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We use this protocol and it's working

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80066

Ovarian tissue processing from organ donor V.1

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Hannah McDowell

ABSTRACT

Purpose: This protocol is intended to be used for human ovarian tissue processing from organ donors in a research setting. This details initial processing of the female reproductive tract, isolation of the ovary, and preparation of tissue sections for fixation, decellularization, freezing (for protein assays), and cryopreservation (by controlled-rate or slow freezing).

GUIDELINES

****CRITICAL****Personnel who process and handle human specimens must adhere to Office of Research Safety guidelines at their institution. They must be up to date on their original and refresher courses for the following training (or equivalent):

1. Biosafety Certification
2. Bloodborne Pathogens Certification
3. Training in Filling and Maintaining Liquid Nitrogen Tanks (as appropriate for LN_2 use)
4. Working with Formaldehyde Certification (as appropriate for fixation)
5. CITI training (highly encouraged)

Keywords: human ovary, HuBMAP, tissue process, organ donor, ovary

MATERIALS

Human Ovarian Tissue Processing

All materials are listed as Type (Vendor, Catalog Number)

- OFC Holding Media (Sage/Origio, #ART-8040)
  - Alternative: Leibovitz-15, L-15 (Caisson Labs, LVL02-6X500ML or equivalent)
- Stadie-Riggs Tissue Slicer (Thomas Scientific, discontinued)
- Disposable surgical scalpels (Exel International, 29550)
- Carbon Steel Blades (Thomas Scientific, 555TADIEBL)
- 15 mL conical tubes (Fisher Scientific, 14-959-53A or equivalent)
- 0.1% Sodium Dodecyl Sulfate, SDS
  - Sodium Dodecyl Sulfate pellets (Sigma-Aldrich, 75746-1KG)
  - dilute in MilliQ water (or equivalent)
- Modified Davidson's Fixative, MDF (Electron Microscopy Sciences, 64133-50)
- Histology grade ethanol (Bioworld, 40120790-3)
- 60 mm petri dish (CellTreat, 229663 or equivalent)
- 2.0 mL microcentrifuge tubes (Fisher Scientific, 05-408-138 or equivalent)
- 2.0 mL cryovial (TPP, 89020 or equivalent)

SAFETY WARNINGS

Personnel will wear appropriate personal protective equipment. Areas are disinfected with the Dymon **Do-it-all germicidal foaming cleaner** (or equivalent) according to manufacturer's instructions.

ETHICS STATEMENT

This work should take place under an IRB protocol or IRB exemption. The Laronda Lab operates in partnership with organ procurement programs and under the Ann & Robert H. Lurie Children's Hospital of Chicago IRB (2021-0549) and appropriate Materials Transfer Agreements (MTA) with the Gift of Hope Organ and Tissue Donor Network. MTA and Data Use Agreements (DUA) are also in place for transferring materials or unmodified derivatives and associated data within HuBMAP collaborations.

Ovarian Tissue Processing

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Record donor information. This may include: sample ID, location of procurement, date received, age, cause of death, sex, race, ethnicity, infectious disease serologies, weight, height, and body mass index (BMI).

Tissue must be processed within 24 hours of procurement in order to maintain optimal viability

and is kept at 2-4 °C throughout all processing steps unless otherwise indicated. Additionally, tissue must be kept in appropriate holding media buffered for air, such as OFC Holding Media or Leibovitz-15, and at 2-4 °C to prevent the tissue from drying out.

Throughout processing, ensure notation of anatomic location within the ovary consistent with suggested nomenclature as described in these references.

CITATION

Tsui EL, O'Neill KE, LeDuc RD, Shikanov A, Gomez-Lobo V, Laronda MM (2023). Creating a common language for the subanatomy of the ovary..
LINK
https://doi.org/10.1093/biolre/ioac199

CITATION

O'Neill KE, Maher JY, Laronda MM, Duncan FE, LeDuc RD, Lujan ME, Oktay KH, Pouch AM, Segars JH, Tsui EL, Zelinski MB, Halvorson LM, Gomez-Lobo V (2023). Anatomic nomenclature and 3-dimensional regional model of the human ovary: call for a new paradigm..
LINK
https://doi.org/10.1016/j.ajog.2022.09.040

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2 Image anterior and posterior of the whole female reproductive tract (FRT) (uterus, fallopian tubes, and ovaries).

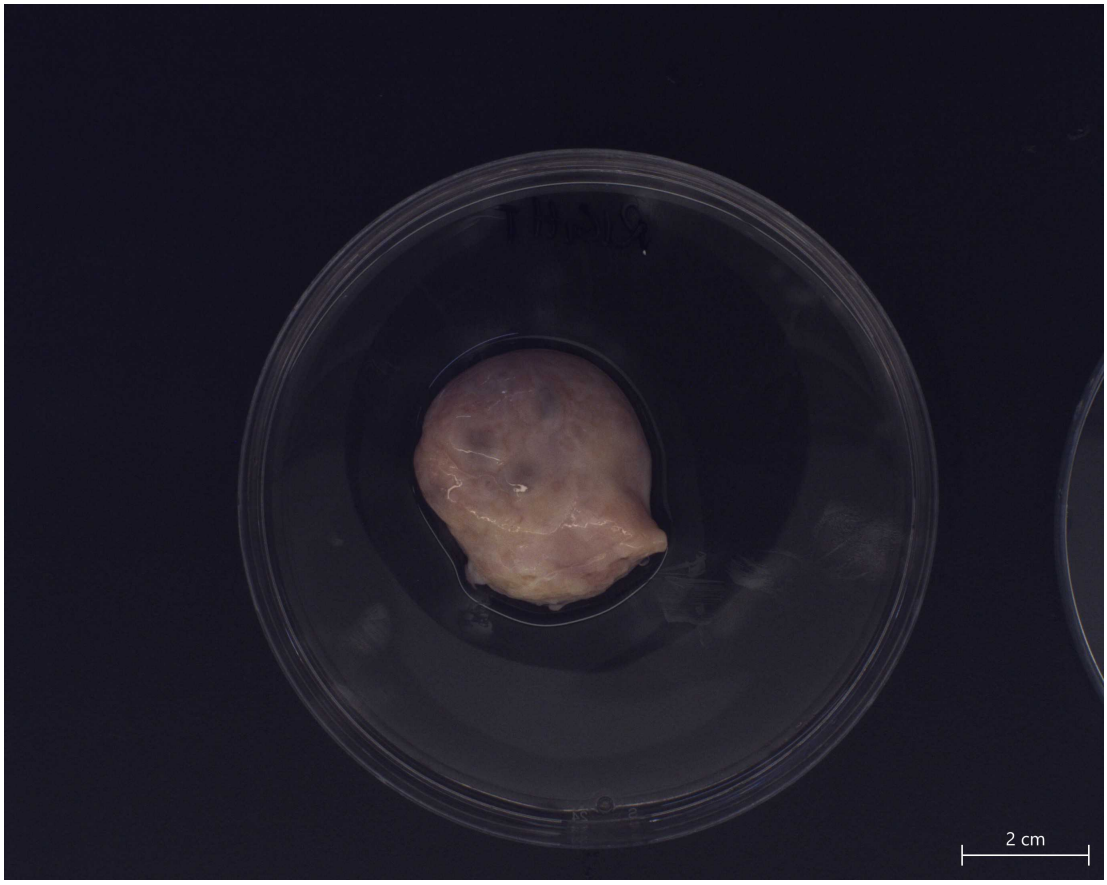
Anterior View

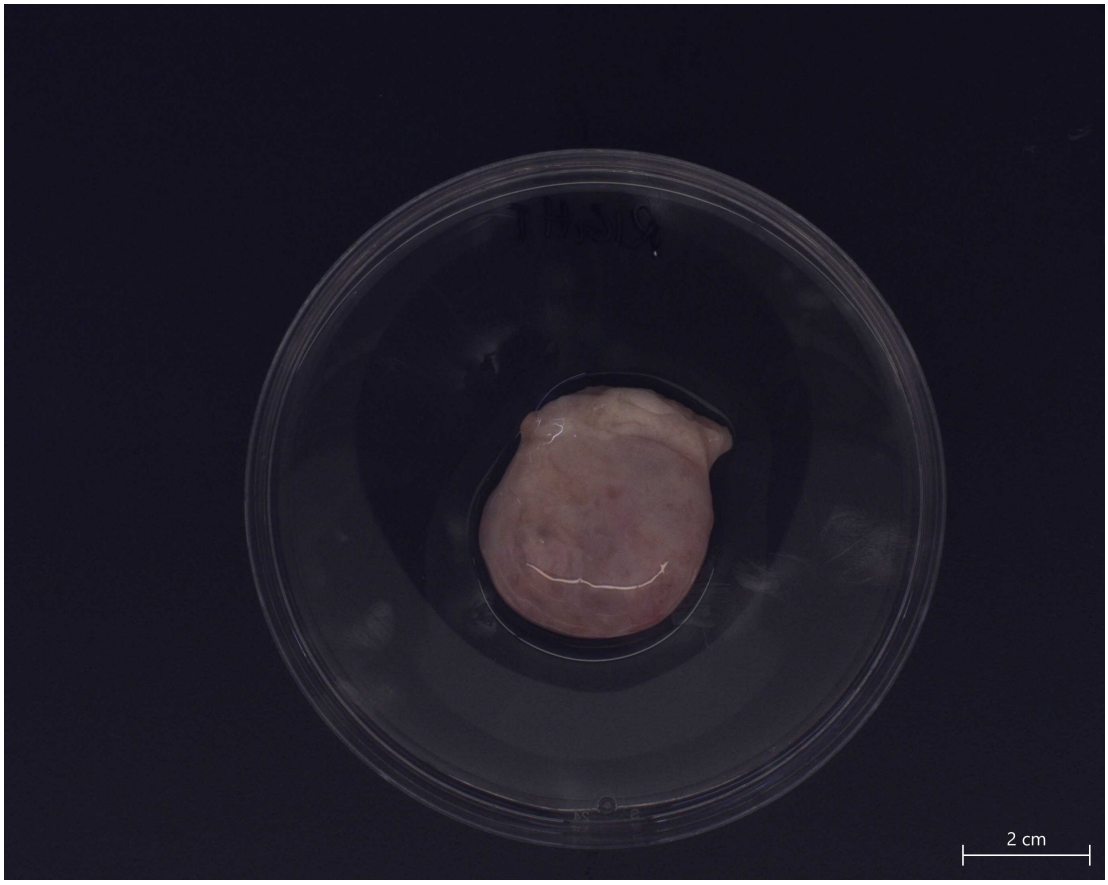
Posterior View

3 Record the weight of the whole reproductive tract.

4 Remove each ovary from the FRT by cutting along the hilum. Each ovary is placed in a 60 mm dish and labeled as the left or right ovary according to anatomic position.

5 Image and record the weight of each isolated ovary.


2 cm


2 cm

6 Bisect each ovary through the hilum (along the long axis) using a straight blade, keeping track of which half is located anatomically superior ("Over") vs. anatomically inferior ("Under").

These regions are named in line with the recently published 3-D regional model of the human ovary described in the following citation.

CITATION

O'Neill KE, Maher JY, Laronda MM, Duncan FE, LeDuc RD, Lujan ME, Oktay KH, Pouch AM, Segars JH, Tsui EL, Zelinski MB, Halvorson LM, Gomez-Lobo V (2023). Anatomic nomenclature and 3-dimensional regional model of the human ovary: call for a new paradigm..

LINK

https://doi.org/10.1016/j.ajog.2022.09.040

Note any defining features of the bisected halves, if relevant (i.e., presence of hemorrhagic cysts, etc...).

7 Using the Stadie-Riggs slicer, prepare a →|← 0.5 mm slice of the internal portion of a bisected half to generate a cross-sectional slice of the ovary across its short axis.

Equipment

Stadie-Riggs Tissue Slicer/Microtome Blades NAME

labForce BRAND

6727C10 SKU

https://cdn.thomassci.com/FetchFile.ashx?id=fa29617d-0b07-42e1-b73a-6d068c5b57f0 LINK

Repeat this step with the other ovary to generate a total of two 0.5 mm tissue sections for tissue sections.
If you are proceeding with paraffin-embedded analyses, fix with Modified Davidson's Fixative (MDF).

If you are proceeding with analyses that require frozen sections for Vanderbilt collaborations, please follow the following protocol.

Protocol

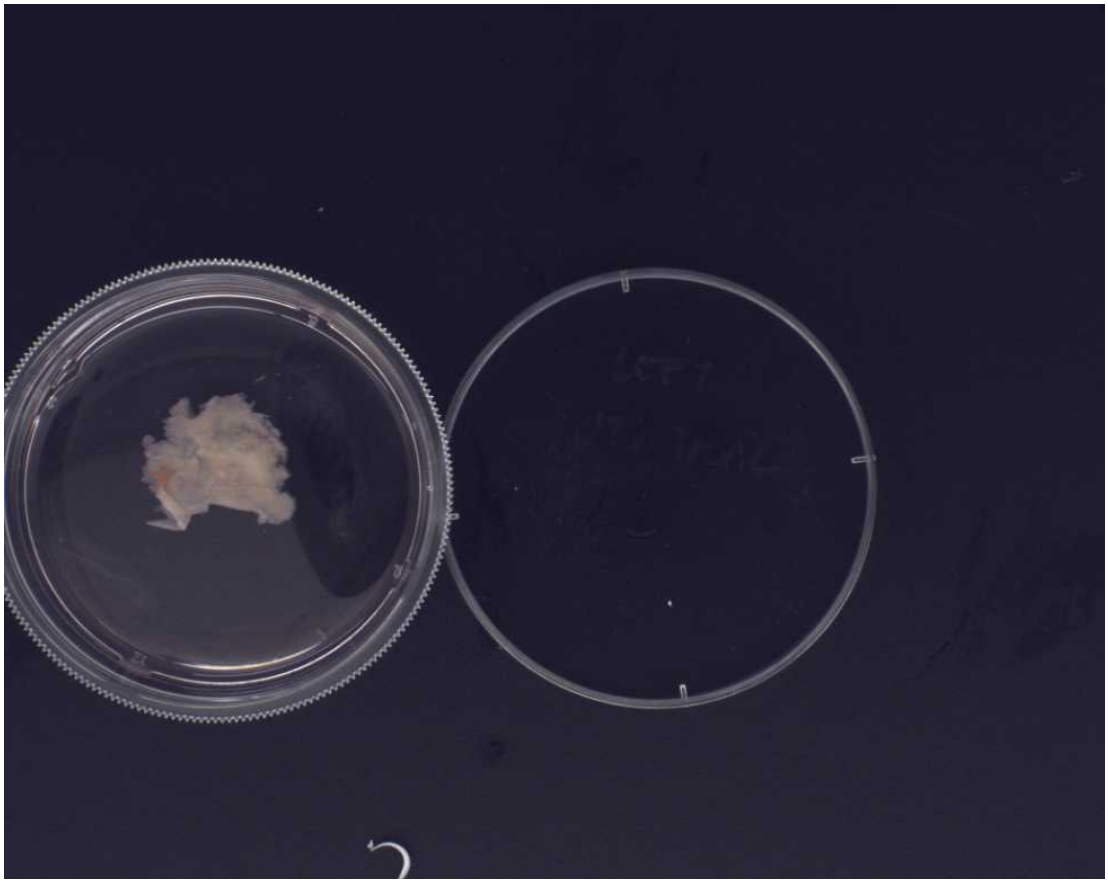
NAME

Freezing and Formalin Fixation of Tissue

CREATED BY

Jamie Allen

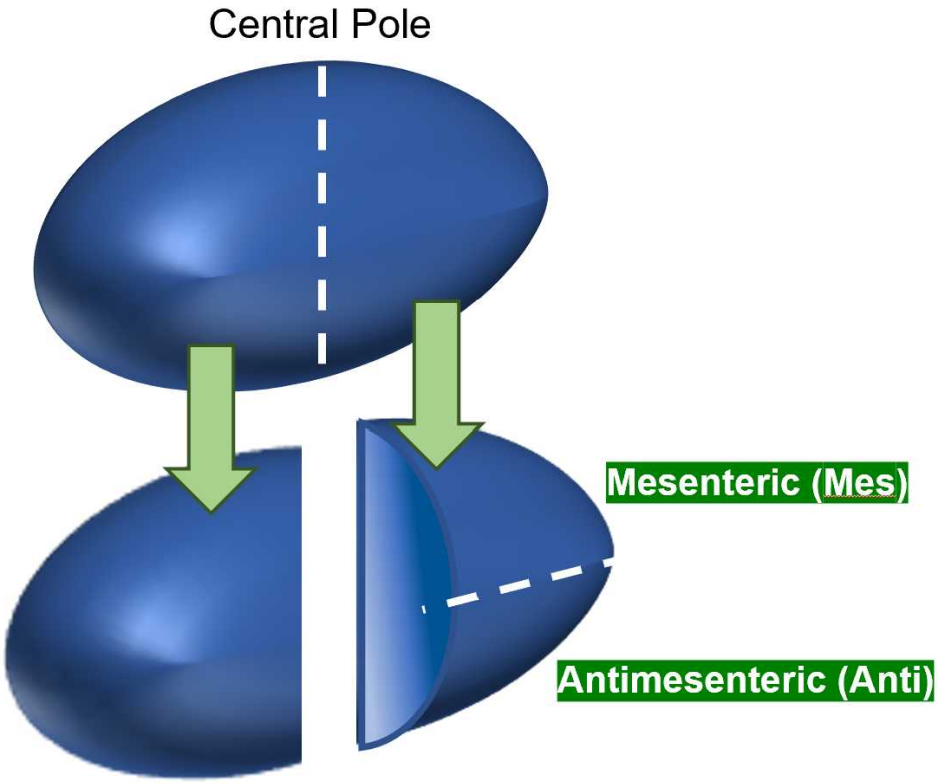
PREVIEW



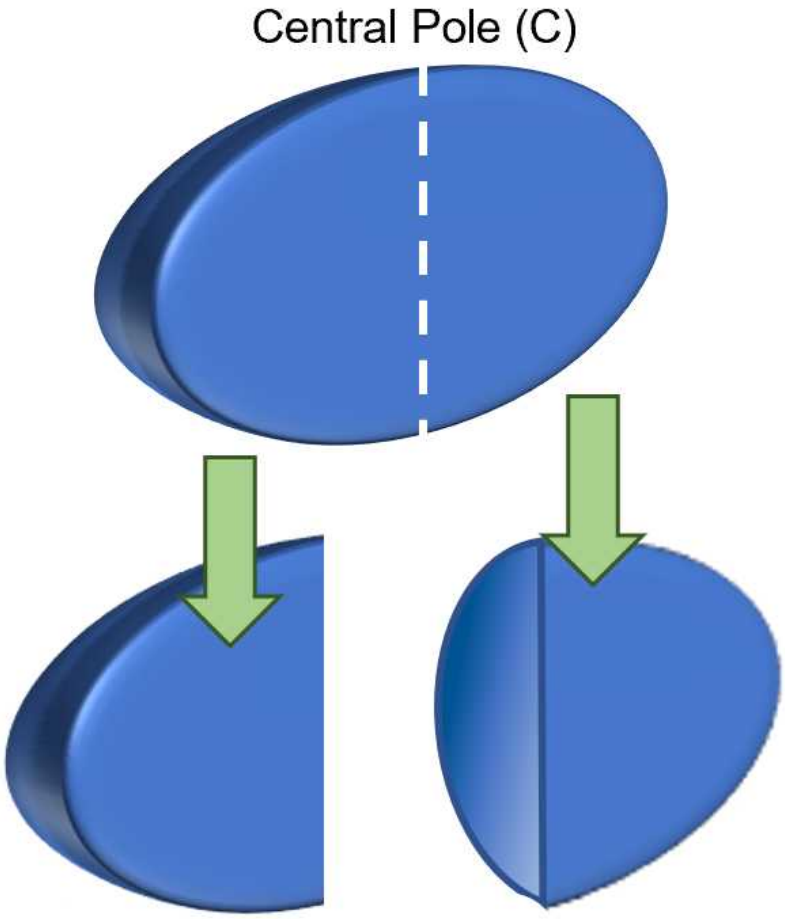
Central 0.5 mm section for fixation

For MDF-fixed sections: after fixation in MDF overnight (< 24 hours), remove fixative and dehydrate tissue by washing in increasing amounts of histology grade ethanol. Begin at 50% ethanol (repeat 2x), then 60% ethanol (repeat 2x), and finally 70% ethanol. Package tissue into histology cassettes and store cassettes submerged in 70% ethanol at 4 °C until processed and embedded in paraffin by histology core.

8 Return to the bisected halves (over and under portions) and cut along the central pole to generate two quarter spheres (representing lateral and medial regions) per bisected half.



Bisected "Over" portion



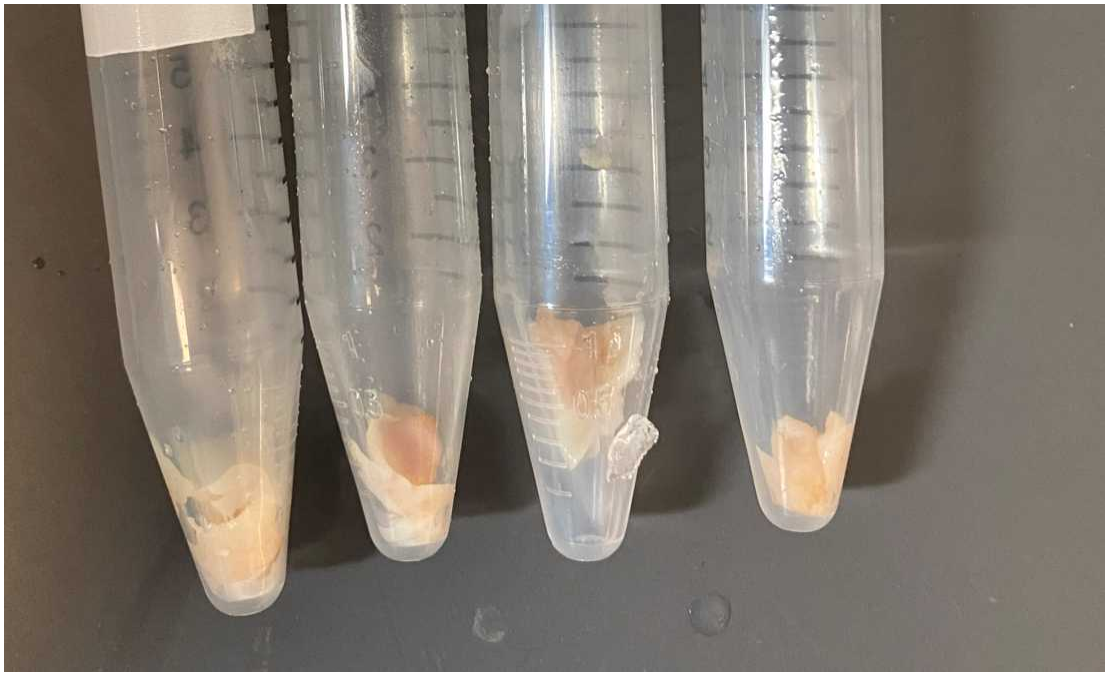
Bisected "Under" portion

9 Generate tissue sections for *decellularization.*

With the **lateral under** portion from each ovary, use the Stadie-Riggs slicer to generate 0.5 mm sections.

Place each section into a labeled 15mL conical, noting anatomic location within the ovary. For example, the first 0.5 mm slice (most superficial) taken from the left ovary is labeled: Left Under, Lateral dECM-1.

Add the appropriate volume (10-20 x tissue volume) of 0.1% SDS to each conical and allow to nutate at 4 °C for 48 hours to decellularize. Replace SDS solution within the 48 hour incubation, if cloudy.



Conicals for decellularization (LU 1-4) corresponding to the 0.5mm slice - 2.0mm slice

9.1 Following incubation in 0.1% SDS solution, rinse decellularized samples in double distilled water for two minutes. Repeat 3 times total and proceed to downstream applications or store at -80 °C .

10 Generating *banked tissue sections for slow freeze/cryopreservation.*

With the **medial under** portion of the ovary, slice coronally into sections no larger than 5 mm in any dimension to allow for adequate penetration of cryoprotectant. A typical section is usually 4 mm x 2 mm x existing depth.

Note anatomic location as described above and proceed with cryopreservation protocol.

Protocol

NAME

Slow freeze (cryopreservation) protocol for human ovarian tissue

CREATED BY

Elizabeth L Tsui

PREVIEW

11 Generating tissue sections for *protein analysis (flash freeze)*.

With the **lateral over** portion of the ovary, use the Stadie Riggs slicer to generate 0.5mm slices from the ovarian surface epithelium to the medulla.

Place into labeled 1.5 mL eppendorf tubes, noting anatomic position as described above.

When complete, place tubes in liquid nitrogen until frozen. Once frozen, transfer to -80 °C .

Safety information

Use appropriate personal protective equipment when using liquid nitrogen.

12 Generating *slow frozen tissue sections for downstream RNAseq*.

Using the **medial over** portion of the ovary, divide the quarter sphere into mesenteric (closest to hilum) and antimesenteric portions. For both mesenteric and antimesenteric pieces, slice the tissue coronally into sections no larger than 5 mm in any dimension to allow for adequate penetration of cryoprotectant. A typical section is 2 mm x 2 mm x existing depth.

Note anatomic location (*make sure to include mesenteric vs. antimesenteric information*) and proceed with cryopreservation protocol.

Protocol

NAME

Slow freeze (cryopreservation) protocol for human ovarian tissue

CREATED BY

Elizabeth L Tsui

PREVIEW

Overall Protocol Schematic

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