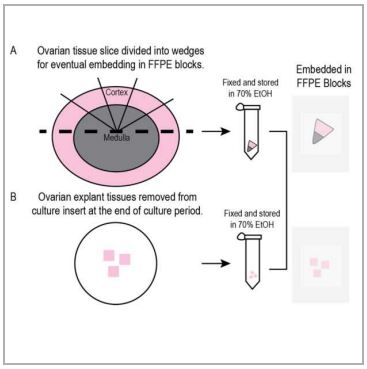

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Protocol status: Working
We use this protocol and it's working

Created: Jul 27, 2023

Human ovarian tissue fixation

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ABSTRACT

Purpose: This protocol is intended for use in fixing human ovarian tissue in a research setting. This protocol supplements the steps detailed in the collection, processing, and long-term storage of human ovarian tissue as formalin-fixed paraffin-embedded (FFPE) samples in protocol: dx.doi.org/10.17504/protocols.io.e6nvwdk29lmk/v1.

GUIDELINES

Researchers working with human specimens will adhere to all safety and training protocols required by the institution (Northwestern Medicine/Northwestern University) including but not limited to:

1. Biosafety Certification
2. Bloodborne Pathogens Certification
3. Working with Formaldehyde Certification
4. Collaborative Institutional Training Initiative (CITI program) certification

MATERIALS

1. Modified Davidson's Fixative, MDF (Electron Microscopy Sciences, 64133-50)
2. Histology grade ethanol (Fisher Scientific 04-355-223)
3. Invitrogen™ RNase-free Microfuge Tubes (ThermoFisher Scientific, AM12400)

Last Modified: Jul 27, 2023

PROTOCOL integer ID: 85594

Keywords: ovary, fixation, FFPE, explant

SAFETY WARNINGS

Researchers will wear personal protective equipment when working with human specimens, including gloves, masks, and lab coats.

ETHICS STATEMENT

This protocol takes place under approved IRB protocol through Northwestern University (NU12G09) for collection of human ovarian tissue through Northwestern Medicine.

Ovarian tissue fixation for fixed wedges and ovarian explan..

1 Fill 1.5 mL micro centrifuge tube with 1 mL of Modified Davidson's Solution (Cat. #64133- 50; Electron Microscopy Sciences, Hatfield, PA)

2 Add one ovary wedge or all tissue pieces (ovarian explant) from one experimental well per 1.5 mL micro centrifuge tube.

3 Allow all tubes to rock @ room temperature (RT) for 2-4 hrs.

4 Transfer tubes to cold room and rock at 4°C overnight (Fig. 1).

5

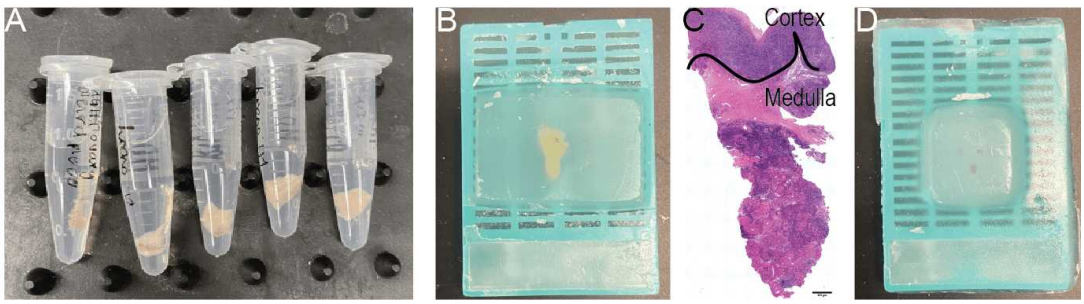

Fig. 1. Ovarian tissue wedges resting on rocker at 4°C overnight (A). Ovarian tissue wedges are embedded in paraffin blocks so that the cortex and medulla will be present in every section (B). A representative tissue section is shown for the block in B which includes both cortex and medulla approximated by a black line (C). Ovarian explants from one tissue well can be embedded together so that they are sectioned in one plane (D).

6 Wash in 1 mL 70% EtOH 3x10 minutes, rocking in between each, and then store in 70% EtOH @ 4°C until processing/paraffin embedding, no longer than 1 week.

7 Ovarian tissue wedges containing both cortex and medulla should be embedded so that the cortex and medulla will be sectioned in one plane (Fig. 1B). A representative H&E tissue section indicates the proper orientation (Fig. 1C).

8 Ovarian tissue pieces from explant culture should be embedded together, if cultured in the same well (Fig. 1D). While embedding try to ensure that tissues sit as evenly as possible, so that they are sectioned in the same plane.