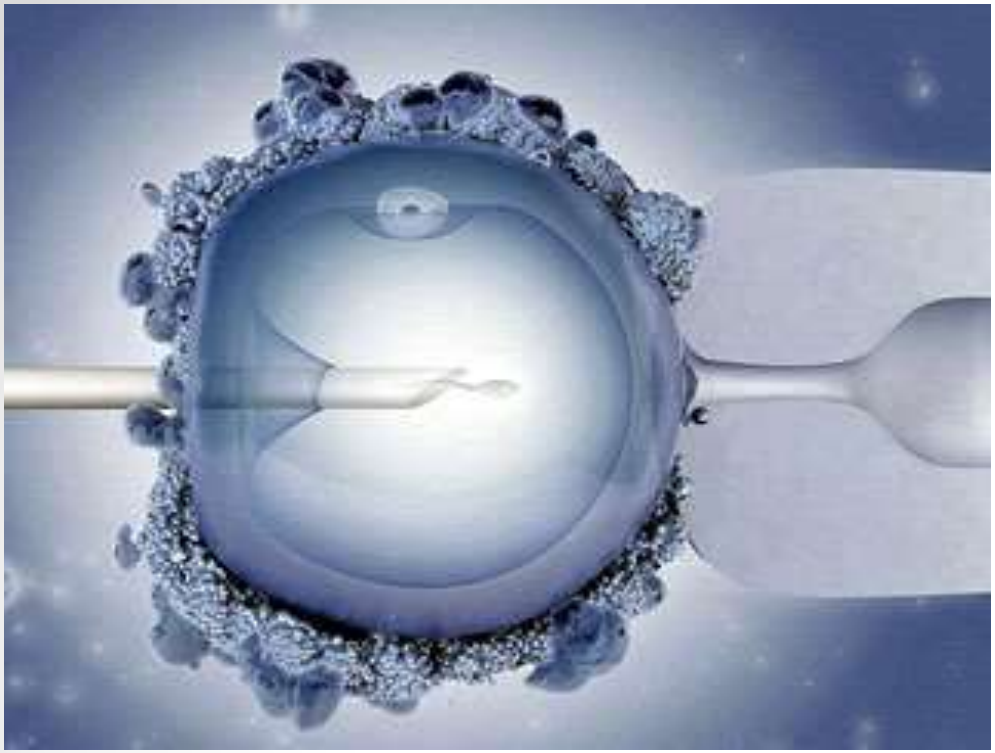

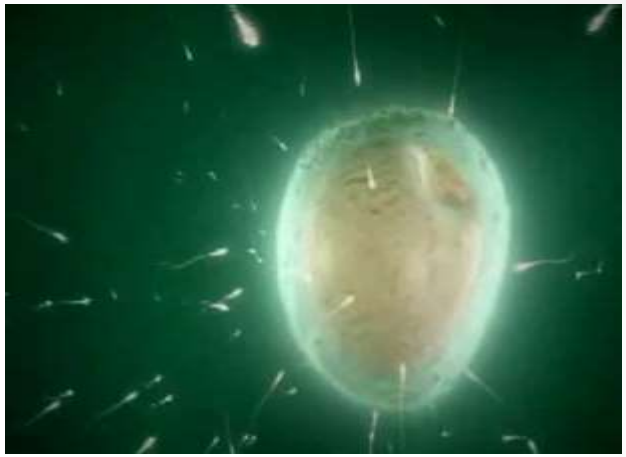

In-vitro Fertilization

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Introduction

- In vitro fertilisation (IVF) is a process by which an egg is fertilised by sperm outside the body: in vitro.
- Evolved as a major treatment protocol for infertility.
- The term in vitro, from the Latin meaning in glass, is used, as early biological experiments involving cultivation of tissues outside the living organism were carried out in glass containers such as beakers, test tubes, or petri dishes.
- A colloquial term for babies conceived as the result of IVF, is "test tube babies".

History of IVF

- **1890** - The very first in vitro manipulation of eggs/ embryos was performed by **Walter Heape** , when he transferred in vivo fertilized eggs from one female rabbit to another.
- **1959** - **M.C. Chang**, successfully conducted IVF in rabbits.
- **February 15, 1969**, the journal *Nature* published a paper authored by **R.G. Edwards**, B.D. Bavister and P.C. Steptoe: "Early stages of fertilization in vitro of human oocytes matured in vitro"
- **1977**, the first IVF pregnancy in human and the birth of **Louise Brown.**

History of IVF

Worlds first test tube baby, Louise Brown turns 36 in 2014

In 2010, Sir Edwards was awarded the Nobel Prize in Physiology or Medicine "for the development of in vitro fertilization"

History of IVF

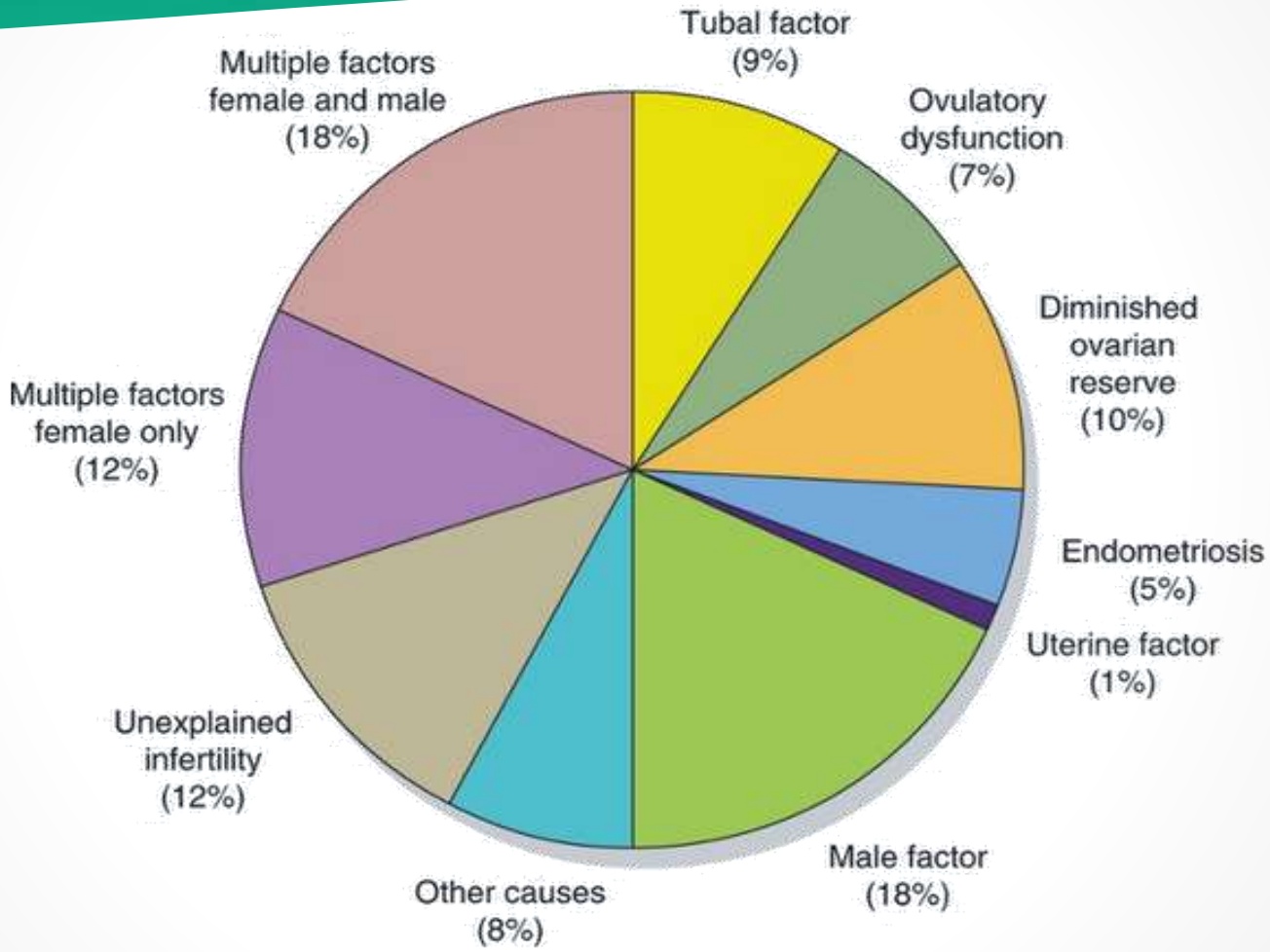
The Indian angle

- **Dr. Subhash Mukhopadhyay** (16 January 1931 – 19 June 1981) was a physician from Kolkata, India, who created **the world's second and India's first child using in-vitro fertilisation, Durga** who was born 67 days after the first IVF baby in United Kingdom.
- Unfortunately, he was harassed by the state government, and not allowed to share his achievements with the international scientific community.
- Dejected, he committed suicide on 19 June 1981.
- The critically acclaimed film **Ek Doctor Ki Maut (1990)** was made on his life

Indications of IVF

- IVF is most clearly indicated when infertility results from one or more causes having no other effective treatment;
  - **Tubal disease.** In women with blocked fallopian tubes, IVF has largely replaced surgery as the treatment of choice.
  - **Endometriosis.** Patients with endometriosis, often have tubal involvement and ovarian cysts (endometrioma).
  - **Ovulatory dysfunction.** In patients with polycystic ovarian disease (PCOS) and other ovulatory problems.
  - **Age Related Infertility.** In normal reproductive life, a woman's ovarian function is diminished with age. In many cases, this reduced function can be overcome through the use of IVF
  - **Male Factor Infertility.** Azoospermia, oligozoospermia, asthenozoospermia, anti-sperm antibody etc.
  - **Preimplantation Genetic Testing (PGT).** Genetic testing on pre-implantation embryos may be indicated for patients who are at risk for genetic disorders such as Cystic fibrosis and Thalassemia

Indications of IVF



Diagnosis among couples using IVF technology

(Centres for Disease Control and Prevention, 2013 IVF Success Rates. National Summary and Fertility Clinic Reports. Atlanta, GA, 2013.)

Steps in IVF (the IVF cycle)

- Initial evaluation
- Suppression of natural hormonal cycle
- Ovarian stimulation
- Collection of oocytes
- Collection of sperms
- In vitro fertilization of oocytes
- Embryo transfer

Initial Evaluation

- Blood tests
- Seminal Fluid examination
- Hysterosalpingogram
- Trans vaginal ultrasound

Suppression of natural hormonal cycle

- Suppression drugs prevent spontaneous ovulation.
- In an IVF cycle, it is important that natural ovulation should not occur - if the eggs leave the ovary, the doctor will not be able to retrieve them.
- Drugs used :
  - **Oral contraceptive pills** – each day starting cycle day 1, 2 or 3 for approximately 2-4 weeks
  - **Lupron / Leuprolide Acetate** – GnRH Agonists. It suppresses LH surge preventing eggs from being released before they are mature for egg retrieval. Subcutaneous inj every morning
  - **Nafarelin** - GnRH Agonists, three times a day nasal spray.
  - **Ganirelix Acetate Injection / Cetrotide** - GnRH antagonists -

Ovarian stimulation

- Ovarian stimulation is used to produce multiple mature follicles, rather than the single egg normally developed each month.
- Produces many good follicles to be Fertilized.

- Multiple eggs are stimulated because some eggs will not fertilize or develop normally after fertilization.
- Regular monitoring by ultrasound scan is done.

Ovarian stimulation

Medications for Ovarian Stimulation

- human menopausal gonadotropin (hMG)
- follicle stimulating hormone (FSH)
- luteinizing hormone (LH) (used in conjunction with FSH)
- human chorionic gonadotropin (hCG)
- clomiphene citrate

Medications to Prevent Premature Ovulation

- Gonadatropin releasing hormone (GnRH) agonists
- GnRH antagonists

- Generally, eight to 14 days of stimulation is required.

Ovarian stimulation

Ovarian follicles, stimulated by ovulation medications, visible on ultrasound. The dark, circular areas are the follicles.

Possible Side Effects of ovarian stimulation

- Discomfort, bruising or swelling at injection site
- Rash
- Allergic sensitivity
- Headache
- Mood swings
- Abdominal discomfort and bloating
- Chance of multiple pregnancies
- **Ovarian Hyperstimulation Syndrome (OHSS)**

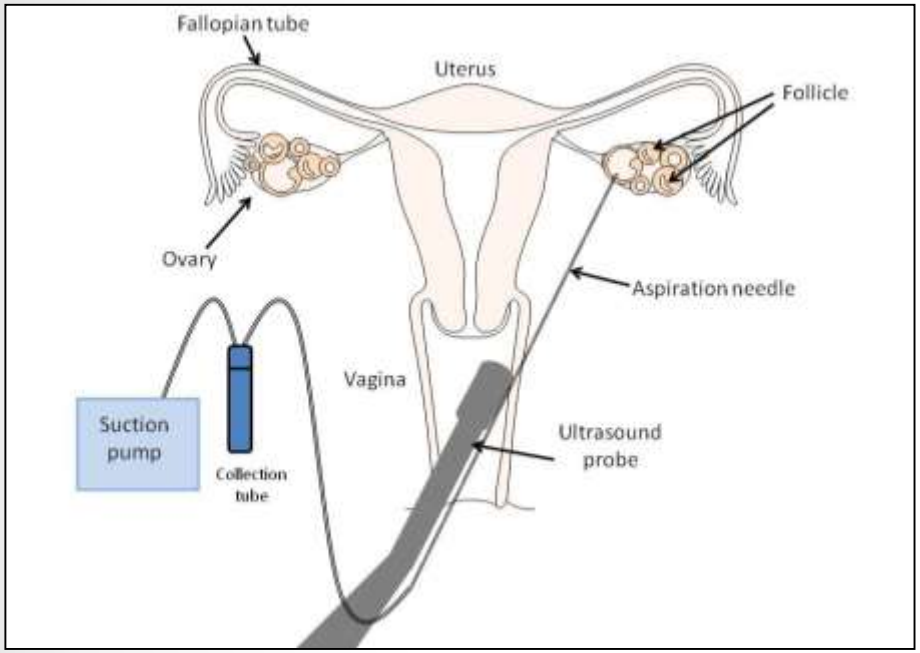
Ovarian stimulation
Ovarian Hyperstimulation Syndrome (OHSS)

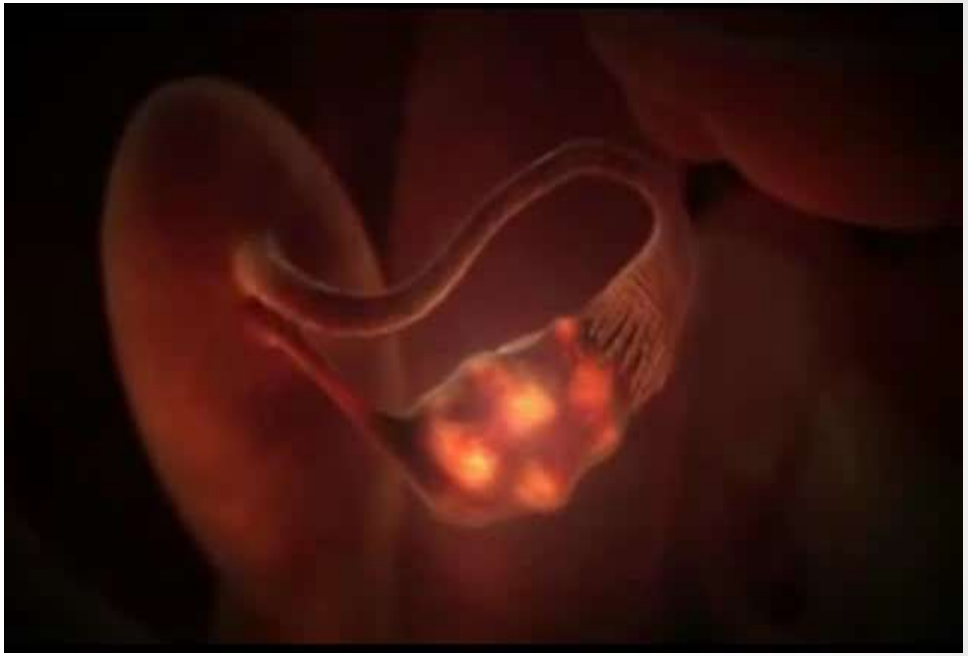
- Ovarian hyperstimulation can result in enlargement of the ovaries with leakage of fluid into the abdomen and rarely into the lungs.
- Most cases are mild, but a small proportion are severe and fatal.
- Treatment of OHSS depends on the severity of the hyperstimulation. Mild OHSS can be treated conservatively
- If moderate to severe OHSS develops the IVF process should be postponed since establishment of pregnancy can lengthen the recovery time or contribute to a more severe course.

Collection of oocytes

- The oocyte maturation is performed, generally by an injection of human chorionic gonadotropin (hCG). Commonly, known as the "trigger shot.“
- The egg retrieval is performed at a time usually between 34 and 36 hours after hCG injection.
- Egg retrieval is usually accomplished by transvaginal ultrasound aspiration.
- It is done under short general anesthesia, and is 20 to 30 minutes procedure.
- In some circumstances, one or both ovaries may not be accessible by transvaginal ultrasound. Laparoscopy may then be used to retrieve the eggs using a small telescope placed in the umbilicus.

Collection of oocytes

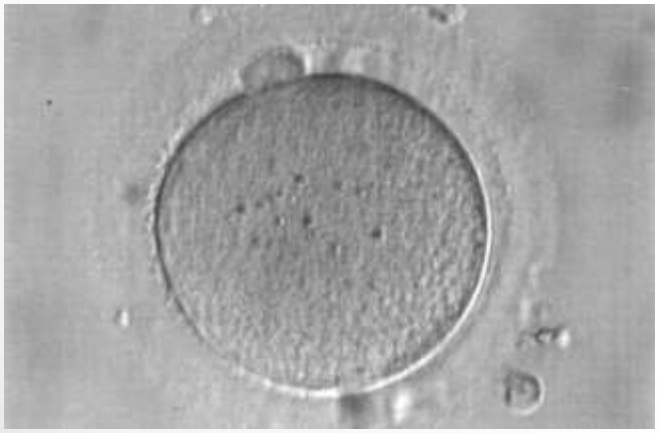


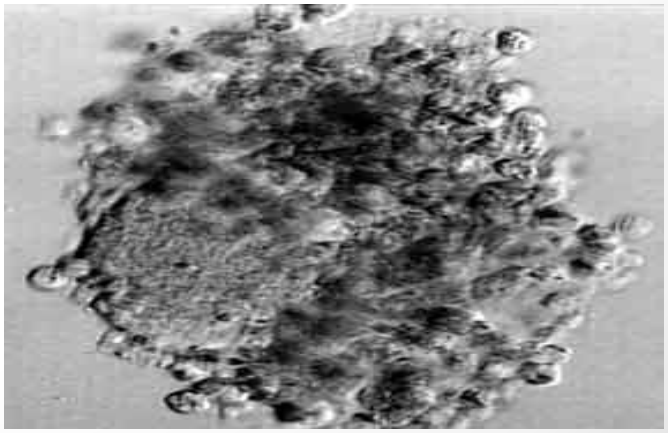


- The eggs are aspirated (removed) from the follicles through the needle connected to a suction device.

Collection of oocytes

- Usually around 10-15 oocytes are aspirated
- The eggs are prepared and stripped from the surrounding cells.
- After the eggs are retrieved, they are examined in the laboratory for maturity and quality.
- Mature eggs are placed in an IVF culture medium and transferred to an incubator to await fertilization by the sperms



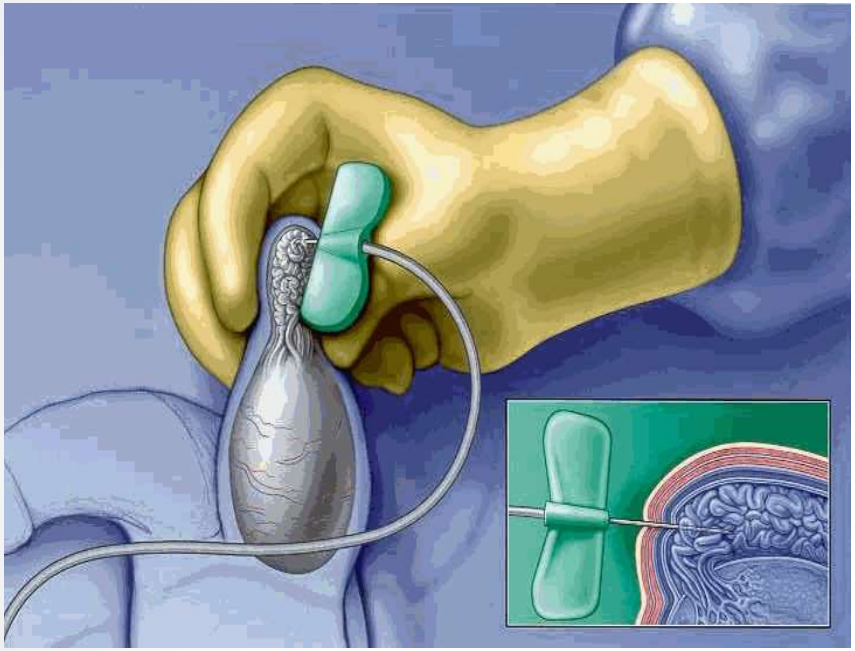


Collection of sperms

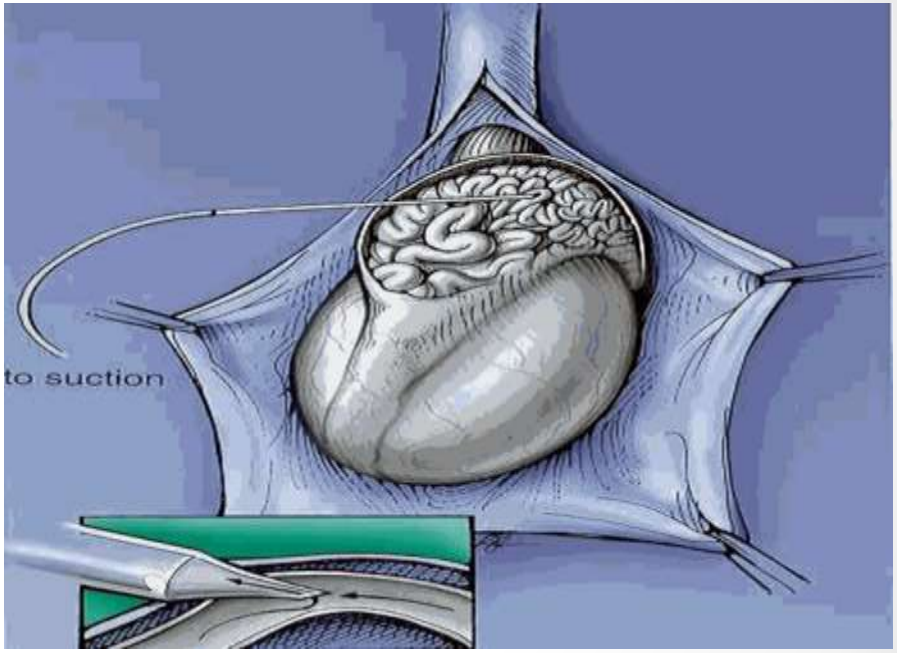
- Shortly before or after the oocyte collection the male partner will be asked to give a sperm sample.
- Sexual abstinence of 3-4 days should b exercised.
- Collected about 60-90 minutes prior to fertilization,.
- Liquefied ,centrifuged, suspended in culture medium, and incubated for 30-60 mins at 37º C.
- The most active sperms are located in the surface of the medium.
- Sperm may be obtained from the testicle, epididymis, or vas deferens from men whose semen is void of sperm either due to an obstruction or lack of production.

Collection of sperms

Surgical sperm retrieval



Percutaneous epididymal sperm aspiration (PESA)



Microsurgical epididymal sperm aspiration (MESA)

In vitro fertilization of oocytes

- Fertilization is started by adding 10,000-50,000 motile sperms to about 100 µl to 1 ml culture medium in which the oocytes is being incubated.

- Intra-cytoplasmic sperm injection (ICSI) is indicated in cases where semen fluid does not contain sperm.

In vitro fertilization of oocytes

Intra-cytoplasmic sperm injection (ICSI)

- During the process of ICSI, an embryologist isolates a sperm cell, draws it up into a microscopic needle and injects it inside an oocyte using a high power microscope.

An oocyte is held in place with the large holding pipette on the left, while the smaller pipette is used to pick up a single live sperm.

This pipette is then manipulated to pierce the oocyte cell membrane and the sperm is injected into the cytoplasm .

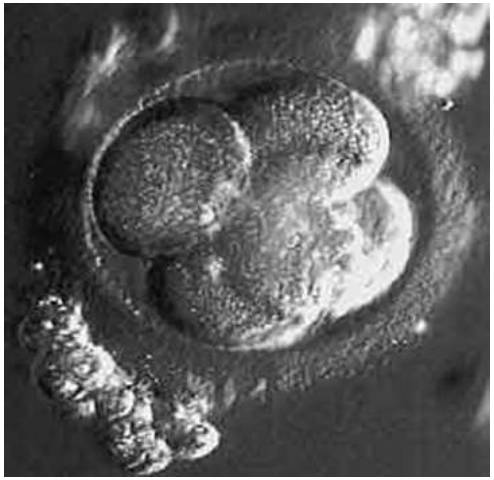
In vitro fertilization of oocytes

- Fertilisation check is performed the next day approximately 18 hours after sperm injection or insemination of the eggs.
- Usually 65% to 75% of mature eggs will fertilize after insemination
- They are cultured in special incubators to support division and development.
- If the couple has a history of certain genetic disease and the gene that is causing that problem is identified, a pre-implantation genetic diagnosis may b done.

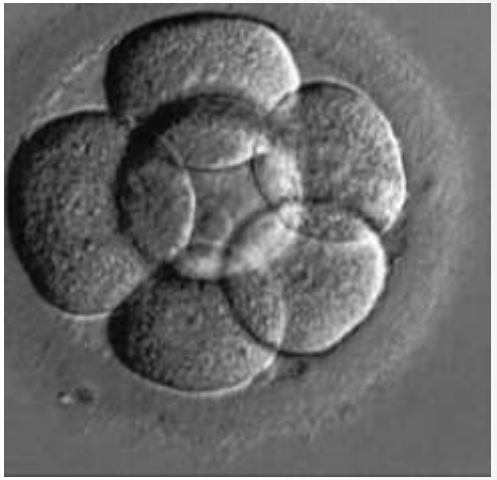
In vitro fertilization of oocytes

Development of embryo

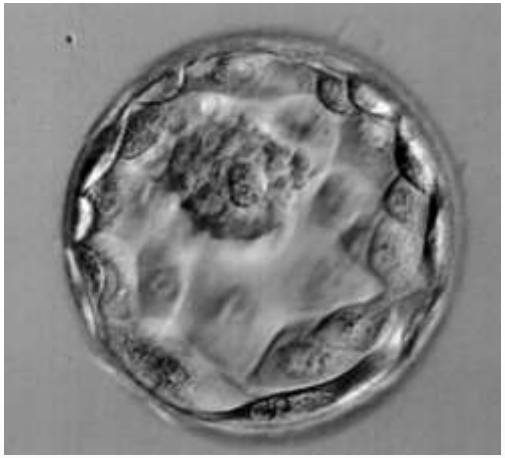
Day 1: 2 cell stage
male and female pronuclei



Day 2: 4 cell stage



Day 3: 8 cell stage

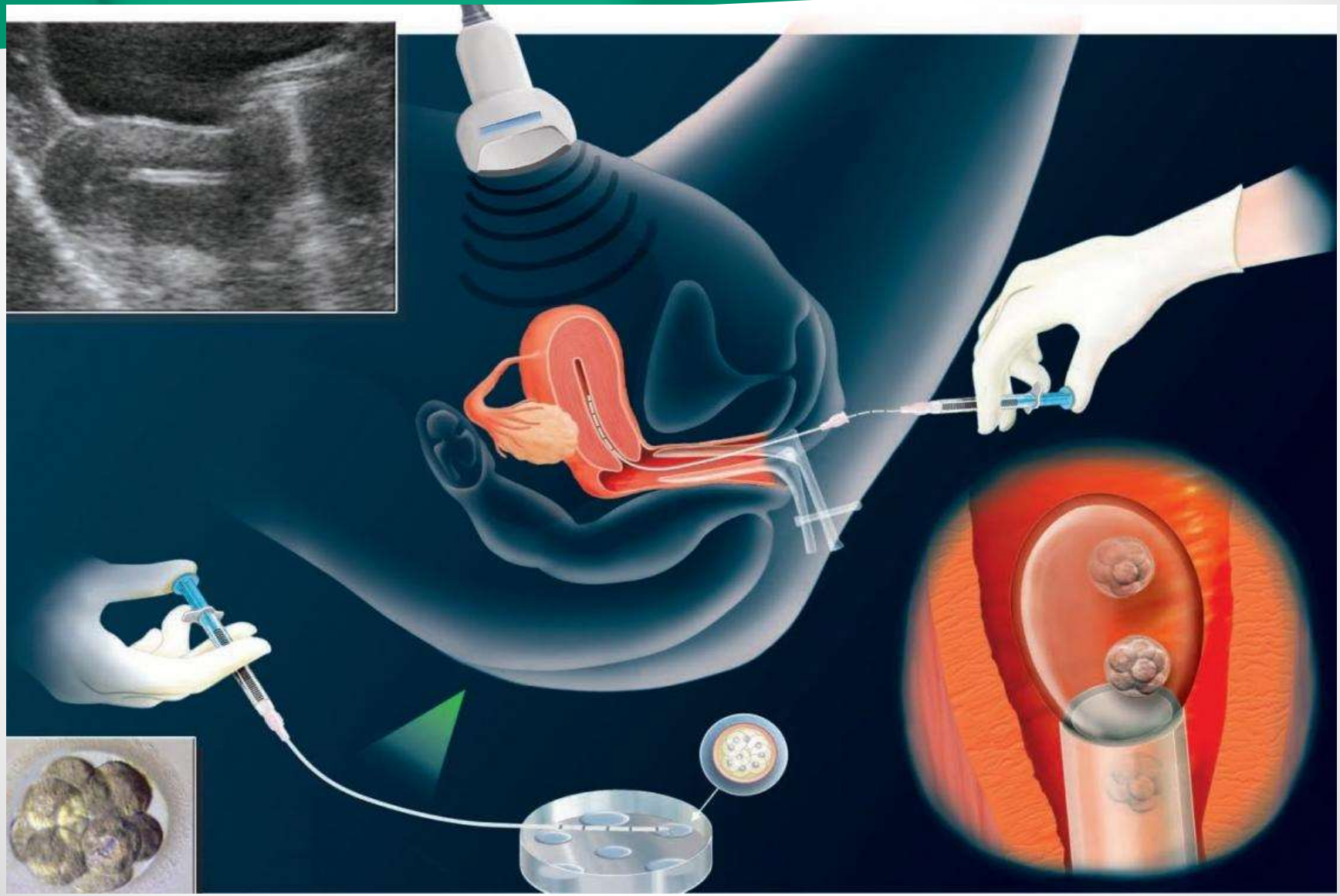


Day 5: blastocyst (200-300 cell)

Embryo transfer

- Embryo transfer may be performed on day 2, 3 or 5 post fertilization.
- One or more embryos suspended in a drop of culture medium are drawn into a transfer catheter, a long, thin sterile tube with a syringe on one end.
- The physician gently guides the tip of the transfer catheter through the cervix and places the fluid containing the embryos into the uterine cavity

Embryo transfer



Embryo transfer

How Many Embryos are Transferred?

- Related to age and embryo quality
  - <35 = 2
  - 35-37 = 2-3
  - 38-40 = 3-4
  - >40 = up to 5

- For patients with 2 or more failed IVF cycles, or a poor prognosis, can add more based on clinical judgement

What are the percentages of success?

Women's age

- Less than 35 years old (41-43%). In our center, 65-70%.
- Between 35-37 years old (33-35%). In our center, 50- 55%.
- Between 38-40 years old (23-27%). In our center, 30-35%.
- After 41 years old (13-18%). In our center, 20-22%.

Other Statistics

- Repetition of four trials: success rate (70-80%).
- Repetition of after three trials: success rate (60-65%).
- If four embryos are transferred the percentage of success becomes 40%.
- If three embryo transfer the percentage 35%.
- If two embryo transfer the percentage 25%.
- One embryo carry 17% success rate.

Other Factors

- increasing the number of embryos transferred, the success rate increases
- risk of multiple pregnancies increases and miscarriage increases
- Quality of the eggs and sperm.
- Number and quality of embryos

Variations of IVF

- **Gamete intrafallopian transfer (GIFT)** is similar to IVF, but the gametes (egg and sperm) are transferred to the woman's fallopian tubes rather than her uterus, and fertilization takes place in the tubes rather than in the lab.
- **Zygote intrafallopian transfer (ZIFT).** This technique differs from GIFT in that fertilization takes place in the lab rather than the fallopian tube, but is similar in that the fertilized egg is transferred to the tube rather than the uterus.
- They both comprises less than 1% of IVF procedures performed worldwide.

.....its over!!!!

Thank you!