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Post-Surgical Dissection of Ovaries

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ABSTRACT

This protocol details dissection of the ovaries in preparation for 10X Visium, 10X Multiomics, pathology review, and biobanking. Care must be taken to systematically divide the ovary and to maintain orientation of the specimens as they are removed using the three points of fixation for the ovary (the uteroovarian ligament, the infundibulopelvic ligament and the mesosalpinx). Once the ovary is isolated it is difficult to discern which aspect of the ovary is superior to the ovary's attachment to the mesosalpinx and which is inferior. Colored marking dyes can be used to help maintain orientation provided they do not interfere with downstream analyses.

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MATERIALS TEXT

- Ice
- Clean cutting surface
- Disposable scalpels
- Kimwipes
- 3D Mold (pre-chill) (see Figure 2)
- Disposble rulers (with centimeter measurements)
- Ice bucket
- Biohazard bags and container
- Nuclease-free Water **Contributed by users**

  DMEM/F-12 **Thermo**
- **Fisher Catalog #11320033**

  RPMI 1640 Medium **Thermo**
- **Fisher Catalog #11875085**

  MACS® Tissue Storage Solution **Miltenyi**
- **Biotec Catalog #130-100-008**
- DPBS (10X), no calcium, no magnesium **Thermo Fisher Scientific Catalog #14200075**

  - dilute to 1X with nuclease-free water

  Marking dye **Cancer**
- **Diagnostics Catalog #03000P**

VWR® Pour-Boat Weighing Dishes
flat-bottomed dish
VWR International **10803-166**

BEFORE STARTING

Dilute phosphate buffered saline (Gibco; 14200-075) to 1X with nuclease-free water.

Receive excised female reproductive system on ice en bloc. Orient the specimen with the

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1 anterior aspect of the uterus facing up based on presence of vesicouterine reflection.

2 Using a disposable scalpel, incise the mesovarium. Orient ovary with the uteroovarian ligament on the right, infundibulopelvic ligament on the left, and mesovarium on top for the right ovary (Figures 6).

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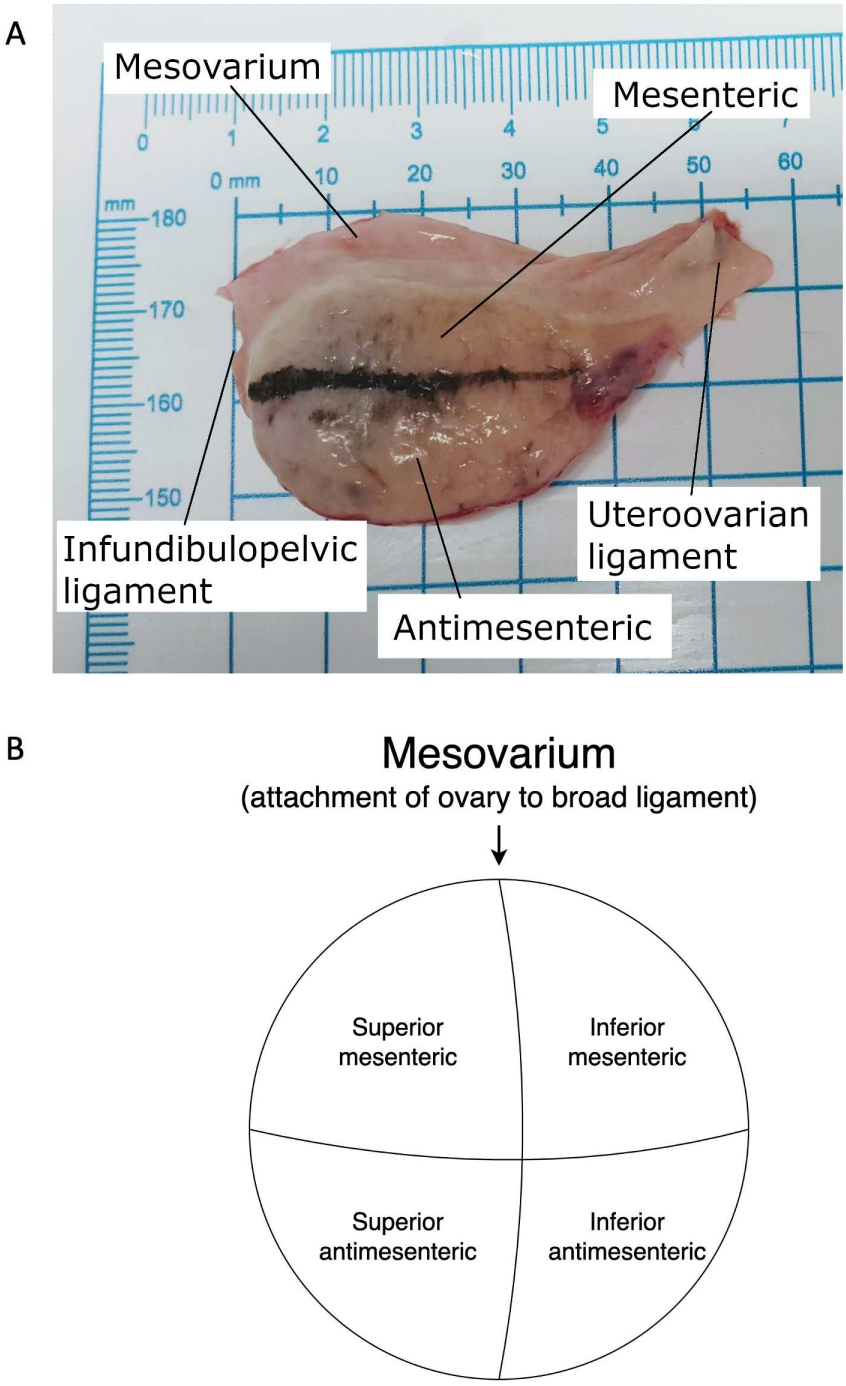

Figure 1. Use of fixed anatomic structures in the ovary. There is laxity in the structures supporting the position of the ovary which results in both intra- and inter-individual variations in the organ's orientation within the pelvis. In order to standardize collection and maintain orientation of tissue sections, a model proposed at a NICHD-sponsored workshop that used fixed anatomic structures to create a regional map of the ovary, was applied. Here the black line was drawn on the inferior half of the ovary when it is divided by its attachment to the mesovarium. The line delineates the mesenteric and antimesenteric portions of the inferior aspect of the right ovary.

3 Dry the ovary thoroughly with a Kimwipe.

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4 Use marking dye to maintain orientation of the ovary with the uteroovarian ligament on the right, infundibulopelvic ligament on the left, and mesovarium on top.

5 Take the weight and, using a disposable ruler, take spatial measurement of each ovary.

6 Return ovaries to ice.

7 Place the first ovary in the pre-chilled mold (Figure 2).

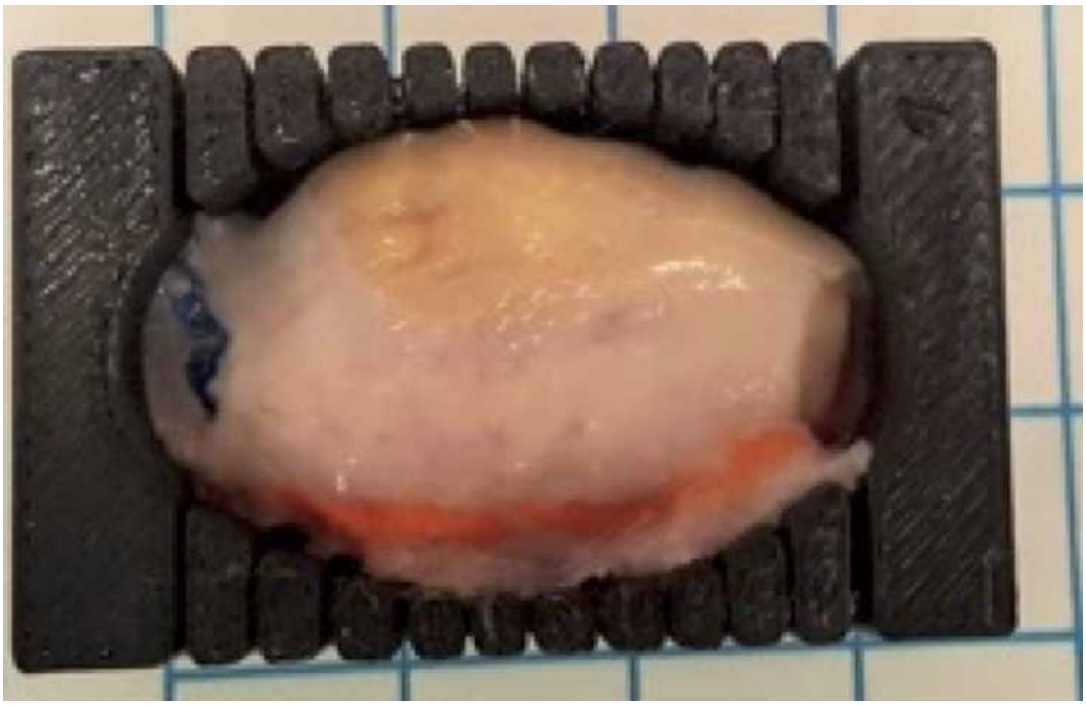

Figure 2. Pre-Chilled Ovary Mold. A 3D mold for long-axis sectioning of the ovary was designed in Autodesk Fusion 360 and printed in polylactic acid (PLA) using a Stratasys F170 Series 3D printer. A razor blade or dissecting knife is passed sequentially through each fenestration which allows for standardization in the width of ovarian slices obtained as well as maintains orientation of individual tissue pieces.

Dr. Alison Pouch, Dr. James Gee, Olivia Sandvold, and Kate da Silva were responsible for the design and execution of the 3D ovarian molds used in Basic Protocol 3. 3D molds were printed courtesy of the University of Pennsylvania Libraries' Biotech Commons.

8 Divide the ovary along the long axis of the ovary from the uteroovarian to the infundibulopelvic ligament using the guidelines of the mold (Figure 1a).

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9 Take each slice and orient mesovarium ridge at 12 o'clock.

10 Divide each slice along the line of the mesovarium ridge to divide the ovary into portions superior to and inferior to the mesovarium.

11 Take each half and divide it again in half to separate the ovary into the mesenteric and antimesenteric portions (Figure 1b).

12 For later 3D anatomical positioning, the quadrant of the tissue piece and the slice number along the long axis should be tracked for each tissue specimen.

13 Tissue can be processed with protocols OCT-Embedded Tissue Preparation, Tissue Fixation Preparation, or Snap-Frozen Tissue Preparation depending on the desired downstream processing.

14 Repeat steps with the contralateral ovary, using a fresh, ice-cold ovary mold.

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