Novel in Vitro Model to Grow and Culture the Culture the Ovaries Outside the Body
Product Design Specifications
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Function:
Oftentimes female patients lose their reproductive capabilities as a consequence of having cancer or from undergoing cancer chemotherapy. Research has shown that doxorubicin (DXR) chemotherapy causes ovarian insult, ultimately leading to ovarian failure. Currently, no systems exist to grow adult ovaries in vitro to test chemotherapy toxicity and protection. This situation greatly limits ovarian research. A system to test the effects of the chemotherapy agent DXR on the ovaries needs to be developed. This project will establish a novel ex-vivo ovary culture system to maintain cell viability in order to facilitate assessment of chemotherapy toxicity and protection. The device will utilize ovarian vasculature, providing the ovary with necessary nutrition in a physiologically accurate manner to prevent necrosis. The fluid flow rates and pressure of the infusion lines will be adjusted to physiological conditions to ensure ovarian health. The ovary scaffold will be contained within a sealed environment capable of maintaining internal sterility and cell viability.

Client Requirements:
- Establish novel technique to grow ovaries outside the body
- A method that mimics ovarian vasculature must be developed to properly deliver nutrients to the ovary in a physiologically accurate manner
- Bioreactor must provide sufficient nutrients to facilitate the transition from primordial follicle to primary follicle and prevent necrosis
- Fluid flow rate and pressure must be accurately monitored using digital controls to ensure optimal ovarian health
- All components of the bioreactor must be biocompatible and non-toxic
- Bioreactor must be durable and be able to function for experiments last up to 3 months
- Price of device should be as low as possible

Design Requirements:

1. **Physical and Operation Characteristics**

    a. *Performance Requirements*: This project will involve culturing ovarian tissues/ovaries. The bioreactor will culture individual cow ovaries (35x25x15mm in size) [1]. The bioreactor will need to maintain significantly high cell viability

(~90% – 100% viability) and sterility for ovarian follicle cells. Bioreactor must provide sufficient nutrients to facilitate the transition from primordial follicle to primary follicle and prevent necrosis. Infusing media in the ovarian artery, or creating an artificial circulation system through the ovary will achieve this. The ovary bioreactor device needs to accurately detect and measure fluid flow rate and pressure when ovaries are present and when they are not. Flow rate must approximately 30 mL/min [2] and blood pressure should be approximately 203/189 mmHg [3]. The device will have the ability to be used multiple times, for multiple experiments with experiments lasting up to 2 weeks. All opening to the bioreactor must be either sealed or outfitted with a filter to maintain sterility

b. *Safety*: All materials in the device must be safe for handling under basic laboratory safety procedures and National Institute of Health (NIH) protocols. The device should be in compliance with mammalian cell culture standard operating procedures. The bioreactor must create an enclosed environment to ensure all materials/fluids that contact cells are secured and do not contact user.

c. *Accuracy and Reliability*: The bioreactor must be able sustain ovary follicle cell viability for 2 weeks at approximately 90%. To assess ovarian tissue viability, H2W staining of ovarian slices and TUNEL assays will be performed. Additionally, this device will need a great deal of precision (repeatability) and accuracy in creating and monitoring of fluid flow and pressure similar to physiological values, as this can greatly affect cell culturing.

d. *Life in Service*: The bioreactor device will need to support cell viability and function accurately for approximately 2 weeks for short term testing. Long term testing will require the device to maintain ovary tissue viability for approximately 3 months. This will provide adequate time to facilitate the transition from primordial follicle to primary follicle.

e. *Shelf Life*: The device should be able to function accurately for approximately five years, so that it can be used for a multitude of experiments and stored for future use. Once in use, the device must persist and maintain accurate functionality throughout an entire experiment and work effectively in the presence of cell culture media and cells.

f. *Operating Environment*: The ovary bioreactor will be used in an incubator to create an environment (37°C and 5% CO_2) that mimics facets of the in-vivo environment of ovary follicle cells and bathed in standard cell culture media (Ham's F-12/DMEM). For cell culturing, the bioreactor will need to be easily sterilized using ethanol, or an autoclave, so that it can be used in a biological safety cabinet (BSC). The inside of the bioreactor should create a sealed, sterile environment for cell culture.

g. *Ergonomics*: The ovary bioreactor device should be easy to use, in order to ensure a high level of repeatability in experiments among different users. The device

should be able to be used with limited experience, as well as by different and multiple users.

h. *Size*: The size of the ovary bioreactor device should be slightly larger than a cow ovary (35x25x15 mm) at least 45x35x25 mm in size. This will eliminate the potential problem of varying ovary size. In order to be housed within an incubator, the bioreactor and other device components should all fit within an incubator, occupying limited size; however, the pump for the device may be placed outside the incubator.

i. *Weight*: The weight of the device should be kept to a minimum in order to maximize ease of use and efficiency; however, weight is not critical in this design and is a low priority consideration.

j. *Materials*: The materials of the device should have no negative effects on cells and need to be non-cytotoxic. Specifically, the material used to mount the ovary should promote cell adhesion and not limit or inhibit the direction/orientation of ovary follicle cell growth. These factors must also be applied to the fluid flowing through the device. Additionally, the infusion lines mimicking ovary capillaries and vasculature should be small in diameter and create a sealed environment for cell culture media to flow through. If the media will be simply diffused to the ovary, the biocompatible and permeable membrane must be used to transport cell culture media.

k. *Aesthetics, Appearance, and Finish*: Ideally, the device should be clear and transparent, in order to allow the user to properly observe the ovaries being cultured. The entire bioreactor will be a hollow cylinder that will stand vertically on its axis. Aesthetics and appearance are not critical in this design and are a low priority consideration.

2. Production Characteristics

a. *Quantity*: There should be one ovary bioreactor device capable of housing one ovary.

b. *Target Product Cost*: The cost of the device should be kept to a minimum (below $3000); however, if a novel and repeatable method is developed, a higher product cost will be considered. Additionally, the client has shown great willingness to invest in the project.

3. Miscellaneous

a. *Standards and Specifications*: This device is not drug related, and therefore does not need approval by the FDA for use or testing. However, animal, and eventually human ovary, will be tested using this device so standard mammalian cell culture

protocols and protocols must be adhered to. Additionally, all National Institute of Health (NIH) cell culture and biological safety protocols must also be adhered to.

b. *Customer:* The device is created for Dr. Sana M. Salih, MD, MMS. The overall goal of Dr. Salih's laboratory is to identify novel mechanisms to improve fertility and reproductive health in women. Specifically, fertility preservation in cancer patients. The device should be easy to use so that other members of the Salih Laboratory can use it. The highest priority for the customer is ensuring ovary viability over an extended 3-month period of time.

c. *Patient-related Concerns*: The device will be used with ovarian follicle cells and will thus need to be sterile for all uses. There are no concerns regarding data storage or confidentiality with this device, as the subjects are not patients.

d. *Competition*: Currently, no systems exist to grow adult ovaries in vitro to test chemotherapy toxicity and protection. However, there are methods used for follicle cell culturing and ovarian tissue culturing [4]. For follicle cell culture, methods include matrix encapsulation, suspension culture in rotating systems, serial culture, and microfluidic culture [5]. Organ bioreactors have been successfully developed, though not for ovaries. Systems for isolating lungs, livers, and kidneys of large animals are commercially available and have been shown to be highly beneficial for the research of how that organ responds to alterations of physiological variables [6].

References:

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[4] Desai, N., Alex, A., AbdelHafez, F., Calabro, A., Goldfarb, J., Fleischman, A., & Falcone, T. (2010). Three-dimensional in vitro follicle growth: overview of culture models, biomaterials, design parameters and future directions. Reproductive Biology and Endocrinology, 8(1), 119.

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