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# Reproductive Tissue Collection (Mammals): Post-mortem Sampling V.2

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ABSTRACT

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The following protocol illustrates how to collect and ship living reproductive tissue from a deceased wild or captive mammal for long-term cryopreservation. Collected tissues can either be immediately cryobanked at -196°C or processed for gamete collection and later cryobanked at -196°C.

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**Protocol status:** In development
We are still developing and optimizing this protocol

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## GUIDELINES

The collection of samples should be opportunistic and follow all applicable regulations. Reproductive tissues collected from deceased animals should be collected **as close to the time of death as possible** to avoid tissue decay. Temperature, body condition, and time are factors that accelerate or decelerate tissue decomposition, dictating the chances of collecting living cells.

If the animal dies suddenly or if the animal must be euthanized, the carcass or any tissues **must not be frozen**. The carcass can be kept in a cool area and reproductive tissues must be dissected immediately and shipped the same day as collection to a biobanking facility.

***Testes*** maximum time after collection before arrival at facility: **24 hours, at 4-15°C**
If testicular tissue is warm at time of collection, it should be kept at room temperature during the collection process. If the tissue is cold, it must be kept cold throughout the process to avoid rewarming and recooling. Testicular tissues must be kept at 4°C during shipment.

***Ovaries*** maximum time after collection before arrival at facility: **4 hours, at 15-20°C**
Ovaries held at low temperatures (less than 15°C) for more than 24 hours will begin to rapidly degrade the oocytes inside. Ovarian tissue must be kept at 15-20°C at all times. Contact receiving facility to discuss transport of samples prior to collection.

For any animal carcass found in the wild, time, ambient temperature, and storage methods are critical factors that can impact how quickly a sample must be collected. If the time of death is unknown, tissues can be harvested as long as it has not been frozen, become necrotic, or started decomposing. In such cases, tissues should still be shipped immediately.

For urgent questions regarding protocol steps or collection guidelines, please contact the Revive & Restore Biobanking Team at informedbiobanking@reviverestore.org

## MATERIALS

**User-supplied Materials:**

Sample containers
Phosphate buffered saline (PBS) with antibiotics (Penicillin, Streptomycin, Gentamicin)
Sterile scalpel
Sterile scissors
Disposable gloves
Gauze
Rubbing alcohol
Field notebook
Pencil/Pen
Permanent/alcohol-resistant marker
Newspaper or bubble wrap
Ice packs (*testicular tissue only*)
Styrofoam shipping box
Sample metadata form
Pre-paid FedEx shipping label

## SAFETY WARNINGS

Steps for personal safety must be considered before going into the field. Some wildlife diseases are transmissible to humans. Refer to your agency's health and safety guidelines for personal protective equipment (PPE). At a minimum, field personnel should wear disposable gloves and a fresh pair should be used between handling different specimens to avoid cross contamination. Sampling instruments and equipment should be thoroughly cleaned and disinfected or disposed of after use.

BEFORE START INSTRUCTIONS

**Tissue Handling**

Collected specimens are stored in vials containing a nutrient-rich media to keep the cells alive and a mixture of antibiotics to prevent bacterial growth. All samples must be collected under aseptic conditions to avoid contamination. Using sterile tweezers, scalpels, and scissors, as well as cleaning the sampling site with alcohol or surgical scrub (e.g. chlorhexidine) will decrease the chances of contamination. Collect testes and/or ovaries in a sterile container large enough to hold the entire organ, such as a large 50ml centrifuge tube, a clean Tupperware, or ziplock bag.

**Cold Storage**

Avoiding temperature fluctuations is very important for preserving the tissue samples. Use ice packs for transport of *testicular tissues* ***only*** from the field and ensure refrigeration is available immediately after returning from the field site. Do not use wet ice or other commercial therapeutic packs. Chill ice packs in the freezer the night before collection. Frozen ice packs will remain cold in an insulated container for up to 24 hours. If the field site is more than 24 hours from refrigeration, plan to bring a portable electric refrigerator. Vials containing tissue should not have direct contact with the ice packs. **Never freeze tissue biopsies** before shipping to a biobanking facility.

**Shipping**

All testicular tissue samples must be shipped to a biobanking laboratory via overnight express. Shipment Monday through Wednesday will guarantee arrival at the receiving facility the next day. **Do not ship samples on Friday.** Immediate transport of ovaries to the receiving facility is highly advised. Overnight shipment of ovarian tissue is not recommend but may be done if it is the only option available. Consult with the receiving facility for any questions regarding shipping timelines before collecting or sending samples.

## Preparation

1 Pre-chill icepacks in the freezer the day before planning to collect and ship samples (*testicular tissues only*).

2 Record all information about the species and individual, including a picture of the animal for identification and GPS location where the animal was found.

3 Proper protective equipment must be worn (gloves, etc). Sterility must be maintained as much as

possible.

## Tissue Collection

4 Use a sterile container large enough to hold the entire organ, such as a large 50ml centrifuge tube, a clean Tupperware with a tight-sealing lid, or a durable ziplock bag. Gather containers and have them readily available.

Step 4 includes a Step case.

**Ovary Collection**

**Testes Collection**

step case

### Ovary Collection

The figure below demonstrates tissue decay of a reproductive tract between time of death and removal from the body.
(A) Two hours after death (four-horned antelope). This is an ideal sample for biobanking.;
(B) Thirteen hours after death (gaur). Visible black patches indicate necrotic change (cell death);
(C) Eighteen hours after death (marmoset). Further black discoloration indicates necrotic change.

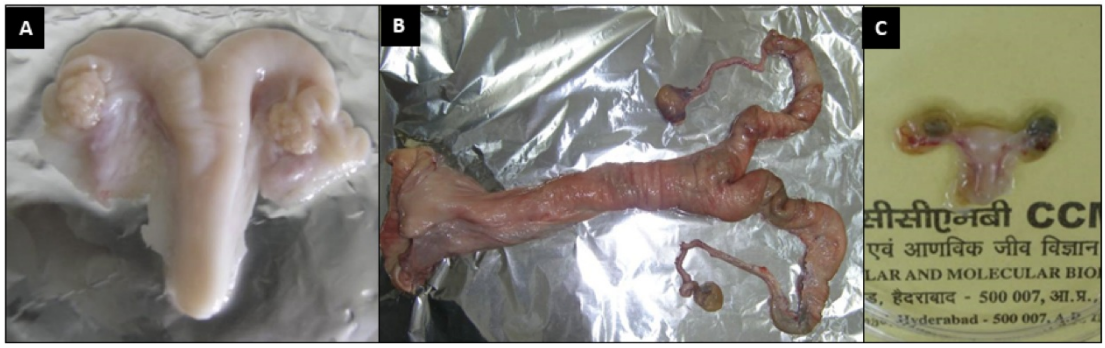


*Photo credit: Brahmasani et al., 2021*

5 Wet the entire sampling area of the carcass in rubbing alcohol and blot with sterile gauze.

6 With sterile scissors or scalpel, dissect open the pelvic cavity. The reproductive tract will lie under the large intestine/rectum.

7 Collect the entire reproductive tract, including both ovaries, the uterine body, and oviduct. For

larger animals, collect only the ovaries.

## Tissue Preservation

8 Thoroughly soak gauze in phosphate buffered saline (PBS) and antibiotics and gently wrap around the tissue. Ensure that the gauze fully covers the tissue, keeping it moist. Place in sample container and close the cap tightly. If the specimen is too large for the container, you may use a clean ziplock bag.

9 Using an alcohol-resistant marker, label the container with an identifier that matches ***exactly*** the recorded metadata. Check to make sure that each container is easy to identify with the information provided.

**Required information to record:**

- Scientific name of animal
- Sex of individual denoted as♀(female)
- Date of tissue collection
- Tissue type
- Any other identification number of individual

10 Ovarian tissue must be kept at 15-20°C. **Do not refrigerate or freeze samples.**

*****Notify the receiving facility immediately.*****

## Transport

11 Place the containers with samples in an insulated shipping box. Add additional 3 inches of bubble wrap or newspaper between the samples to ensure that the samples do not move or become damaged during transport.

12 Enclose all paperwork and sample/animal information in the insulated shipping container.

13 Contact the receiving facility to arrange immediate pick up or shipment.