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Biomedical Engineering +
Leonard Gelfand Center

Artificial Ovaries Design

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This educational resource for high school audiences was developed as a project by Carnegie Mellon student, Meghan McGraw, for the course *Experiential Learning through Projects*, Section O, taught by Dr. Conrad Zapanta and Dr. Judith Hallinen during the summer of 2020.

Editing and additional project development was completed by Carnegie Mellon student Claire Kenny.

CAUTION: If you are attempting an experiment, it is important to make sure that you are following all safety steps. All experiments should be completed with supervision of a adult. Weather permitting, we recommend taking messy experiments outside. Remember to wear safety gear like gloves, aprons, and goggles, especially for experiments with chemical reactions!

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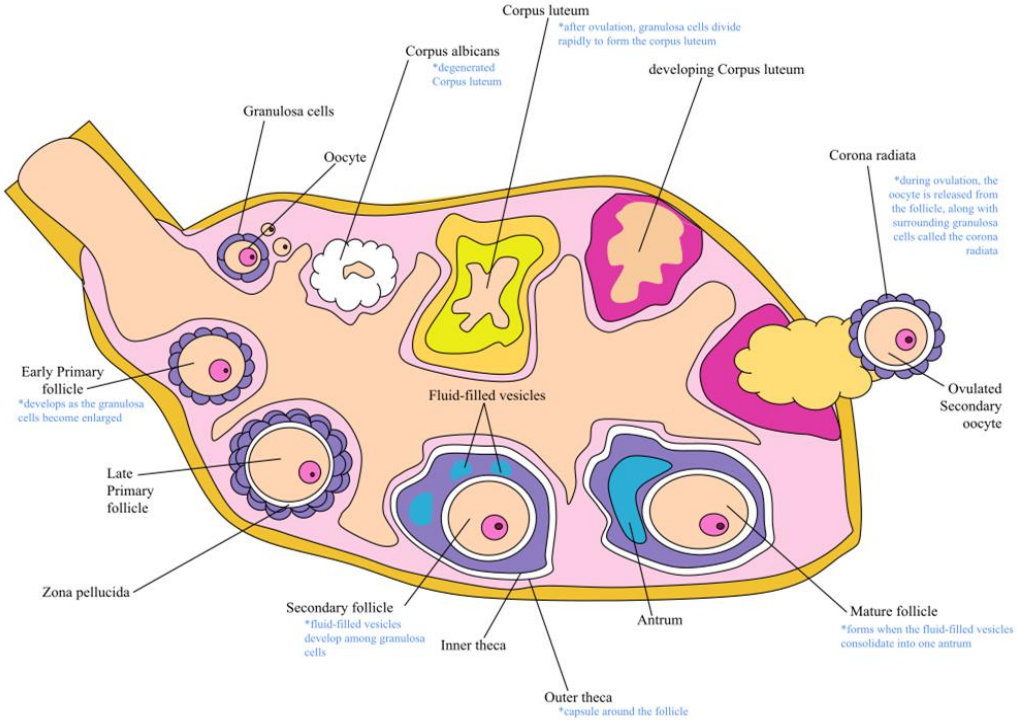
Learning Goals

1. Demonstrate knowledge of basic ovarian anatomy and physiology.
2. Identify the causes and consequences of ovarian damage
3. Describe the stages of tissue engineering and identify how it can be used to solve infertility problems
4. Name the components of an artificial ovary
5. Discuss who can benefit from the technology

What are the Ovaries?

Overview of the Ovaries: What?



An organ in the female reproductive system (and endocrine system) that…

- Produces/ develops eggs (oogenesis)
- Releases eggs (ovulation)
- Produces progesterone and estrogen

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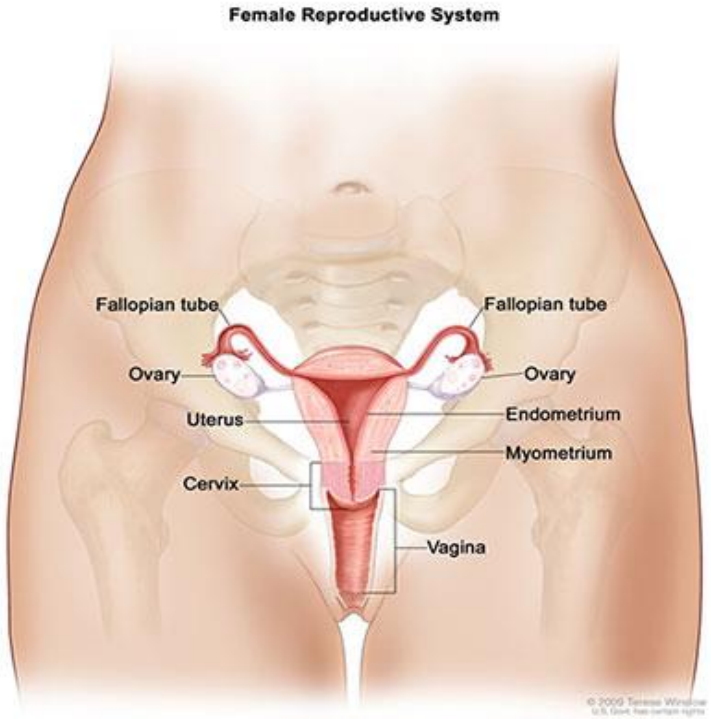
Note: See speaker notes for image citations

Overview of the Ovaries: Where?

- Right below the belly button
- Protected by the pelvis
- Almond-sized organs on either side of the uterus



Overview of the Ovaries: Ovarian Tissue Anatomy

Surface

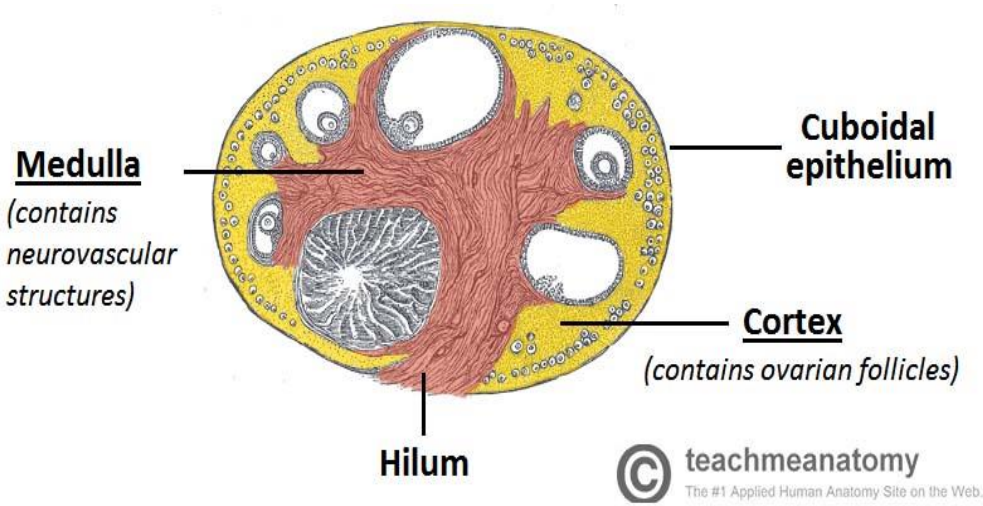
- Epithelium and connective tissue

Cortex

- Connective tissue and follicles

Medulla

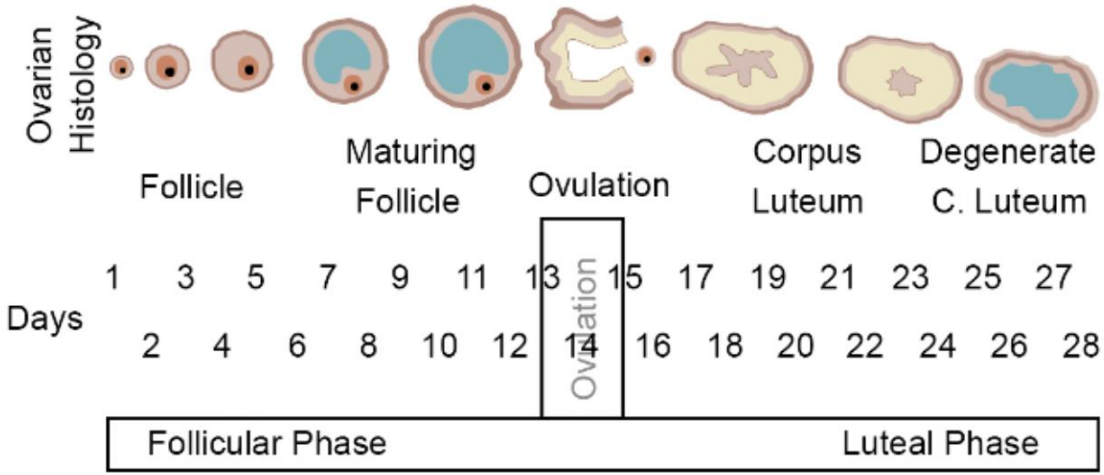
- Connective tissue and neurovascular structures



The Menstrual Cycle

The menstrual cycle can be divided into four main phases:

1. Menstrual Phase
2. Follicular Phase
3. Ovulation
4. Luteal Phase



1. Menstrual Phase

This phase is when a female's period occurs:

- Begins when an egg from the previous cycle isn't fertilized
- The thickened lining of the uterus is shed through the vagina and a combination of blood, mucus, and tissue from the uterus
- Estrogen and progesterone levels *drop*
- ***Length***: On average 3-7 days

Note: See speaker notes for image citations

2. Follicular Phase

Starts on the first day of a woman's period (some overlap with the menstrual phase) and ends during ovulation.

- Follicle-stimulating hormone (FSH) is released to mature follicles
    - This leads to about 5-20 follicles being produced, each containing an immature egg (oocyte)
- 1 healthy egg (ovum) matures
- ***Length***: On average 11-27 days

Note: See speaker notes for image citations

3. Ovulation

Occurs when luteinizing hormone (LH) encourages the release of the ovum into the Fallopian tubes.

- The ovary releases a mature egg, which travels down the fallopian tube toward the uterus to be fertilized by sperm
- After a day, the egg will die or dissolve if it isn't fertilized.
- Occurs around day 14 of a 28-day cycle (right in the middle of the menstrual cycle)
- ***Length***: On average 24 hours

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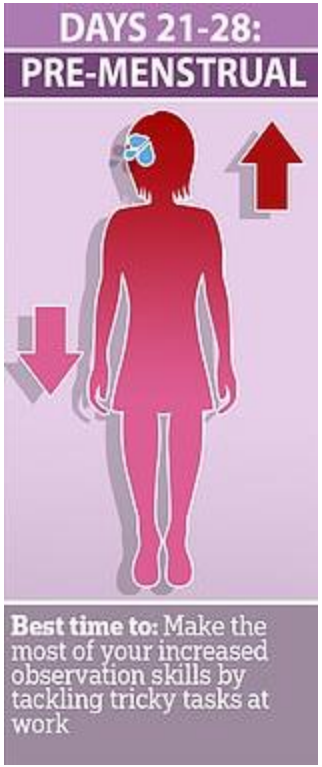
4. Luteal Phase

Involves the leftover follicle being turned into the corpus luteum, a structure in charge of releasing hormones.

- Progesterone and estrogen are released to keep the uterine lining thick and ready for a fertilized egg to be implanted
- If fertilization does not occur,
  - Human chorionic gonadotropin (hCG) is released
  - The corpus luteum will shrink and be resorbed
  - Levels of estrogen and progesterone decrease, leading to a woman's period
- ***Length***: On average 11-17 days



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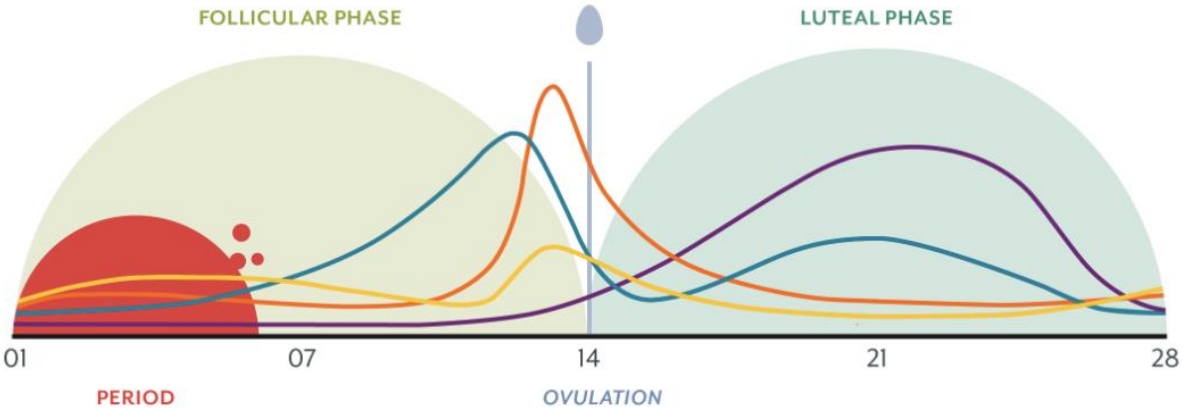
Let’s talk about hormones,



Ovarian Cycle

FSH (Follicle Stimulating Hormone)

E2 (Estrogen/Estradiol)

LH (Luteinizing Hormone)

PG (Progesterone)

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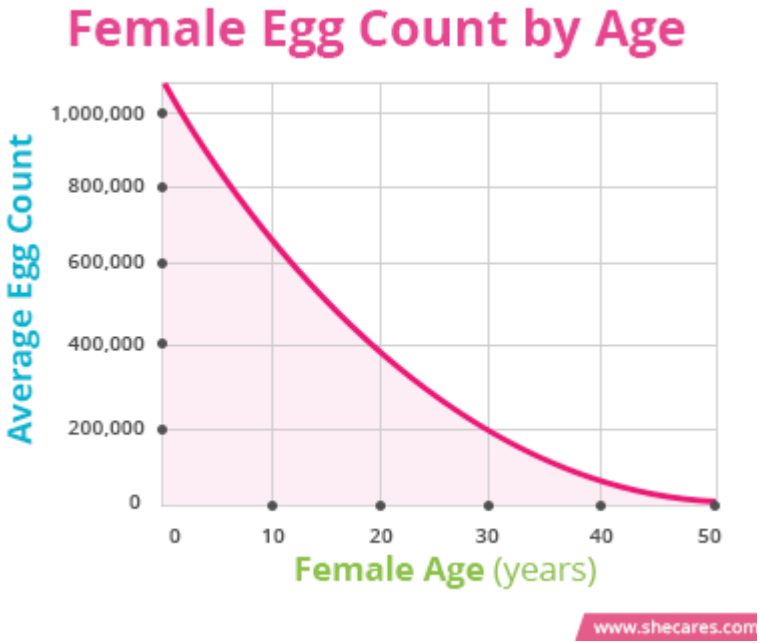
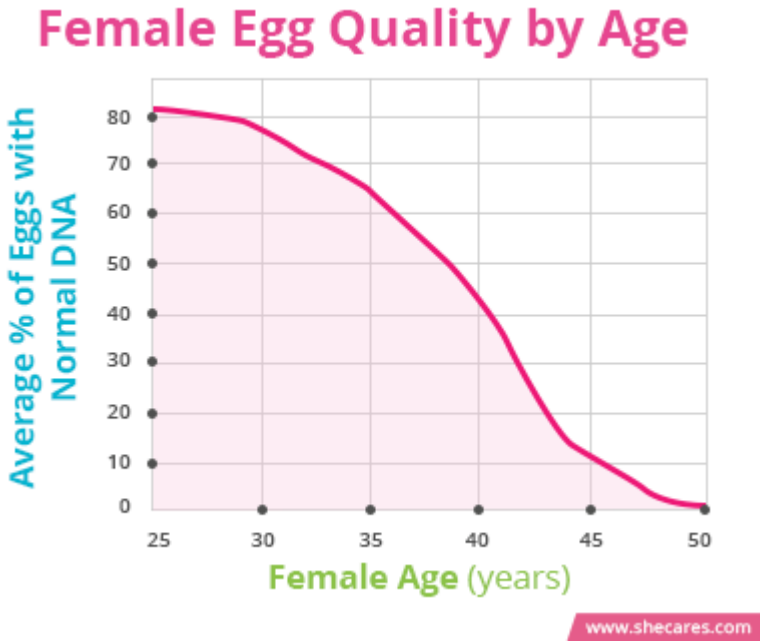
IMPORTANT FACT: Females are born with a limited number of oocytes, no more are created throughout their lifetime, with the count and quality decreasing with age





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What is female fertility?

By definition, fertility is the ability to conceive a biological child

Biological, environmental, and lifestyle factors may cause ovarian infertility

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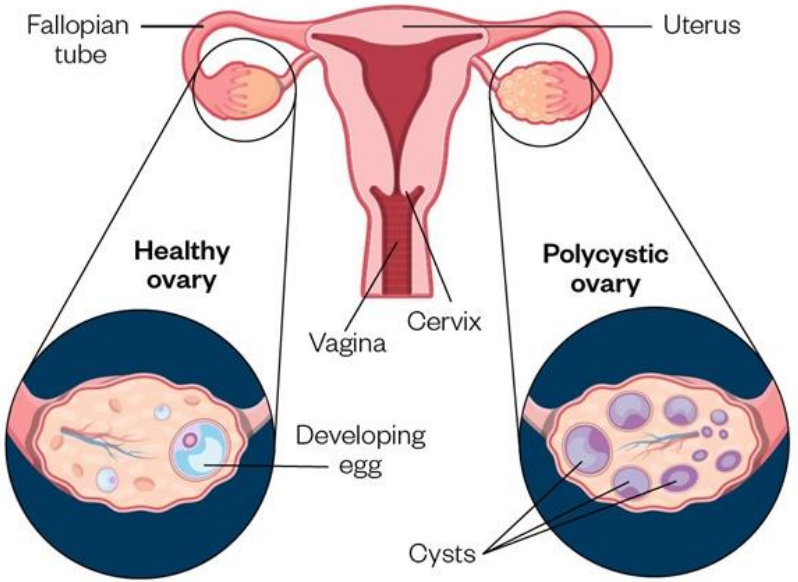
Ovarian Infertility: Polycystic Ovary Syndrome (PCOS)

Syndrome that *affects the ovaries and ovulation.*

Has 3 main features/symptoms:

1. ***Women produce excess amounts of androgens*** (male hormones)
    - Disrupt periods/ovulation
2. ***Presence of 'cysts'*** in ovaries
    - Cysts are follicles whose growth is incomplete, leading to large fibrous structures
3. ***Irregular or skipped periods***



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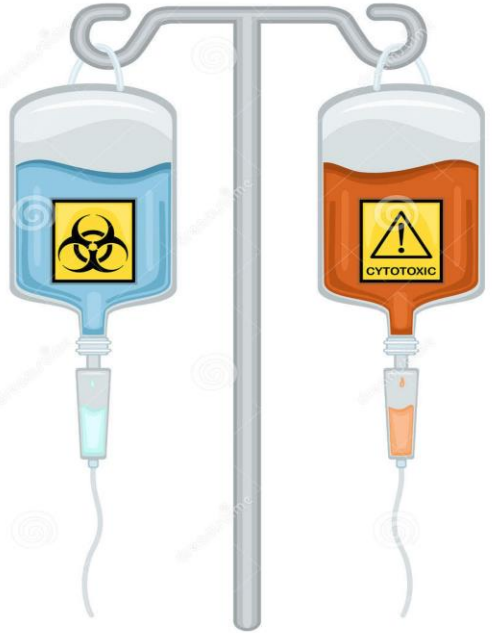
Ovarian Infertility: Cancer Treatments

Cancer treatments may lead to infertility issues.

1. **Radiation**: application of X-ray light to parts of the body
   - Pelvic radiation leads to the direct killing of the ovarian cells
   - Brain radiation prevents the pituitary (an endocrine gland) from releasing hormones important to the menstrual cycle
2. **Chemotherapy**: infusion of anti-cancer drugs into the body
   - Chemotherapeutic drugs target fast dividing cells, like oocytes



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Ovarian Infertility: Scarred Ovaries

May lead to complications in fertilization or a blocked fallopian tube (egg cannot travel to the uterus).

Caused by:

- Multiple surgeries
- Autoimmune disorders
- ***Endometriosis***: Growth of uterine tissue outside of the uterus

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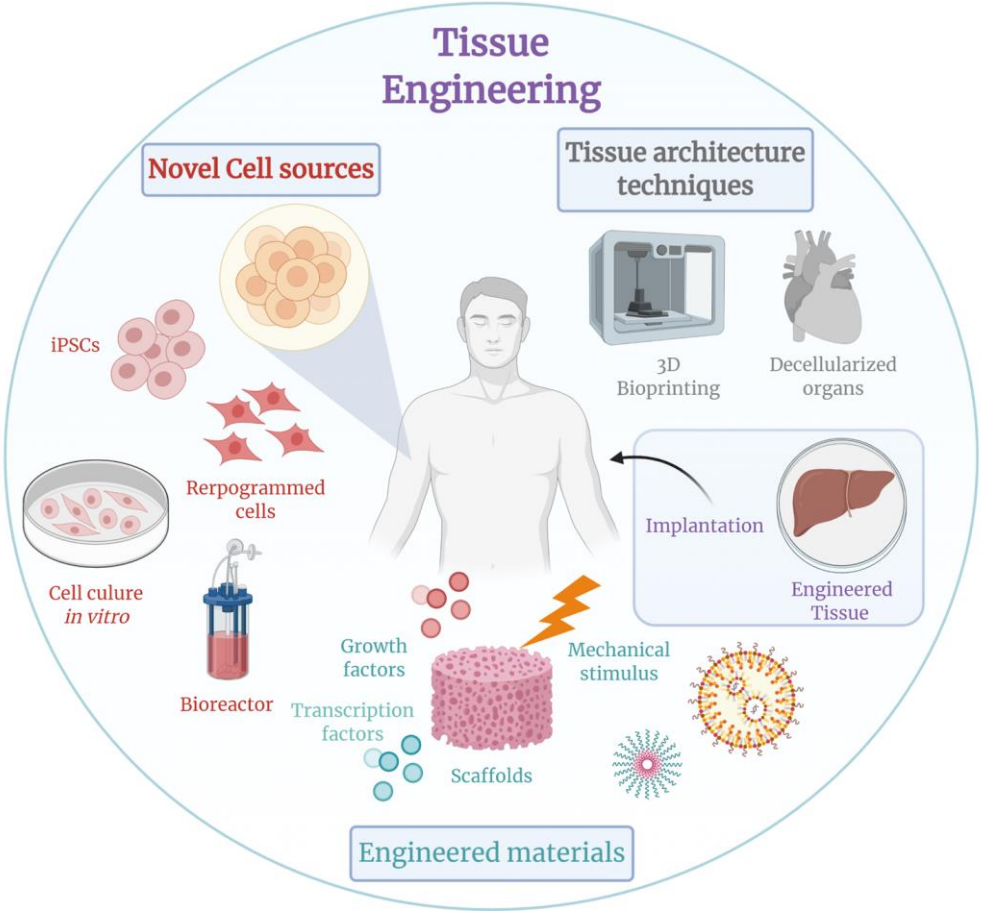
What is Tissue Engineering, and how can we use it?



Tissue Engineering

Involves: Combining scaffolds, cells, and biologically active molecules into functional tissues

Goal: Restore, maintain, or replace damaged tissue or whole organs

Examples:

- Artificial skin and cartilage
- Skin grafts
- Supplemental bladders
- Small arteries

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Artificial Ovaries and Tissue Engineering

Tissue engineering may address the diseases and disorders that can lead to infertility.

Why?

- Decreases complications of organ transplantations (long waiting lists) and medical devices (tissue rejection, bridge to transplant solutions)

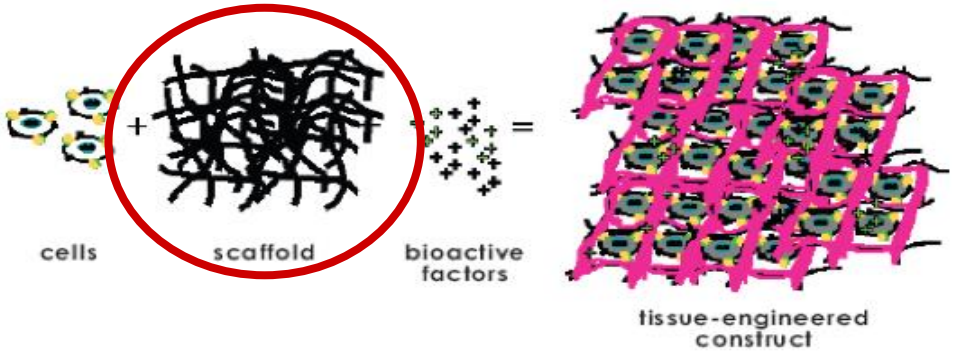
How?

- Aim to create transplantable scaffolds or/and ovarian hydrogels that imitate the ovarian microenvironment and/or achieve in vitro folliculogenesis
- Five steps of the process:
    1. Scaffolding
    2. Isolation of oocytes
    3. Seeding
    4. Growth
    5. Surgical placement inside of patient

Scaffolding

Structures consisting of artificial or natural substances that act as a shape on which cells can grow

- Can be **inert** (do not interact with cells growing on/within) or **active** (actively help cell grow using chemical signals)
- Quite useful in helping rebuild or replace lost tissue



Scaffolding (Cont.)

Two main ways to create a scaffold:

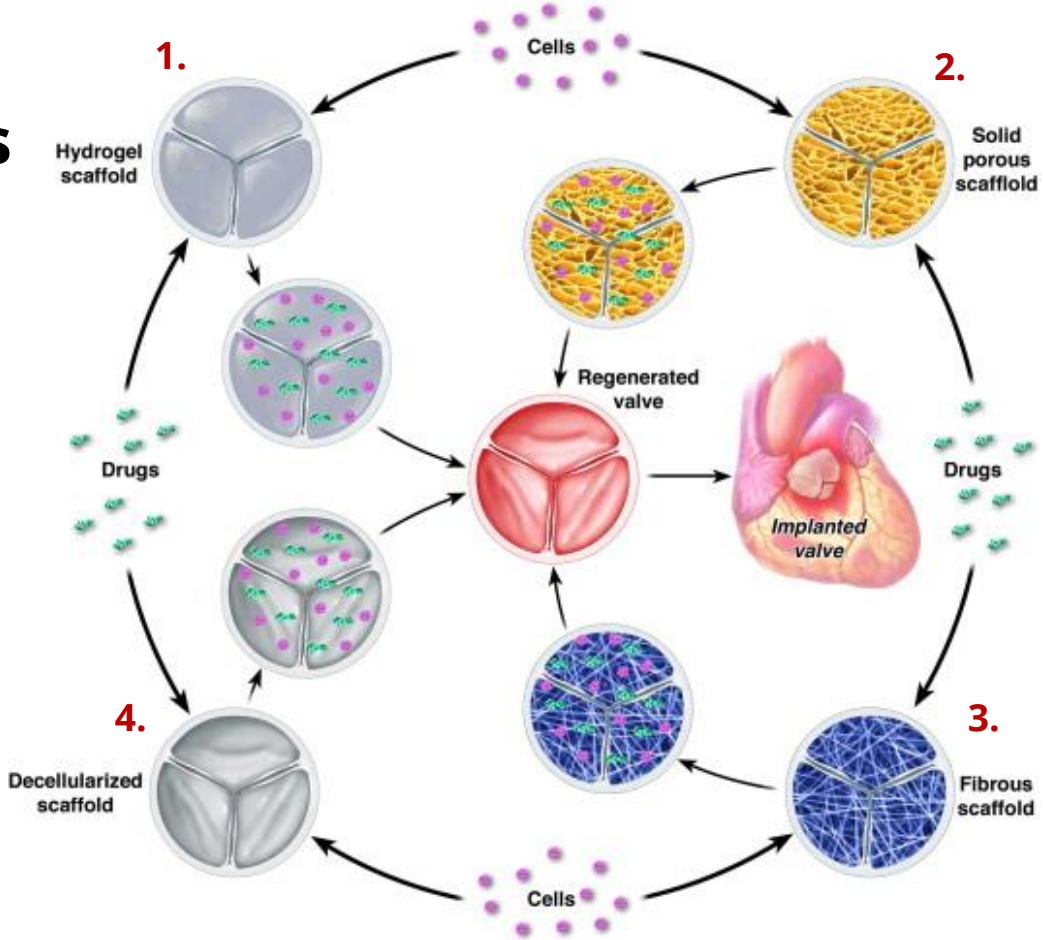
3D Printing

- Print different materials into desired shape
- Can use materials such as:
  - Gelatin
  - Polyethylene glycol (PEG)
  - Collagen
  - Fibrin

Decellularization

- Isolating the extracellular matrix from preexisting ovaries
- Can use detergents or enzymes to do so

Types of Scaffolds



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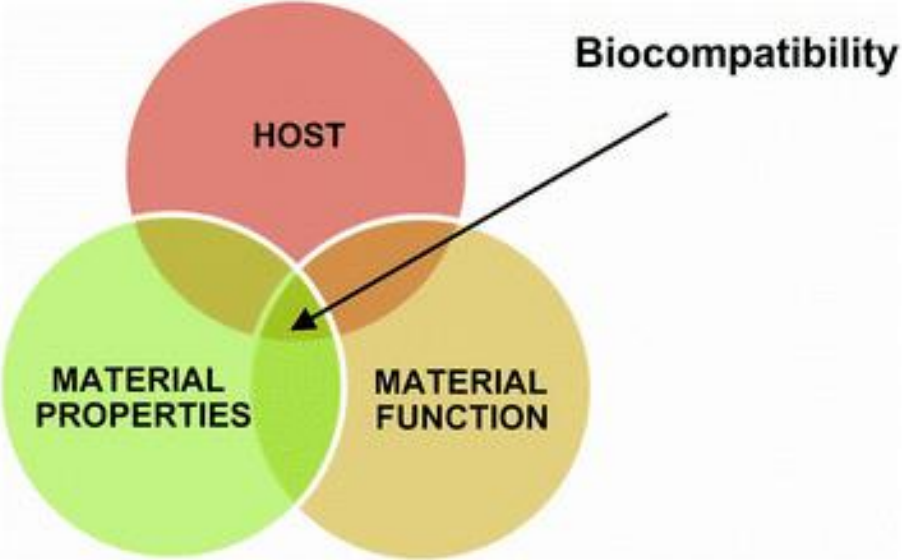
Scaffolding Considerations

When producing a scaffold there are numerous points to be considered

- Shape and size of the implant
- Chemical reactivity
- Mechanism, rate and by-products of degradation
- Material choice and characteristics
- Oocytes radically change in size
- ***Biocompatibility***: "the ability of a material to perform with an appropriate host response in a specific application"



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Isolation of Oocytes

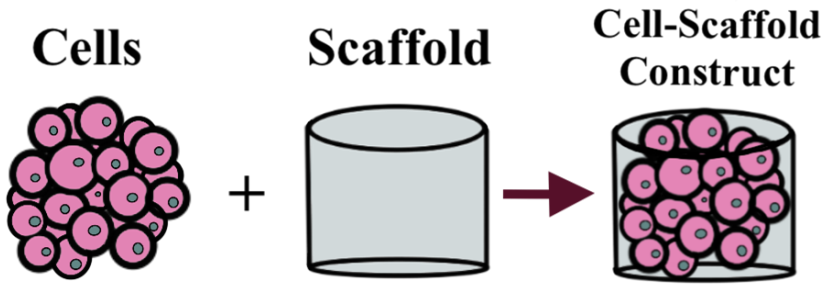
Mechanical and chemical breakdown of patient's ovarian tissue to obtain oocytes

- Can be done manually or using filtering and enzyme treatments
- Living cells can be fluorescently marked

Seeding

Aims to adhere cells on scaffold for cell culturing

- Oocytes are pipetted onto the scaffold
- The cells attach to surfaces via surface receptors called integrins

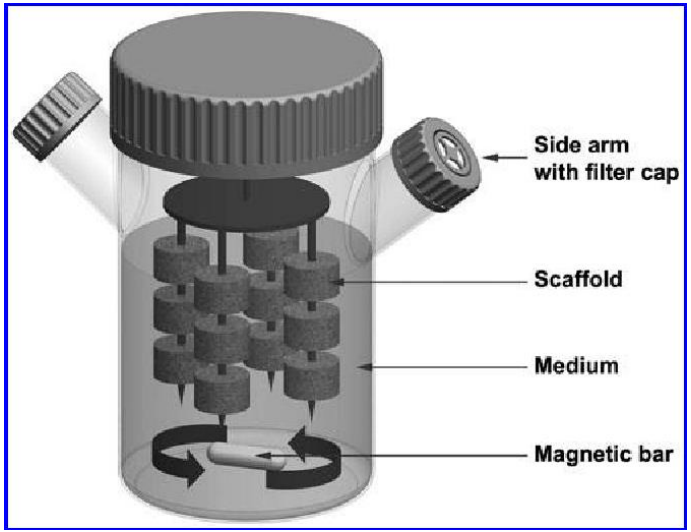


Growth

Bioreactor: Chamber that supports cell growth under specific conditions that simulate the human body environment

For tissue engineering, bioreactors are used in 3 main ways:

1. *In vitro*: Mimic in vivo environment so as to understand normal cell and molecular physiology
2. *Expand cells for potential clinical use*: Cell scaffolds, recellularization and decellularization of tissues
3. *Serve as more realistic setting than simple in vitro conventional culture:* Help establish new therapeutic targets and test potential new treatments



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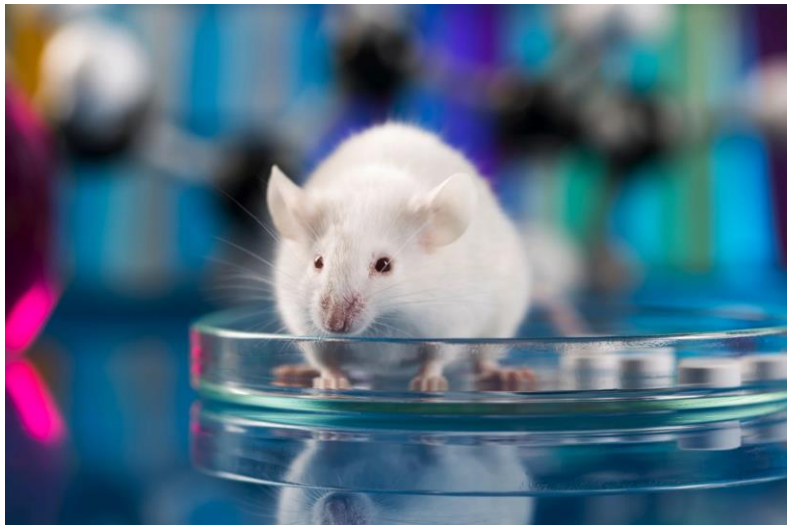
In real life...

- In 2017, researchers at Northwestern University 3D-printed a gelatin matrix, and seeded follicles on it.
- The ovary was implanted into an infertile female mouse, who was then able to ovulate and have healthy litters.
- Hormonal levels were also altered by the presence of the ovaries.
- In Copenhagen, a team used a chemical to strip a mouse ovary (decellularize) and implant it with human follicles



Problems

- The follicles don't live very long
    - Due to the shape of the scaffold, cells die somewhat quickly (within weeks) because the eggs are not able to be supported by the stromal (support) cells.
    - Younger patients would need the follicles to last several years!
- Mouse tissue does not behave exactly like human tissues
- Ethical and moral conflicts

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Possible Recipients

- Cancer patients
- Patients with diseases that render them infertile
  - Polycystic ovary syndrome
  - Endometriosis
- Patients in need of hormone therapy
- Transgender patients

Note: See speaker notes for image citations

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