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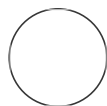
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**Protocol status:** Working  
We use this protocol and it's working

## Human Follicular Fluid Procurement and Processing

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### ABSTRACT

**Purpose:** This protocol is intended for use in the collection and storage of human follicular fluid in a research setting. The protocol details the collection, processing, and long-term storage of follicular fluid for downstream analysis.

### 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. Collaborative Institutional Training Initiative (CITI program) certification

### MATERIALS

1. 50 mL conical tubes (Globe Scientific, #6254)
2. Corning 2 mL and 5 mL Internal Threaded Polypropylene Cryogenic Vial, Self-Standing with Round Bottom (Fisher Scientific, 03-374-059 and 09-761-69 or equivalents).

**Created:** Jul 07, 2023

## SAFETY WARNINGS

**Last Modified:** Jul 27, 2023

**PROTOCOL integer ID:**  
84695

**Keywords:** follicular fluid,  
human



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 NU (NU12G09) for collection of human follicular fluid and associated biofluids through the Fertility and Reproductive Medicine clinic within Northwestern Medicine.

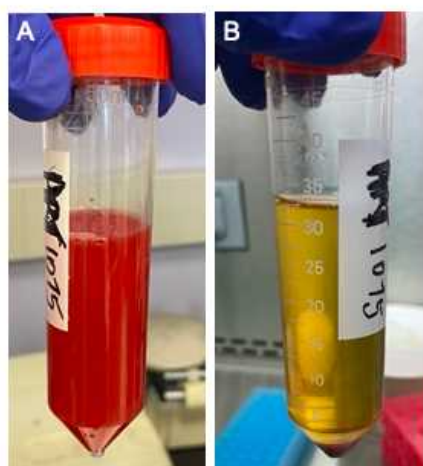
## Collection and processing fluids for storage

- 1 Follicular fluid is collected from an individual undergoing an egg retrieval procedure in 50 mL conical tubes at the Fertility and Reproductive Medicine clinic within Northwestern Medicine and stored at 2-4 °C immediately following collection. Research coordinator will collect and bring all tubes of follicular fluid to the lab kept on ice within 1 hour of an egg retrieval procedure. Samples should be transported in blue, labeled biohazard cooler bag (Fig. 1A, B).



**Fig. 1.** Follicular fluid is collected from clinic and then passed to research coordinator. Samples are brought to the lab once and kept at 2-4°C until processed (A, B).

- 2 Images are taken of follicular fluid before (Fig. 2A) and after (Fig. 2B) centrifugation and color of samples are noted. Samples will range from straw-colored, which is most ideal, to having a red tint which may indicate blood contamination from the process of egg retrieval and samples can still be stored in this case. Samples are of the pooled follicular fluid in all antral follicles from one patient ovary.



**Fig. 2.** Pooled follicular fluid is collected in a large 50ml conical tube. Samples are red in appearance before centrifugation because sample will contain blood and other debris due to collection process in which the needle goes between all growing follicles in the ovary (A). Fluid is centrifuged to yield a transparent yellow aspirate (B).

- 3 Follicular fluid is centrifuged at 300g for 20min at 4 °C and then the top, clear layer (supernatant) is immediately aliquoted into 2 mL and 5 mL cryovials which are subsequently frozen and stored at -80°C.