NOVEL IN VITRO MODEL TO GROW AND CULTURE OVARIES OUTSIDE THE BODY

Matthew Zanotelli, Patrick Hopkins, Joseph Henningsen, Aaron Dederich

Client: Sana M. Salih, MD, MMS

Advisor: John P. Puccinelli, PhD

Biomedical Engineering Design

University of Wisconsin – Madison

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UNIVERSITY OF WISCONSIN-MADISON COLLEGE OF ENGINEERING

OVERVIEW

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PROBLEM STATEMENT

- Doxorubicin (DXR) chemotherapy causes ovarian insult
- No system to grow mature ovaries *in vitro*
- Need to develop a novel ovary culture system that:
  - Maintains cell/tissue viability *in vitro*
  - Has a sterile and biocompatible environment
  - Facilitates assessment of chemotherapy toxicity to an ovary
  - Enables future investigations on ovarian protection from chemotherapy

BACKGROUND: OVARY ANATOMY

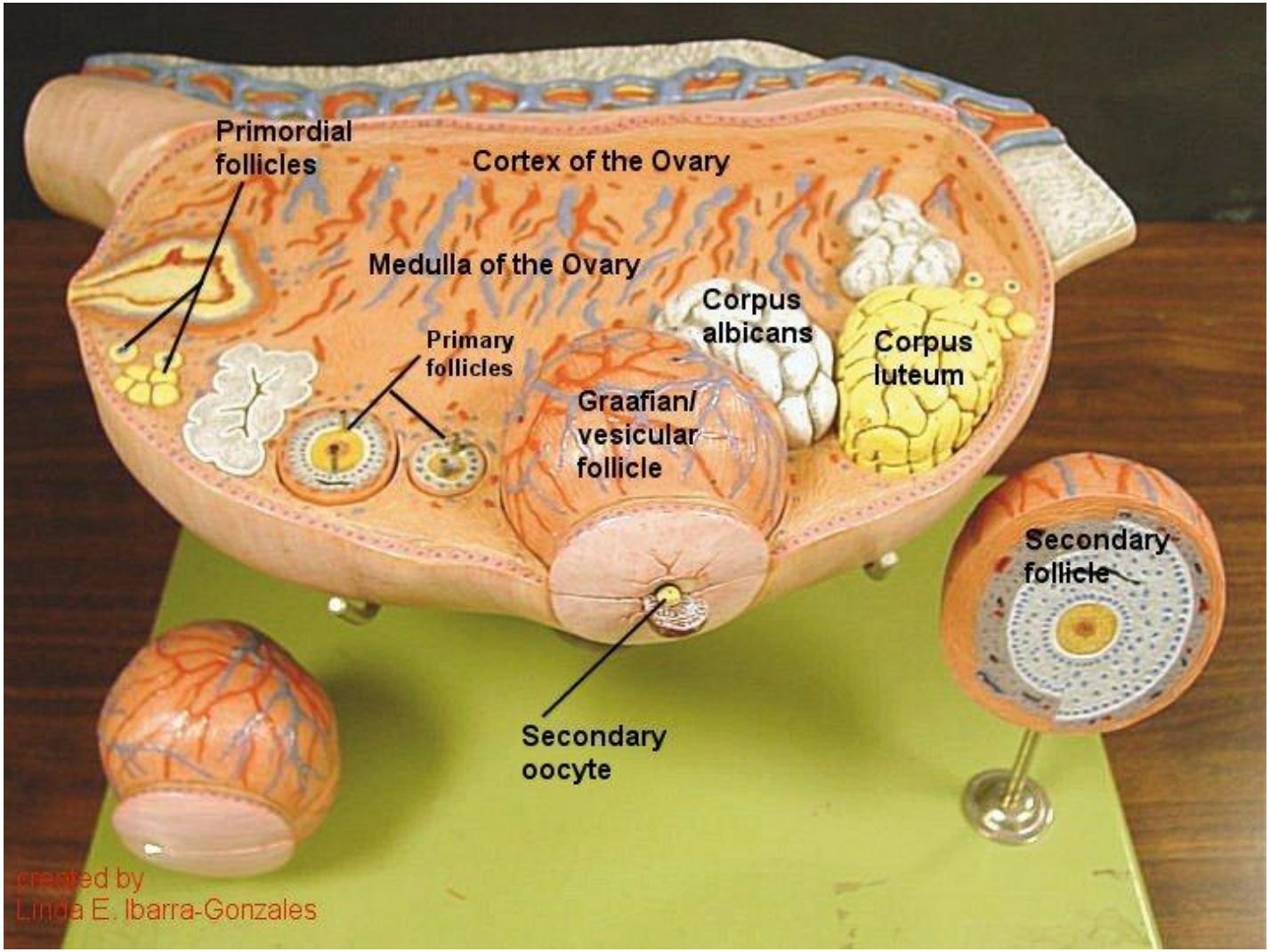


Figure 1: Basic anatomy of the ovary [1].

BACKGROUND: CHEMOTHERAPY

- **Chemotherapy causes ovarian insult:**
  - Primary Ovarian Insufficiency (POI) [2]
    - 40% of reproductive age breast cancer survivors [2]
    - 8% of childhood cancer survivors [2]
  - Increases patient's risk of:
    - Osteoporosis
    - Cardiovascular disease
    - Infertility
    - Premature menopause [4]

Figure 2. Cancer patient receiving chemotherapy [3].

BACKGROUND: CHEMOTHERAPY

- **Doxorubicin (DXR):**
  - Used to treat roughly 50% of premenopausal cancer patients
  - Cells commit to apoptosis based on dosage
  - Reduction of
    - Follicles
    - Ovarian size

- **Mode of follicle and oocyte demise is not well understood [5]**

CURRENT DEVICES: OVARIAN CULTURE

- **Neonatal Mouse Ovary Culture:**
  - Follicle formation
  - Ovary placed on membrane over medium [6]
  - Used to assess reasons for follicle loss

- **Limitations:**
  - Ovaries can only be cultured for 1-15 days [7]
  - Only works with neonatal ovaries

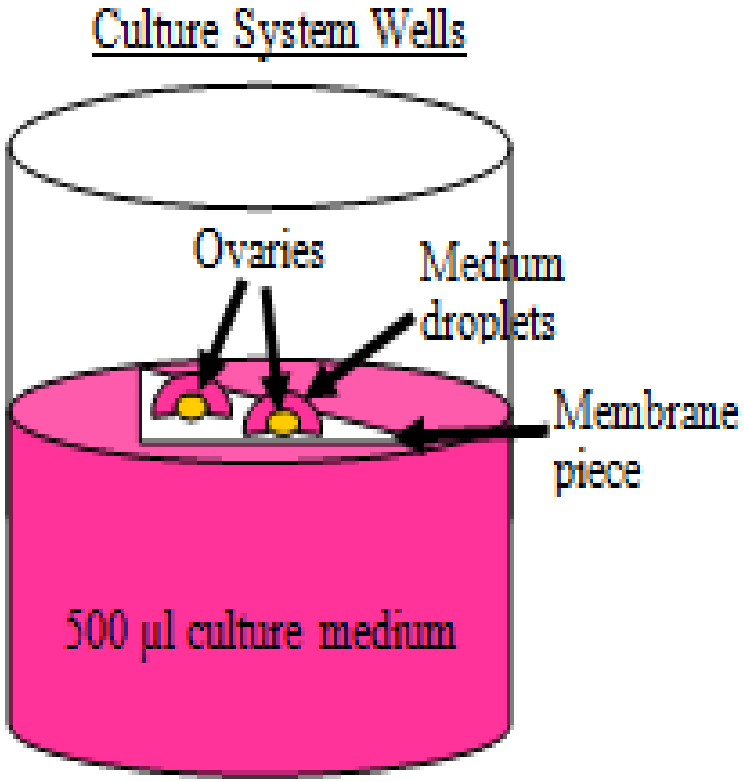


Figure 3. Isolation of neonatal ovaries and establishment of whole ovarian culture system [7].

CURRENT DEVICES: TISSUE BIOREACTORS

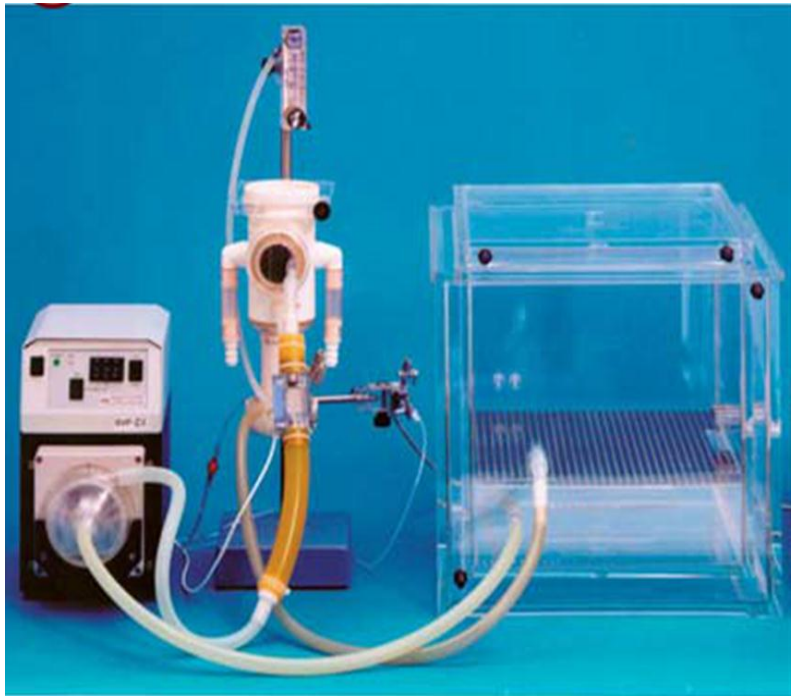


Figure 4. Example of bioreactor used for a pig kidney [9].

- **No current method for extended culturing of ovaries**
- *Tissue Bioreactor Types*:
  - Fixed-wall
  - Rotating Wall [8]
- *Culturing Organ Tissue:*
  - Kidney
  - Liver
  - Lung

CURRENT DEVICES: LANGENDORFF HEART

- **Isolated working heart model**
  - Aortic and atrial cannulas
  - Peristaltic pump
  - Oxygenation of chamber
- Example of *ex vivo* maintenance of organ

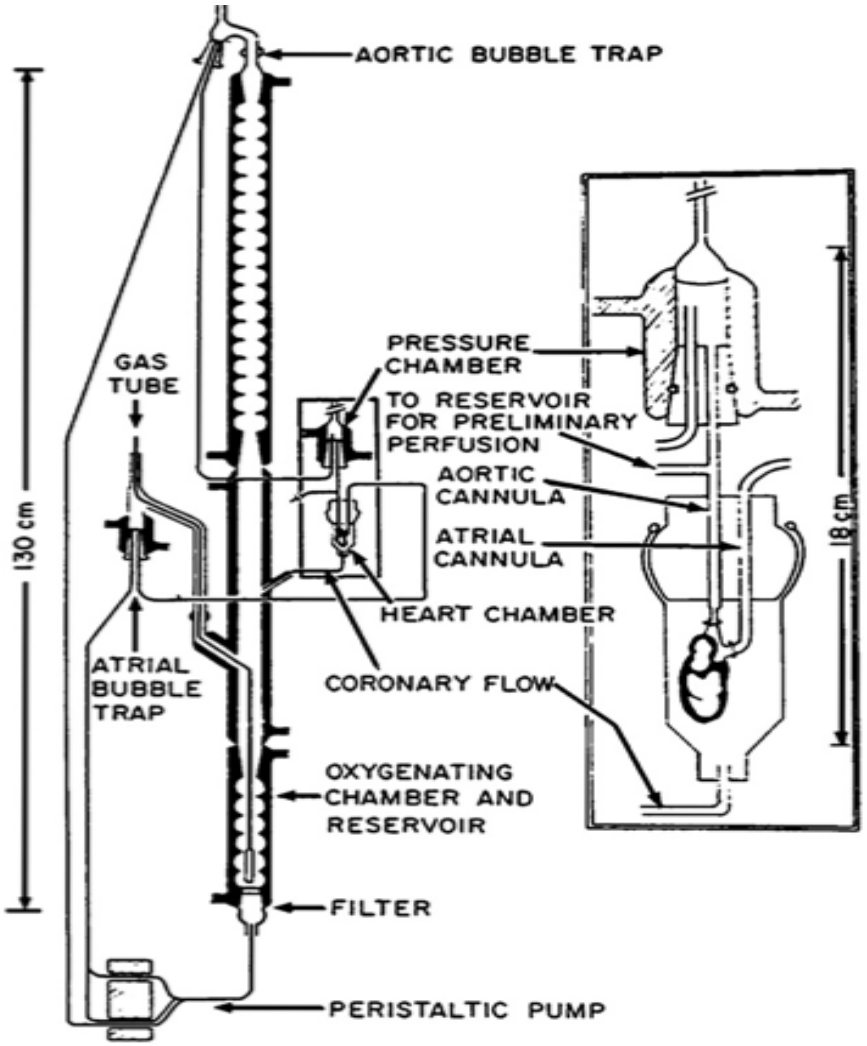


Figure 5. Isolated working heart model. Modification of the isolated heart perfusion model (Langendorff), which allows measurement of left ventricle work [9].

PRODUCT DESIGN SPECIFICATIONS

- **Performance Requirements**
  - Provide environment suitable to bovine ovary growth
  - Detect and measure fluid flow rates and pressures
  - Experiments from 2 weeks to 3 months
- **Accuracy and Reliability**
  - ~90 – 100% ovary cell viability
  - Precise monitoring of flow and pressure
- **Life in Service/Shelf Life**
  - Repeated use over many years
- **Operation Environment**
  - Incubator environment (37°C and 5% CO_2)
  - Easily sterilized
- **Ergonomics**

DESIGN PROCESS

- **1st consideration**: Biological Scale (Follicle, Tissue, or Organ)
  - What is most feasible?
  - What has most clinical relevance?
  - What is applicable for future testing?

- **2nd consideration**: Bioreactor/Culturing Technique
  - Maintain physiological conditions
    - Provide nutrients to follicles

BIOLOGICAL SCALE: DESIGN ALTERNATIVE 1 FOLLICLE CELL CLUSTER

- Culture cluster of primordial follicle cells
- Viability for up to 14 days [10]
- Widely done already
  - Encapsulation in hydrogel [10]
  - Microfluidic culture [10]
- Little clinical and physiological relevance

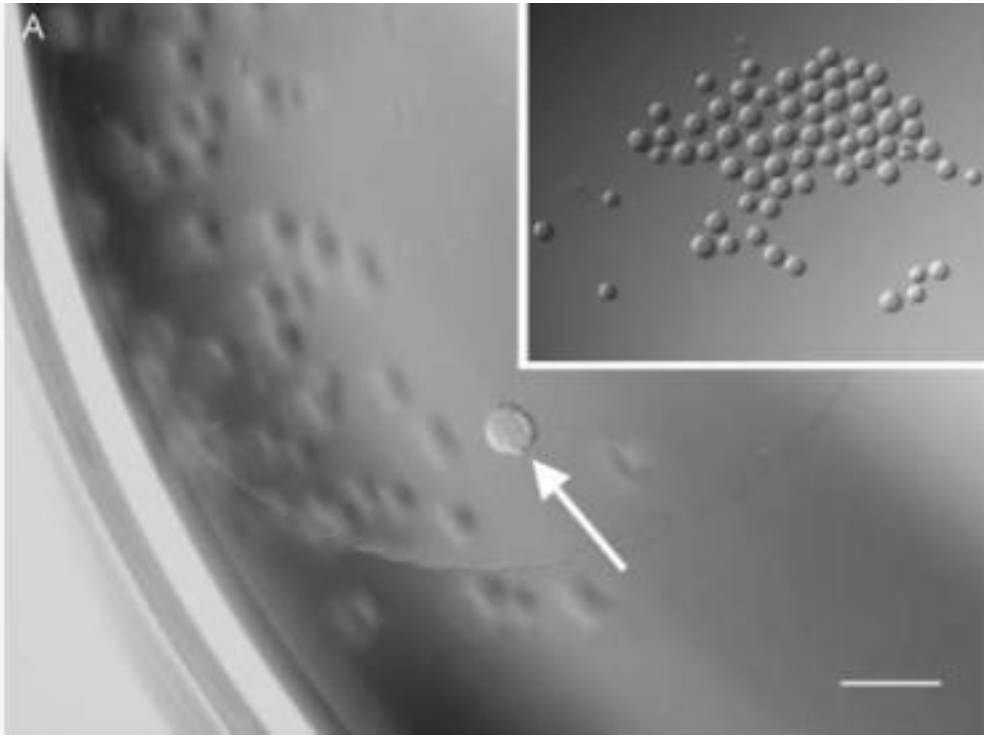


Figure 6. Representative image of follicle in alginate hydrogel bead in culture well with oocytes [11].

BIOLOGICAL SCALE: DESIGN ALTERNATIVE 2 OVARIAN TISSUE

- Culture outer segment of ovarian tissue
- Batch-to-batch variation
  - Location of follicle cells
- Limited clinical and physiological relevance

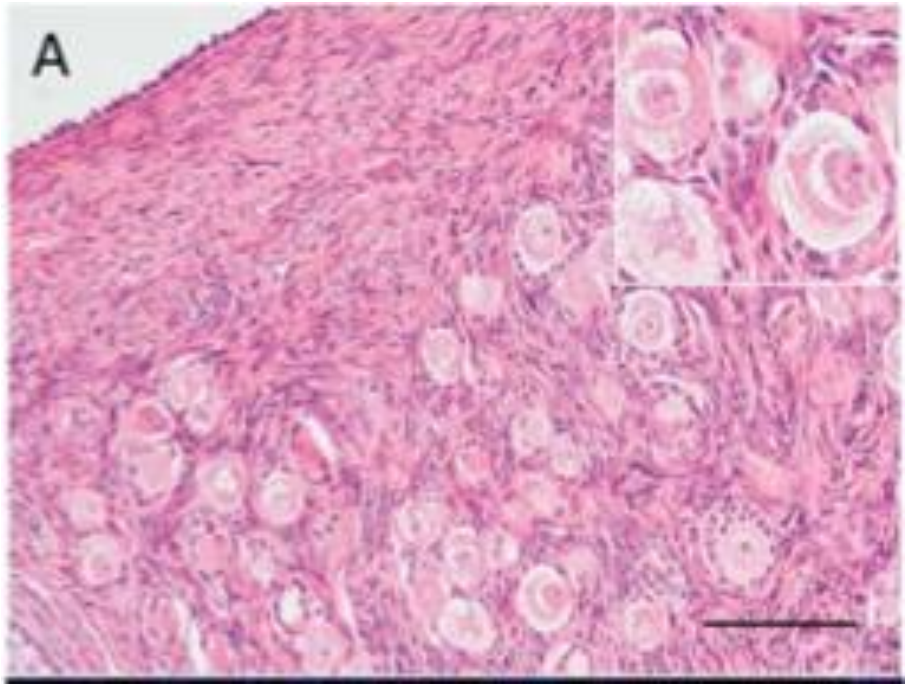


Figure 7. Morphology of fresh ovarian tissue. Representative histological sections of ovarian tissue stained with hematoxylin and eosin [12].

BIOLOGICAL SCALE: DESIGN ALTERNATIVE 3 COMPLETE OVARY

- Culture entire ovary
  - Cow ovary
    - On average 35x25x15 mm
    - More pronounced features
- **Significant clinical and physiological relevance**
  - Accessible vasculature
- Very difficult
  - Complete ovaries have never been cultured

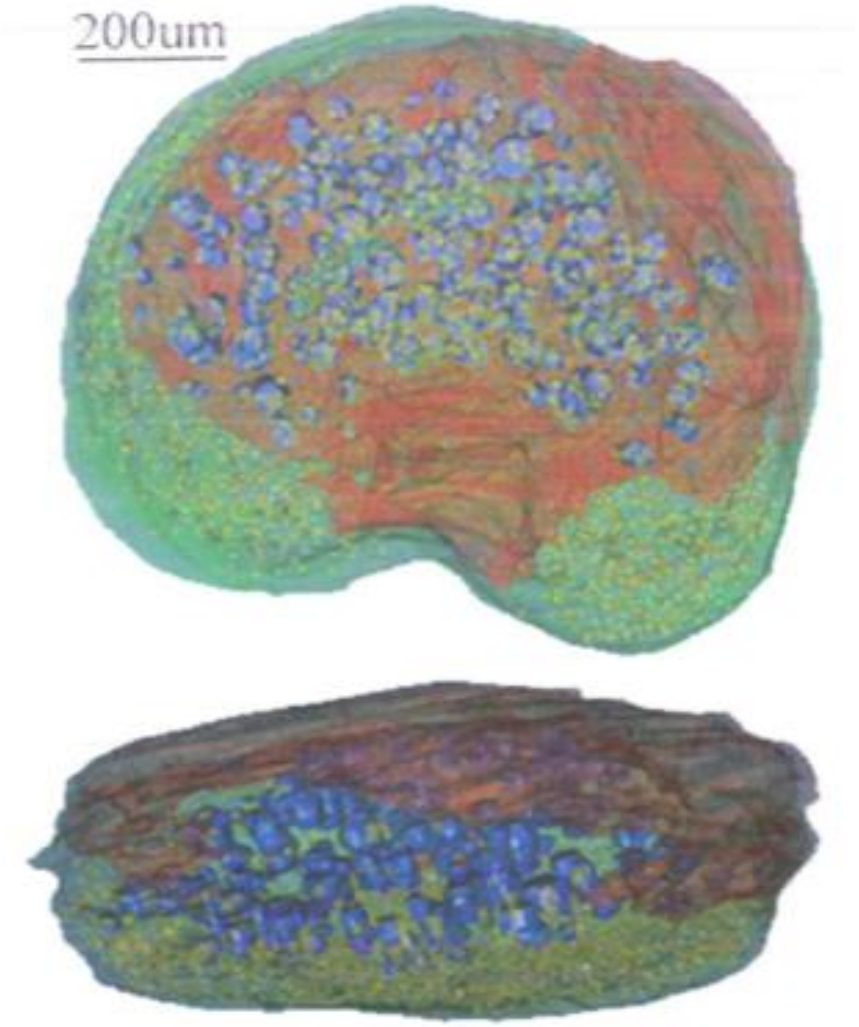
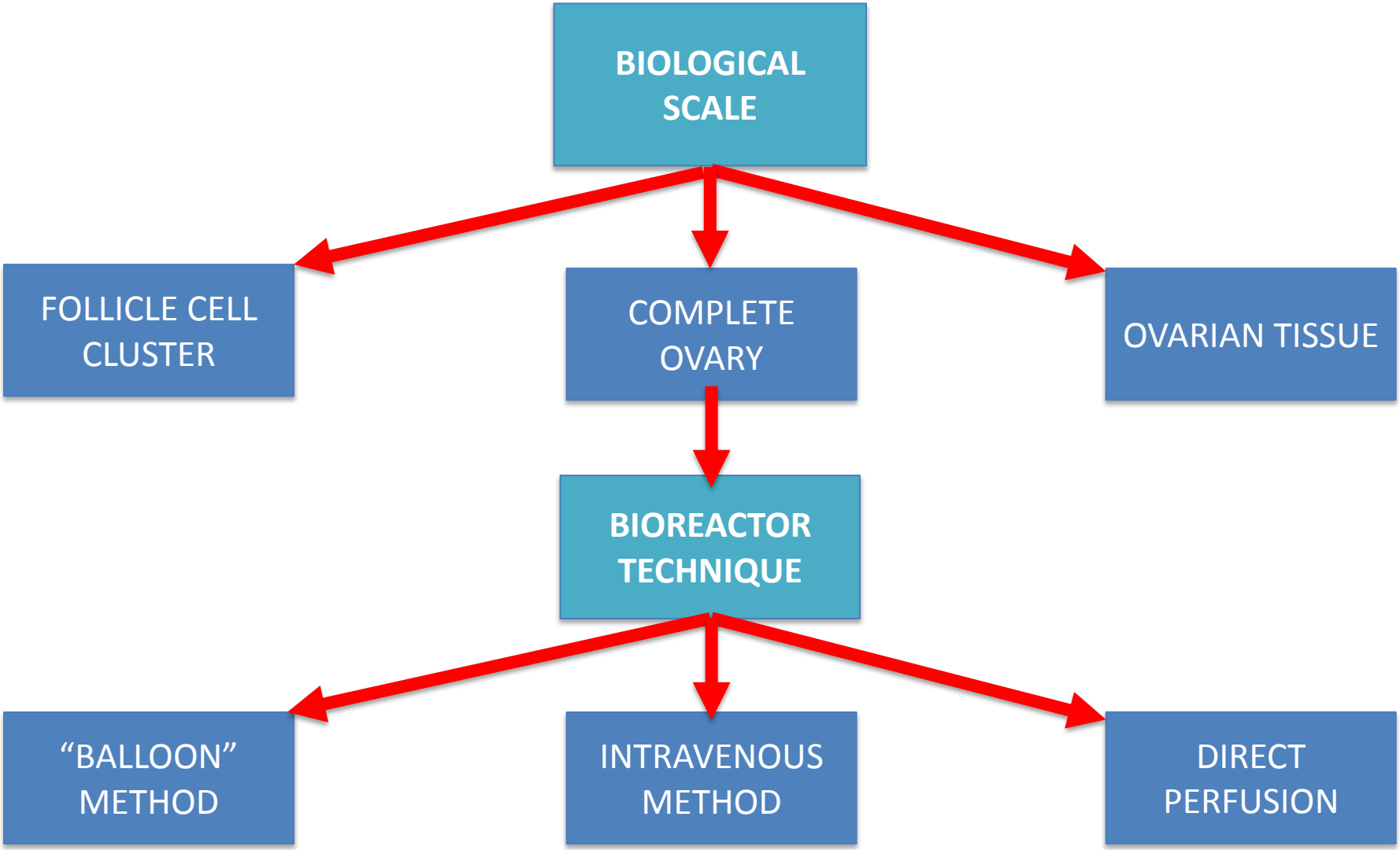


Figure 8. Morphological characterization of neonatal rat ovaries cultured in vitro (yellow = primordial and small primary) [13].

DESIGN MATRIX: BIOLOGICAL SCALE

FACTORS	WEIGHT	FOLLICLE CELL CLUSTER	OVARIAN TISSUE	COMPLETE OVARY
Feasibility	30	27	23	22
Clinical Relevance	30	18	22	30
Ease of Culturing	20	18	15	15
Consistency	15	12	10	15
Cost	5	3	4	5
TOTAL POINTS	**100**	**78**	**74**	**87**

DESIGN PROCESS



BIOREACTOR: DESIGN ALTERNATIVE 1 "BALLOON" METHOD

- Interior of ovary removed
- Internal chamber:
  - Filled with medium
  - Placed inside ovary
  - Connected to inflow and multiple outflow tubes
  - Provides structural support
- Entire ovary placed in large chamber
  - Filled with medium

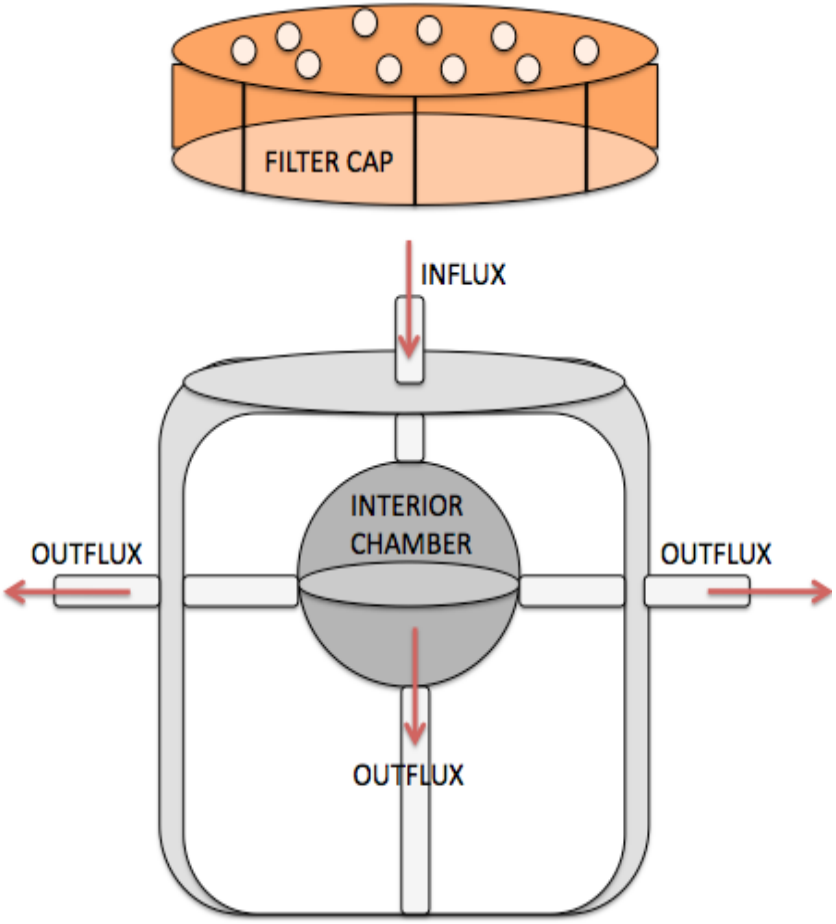


Figure 9. Conceptual diagram of the "Balloon" method.

BIOREACTOR: DESIGN ALTERNATIVE 2 INTRAVENOUS METHOD

- Utilize vasculature of ovary
  - Supply nutrients in physiologically accurate method
- Cannulas put into ovarian artery and vein
  - Artery = inflow
  - Vein = outflow
- Pump used to transport media in and out of ovary

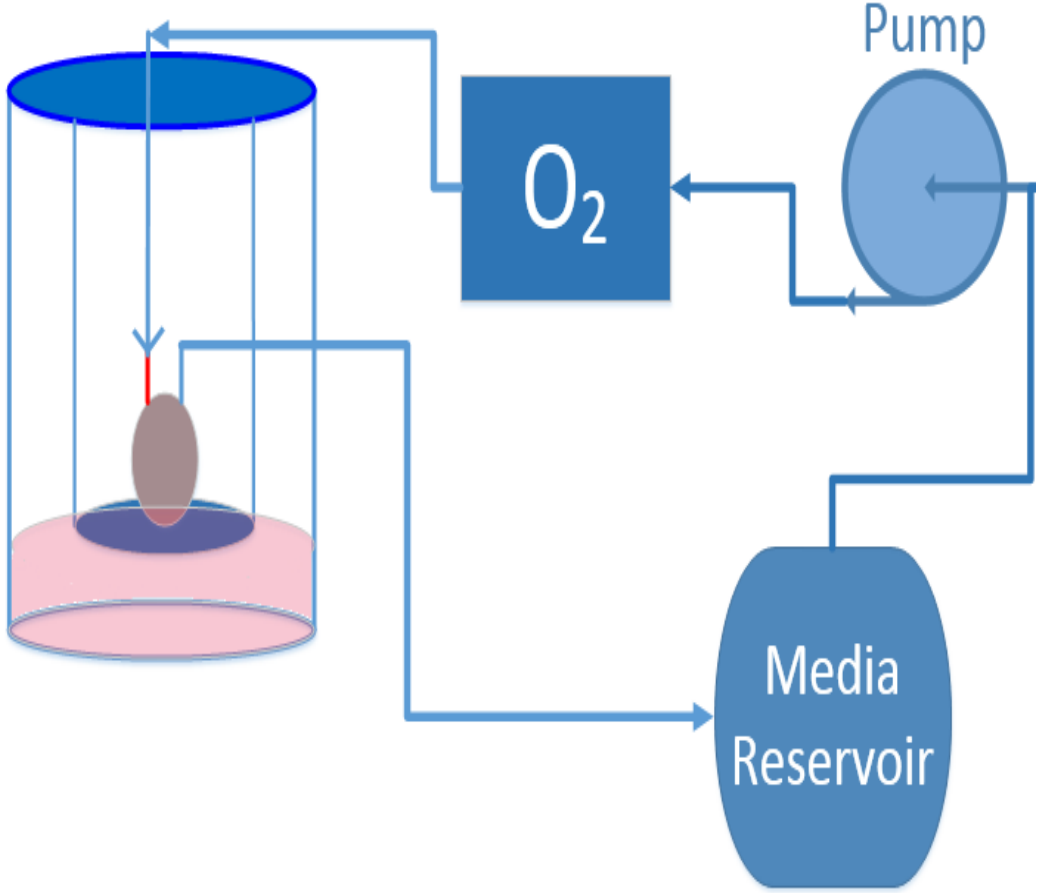


Figure 10. Conceptual diagram of the intravenous method for ovarian culture.

BIOREACTOR: DESIGN ALTERNATIVE 3 DIRECT PERFUSION

- Medium flows directly through ovary
  - Interior of ovary removed to increase diffusion
- Enhances mass transfer at periphery and internal pores [15]
- Low cost
- Widely used in tissue engineering

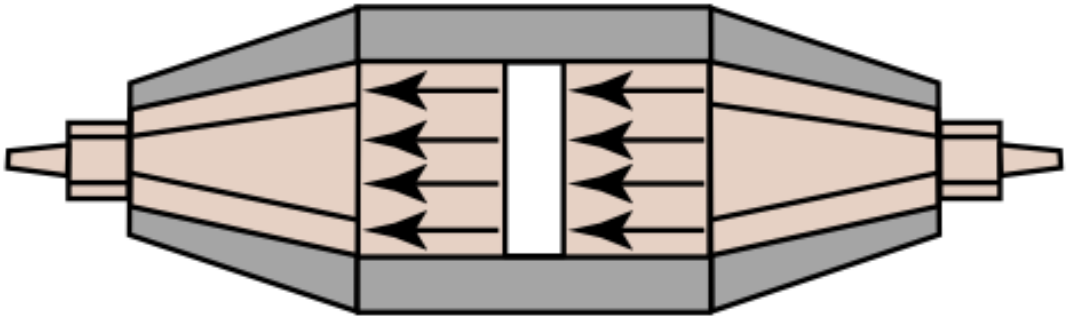


Figure 11. Example of a direct perfusion bioreactor in which the medium flows directly through the scaffold [16].

DESIGN MATRIX: BIOREACTOR

FACTORS	WEIGHT	"BALLOON" METHOD	INTRAVENOUS METHOD	DIRECT PERFUSION METHOD
Cell Viability	20	15	18	10
Physiological Accuracy	20	15	20	13
Ease of Use	15	13	12	14
Biocompatibility	15	14	14	14
Repeatability	10	7	9	8
Versatility	10	6	8	3
Cost	5	3	2	4
Ease of Assembly	5	2	2	4
TOTAL POINTS	**100**	**75**	**85**	**70**

FINAL DESIGN SELECTION

- **Complete Ovary:**
  - Cow ovary
  - Ovary will rest on removable platform

- **Intravenous (IV) Method**:
  - 250 mL Pyrex bottle
    - Autoclaveable and sealed
  - GL 45 cap (45mm) with 3 outlets:
    1. Inflow
    2. Outflow of media
    3. Air filter
  - Media → Oxygenator → Ovary → Media
  - Controlled, constant flow

Figure 12. Omnifit "T" series bottle cap with built-in check valve and inlet filter with two ports [17].

FINAL DESIGN: BIOREACTOR

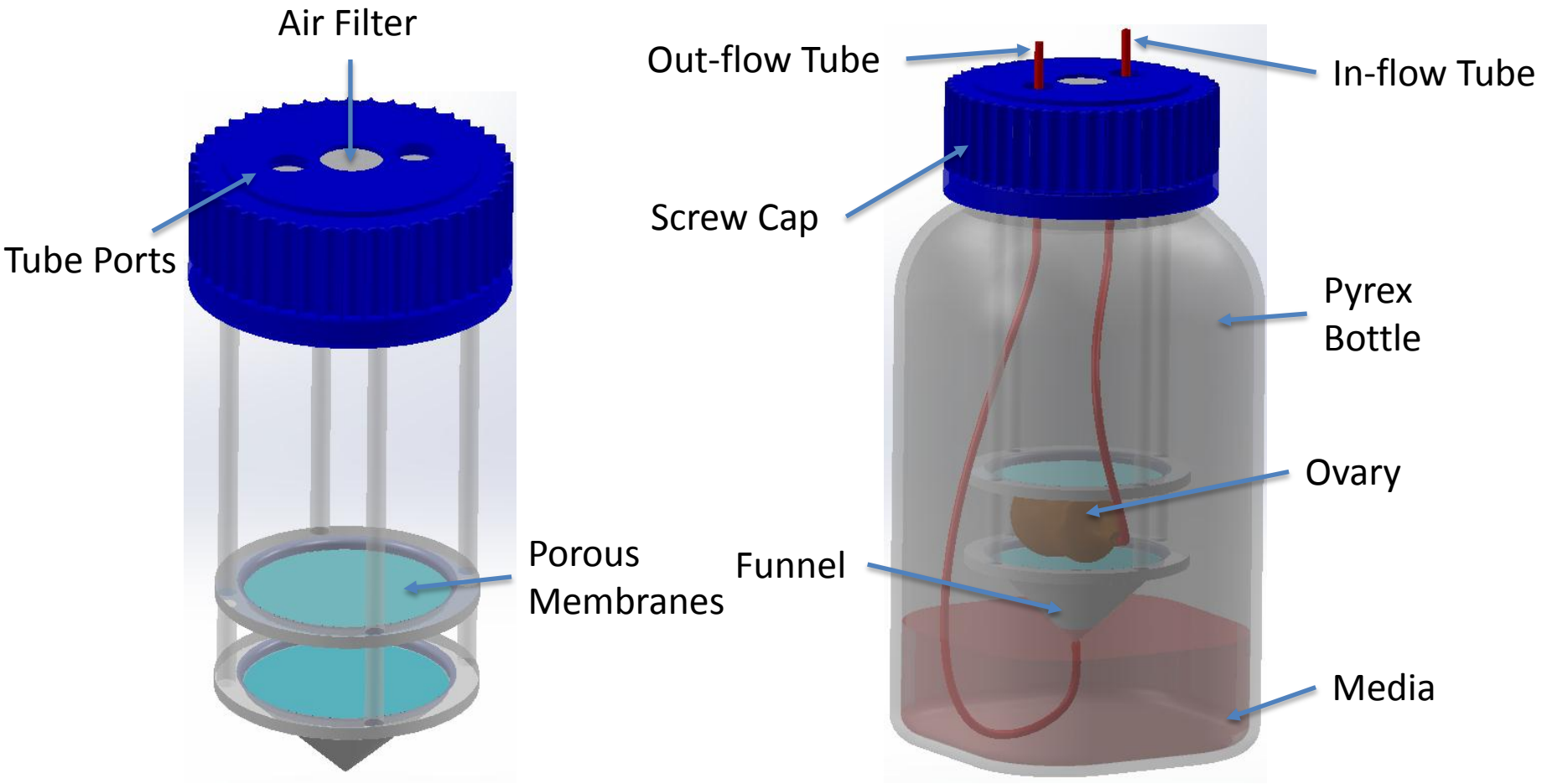


Figure 13. Solidworks rendering of removable cap apparatus (left) and assembled bioreactor (right).

FUTURE WORK

- **This Semester:**
  - Bioreactor assembly
  - Cell viability testing

- **Future Semesters:**
  - Monitoring system for real-time internal condition levels
    - Flow
    - pH
    - Temperature
    - Hormone concentrations

  - Integration with Chemo Bag Project
    - Test chemotherapy toxicity (DXR) on ovaries

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QUESTIONS

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