

Ovary Flow Testing Protocol

Hypotheses

- An ovarian artery or arteries can be consistently isolated and cannulated allowing for controlled flow into the ovary
- A flow rate within the range of the pump will be optimum for flow of media into the ovary for extended periods of time
- Out flow of the media will be observed but it will be a slower rate and ratio of outflow to inflow will be less than 1

Materials

- 4 lbs bovine ovaries
- Glass Dish (2x)
- Scissors
- Ruler
- Balance
- Variable Flow Mini-Pump II
- Tubing (1/4" and 1/8") ID
- Luer stub
- 1/8" tubing to luer adapter
- 1/8" to 1/4" tubing reduction adapters
- 1/4" splitters
- 100 mL graduated cylinder
- 500 mL beaker
- Scalpel
- Nylon thread
- Tweezers
- Water
- Trypan Blue

Ovary preparation

1. Gather cow ovaries to be prepared and place ovaries in glass dish (Note: use ovaries as fresh as possible in order to avoid clotting in the vasculature).
2. On one ovary, locate large connection of excess connective tissue separate from the organ. Apply pressure on connective tissue located close to the ovary with thumb and forefinger, feeling for vasculature and isolating unneeded tissue. The most prominent vasculature will be roughly 2 inches from the ovary consisting of a thick nesting of arteries known as a hilum.
3. Find an artery in the hilum that appears to be curling like a hook towards the ovary. This artery can be straightened out and is sufficiently long enough for inserting the cannula.
4. The artery may need to be further isolated for easier cannulation. The artery is enclosed in an envelope of tissue. Removal of this envelope can be done far from the rest of vasculature by pinching top layer with tweezers and making a shallow incision into the top layer with a scissors. The top layer can then be dissected and removed with scissors exposing vasculature. Take caution not to damage the ovary or its vasculature.

5. Carefully remove the remaining excess connective tissue surrounding vasculature and ovary with scissors or scalpel. At this point all that remains is the ovary connected to the hilum with a main artery extending from the hilum.
6. Using ruler, take and record length, width, and height dimensions of ovary
7. Using balance, tare a glass dish, and record mass of prepared ovaries.

Testing Procedure

1. Assemble pump with 1/4" inner diameter tube connected to 1/8" inner diameter tube with reducer (split at the 1/4" size if necessary). Connect 1/8" tubing to luer stub with luer adapter.
2. Run dyed water through the pump until no air bubbles are present.
3. With the pump off, carefully insert luer needle into the isolated artery of the ovary and tie off with a nylon thread.
4. Place ovary in 500mL beaker to be used to collect the outflow of water from the ovary
5. Record the starting time and begin a steady, controlled flow rate into the ovary on the low setting with a power of 5. Observe any ovarian and vasculature responses throughout the testing.
6. After 2 minutes halt the flow of water into the ovary and record how much of the dyed water has accumulated in the 500 mL beaker.
7. Carefully disconnect the luer stub from the luer adapter and record the mass of the ovary at this time.
8. Reconnect the luer stub to the pump apparatus and repeat steps 7-9 for ten times (20 minutes total) and observe the ovary response.
9. After 20 minutes increase the duration between measurements to ten minutes to obtain long-term observations.
10. Adjust for failures or successes found in earlier tests. Ovary dissection and cannulating techniques should be maintained to keep as much consistency between experiments as possible.