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Human Pregnant Fallopian Tube Tissue Collection and Preservation Methods - UCSD Female Reproductive TMC

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Human pregnant fallopian tube tissue collection and storage protocol for HuBMAP's UCSD Female Reproductive TMC.

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HuBMAP, UCSD Female Reproductive TMC

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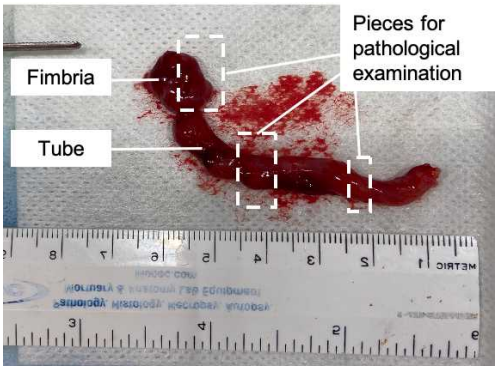
1. Sterile surgical dissecting instruments (scissors/forceps)
2. Gauze pad
3. PBS (cold)
4. Liquid Nitrogen
5. Empty microfuge tubes x8
6. Microfuge tubes pre-filled with RNAlater x8
7. 10% Formalin filled tubes x4

Preparation

1 Fallopian tubes should be collected into empty sterile cups right after sterilization (salpingectomy or tubal ligation). Keep left and right tubes separate.

2 Take a photo of the tubes prior to sampling. If pieces of the tissue were collected for clinical pathological examination, maintain the original orientation of tubes from the fimbrial (distal) end to the tubal (proximal) end after such sampling.



3 Wash the tissues in cold PBS while maintaining the original orientation.

Preserving the fimbria

4 Separate the fimbriated end from the tube (if not already separated).

4.1 Divide the fimbriated end lengthwise into 2 or 3 equal sized sections (depending on size).

4.2 Place one fimbriated piece into a 10% formalin-filled tube (1st priority), the other into RNAlater (2nd priority), and the third to be snap-frozen (3rd priority).

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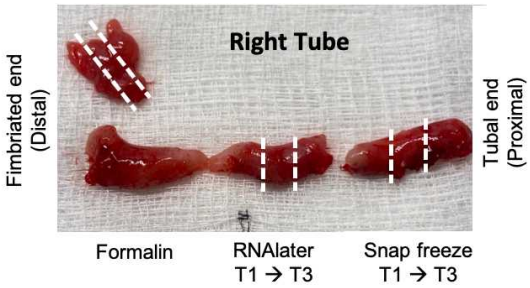
Preserving the tube

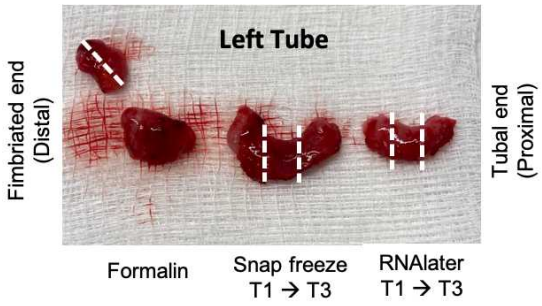
5 Collect cross sections of the tube into 10% formalin (x1), RNAlater (x3) and to be snap-frozen (x3). Each piece should be about 5mm in length, depending on the total length of the tube. If there is an excess of tissue, the formalin piece(s) can be larger.

5.1 Collect the formalin-preserved piece from the most distal end (closest to the fimbria).

5.2 For the right tube, collect the RNAlater pieces from the center of the tube and the snap frozen pieces from the proximal end. T1 should be most distal, then T2, then T3 should be most proximal.



5.3 For the left tube, collect the snap frozen pieces from the center of the tube and the RNAlater pieces from the proximal end. T1 should be most distal, then T2, then T3 should be most proximal.



Flash-freezing

6 Drop the snap frozen labeled tubes into liquid Nitrogen. Leave for ~2-10 minutes, then store in -80C freezer.

Processing with RNAlater

Place RNAlater tubes into a 4C fridge. Let these sit for 24-48 hours, then remove the RNAlater

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7 with a sterile transfer pipette and store in a -80C freezer.

Formalin fixing

8 After one week in formalin, place each formalin-fixed piece into a labeled cassette to be processed into FFPE blocks.

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