Novel In Vitro Model to Grow and Culture Ovaries
Design Excellence Award Executive Summary

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As cancer chemotherapy treatments become more sophisticated, there has been an associated increase in patient survival time. As a result, the severity of long-term side effects of chemotherapy has become apparent. One of these side effects is primary ovarian insufficiency (POI), which is seen in 40% of reproductive age breast cancer survivors. Specifically, studies have shown that doxorubicin (DXR) chemotherapy is associated with ovarian insult. The exact model of this insult has not been determined. To investigate, study, and ultimately solve this problem, the design of a novel device for studying ovaries *in vitro* must be developed, as no systems exist to grow adult ovaries *in vitro* for extended period to allow for extensive testing and study. Thus, we have designed a custom bioreactor to study the effects of DXR on ovarian tissue

To provide extended ovary culture, a system was developed that utilizes ovarian vasculature to provide necessary nutrition in a physiologically accurate manner and prevent necrosis. Similar to the Langendorff Heart, the ovary is dissected and the ovarian arteries isolated, which are then cannulated using a luer stub and tied off with nylon thread to provide a sealed connection. The luer stub is connected to inflow tubing, which is preceded by an oxygenator and media reservoir with a pump providing steady, regulated flow through the entire closed-loop system. A polypropylene funnel was machined to hold the ovary during culturing and allow for fluid collection as media flows through ovarian vasculature and diffuses out of the entire ovary. The funnel was designed to catch all outflow media to be returned to the reservoir, maintaining the natural chemical environment produced by the ovary during maturation. The funnel is connected to outflow tubing allowing the media to be pumped back to the reservoir. The ovary is housed in a 500 mL custom bioreactor with inflow and outflow of media, air filtration, and a controlled, sterile environment. The entire system can be placed within an incubator, mimicking conditions of the body (37˚C and 5% CO_2) and providing the ovary with an environment conducive for growth. Overall, the device allows for simple ovary culture in a closed system that requires little maintenance, high ease of use, and high repeatability.

This device and method has been proven as a viable system for providing nutrients to the ovary in a physiologically accurate manner, while providing a controlled environment. Testing has yielded a simplified and successful method of ovarian artery isolation, ovarian artery cannulation, as well as proven concept of method. Fluid has been successfully flown through the vasculature, saturated the ovary, and ultimately exited via diffusion for extended periods of time. By achieving *in vitro* growth and culture of ovaries, extensive investigation can be conducted to gain vital knowledge on important ovarian issues including the effects of DXR on ovarian insult, follicular development, and hormone and factor influence over time. Ultimately, our device will aid in determining the mechanism of ovarian insult, knowledge which will help reduce or even prevent primary ovarian insufficiency in surviving cancer patients. The *in vitro* culturing and maturation of tissues shows vast potential, and our device will enable further strides forward in this groundbreaking frontier.