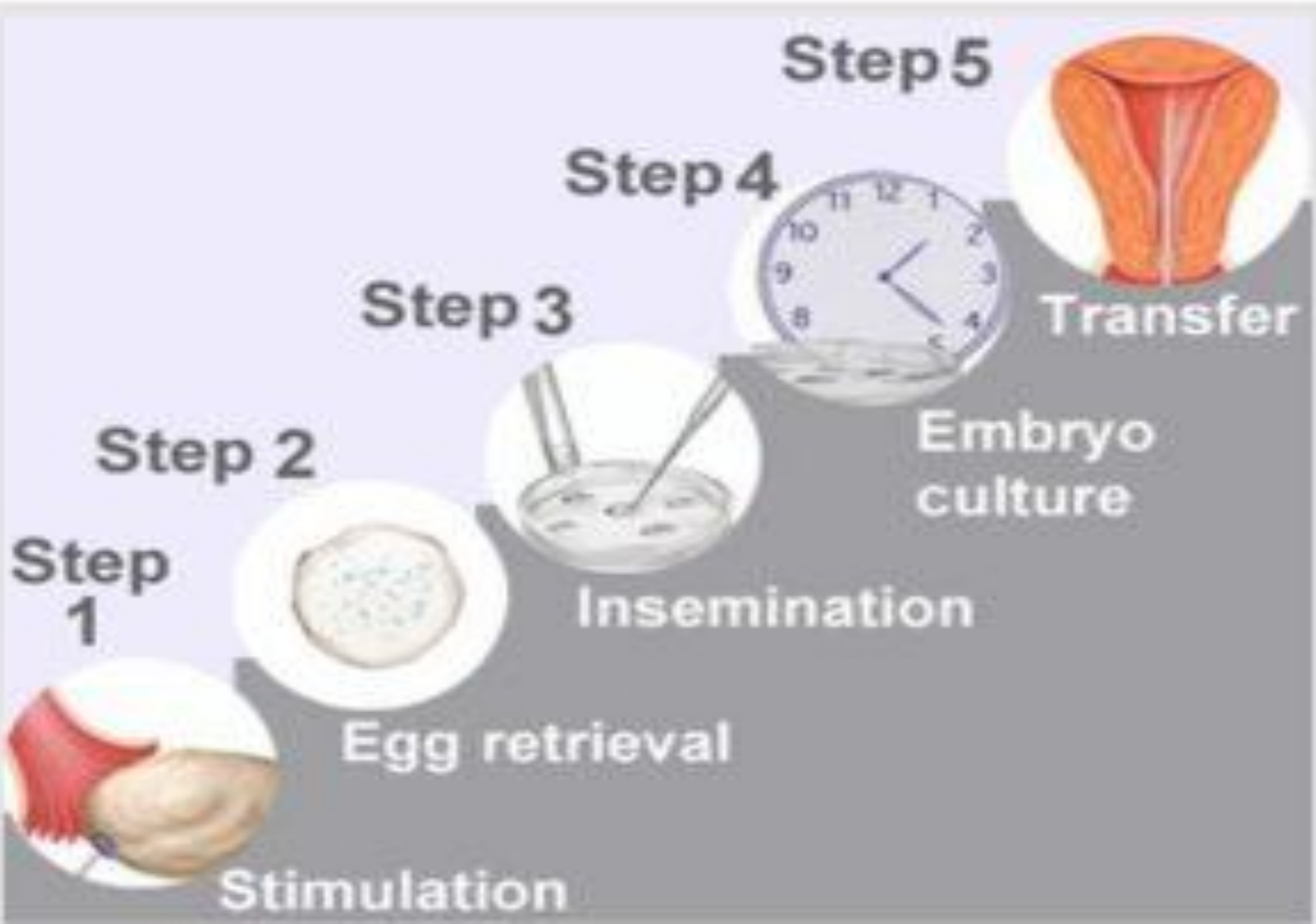
Embryo Development




Day-1
Day-2
Day-3
(A)
(B)
(C)
Day-4
Day-5
Inner Cell Mass
(ICM)
Blastocoel
Trophectoderm
(TE)
(D)
(E)


Embryo Development
Day 1
Day 2
Day 3
Day 4
Day 5
Day 5

IVF is 5 Steps Procedure



Component	A Most Preferred Concentration	B Preferred Range
NaCl	90.08	80.0-100
KC1	5.5	3.5-7.5
NaH_2PO_4	0.25	0.05-1.5
$MgSO_4$	1	0.2-2.0
$NaHCO_3$	25	15.0-30
$CaC1_2$	1.8	0.8-2.8
Glucose	0.5	0.05-5.0
NaLactate (L-isomer)	10.5	5.0-20.
NaPyruvate	0.32	0.1-1.0
Alanine	0.1	0.01-0.5
Asparate	0.1	0.01-0.5
Asparagine	0.1	0.01-0.5
Glutamate	0.1	0.01-0.5
Alanyl - Glutamine	0.5	0.1-1.0
Glycine	0.1	0.01-0.5
Proline	0.1	0.01-0.5
Serine	0.1	0.01-0.5
Taurine	0.1	0.01-10.0
EDTA	0.01	0.005-0.20
HSA	5 mg/ml	1-10.0 mg/ml
Hyaluronate	0.1 mg/ml	0.02-0.5 mg/ml

Differences in the physiology of the mammalian embryo for development from the zygote to the blastocyst stage.

Precompaction stage

- Low biosynthetic activity
- Low QO2(Metabolic Quotient)
- Pyruvate-based metabolism
- Maternal genome
- Single cell
- Low ability to maintain cellular homeostasis
- Totipotent

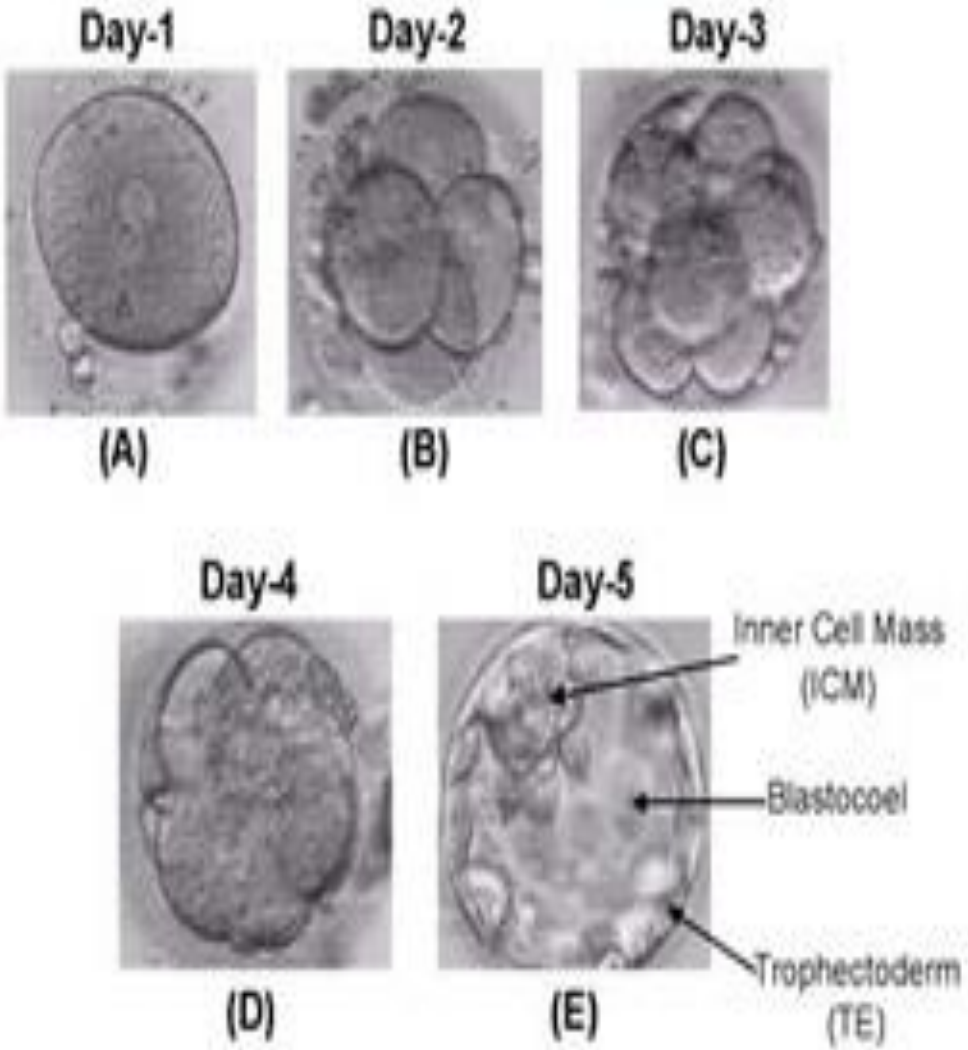
Postcompaction stage

- High biosynthetic activity
- High QO2(Metabolic Quotient)
- Glucose-based metabolism
- Embryonic genome
- Complex systems for maintenance of cellular homeostasis
- Differentiation into inner cell mass and trophectoderm

EMBRYO CULTURE

Four protocols can be used for the culture from fertilization to the blastocyst stage in an ART laboratory:

- **1. Sequential media protocol,** with an interrupted culture where two media of different compositions are used sequentially, change of medium occurs on day 3 of embryo culture
- **2. Sequential media protocol with fresh medium change every day**
- **3.Monoculture – uninterrupted- culture using one medium throughout the 5 days of embryo culture**
- **4.Monoculture- interrupted- where a monoculture medium is used throughout but is renewed on day 3 of embryo culture.**

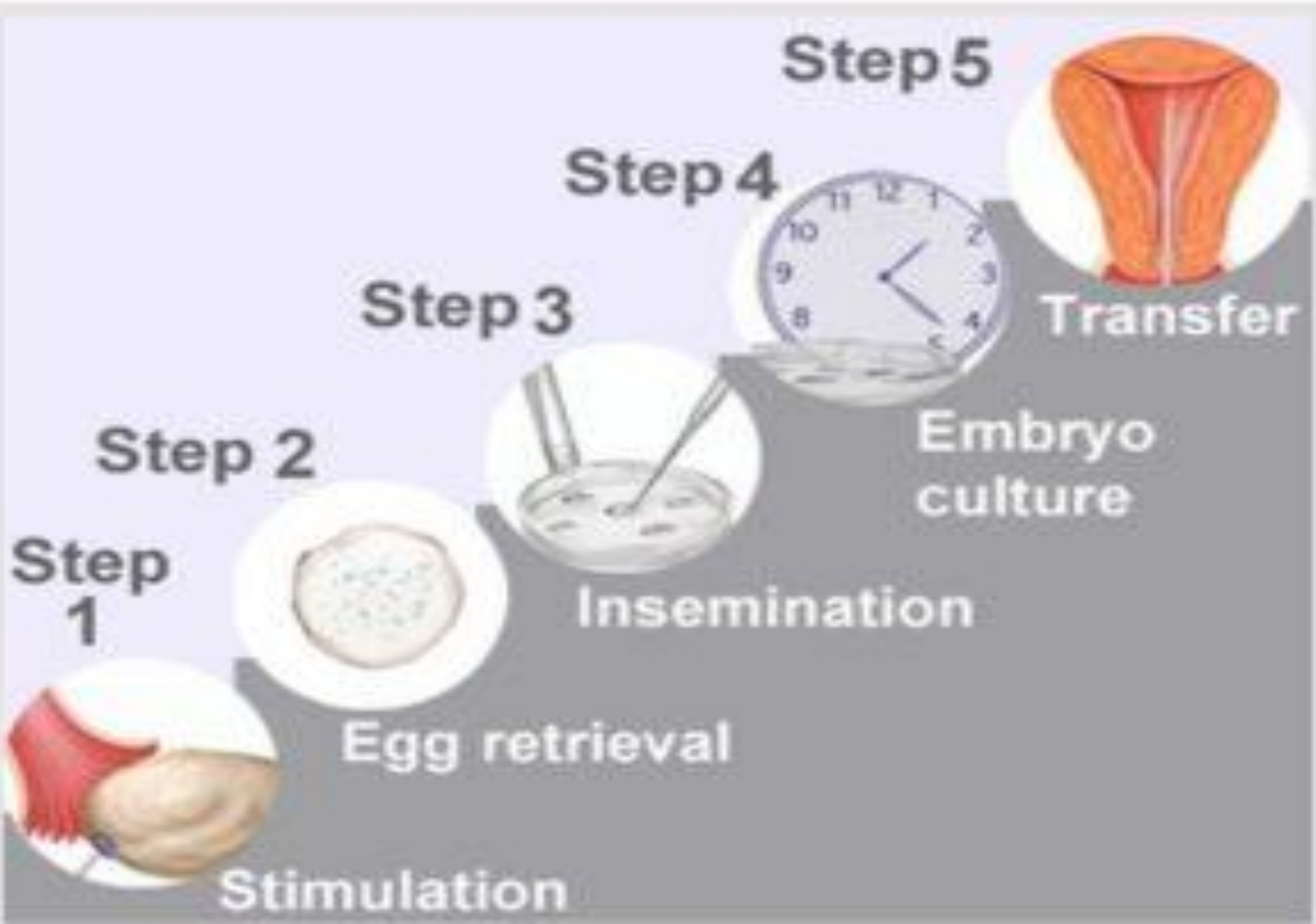

Day-1
Day-2
Day-3
(A)
(B)
(C)
Day-4
Day-5
Inner Cell Mass
(ICM)
Blastocoel
Trophectoderm
(TE)
(D)
(E)


Embryo Development
Day 1
Day 2
Day 3
Day 4
Day 5
Day 5

Embryo culture media and IVF/ICSI success rates: a systematic review Human Reproduction Update Vol 19-2012

- **BACKGROUND** The media that are used to culture human preimplantation embryos are considered to be an important factor for the success rates of IVF/ICSI. Here, we present a systematic review of randomized controlled trials (RCTs) on the effect of culture media on IVF/ICSI success rates.
- **METHODS** RCTs published between January 1985 and July 2012 were eligible for inclusion. The primary outcome was live birth.
- **RESULTS** Twenty-two RCTs were included. **Pooling the data did not reveal a superior culture medium.**
- **CONCLUSIONS** It is yet unknown what culture medium leads to the best success rates in IVF/ICSI. Given the potential importance of culture media for treatment outcome, rigorously designed RCTs are needed for currently available, as well as newly introduced culture media

IVF is 5 Steps Procedure



Τεχνικές και εφαρμογές της εξωσωματικής γονιμοποίησης

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- **ΠΡΟΕΜΦΥΤΕΥΤΙΚΗ ΔΙΑΓΝΩΣΗ (PGD)**
  Τι ειναι? Ποτε? Και Πως?
- ΚΑΤΑΨΥΞΗ
  ΕΜΒΡΥΩΝ (Embryo Freezing)
  ΩΑΡΙΩΝ(Oocyte Freezing)
  ΣΠΕΡΜΑΤΟΣ (Sperm Freezing)
- ΚΛΩΝΟΠΟΙΗΣΗ (CLONING)

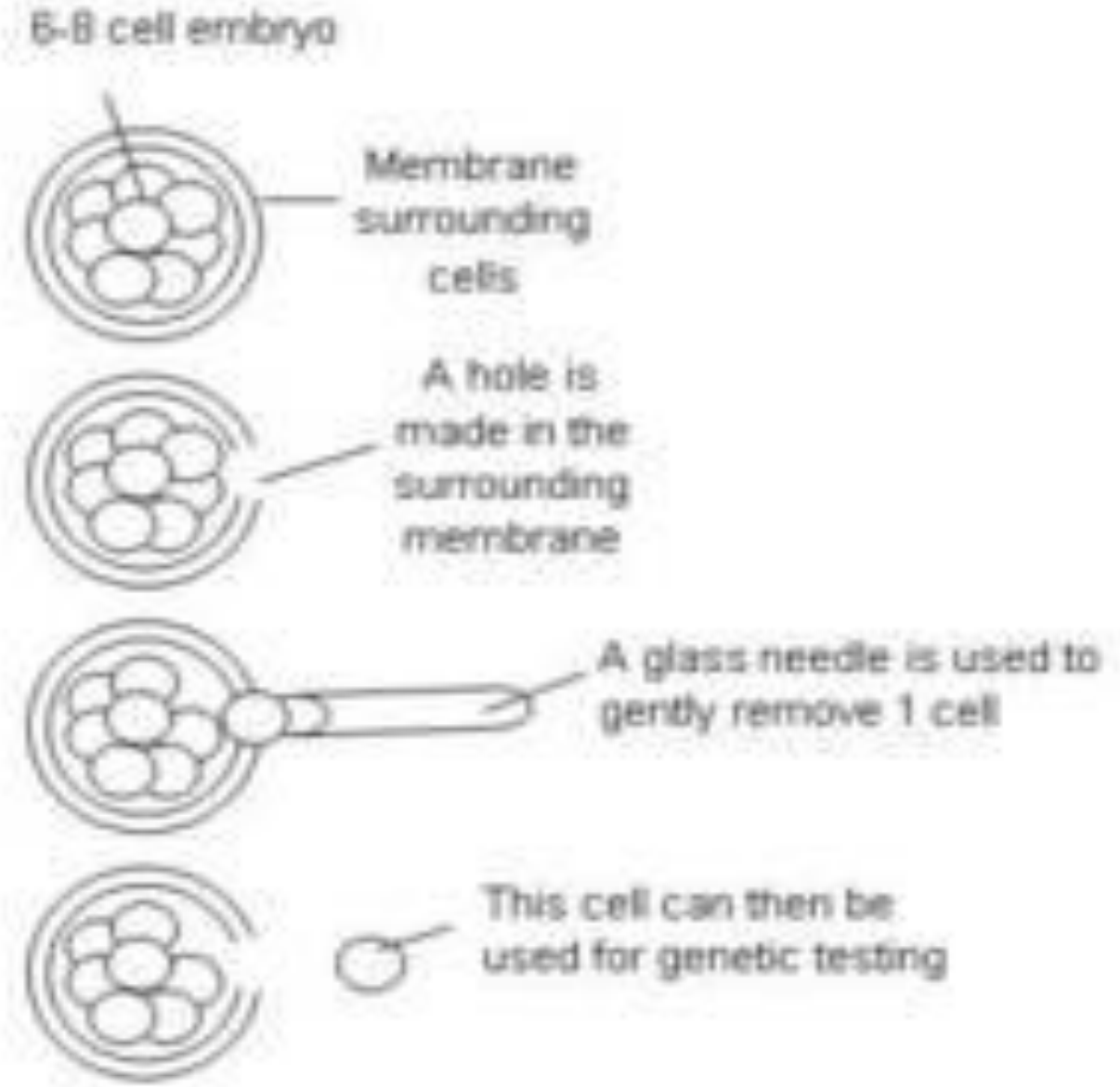
Preimplantation Genetic Diagnosis

Preimplantation genetic diagnosis (**PGD**) is a procedure which is done

- WHEN? prior to implantation of the embryo
- AIM? to identify **genetic** defects within embryos created through in vitro fertilization
- WHY? to prevent certain diseases or disorders from being passed on to the child.

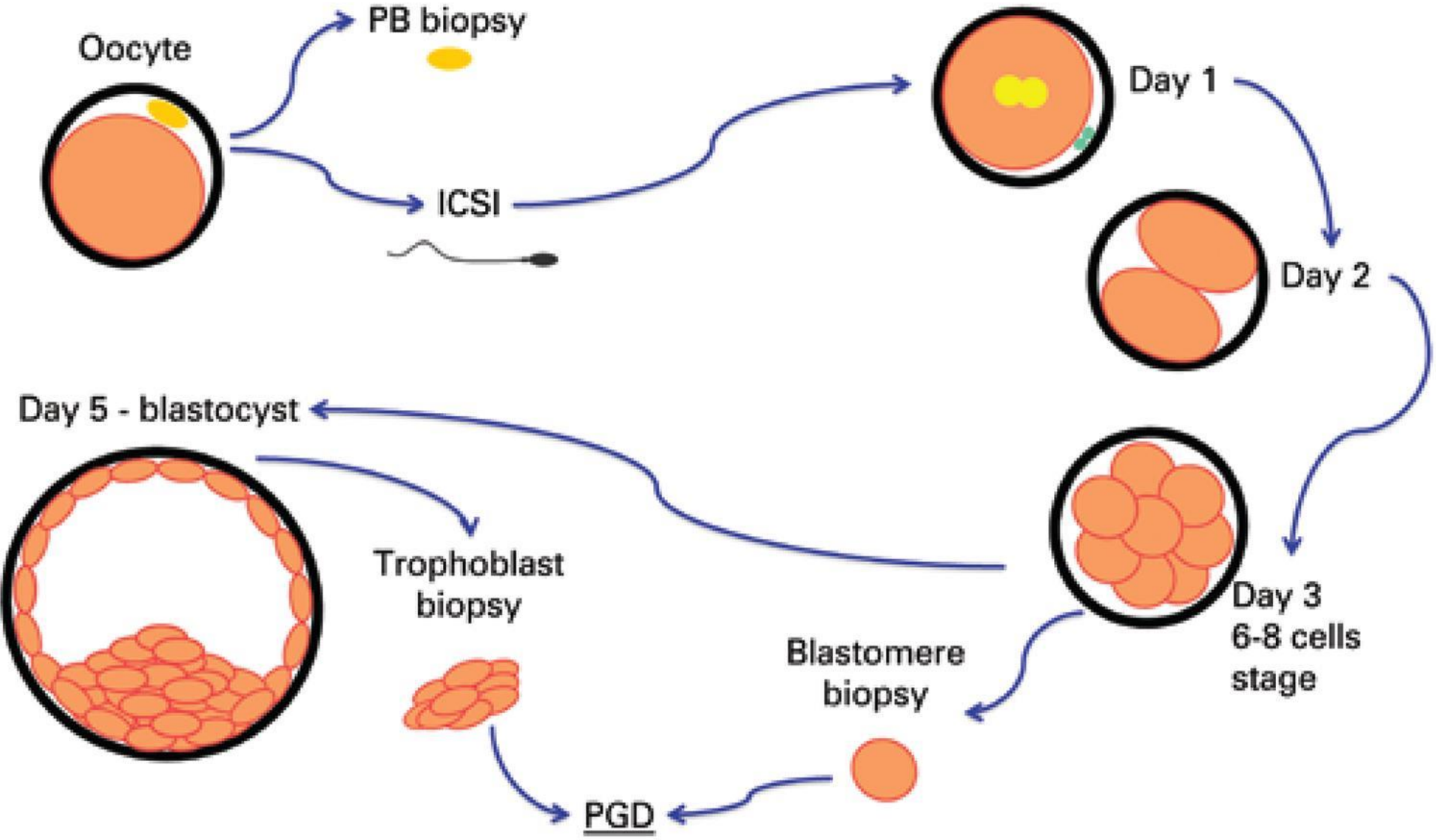
DR. LUKAS KLENTZERIS

PREIMPLANTATION GENETIC DIAGNOSIS



PGD ON DAY 3 EMBRYO

ORIGIN OF CELLS FOR PGD



ICSI: intra-citoplasmic sperm injection; PGD: preimplantation genetic diagnosis.

INDICATIONS FOR PGD 1/4

Monogenic disorders

- PGD is available for a large number of monogenic disorders—that is, disorders due to a single gene only (autosomal recessive, autosomal dominant or X-linked)
- chromosomal structural aberrations (such as a balanced translocation).
- The most frequently diagnosed **autosomal recessive** disorders are cystic fibrosis, Beta-thalassemia, sickle cell disease and spinal muscular atrophy
- The most common **dominant** diseases are myotonic dystrophy, Huntington's disease and Charcot–Marie–Tooth disease;
- X-linked diseases, most of the cycles are performed for fragile X syndrome, haemophilia A and Duchenne muscular dystrophy.
- PGD for mitochondrial disorders or two indications simultaneously.

INDICATIONS FOR PGD 2/4

Improve Pregnancy chances???

- Preimplantation genetic profiling (PGP) has been suggested as a method to determine embryo quality in in vitro fertilization, in order to select an embryo that appears to have the greatest chances for successful pregnancy.

PGS (Preimplantation Genetic Screening)
BUT……mosaicim plus damage to the embryos….made results worse!!!!

INDICATIONS FOR PGD 3/4

HLA matching

- Human leukocyte antigen (HLA) typing of embryos, so that the child's HLA matches a sick sibling, availing for cord-blood stem cell donation.
- The child is in this sense a "savior sibling" for the recipient child.
- HLA typing has meanwhile become an important PGD indication in those countries where the law permits

INDICATIONS FOR PGD 4/4

Sex selection

- Medical reasons specific gene testing for monogenic disorders, which can be very useful for genetic diseases whose presentation is linked to the sex, such as, for example,X-linked diseases – Hemophilia A and B—Duchene Muscular Dystrophy
- Social reasons!! A 2006 survey found that 42 per cent of clinics that offer PGD have provided it for sex selection for non-medical reasons. Nearly half of these clinics perform it only for "family balancing",

PURPOSE OF PGD

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  Ωαριων(Oocyte Freezing)
  Εμβρυων (Embryo Freezing)

- ΚΛΩΝΟΠΟΙΗΣΗ (CLONING)

Cryopreservation of spermatozoa

- The history of human sperm cryobiology dates from the late 1940s.
- The discovery that glycerol protected spermatozoa against damage from freezing led to the use of human spermatozoa stored on dry ice at –79 °C (Polge et al., 1949)
- Subsequently, liquid nitrogen (-196 Celcius) was used and semen cryopreservation developed rapidly in many countries with the establishment of commercial sperm banks or coordinated national services (Perloff et al., 1964; David et al., 1980; Leibo et al., 2002).

Cryopreservation of spermatozoa

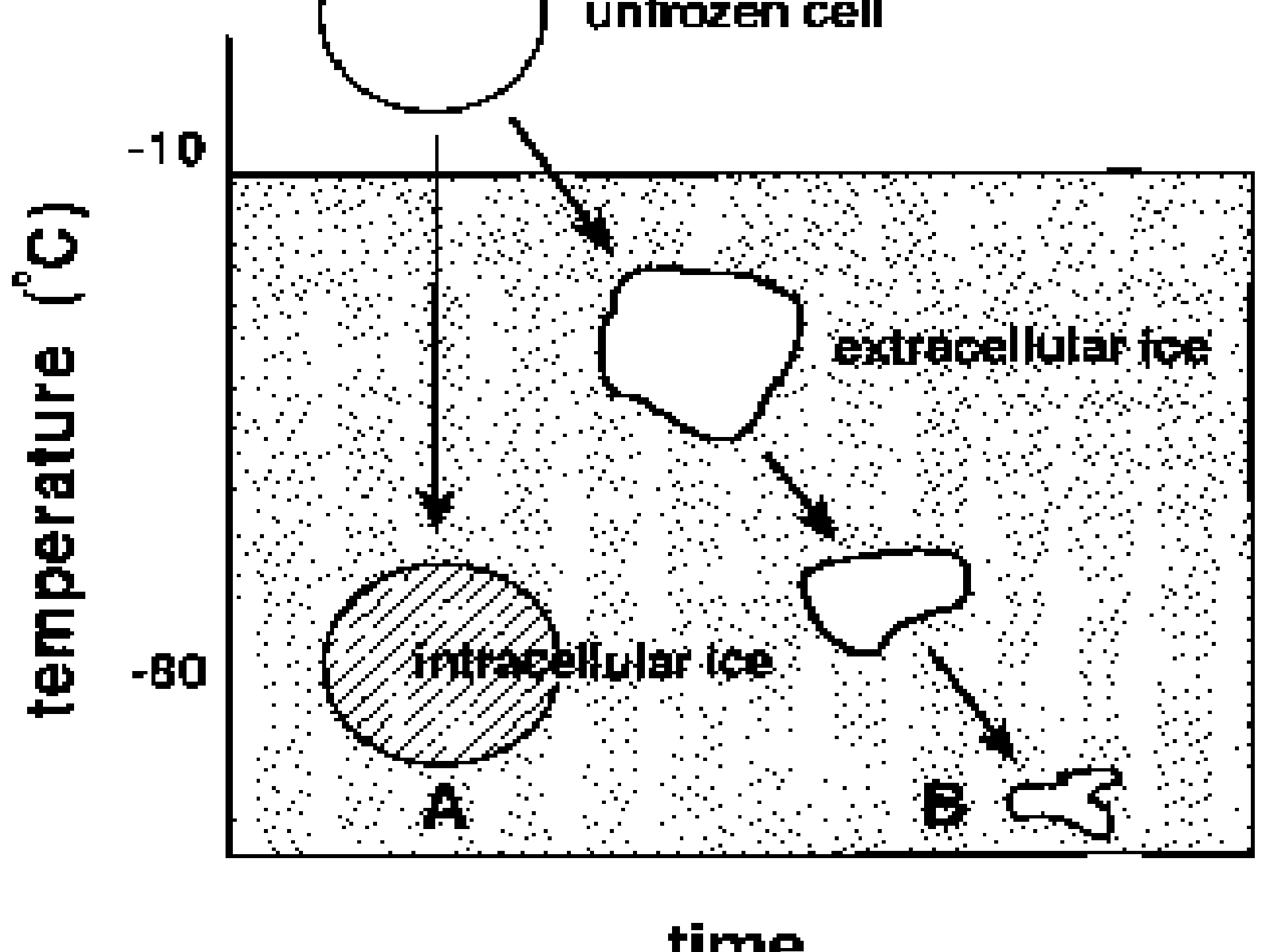
- Cell survival after freezing and thawing depends largely on minimization of intracellular ice crystal formation. This is done by using

  A. appropriate cryoprotectants and

  B. applying rates of cooling and warming that minimize the amount of intracellular water subject to ice formation

- Spermatozoa are not very sensitive to damage caused by rapid initial cooling (cold shock), possibly because of

A. High membrane fluidity from the unsaturated fatty acids in the lipid bilayer (Clarke et al., 2003).
B. Low water concentration in the intracytoplasmic area (about 50%).


unfrozen cell
-10
-80
temperature (°C)
extracellular ice
intracellular ice
A
B
time

THE HUMAN BODY

Techniques for Sperm Cryopreservation

Slow Freezing

- The slow freezing technique consists of progressive sperm cooling over a period of 2–4 h in two or three steps, either **manually** or automatically using a semiprogrammable freezer.
- The manual method is performed by simultaneously decreasing the temperature of the semen while adding a cryoprotectant .
- It has been shown that the optimal initial cooling rate of the specimen from room temperature to 5°C is 0.5–1°C/min
- The sample is then frozen from 5°C to −80°C at a rate of 1–10°C/min. The specimen is then plunged into liquid nitrogen at −196°C

Behrman and Sawada 1966

Techniques for Sperm Cryopreservation

Rapid Freezing

- Rapid freezing was first proposed by Sherman 1990 and has 4 stages

STAGE 1. The sample is initially mixed in dropwise manner with equal volume of cold cryoprotectant

STAGE 2. The mixture is loaded into the straws and left to incubate at 4°C for 10 minutes.

STAGE 3. The straws are then placed at a distance of 15–20 cm above the level of liquid nitrogen (−80°C) for 15 min

STAGE 4. The straws are immersed in liquid nitrogen -196. During cooling it is preferable to place the straws in horizontal position to minimize the heat difference between the two ends.

Techniques for thawing cryopreserved sperm

- The thawing procedure is an equally important step. The cell must be allowed to recover its normal biological activities trying to avoid abrupt thermal changes as far as possible.

A. Thawing at room temperature for 10 min and subsequent thermostat pass at 37°C for another 10 min

B. Thawing in a thermostat and water-bath at 37°C for 10 min

C. Thawing at room temperature for 15 min.

- **Once the semen is thawed, it is separated from the cryopreservation medium by washing in culture medium and centrifugation**

Cryoprotectants

- Cryoprotectants are low-molecular-weight and highly permeable chemicals used to protect spermatozoa from freeze damage by ice crystallization.
- There are four main well-known cryoprotectants: glycerol, **ethylene glycol**, dimethyl sulphoxide, and 1,2-propanediol.
- Cryoprotectants act by decreasing the freezing point of a substance.
- Usually the cryoprotectants are added in an equal volume of semen in a dropwise manner, gently mixed at room temperature, and then placed at 37°C for 10–15 minutes to allow for proper equilibration between the cells and the medium.

Reasons for cryopreservation of sperm

- **Treatment with donor sperm**

Azoospermia- to prevent transmission of an inherited disease- to prevent fetal hemolytic anemia from blood group incompatibility- treat women without partners!

- **Fertility preservation**

Prior to cytotoxic therapy or radiotherapy for cancer-prior to vasectomy

- **Infertility treatment**

Severe oligoasthenospermia-intermittent azoospermia

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OOCYTE FREEZING

Who should consider oocyte freezing?

- Women who want or need to delay childbearing in order to pursue educational, career or other personal goals.
- Women diagnosed with cancer- prior to chemo/radiotherapy
- Women with objections to storing frozen embryos for religious and/or moral reasons.

OOCYTE FREEZING

- What does it mean for the woman?

  An IVF cycle!

A. Stimulation of the ovaries

B. Monitoring ovarian response to hormones

C. Oocyte recovery

- The oocytes can remain frozen for 5 years,initially!!
- Number of babies born with frozen eggs 5000!!

Vitrification

- Process that produces a glasslike solidification of living cells that completely avoids ice crystal formation during cooling. It completely avoids ice crystal formation in cryopreserved cells during warming to recover the cells for biological applications

VITRIFICATION

The process of vitrification has 3 critical components

- First, eggs or embryos are exposed to high concentrations of cryoprotectants to allow rapid dehydration of cells.
- Second, the eggs or embryos are loaded into tiny storage devices (usually straws) that will facilitate ultra-rapid cooling
- third, the straws containing the eggs/embryos are cooled as fast as possible, typically at thousands of degrees per minute.

Midland Fertility Services

Egg Freezing (Vitrification)

'Building futures,
transforming lives'

Differences of slow freezing and vitrification

Slow-freezing

- low levels of cryoprotectants
- slow controlled rates of cooling (0.3°C/min)
- slow dehydration to minimize ice-crystal formation
- *takes hours*

Vitrification

- high levels of cryoprotectants
- very fast cooling rates
- (~20,000°C/min)
- fast cooling rates result in solidification of solution into glass-like structure (no crystallization)
- *takes seconds*

Stop your biological clock and preserve your fertility!

ASRM lifts "experimental" label from "egg freezing technique" on Monday, October 22, 2012.

Samantha Pfeifer, MD, Chair of the ASRM Practice committee *said, a careful review of the literature indicates egg freezing is a valid technique for young women for whom it is medically indicated..*

"Oocyte cryopreservation is an exciting and improving technology and should no longer be considered experimental. Pregnancy rates and health outcomes of the resulting children are incomparable to those of IVF with fresh eggs", said ***Eric Widra, MD, Chair of Society for Assisted Reproductive Technology (SART) practice committee.***


Egg Vitrification Success Rates
(Cobo 2008*)
100
90
80
70
60
50
40
30
20
10
0
96.1%
73.1%
38.5%
63.2%
Vitrified eggs that survived warming process
Fertilised warmed eggs
Implantation rate per embryo
Pregnancy rate per transfer

Freezing eggs isn't a magic bullet, it's the least worst option!!!

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What is embryo freezing and storage? (HFEA info to patients-modified)

- Often with IVF or ICSI, people have a number of unused embryos after their first cycle.
- Some people choose to freeze them for

A. own use in later treatment cycles
B. donation for use in others' treatment
C. donation for research purposes or training.

- Your chances of becoming pregnant with a thawed frozen embryo are not affected by the length of time the embryo has been stored.
- But not all embryos will survive freezing and eventual thawing when they come to be used. Very occasionally no embryos will survive.

Is embryo freezing and storage for me? (HFEA info to patients-modified)

- You may consider freezing your embryos for the following reasons:

A. It gives you the option of using the embryos in the future.

B. If your treatment needs to be cancelled after egg collection (for example, if you have a bad reaction to fertility drugs), you may still be able to store your embryos for future use.

C. If you have a condition, or are facing medical treatment for a condition, that might affect your fertility (embryo freezing is currently the most effective way for women to preserve their fertility).

D. you are at risk of injury or death (eg, you're a member of the Armed Forces who is being deployed to a war zone).

Vitrification

- Process that produces a glasslike solidification of living cells that completely avoids ice crystal formation during cooling. It completely avoids ice crystal formation in cryopreserved cells during warming to recover the cells for biological applications

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The process of vitrification has 3 critical components

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A typical embryo vitrification protocol is complete in about 10 minutes.

Differences of slow freezing and vitrification

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- slow dehydration to minimize ice-crystal formation
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J Assist Reprod Genet. 2009 Jun; 26(6): 347–354.
Published online 2009 Jun 10. doi: 10.1007/s10815-009-9318-6

Vitrification versus slow freezing gives excellent survival, post warming embryo morphology and pregnancy outcomes for human cleaved embryos

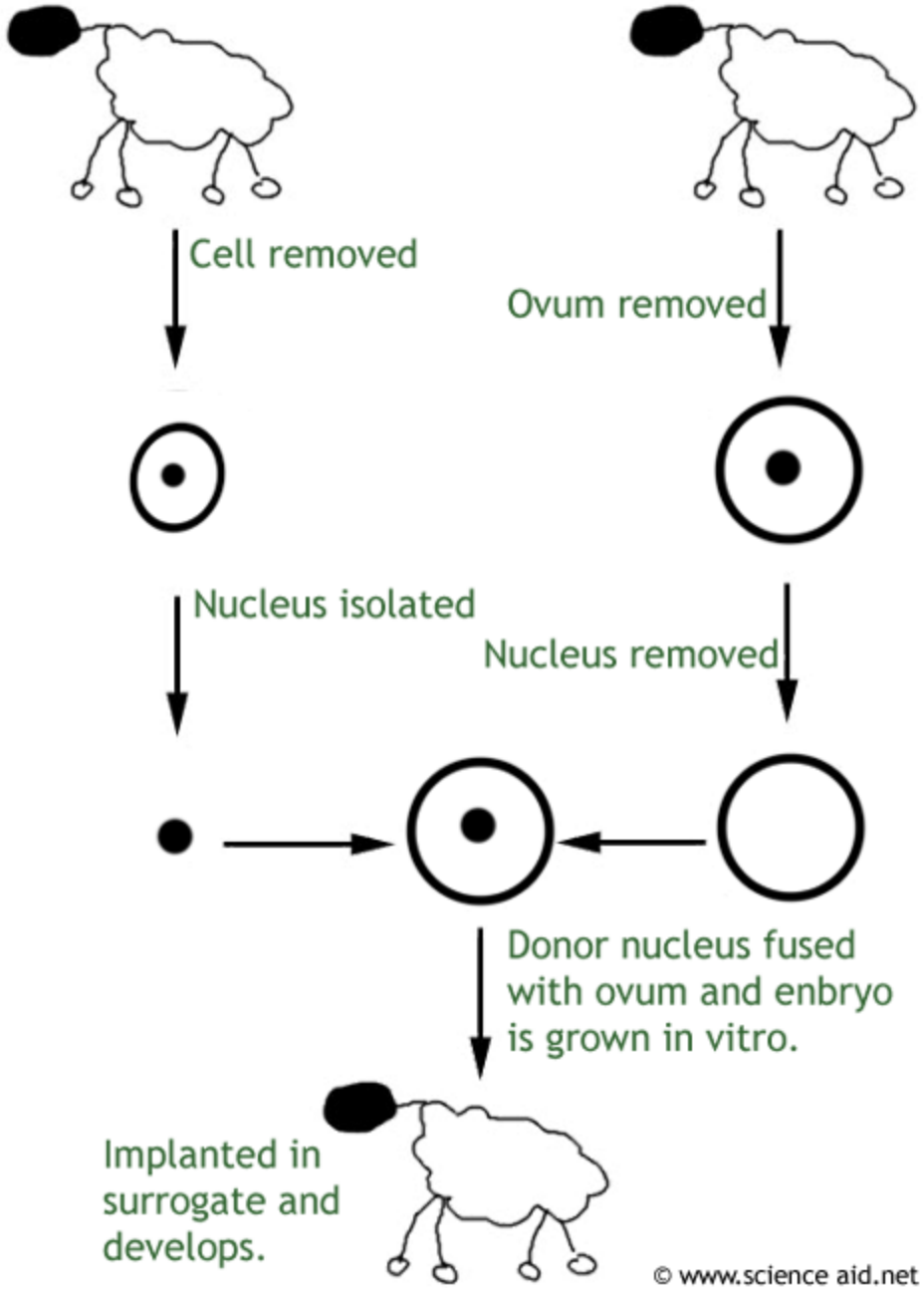
Percentages of Transfers Using Fresh or Frozen Nondonor Embryos That Resulted in Pregnancies, Live Births, and Single-Infant Live Births, 2013-CDC-USA

	PREGNANCY RATE %	LIVE BIRTH RATE %	SINGLE INFANT LBR %
FRESH ET	50	40	31
FROZEN ET	45	37	27

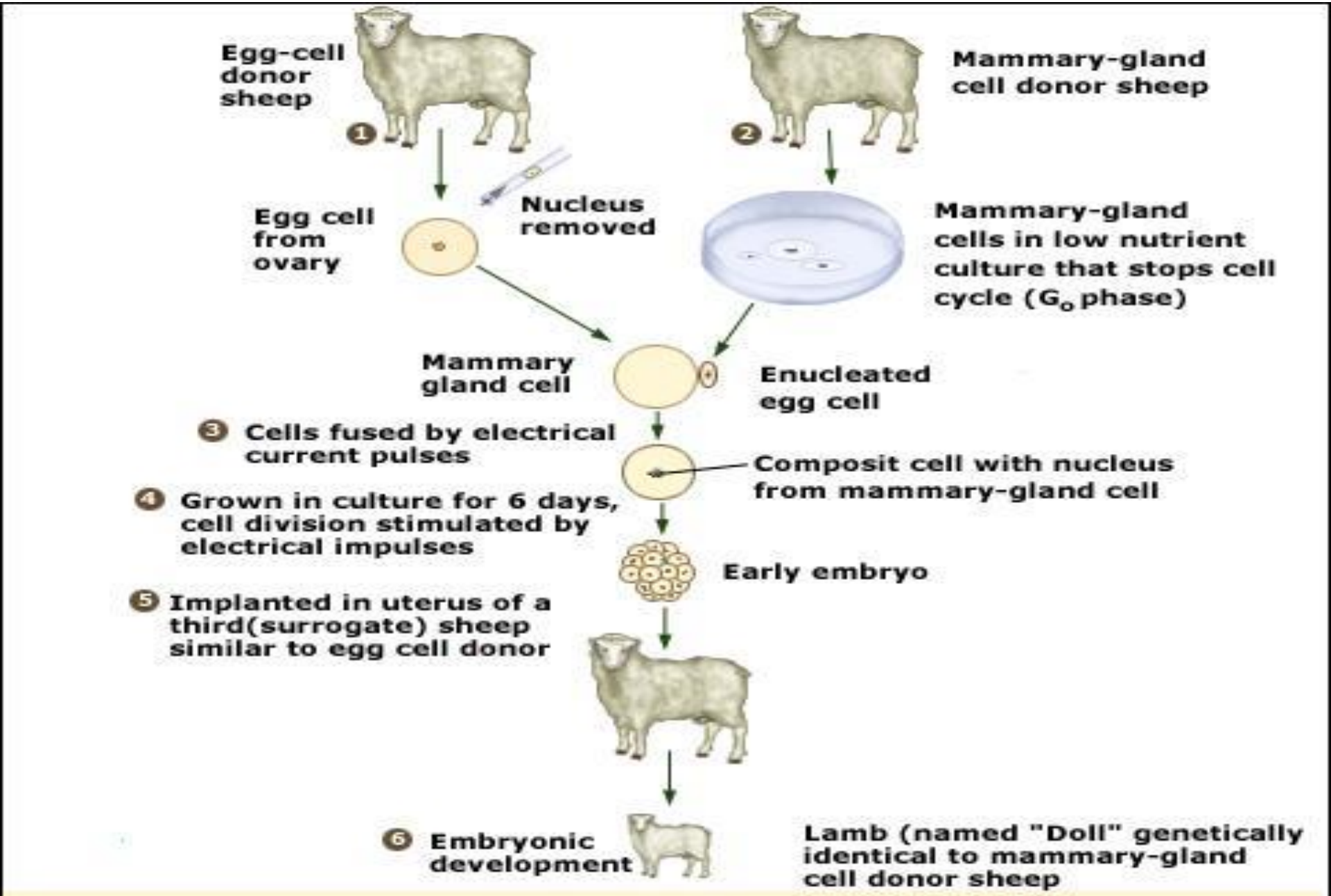
HUMAN CLONING!! TO BE OR NOT TO BE?

What is cloning?

- The term cloning describes a number of different processes that can be used to produce genetically identical copies of a biological entity. The copied material, which has the same genetic makeup as the original, is referred to as a clone.


Cell removed
Ovum removed
Nucleus isolated
Nucleus removed
Donor nucleus fused with ovum and enbryo is grown in vitro.
Implanted in surrogate and develops.
© www.science aid.net

CLONING



Cloning of a sheep

Top 6 facts about clones

- 1. The first cloning of an animal was done in the 1880s by German biologist Hans Driesch who cloned a sea urchin from an embryo cell.
- 2. In 1952, Robert Briggs and Thomas King cloned northern leopard frogs…
- 3. …but it was not until 1963 that biologist JBS Haldane coined the term “clone”.
- 4. The reproductive cloning of humans is banned under the EU Charter of Fundamental Rights.
- 5. Dolly the Sheep, the first mammal to be cloned from an adult cell, was born on July 5, 1996. The cell was taken from a mammary gland...
- 6. She was named ‘Dolly’ as, in the words of the project leader: “We couldn’t think of a more impressive pair of glands than Dolly Parton’s.”

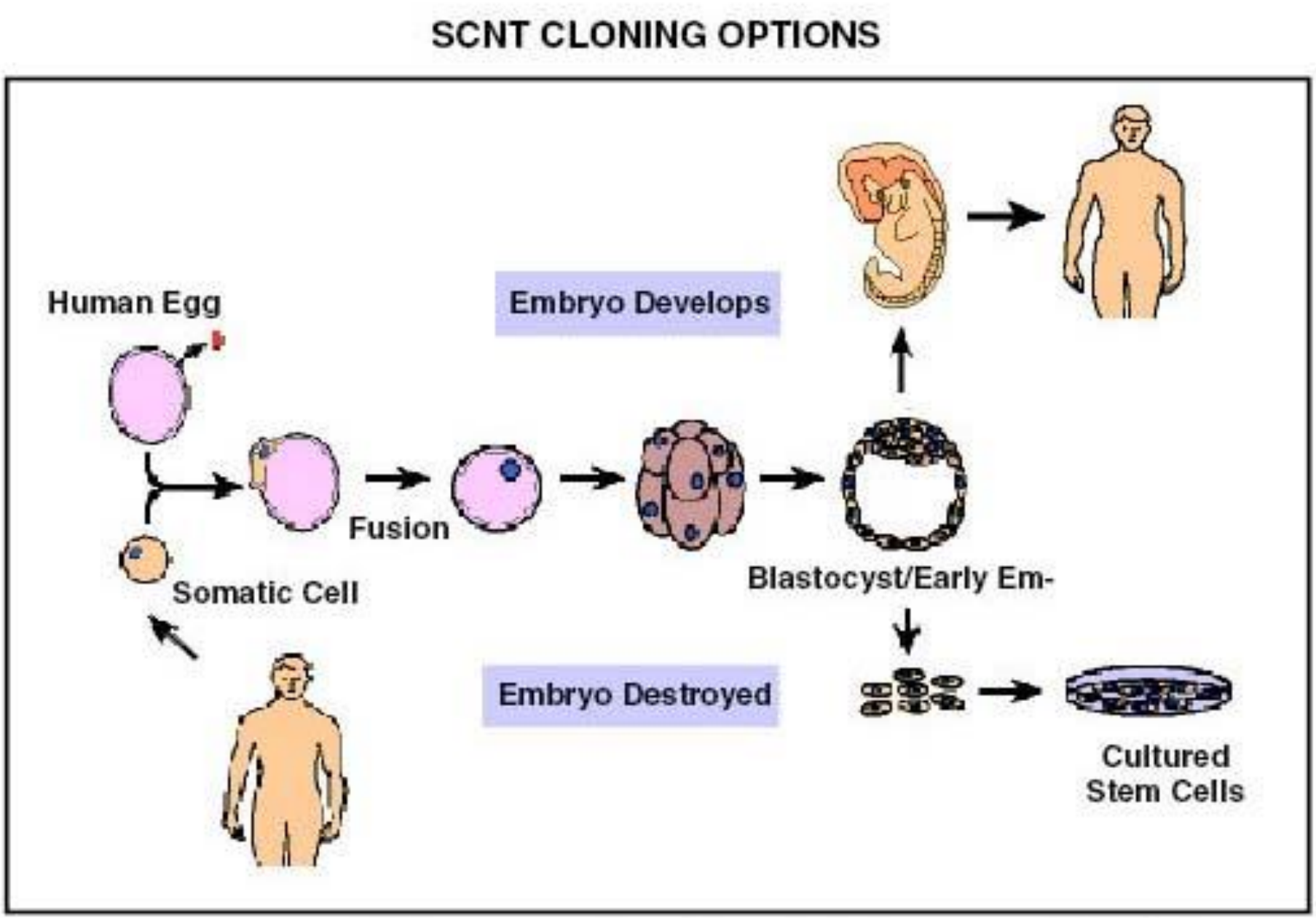
Do clones ever occur naturally?

- YES!!!
- Natural clones, also known as identical twins, occur in humans and other mammals. These twins are produced when a fertilized egg splits, creating two or more embryos that carry almost identical DNA.

Have humans been cloned?

- Despite several highly publicized claims, human cloning still appears to be fiction. There currently is no solid scientific evidence that anyone has cloned human embryos.
- **In 1998, scientists in South Korea** claimed to have successfully cloned a human embryo, but said the experiment was interrupted very early when the clone was just a group of four cells.
- **In 2002, Clonaid**, part of a religious group that believes humans were created by extraterrestrials, held a news conference to announce the birth of what it claimed to be the **first cloned human, a girl named Eve.** However, despite repeated requests by the research community and the news media, Clonaid never provided any evidence to confirm the existence of this clone or the other 12 human clones it purportedly created.
- **In 2004, a group led by Woo-Suk Hwang of Seoul National University** in South Korea published a paper in the journal *Science* in which it **claimed to have created a cloned human embryo in a test tube**. However, an independent scientific committee later found no proof to support the claim and, in January 2006, *Science* announced that Hwang's paper had been retracted.

Reproductive and Therapeutic cloning



Therapeutic cloning of Human embryos….Yes!! 1/2

FOR WHAT PURPOSES?

- To promote advances in the treatment of infertility
- To increase knowledge about the causes of congenital disease
- To increase knowledge about the causes of miscarriages
- To develop more effective techniques of contraception

Therapeutic cloning of Human embryos….Yes!! 2/2

FOR WHAT PURPOSES?

- Increasing knowledge about the development of embryos
- Increasing knowledge about serious disease
- Enabling any such knowledge to be applied in developing treatments for serious disease
- To develop methods for detecting the presence of gene or chromosome abnormalities

HUMAN REPRODUCTIVE CLONING
ETHICAL ISSUES

- Create a human being that is genetically identical to another person who has previously existed or who still exists!!
- Contradicts long-standing religious and social values about human dignity.
- Could possibly interfere with the basic principles of individual freedom, identity,rights and autonomy.
- Even if we create a somatic clone …..probably we will never create a psychological clone !!!

HUMAN CLONING!! TO BE OR NOT TO BE?

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- ΕΛΕΓΧΟΣ ΣΠΕΡΜΑΤΟΣ – WHO CRITERIA 2010
- ΕΞΩΣΩΜΑΤΙΚΗ ΓΟΝΙΜΟΠΟΙΗΣΗ (IVF AND ICSI)
  Ενδειξεις-Πρωτοκολλα-Τεχνικες
- ΠΡΟΕΜΦΥΤΕΥΤΙΚΗ ΔΙΑΓΝΩΣΗ (PGD)
  Ποτε? Και Πως?
- ΚΑΤΑΨΥΞΗ ΓΕΝΕΤΙΚΟΥ ΥΛΙΚΟΥ
  ΕΜΒΡΥΩΝ (Embryo Freezing)
  ΩΑΡΙΩΝ(Oocyte Freezing)
  ΣΠΕΡΜΑΤΟΣ (Sperm Freezing)

- ΚΛΩΝΟΠΟΙΗΣΗ (CLONING)