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We are still developing and optimizing this protocol

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Reproductive Tissue Collection (Mammals): Post-mortem Sampling

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Revive and Restore

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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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 during shipment as well.

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.

MATERIALS

Included in Commercial Sampling Kit:
Sample vials with media
Parafilm
Insulated Tupperware
Vinyl lunch bag
Ice packs
Styrofoam container
Biopsy form
Pre-paid FedEx shipping label

User-supplied Materials:
Sterile scalpel
Disposable gloves
PBS with antibiotics (Penicillin, Streptomycin, Gentamicin)
Battery powered or electric clippers
Gauze
Rubbing alcohol
Disposable tweezers
Sterile scissors (optional)
Field notebook
Pencil/Pen
Permanent/alcohol-resistant marker
Newspaper or bubble wrap

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

Biopsied tissues 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 will decrease the chances of contamination. Collect as many samples as possible.

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 samples must be shipped to a biobanking laboratory via overnight express. You must use an insulated container. Ice packs must be used to ship *testicular tissues only* to maintain a temperature between 4-8°C. Considering that samples may be in queue at the receiving facility for a period of time before they are processed, **ship your samples as soon as possible**. Shipment Monday through Wednesday will guarantee arrival at the receiving facility before the weekend. **Do not ship samples on Friday.** Most facilities will not be available to receive shipments on weekends. If samples are collected Thursday through Sunday, samples must be stored in the fridge until Monday. Consult with the receiving biobanking facility for any questions regarding shipping timelines before 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 indicated in the biopsy form, 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 Remove the biopsy container from the kit 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.

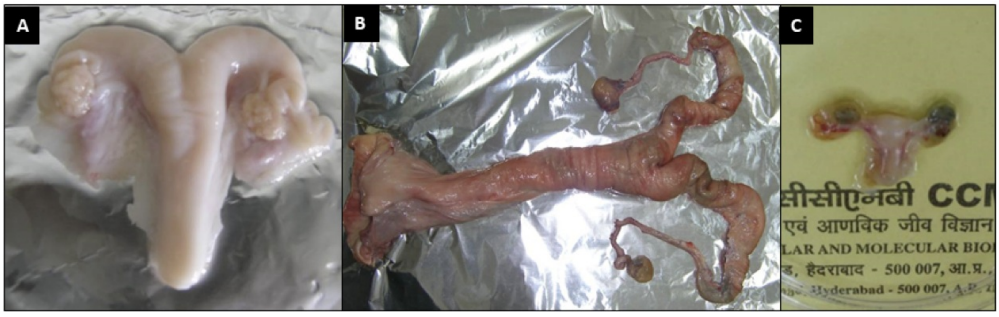


Photo credit: Brahmasani et al., 2021

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, only collection the ovary pair is needed.

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.

9 Using an alcohol-resistant marker, label the biopsy vial with an identifier that matches ***exactly*** what is indicated on the biopsy form. Check to make sure that each vial is easy to identify with the information provided on the form.

Required information to include on the form:

- Scientific name of animal
- Sex of individual denoted as ♂ (male) or ♀(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 freeze samples.**

Immediately ship overnight express. Do not wait to ship ovarian tissues. For samples collected on Thursday-Friday, contact receiving facility to arrange off-hour delivery*

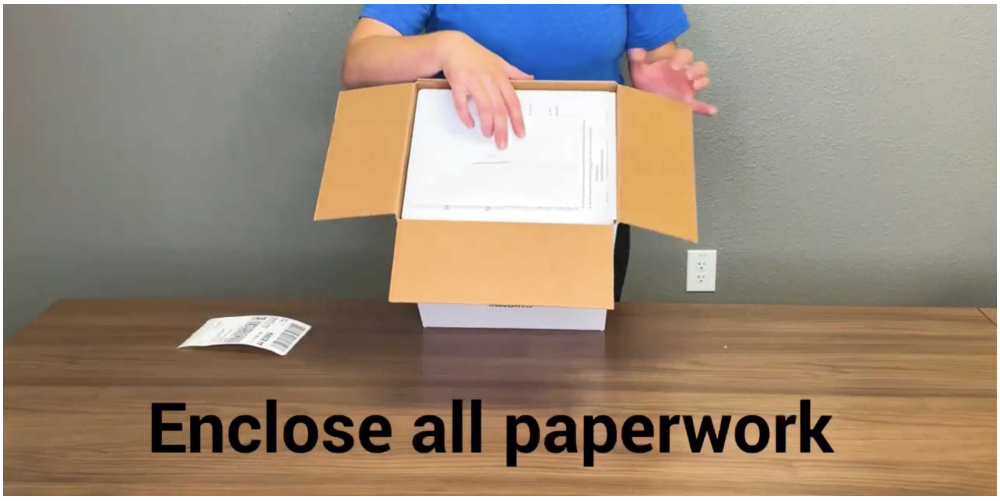
Shipping

11 Place the biopsy vials inside the Tupperware container included in your kit.

12 Close the lid and place the Tupperware container into the vinyl lunch bag provided in your kit.

13 Place the vinyl lunch bag into the styrofoam container.

14 Close the lid to the styrofoam container. Enclose all paperwork to the top, including the Biopsy Form. Close the shipping box and place the prepaid shipping label to the top.



15 Contact the receiving facility when you have shipped your sample and provide the tracking number so that personnel know when to expect your shipment.