The Complete Tiger Necropsy Protocol
Amur, Malayan, and Sumatran Tigers

Notification and Reporting

Time critical gamete and genome collection- Prior to or immediately upon death, contact a reproductive physiology program to discuss gamete collection and preservation. Omaha’s Henry Doorly Zoo and Aquarium’s Center for Conservation and Research (contact below) has done the majority of tiger gamete preservation post mortem, but most zoo reproductive programs and veterinary school theriogenology programs are doing or can do this. Semen preservation is most successful although oocytes may be preserved as well. Ovaries or testicles need to be collected within minutes if at all feasible. A protocol for initial testicle or ovary collection is provided at the end of this protocol if you are unable to make contact with a reproductive program immediately. However recommended procedures change so it would be useful to talk to the person who will be doing the work to determine their current recommendations.

Programs preserving genome cell lines are also in place although less common as of this writing. Preservation of this material also requires quick action. Contact the San Diego Zoo Institute for Conservation Research for the most current instructions for this procedure.

Routine reporting- Any time a tiger managed by a Species Survival (SSP) Plan dies, please notify the Tiger SSP Coordinator, the relevant SSP Vice Coordinator, and the North American Tiger Studbook Keeper (contact info shown below) by email within 48 hours of the event with the following information:

Institution and local contact
Subspecies/Population
Studbook number
Local identification number
Any unique physical identifiers such as tattoos or microchips
Date of Death

For those not using ZIMS, please send a specimen report that includes the tiger’s death to the AZA studbook keeper and the International Studbook keeper (currently Peter Mueller) so that the studbooks can be updated.

The gross necropsy report, histopathology report and any ancillary work such as cultures, electron microscopy, special stains, virus isolation and other diagnostic procedures should be submitted as a single report, annotated with all the identifying information, when all work has been done and the report is complete, to one or both of the Tiger SSP Veterinary Advisors and the Tiger SSP Coordinator. Please include the above identifying information in the final report so all records are well correlated.
Contact List
Current October 2019

AZA Tiger Species Survival Plan Coordinator
Tara Harris, PhD
Director of Conservation & Science
Arizona Center for Nature Conservation/Phoenix Zoo
455 N Galvin Pkwy, Phoenix, AZ 85008
Phone: 602-914-4317
Email: tharris@phoenixzoo.org

Tiger Studbook Keeper- North America
Kathy Traylor-Holzer, PhD
Senior Program Officer
Conservation Planning Specialist Group (SSC/IUCN)
12101 Johnny Cake Ridge Road
Apple Valley, MN 55124
Phone: 1-952-997-9802
Email: kathy@cpsg.org

Tiger International Studbook Keeper
Peter Müller
Leipzig Zoo
Pfaffendorfer Str. 29,
04105 Leipzig, Germany
Phone: +49 341 5933385
Email: pmueller@zoo-leipzig.de

Amur Tiger Subspecies Vice Coordinator
Trista Fischer
Northern Trail Zoologist
Minnesota Zoo
13000 Zoo Blvd.
Apple Valley, MN 55124
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Malayan Tiger SSP Vice Coordinator
Mike Dulaney
Curator of Mammals
Cincinnati Zoo & Botanical Garden
3400 Vine Street
Cincinnati, Ohio 45220
Phone: 513-475-6156
Email: mike.dulaney@cincinnatizoo.org
Sumatran Tiger SSP Vice Coordinator
Karen Goodrowe Beck, PhD
General Curator
Point Defiance Zoo & Aquarium
Metro Parks Tacoma
Phone: 253-404-3680
Email: karen.goodrowe@pdza.org

Generic Tiger SSP Vice Coordinator
Lyn Myers
General Curator
Fresno Chaffee Zoo
894 W Belmont, Fresno, CA 93728
Phone: 559-492-4437
Email: lmyers@fresnochaffeezoo.org

Veterinary Advisor - Tiger SSP
Doug Armstrong, DVM
Retired
1515 Madison St.
Omaha, NE 68107
Phone: 402-676-2200
Email: Tigerdr@outlook.com

Co-Veterinary Advisor - Tiger SSP
Julie Napier, DVM
Email: napvet25@gmail.com

Post-Mortem Gamete Preservation - Tiger SSP
Jason Herrick, PhD
Director of Reproductive Sciences
Center for Conservation and Research
Omaha’s Henry Doorly Zoo & Aquarium
3701 S. 10th Street
Omaha, NE 68107
Email: jason.herrick@omahazoo.com
Necropsy Protocol

This protocol is written as a “complete” necropsy protocol. However, the author fully recognizes resources and time constraints do not always allow for as thorough a necropsy and tissue collection as might be preferred. At a minimum we need everyone to do a “basic” necropsy and tissue collection. Please complete as much of the more thorough “complete” protocol as possible.

There are some population disease concerns that require additional special tissue collections, processing or submission. At this time these include Inflammatory Bowel Disease, Reproductive tract disease and old tiger senescence. Prior to or during necropsy, if possible, please contact the following people to get the most current requests for tissue collection or handling.

Reproductive Tract Disease:
Complete tracts from all tigers needed, male and female, whether diseased or not, implanted or not. Entire tract in formalin.

Dalen Agnew, DVM, DACVP
Michigan State University
Michigan State University
Diagnostic Center for Population and Animal Health
Lansing, Michigan 48910-8104
Phone: 517-432-5806
Fax: 517-353-5096

The following pathologists all have particular interest in and expertise in cats and may be willing to advise you on tissue collection and handling.

D. McAloose, VMD, DACVP
Wildlife Conservation Society
Zoological Health Program
Head of Pathology
2300 Southern Blvd
Bronx, NY 10460
Phone: 718-220-7105
Email: dmcaloose@wcs.org

Karen A. Terio DVM, PhD, DACVP
Zoological Pathology Program
University of Illinois
LUMC Bldg 101 Rm 0745
2160 S First St
Maywood, IL 60153
Ph: 708-216-6183
Email: kterio@illinois.edu
Complete Procedure: The necropsy should be thorough, two sets of formalin tissue should be collected and a liberal set of frozen tissue should be collected. Gross photos of everything possible should be collected and stored where they can be retrieved. Lesions should be measured, and if possible, some tissues should be weighed, such as heart. Remember the common sense part: for instance, if the cat has CNS disease, then a serious effort to get the whole brain and cord out would be a good thing. If it has heart disease, measure the walls, the luminal diameter, photo the valves and so forth. Holding the carcass until histology has been completed is also a good thing if possible.

Record the following information
Institution: _________________________________________________________________
Address: __________________________________________________________________
___________________________________________________________________________
___________________________________________________________________________
___________________________________________________________________________
Pathologist or person completing the necropsy____________________________________
Species/subspecies: ___________________________________________________________
Studbook No: ___________ In house or ISIS number: _____________________________
In-house identity: __________________________ Sex: ___________________________
Date of birth: ___________________________ Weight: ___________________________
Date of death: ________________________ Date of necropsy: _____________________
Necropsy number: _____________________

STANDARD FROZEN (-70°C IF POSSIBLE) TISSUE CHECK LIST: From Karen Terio
Please hold samples at your institution for future toxicological or nutritional analysis if necessary.
  ____ Liver
  ____ Kidney
  ____ Brain (portion of cerebral cortex)

STANDARD FIXED TISSUE CHECK LIST:
Preserve the following tissues in 10% buffered formalin at a ratio of 1 part tissue to 10 parts formalin. Tissues should be no thicker than 1 cm. INCLUDE SECTIONS OF ALL LESIONS AND SAMPLES OF ALL TISSUES ON THE TISSUE LIST. Photograph and measure lesions. Weigh organs that are larger or smaller than expected.
SSP SURVEILLANCE TISSUES and recommended tissue sampling procedures:

- **Liver** - sections from 3 lobes, including gall bladder
- **Spleen** - Cross section including capsule.
- **GI Tract** - 3 cm long sections of:
  - **Esophagus**
  - **Stomach** - multiple sections from cardia, fundus (body), and antrum of pylorus
  - **Small intestines** - duodenum, jejunum, ileum
  - **Large intestines** - cecum, colon
  - **Omentum** - ~3 cm square
  - **Pancreas** - representative sections from two areas including central ducts
  - **Adrenal** - entire gland with transverse incision.
  - **Kidney** -cortex and medulla from each kidney
- **Urinary bladder, ureters, urethra** - cross section of bladder and 2 cm sections of ureter & urethra.
- **Salivary gland**
- **Oral/pharyngeal mucosa and**
- **Tongue** - cross section near tip including both mucosal surfaces.
- **Lung** - sections from several lobes including a major bronchus
- **Trachea**
- **Thyroid/parathyroids** - leave intact.
- **Lymph nodes** - cervical, mediastinal, bronchial, mesenteric and lumbar. Cut transversely.
- **Thymus**
- **Heart** - longitudinal sections including atrium, ventricle and valves from right and left sides.
- **Eye** - both eyes intact. Remove extraocular muscles and periorbital tissues.
- **Brain** - cut longitudinally along midline. Submit entire brain and **pituitary gland**.
- **Spinal cord** (if neurologic disease) - sections from cervical, thoracic and lumbar cord.
- **Diaphragm and Skeletal muscle** - cross section of thigh muscles
- **Opened rib or longitudinally sectioned ½ femur** - marrow must be exposed for proper fixation
- **Skin** - full thickness of abdominal skin, lip and ear pinna.
- **Neonates: umbilical stump** - include surrounding tissues.
The following checklist is also provided as an aid in being thorough and keeping track of tissues, photos, etc.

**Tissue examination and collection checklist- from Dee MacAloose**

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**Gross** = **Gross appearance**: N=normal/no gross lesions; AB=abnormal; NE=not examined; NF=not found; NP=not present

**FF** = **Tissue fixed in formalin**: + = yes

**PHOTO** = **Photograph**: + = yes

**FP** = **Filter paper sample**: + = yes

**Histo** = **Tissue submitted for histology**: +

-20/-80 + = Frozen tissue temperature: list

If ancillary diagnostics, please include list and results with report
Collection and Processing of Testicles or Ovaries
Post-Mortem for Shipment

Testes
As soon as possible after death, remove testicles leaving as much of the spermatic cord, including the vas deferens, as possible. Dissection of the skin and scrotal sac is not necessary. *Place tissue into a clean plastic bag or specimen container with saline-soaked gauze at room temperature and seal tightly.*

Ovaries
Ovaries should be removed as soon as possible after death and placed into a container (plastic bag or specimen container) containing sterile saline at room temperature. *Enough saline should be included to keep the tissue fully submerged at all times.*

Packaging:
Both ovaries and testes should be maintained at 4 to 7 °C during transit. Wrap the container with the tissue in several layers of paper towels and place in a styrofoam cooler with ice packs or wet ice in a sealed plastic bag. Do not place ice or ice packs in direct contact with the container of tissue. **DO NOT USE DRY ICE.** Ship samples for priority overnight (next morning) delivery.

Shipping:
Please notify Omaha’s Henry Doorly Zoo and Aquarium, or other receiving institution, in advance of a planned euthanasia or as soon as possible after an unexpected death. Samples should be sent for priority overnight (next day AM) delivery.

   Jason Herrick
   Reproductive Science
   Omaha’s Henry Doorly Zoo & Aquarium
   3701 S. 10th Street
   Omaha, NE  68107-2200
   Phone: 402-738-2008
   Cell: 720-326-8289
   jason.herrick@omahazoo.com

Participation in this is voluntary, but highly encouraged for the benefit of the species. The long-term storage of the gametes and the processing costs will be the responsibility of Omaha’s Henry Doorly Zoo and Aquarium. We ask that institutions budget for overnight courier (testes) or counter-to-counter (ovaries) shipping costs. If you are unable to fund these costs, please contact us in advance to discuss alternatives.

*The SSP will authorize the use of any rescued gamete material, according to value, genetics, and banked quantity. In the event of successful propagation, institutions will be contacted and breeding loan agreements and documentation will be drafted at that time.*