ONPRC Module 3A:
Assisted Reproductive Technologies (ART)

Guiding Question:

How can we preserve the fertility of cancer patients?

Module Question	Laboratory Questions
How does learning about fertilization, embryonic development and assisted reproductive technologies create options for preserving fertility?	• How does fertilization happen in humans, in vivo and in vitro? • How do embryos develop? • How can we monitor pregnancy using ultrasound?

Learning Outcomes:

Describe the process of fertilization.

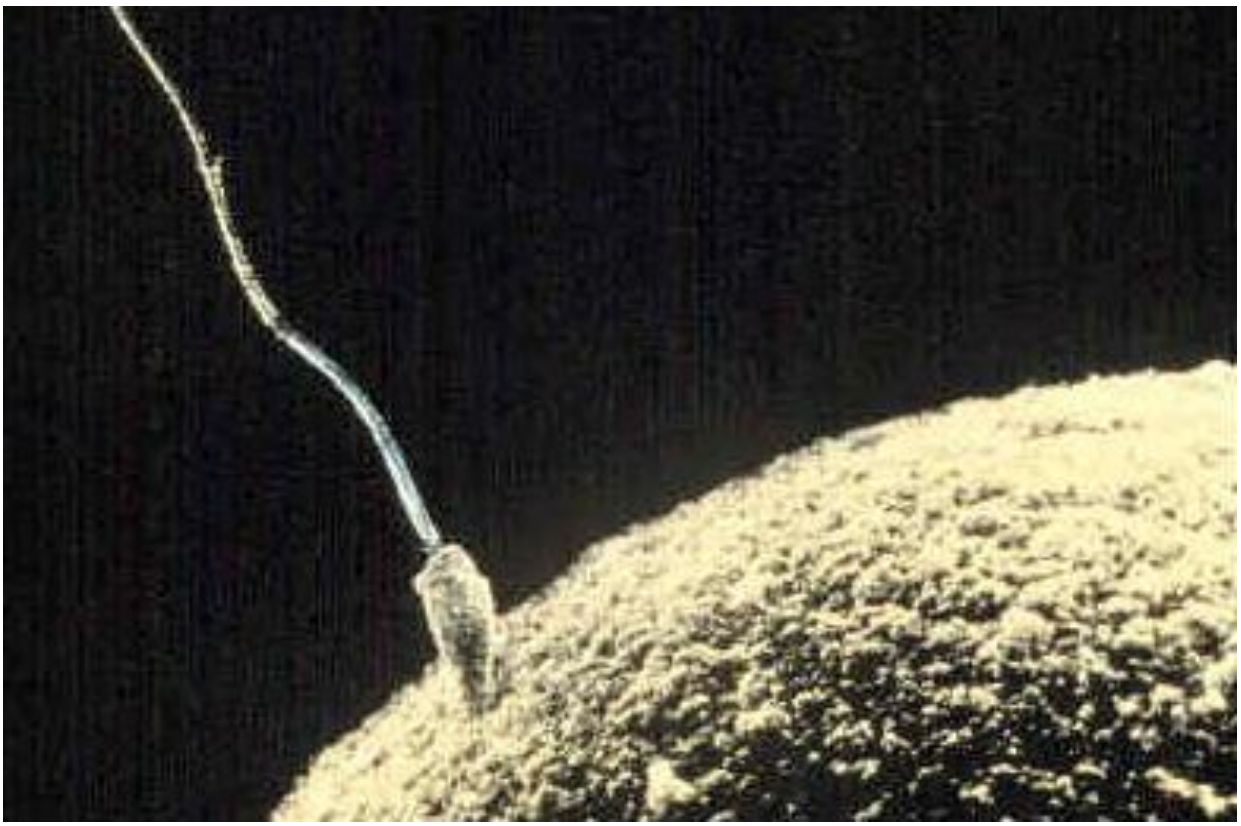
Describe embryonic and early fetal development.

Describe assisted reproductive technologies (artificial insemination, in vitro fertilization, intracytoplasmic sperm injection).

Describe how assisted reproductive technologies can help preserve endangered species.

Describe how assisted reproductive technologies can help preserve the fertility of cancer patients.

Fertilization Process



http://commons.wikimedia.org/wiki/File:Sperm-egg.jpg

Steps for Forming a Fertilized Egg

1. **Sperm <u>bind</u> to zona pellucida proteins (ZPs) – specific to every species except hamster**
2. **Sperm undergoes <u>acrosome reaction</u> so it can digest through the zona pellucida**
3. **Sperm <u>fuses</u> with oocyte plasma membrane = fertilization and enters oocyte; only 1 sperm in 1,000,000 will make it to fertilization.**
4. **<u>Cortical reaction</u> occurs, "hardens" oocyte membrane, no more sperm can enter**
5. **<u>Zona reaction</u> occurs, destroys ZPs so no more sperm can bind**

Fertilization Vocabulary

Fertilization - the process by which a male and female gamete fuse to form a zygote

Fertilization membrane - specialized membrane that form around the fertilized oocyte to prevent entry by additional sperm

Polyspermy - the fusion of more than one sperm with an oocyte

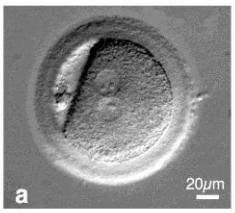
Zona Pellucida - a thick solid transparent outer membrane of a developed mammalian oocyte; can be penetrated by one sperm in the fertilization process; usually remains around the fertilized oocyte until it is implanted in the wall of the uterus

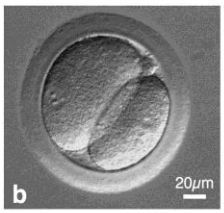
Polar Body - a small cell containing little cytoplasm that is produced along with the oocyte and later discarded

Pronucleus - the haploid nucleus of a sperm or oocyte before fusion of the nuclei in fertilization

Cleavage - a series of synchronized mitotic cell divisions immediately following fertilization that results in the formation of the blastomeres and changes the single-celled zygote into a multicellular embryo

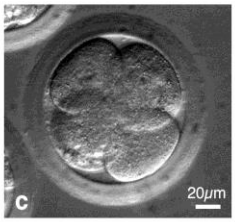
zygote (fertilization)

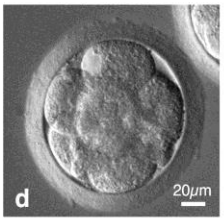




2-cell stage

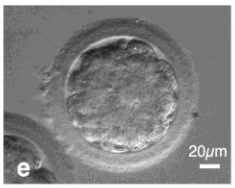
4-cell stage

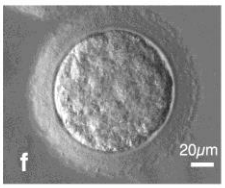




8-cell stage

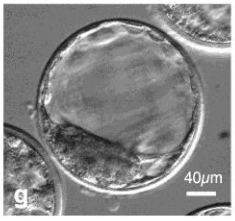
Morula (12-16 Compacted Cells)





Morula

Blastocyst Can implant in uterus



Photos: Dr. Shoukhrat Mitalipov, PhD, ONPRC

Infertility

- **Female**
  a. Cannot ovulate (release oocyte)
  b. Abnormal oocyte
- **Male**
  a. Low sperm count
  b. Immotile sperm
  c. Abnormal sperm
- **Causes**
  a. Genetics
  b. Age – "biological clock"
  c. Disease, such as pelvic inflammatory disease
  d. Environmental toxins

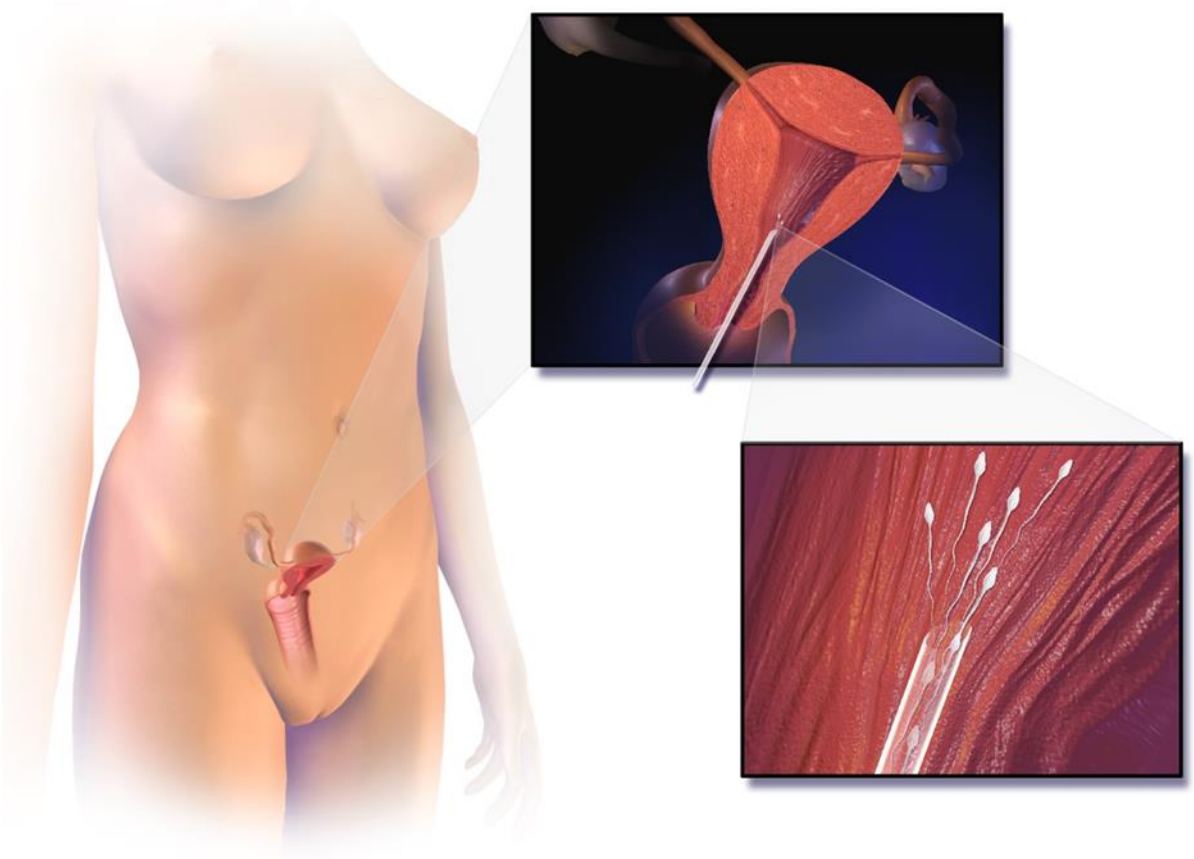
Assisted Reproductive Technologies (ART) helps treat couples who haven't had success having an offspring on their own. Methods include:

a) Artificial insemination

b) *In vitro* fertilization (IVF)

c) Intra-cytoplasmic sperm injection (ICSI)

Artificial Insemination



- Artificial insemination is the placement of sperm into a female's uterus for the purpose of achieving a pregnancy by means other than sexual intercourse.
- The sperm can be collected fresh or it could be frozen-thawed.
- The sperm are deposited into the woman's uterus when she is about to ovulate.
- It is used for couples where there is male infertility and in cases where a single women wants to have a child.
- In addition, this is a common practice in animal breeding, such as in cattle, horses, and zoo elephants.

"Test Tube Babies"

- Test tube babies are actually made in a petri dish rather than a test tube through a process called ***In Vitro* Fertilization (IVF**).
- This is a laboratory technique that can be used to help:

a. People who are unable to have their own children naturally due to problems in their reproductive systems.

b. Non-human primate and other mammalian endangered species.

Cartoon: Microsoft Clip Art

In Vitro Fertilization Process

- First, the woman is given extra estrogen and progesterone over a month or so in order that she will produce multiple oocytes.
- The metaphase II oocytes are then aspirated from the ovaries in a laparoscopic procedure and the oocytes are cleaned of extra tissue and placed in a petri dish.
- The man's sperm is added to the petri dish and the cells are incubated for 3 or so days.
- Then the best 4- to 8-cell embryos are chosen and inserted into the woman's uterus for development.
- Research is being done to determine the most viable one embryo because multiple embryos can cause each to develop in the womb less fully due to crowding.

Cartoon: Microsoft Clip Art

In Vitro Fertilization Babies

1978 - First IVF baby, Louise Brown, was born.

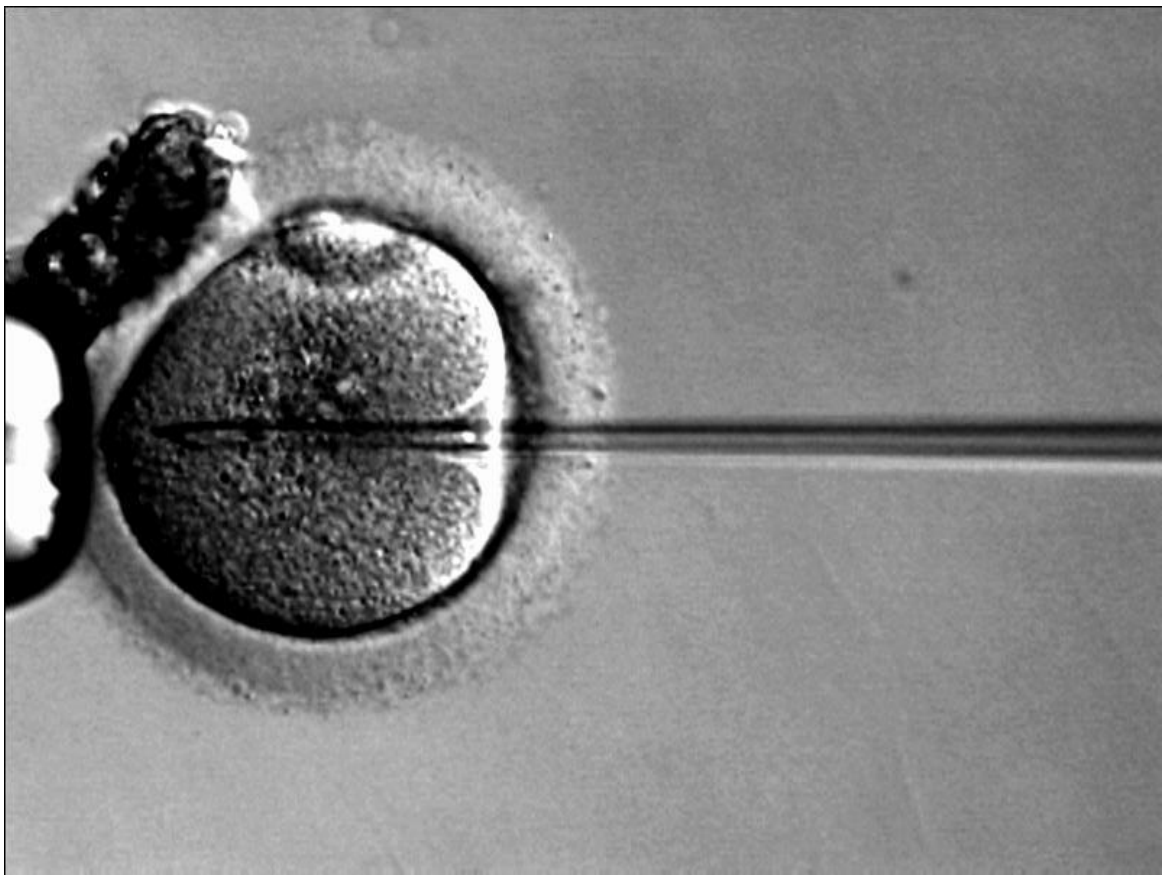
2012 - 5 million IVF babies born

Cartoon & Photo: Microsoft Clip Art

Intra-Cytoplasmic Sperm Injection (ICSI)

•Intra-cytoplasmic sperm injection (ICSI) involves injecting a single sperm directly into an oocyte in order to fertilize it. The zygote then divides to the 4– to 8-cell stage and is transferred to the woman's womb.

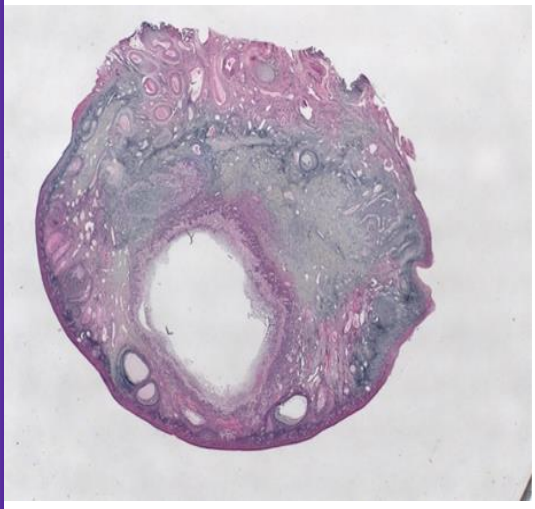
•The major development of ICSI means that fertilization is possible as long as some sperm can be obtained (even in very low numbers).



https://commons.wikimedia.org/wiki/File:Icsi.JPG

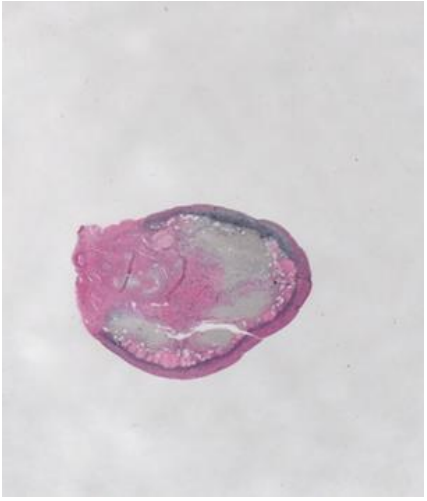
Can Fertility Be Preserved in Female Cancer Patients?

> 70,000 patients exposed to chemotherapy or radiotherapy. More than half are breast cancer patients.



Chemotherapy & Radiotherapy

Causes Apoptosis (cell death in oocytes)



Mature Monkey Ovary

Premature Ovarian Failure Leading to Infertility

Photos: Dr. Mary Zelinski, PhD, ONPRC