An Introduction to Genetic Resource Banks for Wildlife Conservation

Central Zoo Authority
केन्द्रीय चिड़ियाघर प्राधिकरण

Nature Protects if She is Protected

CSIR-CCMB

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Dr. S P Yadav, IFS
Member Secretary

Foreword

India is one of the richest mega biodiverse countries in the world holding 2.4% of the world's land, harbouring 7.5% of the species of the world, including about 92,000 described species of animals. As per the IUCN Red Data List of threatened species, 304 vertebrate species are endemic to India, of which 130 species are listed as threatened.

Reproductive fitness is central to the existence of any species. The reproductive capacity of endangered wildlife species is likely to be affected by habitat loss and fragmentation. Preservation of natural habitats (*in situ*) and captive breeding (*ex situ*) are the best strategies for the conservation of any species. Traditional conservation efforts, hence, need to be complemented with intensive strategies that can manage isolated populations of species and maintain their genetic diversity. Similarly, in captive breeding programs, small numbers of founders and husbandry factors might compromise reproduction. This necessitates the use of reproductive technologies for the efficient management of captive wild animal populations.

Advances made in cryobiology offer new possibilities to preserve germplasm from endangered species for an extended period until suitable methods are developed. Therefore, the establishment of biological repositories, popularly known as Genetic Resource Banks, to store gametes (sperms and eggs), embryos, primary cells, and tissues collected opportunistically from threatened species could be an important step in long-term conservation planning.

The document prepared by the Centre for Cellular and Molecular Biology's Laboratory for the Conservation of Endangered Species (LaCONES) and Central Zoo Authority (CZA) puts forth the role of biobanks in improving conservation outcomes for endangered species. Through this document zoo and wildlife managers of the country will understand the utility of the technology and understand the process of implementing it. As of today, 6 zoos in the country have been identified as pilots to join hands with LaCONES and enhance our capacity in biobanking of wildlife genetic resources. I congratulate LaCONES for taking the necessary steps to initiate biobanking of wildlife genetic resources of endangered species.

I am sure this effort will be successful to complement the initiatives in place for the conservation of endangered species in India.

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प्रकृतिः रक्षिति रक्षिता
Nature Protects if She is Protected


वैज्ञानिक तथा औद्योगिक अनुसंधान परिषद
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INTRODUCTION TO WILDLIFE GENETIC RESOURCE BANKS

Anthropocene has witnessed an extensive alteration of habitats for wildlife, endangering many wildlife species. Several in situ approaches have been used to attempt increasing the population of the selected species. In addition, conservation breeding and assisted reproductive technologies have been used, by adapting techniques used for domestic animals to conserve wild animals (Wildt et al 1997). But their efficiency in wildlife is still evolving when compared to the technology available for domesticated animals. One of the reasons is due to lack of data on their reproductive anatomy and physiology.

Genetic resource banking involves systematic collection, preservation and accession of genetic material, tissues, gonads, gametes, embryos and cells. Genetic Resource Banks (henceforth referred to as biobanks in this document) have become increasingly important for conservation of wild endangered species, by preserving species as their genetic material (DNA/RNA), semen, oocytes, embryos, tissues, and cells at ultra-low temperatures of around -196 ºC (Wisely et al 2015).

The biological samples preserved in the biobanks serve as a biological storehouse. These samples can be used to develop species-specific protocols for reproductive technologies in conservation programs as well as for various studies on genetics and disease investigation in future (Eckerle et al 2014; Comizzoli and Wildt, 2017). These also facilitate enhancing the genetic diversity of existing wild populations (Fig 1).

- The value of biobanks and reproductive technologies for reviving critically endangered wild populations has already been aptly demonstrated by the following examples:

- The critically endangered black-footed ferrets were successfully reintroduced without loss of their genetic diversity by implementing a combination of traditional and modern reproductive technologies (Howard et al 2016).

- At Avantea, Italy on 15 Jan, 2019, northern white rhinoceros (NWR) embryos were generated *in vitro* by intra cytoplasmic injection of frozen-thawed sperm in to oocytes collected from the world's last two NWR females that were declared unfit for natural reproduction (Fig. 2).

- At Columbus Zoo and Aquarium, USA, on 24 Feb, 2020, two cheetah cubs were successfully born from *in vitro* produced embryos. Frozen spermatozoa were thawed and used to fertilize the oocytes of genetically valuable female cheetah to produce these cubs.

- The black footed ferret and Przewalski's horse were recently successfully cloned from the frozen thawed cells of their wild ancestors. Cells of wild female black footed ferret and male Przewalski's horse were cryopreserved in Frozen Zoo, San Diego Zoo Global more than three decades ago (Smithsonian magazine, 2020;2021).

These instances show that the biobanked cells and gametes have been valuable in reviving animals as well as maintaining genetic diversity of the wild populations.

Therefore, as we progress preserving biological materials of endangered species, and making these bio-repositories accessible to the researchers may be a good practice. This opens up several future options for wildlife conservation.

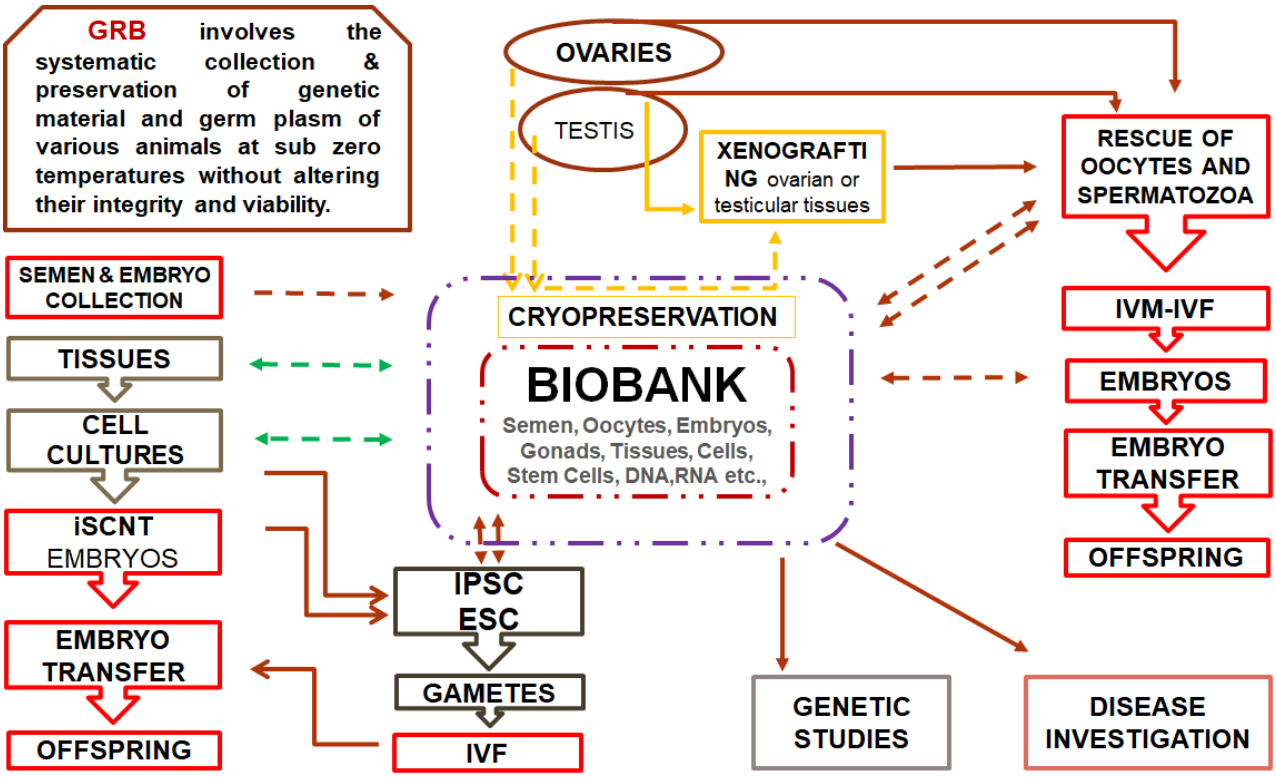
Globally twenty-two institutions (Table 1) currently preserve biological materials for use in Artificial Reproductive Technologies for wildlife. The 'Frozen Zoo', at San Diego Zoo Institute for Conservation Research, USA has one of the largest repositories worldwide including >10,000 living cell cultures, oocytes, sperm and embryos representing nearly 1000 taxa. In India, CCMB-LaCONES is the only research institute with biobanking facility for wild animals as part of National Wildlife Genetic Resource Bank. It collects tissues of various wild animals that have died naturally or due to medical reasons and preserves their genetic material, cells, gametes, gonads and tissues. The stored cells and tissues can be used for various genetic and disease investigation studies in future.

Table 1: Worldwide Genetic Resource Banking Facilities for Wildlife (Andrabi & Maxwell, 2007)

S No	Genetic Resource Bank Facility	Location
01	Ambrose Monell Laboratory, American Museum of Natural History	New York, USA
02	Frozen Zoo, San Diego Zoo Institute for Conservation Research	California, USA
03	Smithsonian Conservation & Research Institute, National Zoological Park	Virginia, USA
04	White Oak Conservation Centre	Florida, USA
05	**CSIR-Centre for Cellular and Molecular Biology**	**Hyderabad, India**
06	Cryobiology Research Group, Inst. of Res. Applied Natural Sciences, The Univ. of Luton	Luton, UK
07	Museum of Natural History	London, UK

08	Zoological Society of London	London, UK
09	North of England Zoological Society	Chester, UK
10	School of Biology, The University of Nottingham	Nottingham, UK
11	Metro Toronto Zoo	Toronto, Canada
12	Museum National d'Histoire Naturelle	Paris, France
13	University of Sassari	Sardinia, Italy
14	Experimental de Zonas Aridas	Almeria, Spain
15	Antwerp Zoo	Antwerp, Belgium
16	Zoological Park of Buenos Aires Buenos	Aires, Argentina
17	Cheetah Conservation Fund	Otjiwarongo, Namibia
18	Wildlife Biological Resource Centre, Endangered Wildlife Trust	Pretoria, South Africa
19	Conservation Genome Resource Bank for Korean Wildlife	Seoul, South Korea
20	Animal Gene Storage Resource Centre, Monash University	Melbourne, Australia
21	Giant Panda Breeding and Research Base	Chengdu, China
22	King Khalid Wildlife Breeding Research Centre	Thumamah, Saudi Arabia

Fig 1: Schematic of the operation and utility of Genetic Resource Banks



The activities associated with biobanking are collection, preservation and accessibility of the samples for various purposes in future (Fig 1). Collections can be done either from live or dead animals. After collection, the biological materials undergo a gradual cooling process called cryopreservation before being stored in biobank repository at -196 ^{O}C. Preserved tissues of gonads, oocytes, spermatozoa and others can be used for the production of offspring by *in vitro* maturation and fertilization. The preserved cells can be used for regeneration of species through somatic cell nuclear transfer. These cells can also be used for the generation of induced pluripotent stem cells (iPSC), which can be used for generation of gametes.

Key step in biobanking: Cryopreservation

Cryopreservation is a critical component of biobanking. It is the process of decreasing the metabolic activity of cells either partially or completely by gradual cooling of the cells/tissues to sub-zero temperatures. The cryopreservation solutions are supplemented with cryoprotective agents to protect the cells from possible damage due to formation of intra-cellular ice crystals.

Protocols for cryopreservation of semen and embryos have been fairly standardized compared to that of oocytes. The efficiency of cryopreservation of the biological materials varies with the type of cell/tissue, cryoprotective agents, cooling and warming rates, and devices used for holding the material during cryopreservation.

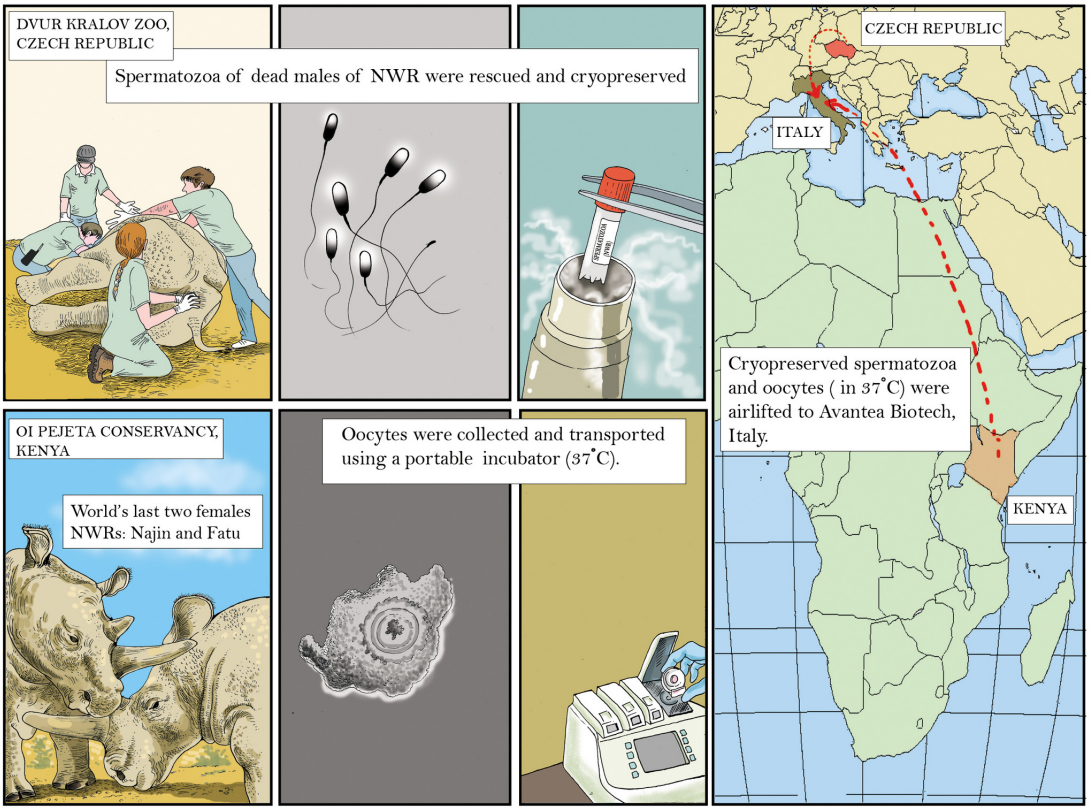
The protocols developed for cryopreservation of biological materials of one species may not be suitable for another species. One needs to optimize the procedures for cryopreservation of tissues/cells for each species in question, according to their biochemical constituents and physiological characteristics.

Biobanking facility at the National Wildlife Genetic Resource Bank, CCMB-LaCONES

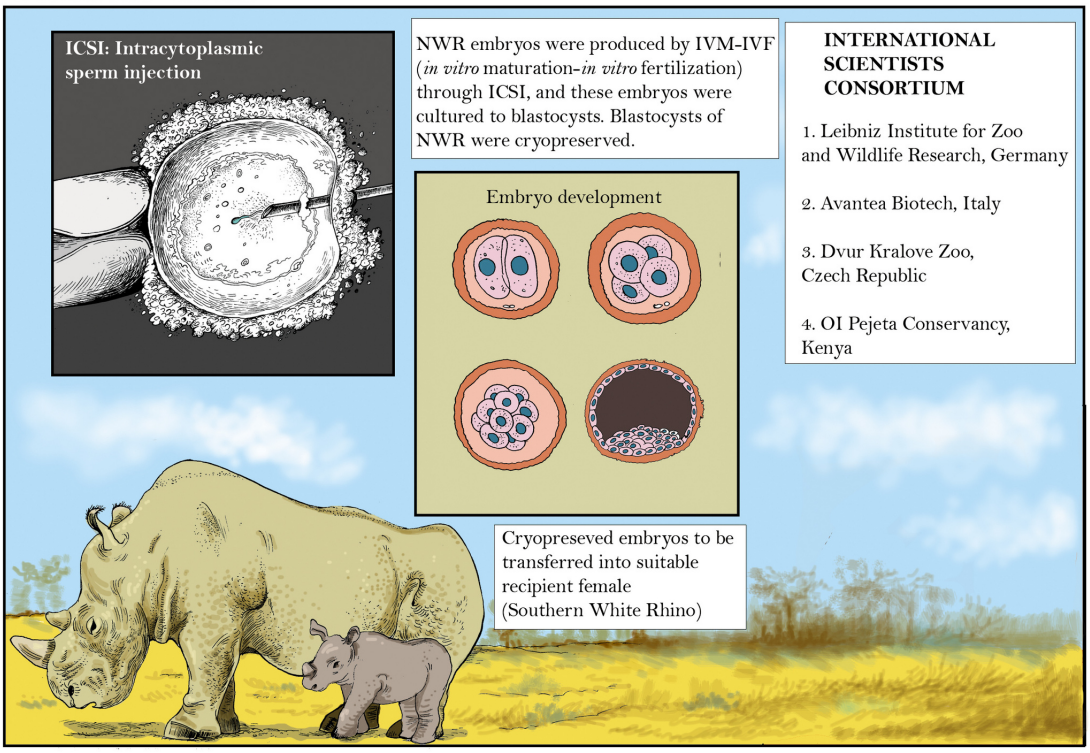
BIOBANKING FOR CONSERVATION OF ENDANGERED ANIMALS

Biobanking of genetic material including gametes, cells, and tissues has tremendous potential in conservation breeding and genetic management of threatened wildlife species. These stored biological samples can be retrieved as and when required for application in variety of assisted reproductive technologies (Fig 1).

Fig 2: Reintroduction program of northern white rhinoceros involving multiple agencies



* NWR: Northern White Rhino



For their effective utilization, zoos, conservation parks, animal research labs and biobanks have to work cohesively, and have access to each other's resources and expertise. The ongoing reintroduction program of northern white rhinoceros (NWR; *Ceratotherium simum ssp. cottoni*) is a striking example of this.

NWR is a subspecies of the white rhinoceros and listed as critically endangered by International Union for Conservation of Nature. For its reintroduction, an International Scientist Consortium led by Leibniz Institute for Zoo and Wildlife Research, Germany was formed with three other institutes.

At present, the Ol Pejeta Conservancy in Kenya has the world's last two females NWR: Najin (30Y) and Fatu (19Y). Pathological lesions in their reproductive tracts have compromised their natural ability to reproduce.

To save NWR from extinction, the consortium decided to implement established procedures of assisted reproduction in southern white rhino to produce viable offspring from these two females before they grow too old. They used ultrasound-guided ovum pick-up method to collect immature oocytes (eggs) from super-ovulated females. These oocytes were air lifted to Avantea Biotech, Italy for *in vitro* maturation and fertilization.

Spermatozoa were collected from the biobank of Czech Republic Zoo that had cryopreserved spermatozoa from testicles of dead NWR males. The frozen spermatozoa were thawed and used for *in vitro* fertilization (IVF).

The fertilized embryos were cultured *in vitro*, and the ones that developed to blastocysts have been cryopreserved. So far, 19 immature eggs have been collected across two sessions: 22 Aug, 2019 & 17 Dec 2019. After *in vitro* maturation, 12 eggs were fertilized using a procedure called Intra Cytoplasm Sperm Injection. Three of these fertilized eggs were developed to viable blastocysts. Embryos are now preserved in liquid nitrogen at -196^{O}C. These cryopreserved embryos will be transferred to suitable surrogate females of southern white rhino.

Biobanks aiding biotechnological tools in wildlife conservation

Artificial insemination: This is the most extensively applied technology used in conservation and genetic management of threatened wild species. Several wild species have benefited from this technology using cryopreserved semen (Table 2).

Table 2: Successes of application of artificial insemination (AI) technology using frozen-thawed semen for wildlife conservation

Species	Preg/AI success (%)	Outcome	Laboratory/Institute	Reference
FELIDS				
Leopard cat (*Prionailurus bengalensis*)	2/2 (100 %)	2 live births	Smithsonian Conservation Research Institute, USA	Wildt et al. 1992
Ocelot (*Leopardus pardalis*)	1/8 (13 %)	1 live birth	Smithsonian Conservation Research Institute, USA	Swanson et al. 1996
Cheetah (*Acinonyx jubatus*)	3/6 (50 %)	6 live births	Sperm were collected from wild-caught males in Namibia Africa, cryopreserved and then imported inter-continentally into the United States for use in the Cheetah SSP Program.	Howard et al. 2002
UNGULATES				
Antelopes				
Addax (*Addax nasomaculatus*)	1/1 (100 %)	1 live birth	Texas A&M University, USA	Densmore et al. 1987
Gaur (*Bos gaurus*)	2/6 (33 %)	2 live births	National Zoological Park, USA	Junior et al. 1990
Scimitar horned oryx (*Oryx dammah*)	2/4 (50 %)	2 live births	Semen collected in 1987 at Toronto Zoo (Canada) and was imported to New Zealand to inseminate oryx females at Orana Wildlife Park in 1988.	Garland et al. 1992

Mohor gazelle (*Gazella dama mhorr*)	3/7 (43 %)	1 stillborn	Zoological Society of London, UK	Holt et al. 1996
Blackbuck (*Antilope cervicapra*)	1/3 (33 %)	1 live birth	Zoological Society of London, UK	Holt et al. 1998
Scimitar horned oryx (*Oryx dammah*)	9/24 (38 %)	7 live births	Smithsonian Conservation Research Institute, USA	Monfort et al. 1999
Eland (*Taurotragus oryx*)	1/4 (25 %)	1 live birth	Rhodes University, South Africa	Bartels et al. 2001
Banteng (Bos *javanicus*)	1/6 (17 %)	1 live birth	Western Plains Zoo, New South Wales, Australia	Johnston et al. 2002
Gerenuk (*Litocranius w.walleri*)	2/8 (25 %)	1 stillborn	White Oak Conservation Center, USA	Penfold et al. 2005
Spanish ibex (*Capra p. hispanica*)	2/8 (25 %)	2 live births	National Wildlife Park, Spain	Santiago-Moreno et al. 2006
Blackbuck (*Antilope cervicapra*)	1/2 (50 %)	1 preterm (dam died before term)	CCMB-LaCONES, India	Sontakke et al., unpublished
Deer				
Red deer (*Cervus elaphus*)	16/39 (41 %)	14 live births	New Zealand	Haigh et al. 1984
Axis deer (*Cervus axis axis*)	7/31 (23 %)	7 live births	New Zealand	Mylrea et al. 1992
Eld's deer (*Cervus eldi thamin*)	9/20 (45 %)	7 live births	National Zoological Park, Smithsonian Conservation and Research Center, USA	Monfort et al. 1993
Fallow deer (*Dama dama*)	26/55 (47 %)	23 live births	University of Sydney, Australia	Mulley et al. 1988
White-tailed deer (*Odocoileus virginianus*)	28/35 (80 %)	57 live births	Mississippi State University, Texas, USA	Jacobson et al 1989

Wapiti deer (*Cervus elaphus*)	102/200 (51%)	100 live births	Semen was collected in Canada in 1983 and was shipped to New Zealand in 1988 to inseminate large number of female Wapiti.	Haigh and Bowen 1991
Black footed ferret (*Mustela nigripes*)	5/14 (36 %)	8 live births	Semen from 8 males was cryopreserved and stored at Smithsonian Conservation Research Institute, USA for 10-20 years and was used for artificial insemination.	Howard et al. 2015
LARGE MAMMALS				
Giant panda (*Ailuropoda melanoleuca*)	5/21 (24 %)	5 live births	China Conservation and Research Center for the Giant Panda (CCRCGP), Wolong Nature Reserve, China	Huang et al. 2012
Pacific white-sided dolphin (*Lagenorhynchus obliquidens*)	5/10 (50 %)	4 live births	SeaWorld and Busch Gardens Reproductive Research Center, San Diego, USA	Robeck et al. 2009
Bottlenose dolphin (*Tursiops truncatus*)	5/8 (63 %)	4 live births	SeaWorld and Busch Gardens Reproductive Research Center, San Diego, USA	Robeck et al. 2005
Beluga whale (*Delphinapterus leucas*)	2/10 (20 %)	1 live birth	SeaWorld and Busch Gardens Reproductive Research Center, San Diego, USA	Robeck et al. 2010
African Savanna elephant (*Loxodonta africana*)	1/1 (100 %)	1 live birth	Leibniz Institute for Zoo and Wildlife Research, Germany	Hildebrandt et al. 2012
Rhinoceros (*Ceratotherium s. simum*)	1/1 (100 %)	1 live birth	Leibniz Institute for Zoo and Wildlife Research, Germany	Hermes et al. 2009

Artificial insemination in leopard via laparoscopic (above) endoscopic (below) guided intra-cervical insemination at CCMB-LaCONES

Somatic Cell Nuclear Transfer: The cells stored in biobanks can be used for cloning. In 1996, Dolly, a sheep was produced from a cultured adult somatic cell by a method called cloning or somatic cell nuclear transfer (SCNT). This method involves transfer of a somatic cell or a nucleus in to an enucleated matured unfertilized oocyte of the same species. This allows the oocyte to develop into an embryo. The embryo is then transferred into a recipient mother of the same species for development.

The success with SCNT technology in various mammalian species has opened up new ways to increase the population size of wild and endangered species. However, the availability of oocytes poses a challenge in the application of SCNT technology in endangered species.

The protocols developed for SCNT in domestic species have been replicated in a few wildlife species (Table 3). These have used fibroblast cells of wild species as nucleus donor and oocytes of closely related domestic species as recipient cytoplasm, and hence, called inter-species somatic cell nuclear transfer (iSCNT).

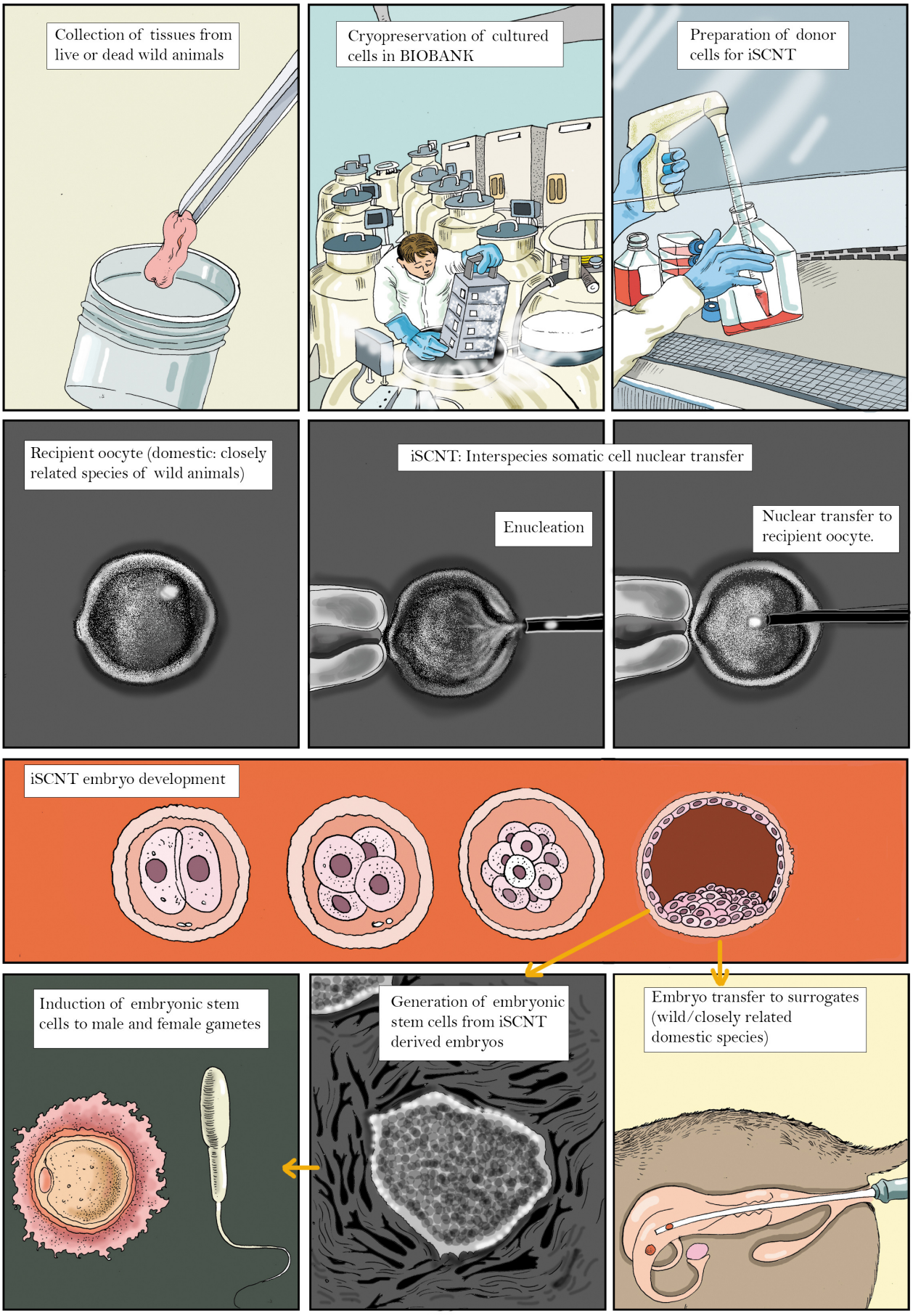
Table 3. Successes of inter-species somatic cell nuclear transfer (iSCNT) in endangered wildlife

Donor species	Somatic cells used from donor (source of genetic material)	Source of oocytes	Outcome (live births and survivability)	Reference
Gaur (*Bos gaurus*)	Dermal fibroblasts from post-mortem male	Domestic cattle (*Bos taurus*)	1 live birth, died on day 2 post-calving	Lanza et al (2000)
Mouflon sheep (*Ovis orientalis musimon*)	Granulosa cells from post-mortem female	Domestic sheep (*Ovis aries*)	1 live birth	Loi et al (2001)
African wild cat (*Felis silvestris lybica*)	Crypreserved fibroblasts	Domestic cat (*Felis silvestris catus*)	2/17 kittens survived	Gomez et al (2004)
Gray wolf (*Canis lupus*)	Dermal fibroblasts from post-mortem animal	Domestic dog (*Canis l. familiaris*)	3/6 pups survived	Kim et al (2007); Oh et al (2008)
Sand cat (*Felis margarita*)	Cryopreserved fibroblasts	Domestic cat (*Felis silvestris*)	5/14 live births, alll died by day 60 post-parturition	Gomez et al 2008

Pyrenean ibex (*Capra pyrenaica pyrenaica*)	Cryopreserved fibroblasts	Domestic goat (*Capraaegagrus hircus*)	1/5 survived but died shortly after birth	Floch et al (2009)
Esfahan mouflon (*Ovis orientalisis phahanica*)	Cryopreserved fibroblasts	Domestic sheep (*Ovis aries*)	2 live births that died post parturition	Hajian et al (2011)
Coyote (*Canis latrans*)	Neonatal fibroblasts	Domestic dog (*Canis familiaris*)	5 live births	Hwang et al (2013)
Wild buffalo (*Bubalus arnee*)	Cryopreserved fibroblasts	Domestic buffalo (*Bubalus bubalis*)	1 live calf	Singla et al (2015)
Bactrian camel (*Camelus bactrianus*)	Cryopreserved fibroblasts	Dromedary camel (*Camelus dromedarius*)	1 live birth but died on day 7 post-parturition	Wani et al (2017)
Przewalski's horse (*Equus ferus przewalskii*)	Cryopreserved fibroblasts	Domestic horse (*Equus caballus*)	1 live birth	Smithsonian magazine (2020)
Black-footed ferret (*Mustela nigripes*)	Cryopreserved fibroblasts	Domestic ferret (*Mustela putorius*)	1 live birth	Smithsonian magazine (2020)

Advancements in regenerative medicine, especially in conversion of somatic cells to stem cells (induced pluripotent stem cells/iPSC), promise to strengthen the existing reproductive technologies of wild/endangered species. iPSCs of the target species can be used to create artificial gametes (Stanton et al 2019). Viable somatic cells are prerequisite for application of iSCNT or iPSC technology for conservation of endangered species.

Fig 3: Conceptual model for inter-species somatic cell nuclear transfer (iSCNT) and its applications for conservation of wild species



Inter-species somatic cell nuclear transfer (iSCNT) involves three steps:

1. Expansion of somatic cells of wild species: Tissues of live or dead animals are cultured in a laboratory. The expanded cells are cryo-preserved and stored in biobank.

2. Preparation of recipient oocyte (cytoplast) for nuclear transfer: Matured oocytes of the closely related domestic species of the wild species are used as cytoplast. The first polar body and nucleus are removed (enucleation) from the oocyte. A whole somatic cell of the wild target species is transferred in to the perivitelline space (space between zona pellucida and cytoplasmic membrane) of the enucleated oocyte. The nucleus of the somatic cell can also be directly inserted in to the cytoplasm of the enucleated oocyte. The membranes of the somatic cell and the cytoplasmic membrane of the oocyte are fused by applying an electrical pulse. Oocytes have the ability to reprogram the nuclear material of the somatic cell to allow normal embryonic development.

3. Embryo transfer: The embryos developed are transferred to surrogate mothers for further development in the uterus. Surrogate mothers either belong to the same species of the donor cell or closely related domestic species to donor cell species.

The iSCNT derived embryos also serve as a source for generation of embryonic stem cells (ESCs) of the wild species used for nuclear transfer. The ESCs derived from the iSCNT embryos can be induced to develop as male and female gametes of the wild species.

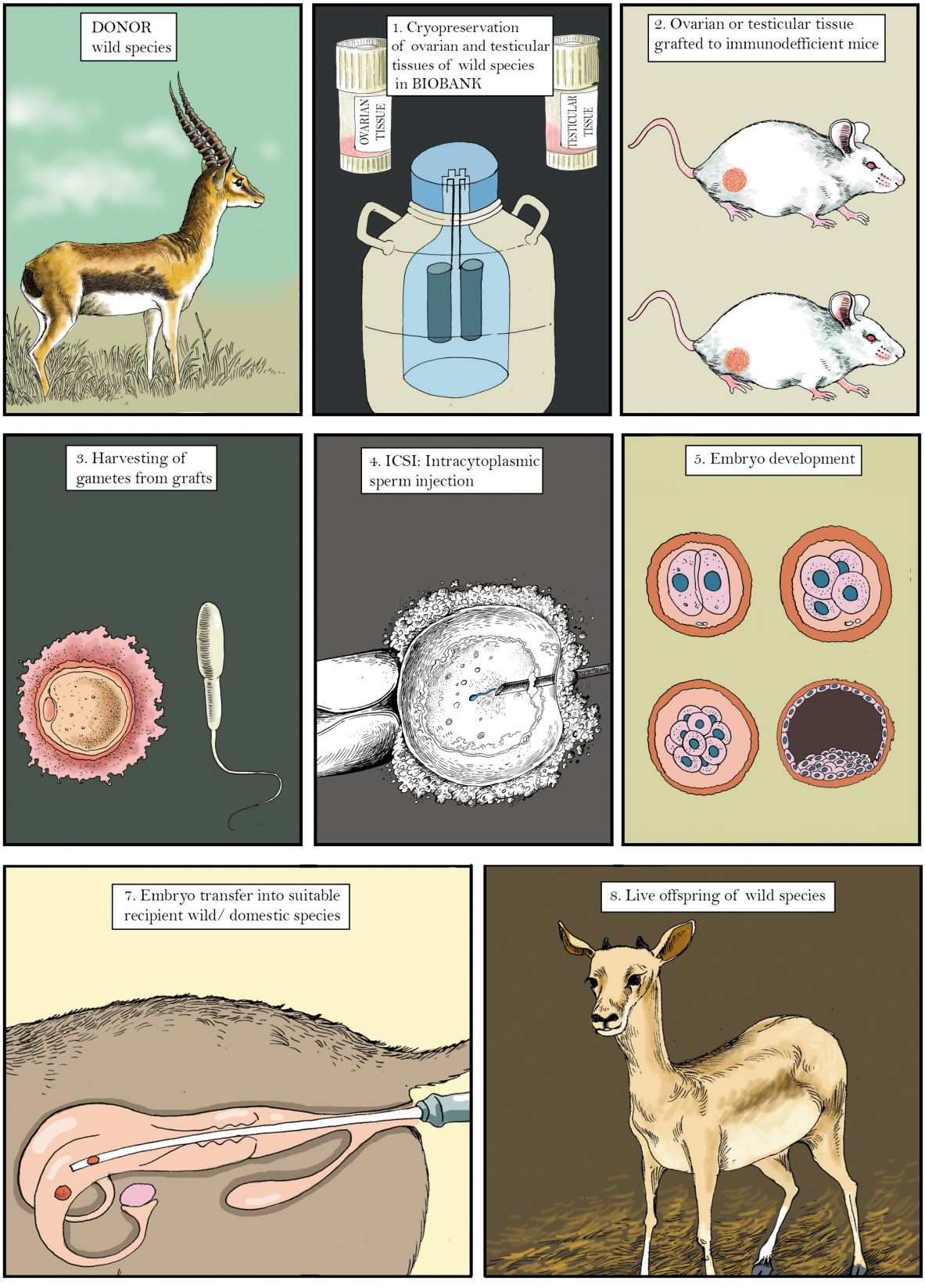
Gonadal tissue grafting: Biobanking of gonads (ovaries) or gonadal tissue (ovarian or testicular tissue) and gametes (spermatozoa and oocytes) of wild species collected postmortem is one of the novel approaches for preservation of the valuable germplasm of wild population even after death of the individuals (Saragusty et al 2006; Comizzoli et al 2019; Thongphakdee et al 2020).

Fernandez-Gonzalez et al (2019) reported generation of nine highly valuable embryos from Asiatic golden cat and northern Chinese leopards by *in vitro* maturation and fertilization using sperms and oocytes. These were collected from the gonads donated to Felid-Gamete-Rescue project, a part of the Leibniz Institute for Zoo and Wildlife Research, Germany. Grafting of gonadal tissue of immature wild animals in to another species (such as in an immunodeficient mice) is a promising approach for the propagation of oocytes and spermatozoa of wild population (Review: Comizzoli et al 2010; Devi and Goel, 2016).

Gonadal (testicular or ovarian) tissue grafting involves transplantation of small tissue pieces. When done:
under the skin of scrotum/testicles or ovarian - it is called orthotopic,
in places other than testicles or ovary - it is called heterotopic,
in the same individual - it is called autologous graft,
in a different individual of the same species or of different species - it is called xenograft.

The graft-derived gametes can help understand early development of gametes as well as for the production of embryos *in vitro* as illustrated in Fig 4.

Fig 4: Steps for propagation of gametes from gonads collected postmortem



Following are the steps for propagation of gametes from gonads collected postmortem:

1. Gonads (ovaries or testicles) from dead wild animals have the potential to produce functional gametes if provided with biological conditions similar to those *in vivo*. For preservation, the ovaries or testicles are collected immediately after the death of animals. The ovarian cortex or testicular tissue is cryopreserved and stored in liquid nitrogen in a biobank for future use.

2. Fresh or frozen-thawed tissue pieces of ovarian cortex or testicular tissue are transplanted to either gonadal region (orthotopic) or non-gonadal region (under the kidney capsule, under the skin of the back, abdominal region (heterotopic) of the prepubertal young immune-deficient mice (such as SCID, nude mice models) for initiation of growth.

3. The grafted tissue produces gametes of the donor wild animal in mice.

4. Graft derived gametes of wild species are subjected to *in vitro* fertilization through intra-cytoplasmic sperm injection to test their functional ability to fertilize and develop in to viable embryos *in vitro*.

5. The viable embryos produced from the graft-derived gametes are transferred to surrogate mother for further development in the uterus. The surrogate mother either belongs to the same species as the donor of the grafted tissue or one of the closely related domestic species.

BIOBANKING FOR CONSERVATION OF ENDANGERED BIRDS

Conservation and management of threatened bird species is a challenging task. *In situ* and *ex situ* conservation or even the conventional assisted reproductive technologies such as artificial insemination may not be adequate to revive sizable numbers of critically endangered birds in a short span of time. It, therefore, necessitates urgent development of innovative breeding technologies to produce individuals with greater fecundity to save it from extinction and to boost long-term survival of their population.

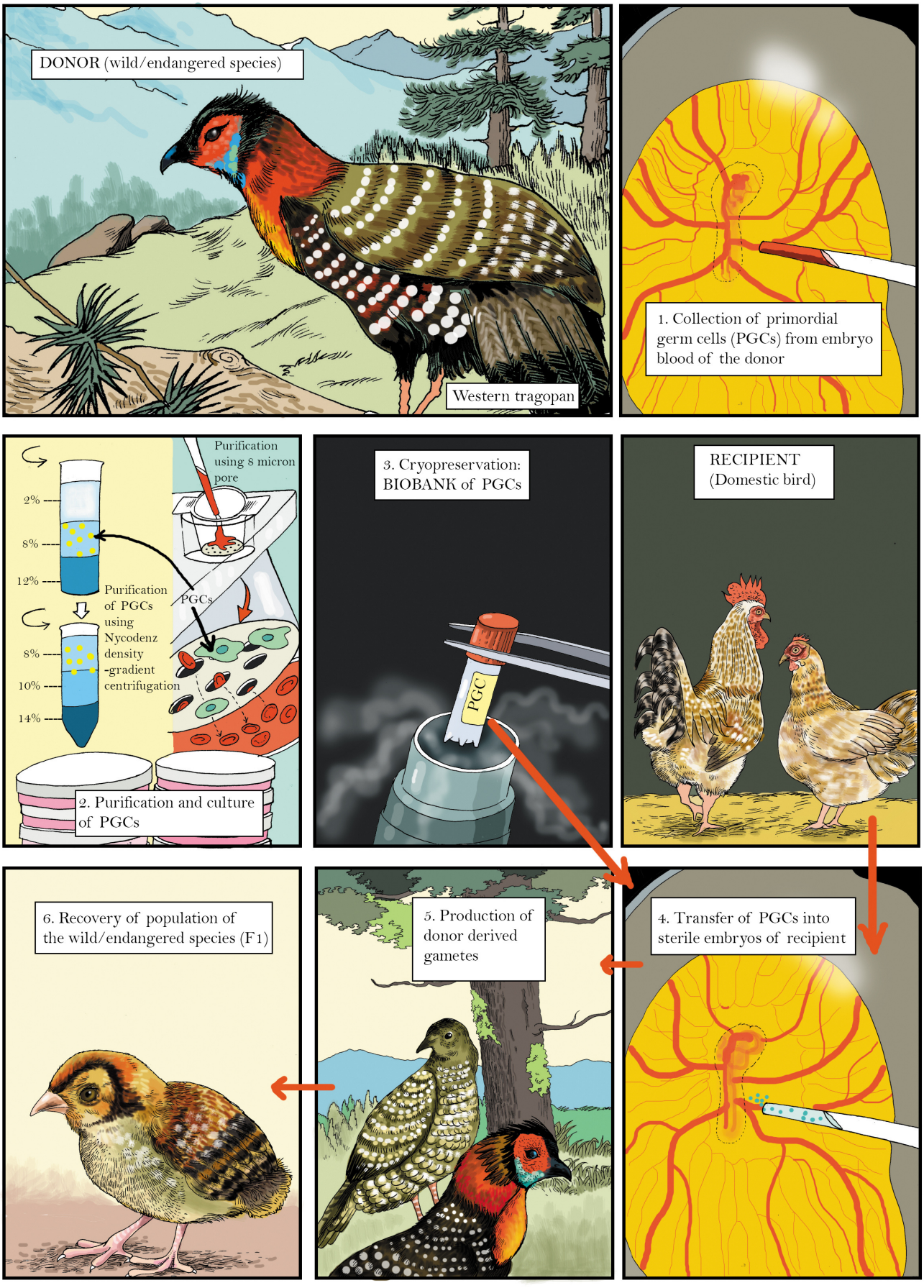
Currently, cryopreservation of semen from endangered birds has been the only avenue. However, there are challenges to preserve the female genetic material. Unlike mammalian oocytes, avian egg has large yolk content. Such eggs cannot be easily cryopreserved. Biobanking of primordial germ cells (PGCs), the precursor cells of oocytes, allows to overcome these constraints.

In birds, PGCs can be harvested either from circulating blood or gonadal ridge of the developing embryo. These germ cells can then be transferred into a common bird species as surrogate to produce the endangered bird.

The avian PGC technology is a promising new approach with great potential to produce viable offspring of endangered birds. This novel technique has already been explored in some of the wild birds such as quail, pheasant and Houbara bustard. The technology promises to revive the Indian wild birds that are facing serious threat of extinction. This includes vultures, the western tragopan, the Great Indian Bustard and the Jerdon's Courser.

This document proposes a plan to use the avian PGC technology in western tragopan (*Tragopan melanocephalus*), a conservation-dependent species found in the Himalayas.

Fig 5: Concept for using PGC technology for conservation breeding of endangered western tragopan



To use avian PGC technology to breed the western Tragopan, following steps should be followed:

1. PGCs can be harvested from the western tragopan embryo, purified and cultured *in vitro*.

2. The cultured germ cells can be transferred into the chicken embryos to produce chimeras of western tragopan. The remaining cells can be biobanked for future purposes.

3. After maturity, chimeras would produce gametes which then be backcrossed to western tragopan birds to produce live chicks of the western tragopan.

COLLABORATIVE ORGANIZATIONS IN BIOBANKING

National Wildlife Genetic Resource Bank at CCMB-LaCONES

CCMB-LaCONES has established India's first wildlife genetic resource bank in Hyderabad. It has deposits of germplasm cells, DNA and RNA from number of wild animals that have died due to natural (such as age) or other reasons (such as roadkill and neonatal mortality).

As part of the genome resource bank, the centre has optimized methods for collection, preservation and culture of oocytes for various wild species: felids, canids, primates, ungulates and, their developmental ability has also been tested (Rao et al 2010; 2011; 2015; Mahesh et al 2011). Protocols for semen cryopreservation from a variety of Indian wild animals including wild felids (Patil et al. 1998, Shivaji et al 1998, Jayaprakash et al 2001), ungulates (Sontakke et al 2007, Umapathy et al 2007, Sontakke et al 2009), and birds (Sontakke et al 2004, Umapathy et al 2005) have also been well established in their laboratory.

Further, the lab has demonstrated the fertilizing capacity of cryopreserved spermatozoa and successful pregnancy in blackbuck and birth of live chicks of blue rock pigeon using artificial insemination (Sontakke et al, unpublished data). They have also demonstrated successful recovery and cryopreservation of testicular spermatozoa from 3-5 days old post-mortem testicles of wild ungulates. Cryopreservation of testicular tissue and the developmental ability of the testicular tissue after grafting in to nude mice were evaluated in various wild species (Devi and Goel, 2016). So far, cell lines have been developed from the skin tissues of various twenty-seven wild species collected postmortem (Mahesh et al 2012; 2016), and have been stored in the genetic resource bank at CCMB-LaCONES (Table 4).

Table 4. List of species for which primary cells were developed from the tissues collected postmortem and stored in the National Wildlife Genetic Resource Bank at CCMB-LaCONES

S No	Species	Preserved material
UNGULATES		
1	Barasingha	Primary (fibroblast) cells
2	Barking deer	Primary (fibroblast) cells
3	Bison	Primary (fibroblast) cells
4	Blackbuck	Primary (fibroblast) cells
5	Chousingha	Primary (fibroblast) cells
6	Mouse deer	Primary (fibroblast) cells
7	Swamp deer	Primary (fibroblast) cells
8	Sambar	Primary (fibroblast) cells
9	Hog deer	Primary (fibroblast) cells
10	Nilgai	Primary (fibroblast) cells
11	Spotted deer	Primary (fibroblast) cells
12	Thamin deer	Primary (fibroblast) cells
13	Wild ass	Primary (fibroblast) cells
14	Wild buffalo	Primary (fibroblast) cells
FELIDS		
1	Jungle cat	Primary (fibroblast) cells
2	Jaguar	Primary (fibroblast) cells
3	Leopard	Primary (fibroblast) cells
4	Lion	Primary (fibroblast) cells
5	Tiger	Primary (fibroblast) cells
CANIDS		
1	Indian wolf	Primary (fibroblast) cells
2	Wild dog	Primary (fibroblast) cells
3	Hyena	Primary (fibroblast) cells
4	Himalayan black bear	Primary (fibroblast) cells

PRIMATES

1	Long-tailed macaque	Primary (fibroblast) cells
2	Rhesus monkey	Primary (fibroblast) cells
3	Chimpanzee	Primary (fibroblast) cells

OTHERS

1	Palm civet	Primary (fibroblast) cells

CCMB-LaCONES and Chattisgarh Forest Department's efforts in wild buffalo conservation

Wild buffalo is listed 'Endangered' in the Red Data book of IUCN due to its declining population trends. It is also listed in Schedule-I of Indian Wildlife (Protection) Act, 1972. It was declared as the state animal of Chhattisgarh in the year 2001. The Ministry of Environment and Forests (MOEF), Government of India included wild buffalo in its Species Recovery Program.

The world's total wild buffalo population is about 4000, and 92% of it is present in India. In India, wild water buffalo population is restricted to Northeast India and Central India. In Central India, most of the wild buffalo population is found in Udanti-Sitanadi Tiger Reserve (USTR), Indrāvati Tiger Reserve in Chhattisgarh, and Kolamarka sanctuary in Maharashtra. The USTR and Indravati tiger reserves are the main habitat in Chhattisgarh, while some animals are moving in Maharashtra near the area adjoining to Indravati Tiger Reserve.

Thanks to concentrated efforts of Chhattisgarh Forest Department, the wild buffalo numbers have increased (from 7) to 14 individuals at USTR. But most of them have originated from the bloodlines of two wild males (Kalia and Shyamu) and one female (Asha). Genetic diversity is one of the important components to keep up a sustainable population in any species management. Although the Indian wild buffalo was successfully cloned by ICAR-NDRI (Singla et al 2015), the application of other reproductive technologies such as artificial insemination with frozen semen, estrous synchronization, super-ovulation, embryo collection, freezing and transfer are not yet explored in this species.

CSIR-CCMB and Chhattisgarh Forest Department have been working since 2009 on conservation of wild buffalo. Recently, CSIR-CCMB submitted a proposal to Chhattisgarh Forest Department for implementation of recent advancements in genetics and reproduction to keep up the genetic diversity in the breeding programs of wild buffaloes in USTR for creating a sustainable and healthy wild buffalo population for reintroduction programs. The main component of this program is to establish the artificial insemination program for wild buffalo. This will include semen collection, cryopreservation and insemination. It will allow genetic exchange between distant populations of wild animals without translocation of the animals.

Central Zoo Authority

The Central Zoo Authority (CZA) is a statutory body of the Ministry of Environment, Forest and Climate Change, Government of India responsible for overseeing the functioning of zoos in the country. Its key mandate is to complement and strengthen the national efforts of biodiversity conservation using *ex situ* approach and promote highest standards of animal welfare in zoos. CZA oversees the functioning of zoos and provides them with technical and financial assistance to attain desired standards in animal management. There are 151 zoos and 8 rescue centres recognized by the CZA today.

Understanding and sustaining a biodiverse planet is a critical task. Historically, genetically diverse species have been preserved by protecting large-size natural habitats. With advancement in science, other conservation approaches such as assisted reproductive techniques (ARTs) and biobanking have also generated much interest and curiosity for its application on critically endangered species.

Many leading zoos in the world support *ex situ* conservation facilities. Indian zoos can also contribute greatly to the advancement of this emerging technology.

For a pilot project, CZA has identified 6 zoos across the country to be a part of a Consortium of Indian Zoos for Biobanking of Wildlife Genetic Resources.

These include:

- National Zoological Park, Delhi,
- Bannerghatta Biological Park, Karnataka,

- Padmaja Naidu Himalayan Zoological Park, West Bengal,
- Nehru Zoological Park, Telangana,
- Sakkarbaug Zoological Park, Gujarat, and
- Sepahijala Zoological Park, Tripura

The primary objective of this project would be to preserve the biological materials of wild animals from zoos by:

- Creating a consortium of zoos that would participate in the program that advances biobanking of wildlife genetic resources;
- Providing basic facilities in participating zoos to enable rescue the targeted genetic resources from wildlife;
- Creating capacity in zoos on collection and preservation of biological materials;
- Creating a network with participating zoos through an e-newsletter on the bio-banking of wildlife, and increase awareness under the aegis of Central Zoo Authority

It is expected that participation by zoos in the consortium would build capacity in zoos in various ways. It will let them access the benefits of implementing ARTs for endangered species and also access to knowledge about reproductive technologies for their breeding programs. It will also acknowledge India's leadership role especially in the South Asia region for advancing the knowledge and application of these technologies for conservation and preservation of endangered wild animal species.

CONSTRAINTS IN BIOBANKING

Collection of tissues, gametes and gonads from live animals is not always possible in wild animals. This is especially true for endangered species.

In wild populations, it is very difficult to collect and process the tissues immediately after the death of animals due to the non-availability of human resources or lack of proper transport facility to reach the sites and conduct postmortem.

Integrity of the genetic material (DNA/RNA), viability and the architecture of tissues/organs collected post-mortem is influenced by time delay between the death of the animal and sample collection and transport to the laboratory for further processing (Fig 6).

The cellular and biochemical changes and environment conditions such as temperature adversely affect the quality of cells and gametes collected from these tissues postmortem (Fig 7). Therefore, a dead animal needs to be shifted immediately after its death to a cool environment at 4-8 ^{O}C.

In addition, the methods of collection, transportation and preservation of tissues play an important role in maintaining the integrity and viability of postmortem tissues for further studies and uses.

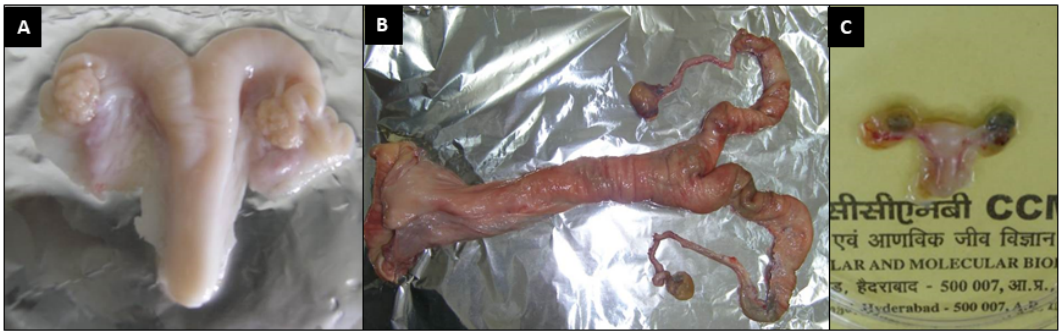


Fig 6: Effect of time delay between death of the animal and removal of reproductive tract from the body: Two hours after death (four-horned antelope); B) Thirteen hours after death (gaur) black patches indicating and C) Eighteen hours after death (marmoset). Black coloration of reproductive tract indicates necrotic changes.

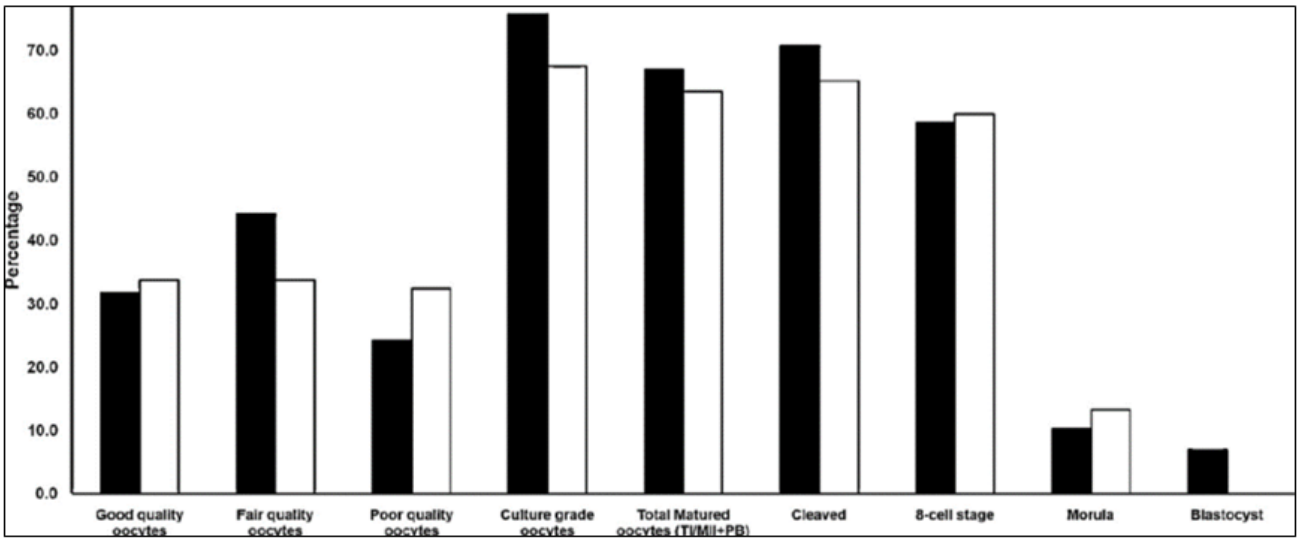


Fig 7: Effect of post-mortem interval on oocyte recovery, *in vitro* maturation and *in vitro* development of activated deer oocytes. Data is presented as percentage. No significant differences (P > 0.05) were found in oocyte quality, maturation. Recovered before (black) or after (white) 12 h after death (Rao et al 2013).

Inauguration of the National Wildlife Genetic Resource Bank at CCMB-LaCONES by Dr Harsh Vardhan, Hon'ble Minister of Science & Technology, Aug 2018

FUTURE PROSPECTS

In India, there are 151 zoos and rescue facilities that are recognized under the Wildlife (Protection) Act, 1972 (CZA Annual Report, 2019-20).

As per the CZA report, more than 50,000 animals including birds, mammals, reptiles and amphibians are housed in Indian zoos (Table 5). The annual mortality of the captive animals is around 5%. (Annual inventory of animals in Indian zoos for 2019-20). The mortality rates in rescue centres are comparatively higher than in zoos due to capture related complications. These dead animals are valuable sources of their genetic material that can be preserved in biobanks.

Table 5: List of animals in Indian zoos (2019-20)

Types of zoos	No. of animals				
	Birds	**Mammals**	**Reptiles**	**Amphibians**	**Total**
Large zoos	10819	9834	5774	27	**26454**
Medium zoos	4981	4981	3875	58	**13895**
Mini zoos	1739	4967	1640	--	**8346**
Small zoos	2837	3129	1445	--	**7411**
Rescue centres	267	928	18	--	**1213**
Total	**20643**	**23839**	**12752**	**85**	**57319**

Resource: Annual Inventory of Animals in Indian zoos -2019-20, CZA. Totals are calculated from closing stock of each zoo

The National Wildlife Genetic Resource Bank at CCMB-LaCONES has an enormous potential in enabling this. Currently, the number of sample deposits there is very low compared to the total number of species present in India. Thus, the biological samples (Table 5) of various species have to be systematically collected from different geographical regions of the country and preserved in optimal conditions. These should be easily accessible to scientists for future studies or applications.

This document strongly encourages zoos to donate biological samples of valuable wild animals to genetic resource banks and consider the potential benefit of developing reproductive technologies for their conservation breeding programs.

The samples collected at the regional biobanks will be consolidated at the National Wildlife Genetic Resource Bank in CCMB-LaCONES. The samples collected at the regional biobanks will be consolidated at the National Wildlife Genetic Resource Bank in CCMB-LaCONES. The zoos that are part of this present consortium have diverse collection and are from varied landscapes, from the Himalayas to the plains. They house several species of native and non-native fauna, and a proportion of these animals are endangered. In addition, these zoos also play a significant role in wildlife conflict mitigation. Often, in these situations the animals cannot be released back into the wild and are housed permanently in the zoos. Thus, zoos become good baselines to get involved with genetic repositories.

Resources at the zoos in the consortium with CZA and CCMB-LaCONES

SAMPLE COLLECTION FOR BIOBANKS

Materials needed for collection of biological samples

It is always better to maintain a kit for collection of biological samples as given in Table 6 and Fig 8 and should be always ready for an emergency call.

Table 6: Biological sample collection kit

S No	Material	Purpose
01	Savlon/soap/alcohol	Cleaning of collection site
02	Shaving blades or clipper	To remove hair on the skin
03	70% alcohol	Disinfectant
04	Sterile tissue forceps	Excision of tissues
05	Sterile scissors and blades	
06	Disposable syringes	Collection of body fluids
07	Sterile polythene zip lock covers of different sizes	Collection and transportation of tissues
08	Sterile swabs and containers of different sizes	Collection and transportation of tissues
09	Sterile test tubes of different volumes	Collection and transportation of body fluids
10	Blood collection vacutainers of coated with different anticoagulants	Blood collection/serum separation
11	Blood collection needles of different gauze and scalp sets	Blood collection
12	PBS solution or tablets/ Normal saline/ DMEM	Storage and transportation
13	Microscopic glass slides and cover slips	Smear for blood protozoan screening
14	Permanent markers	Labeling
15	Ice packs and cool box	Maintain low temperature during transportation

Fig 8: Biological sample collection kit as mentioned in Table 6

Table 7: List of biological samples, purpose and transportation conditions for submitting to Wildlife Genetic Resource Bank

Biological samples	Live/dead	Purpose of collection	Collection medium	Transportation conditions
Blood	Live	DNA/RNA/serum	Blood collection containers/ vacutainers coated with anticoagulants, are available in the market.	Refrigeration temperature (4-8 ^{O}C)
Semen	Live	Cryopreservation, artificial insemination & *in vitro* fertilization (IVF)	Required special media depending on the species	
Skin	Live/dead	Primary cells	Phosphate Buffered Saline (PBS) or Normal Saline (NS) or any cell culture medium (DMEM/M199) supplemented with antibiotics (strepto-peccillin or gentamycin) in 15 or 50 ml test tubes	Refrigeration temperature (4-8^{O}C)
Hair/feather	Live	Blood feather for DNA; others for ICPMS, Stable isotope analyses	in a sterile zip lock polythene covers	Refrigeration temperature (4-8 ^{O}C)
Testicles	Dead	Spermatozoa from epididymis for IVF, cryopreservation of testicular tissue for grafting	Phosphate Buffered Saline (PBS) or Normal Saline (NS) or any cell culture medium (DMEM/M199) supplemented with antibiotics (strepto-peccillin or gentamycin) in 15 or 50 ml test tubes	Refrigeration temperature (4-8 ^{O}C)

Ovaries	Dead	Oocyte collection, cryopreservation & IVF	Phosphate Buffered Saline (PBS) or Normal Saline (NS) or any cell culture medium (DMEM/M199) supplemented with antibiotics (strepto-peccillin or gentamycin) in 15 or 50 ml test tubes	Refrigeration temperature (4-8 °C)
Other tissues: heart, lung, liver, spleen, kidney	Dead	DNA/RNA/disease diagnosis	Sterile swabs are available in the market. If refrigeration facilities are not available, they can be collected in 5 to 10 ml of sterile 50% buffered glycerine solution.	Refrigeration temperature (4-8 °C)
Pregnant uterus	Dead	Amniotic fluid and umbilical cord for developing cells	Sterile zip lock polythene cover or 50 ml test tubes, depends on the size of the gravid uterus	Refrigeration temperature (4-8 °C)
Eyes	Dead	Lens	Sterile zip lock polythene cover or 15 or 50 ml test tubes	Refrigeration temperature (4-8 °C)
Egg shells	Freshly hatched	ICPMS and DNA	Sterile zip lock polythene covers	Refrigeration temperature (4-8 °C)

General precautions for collection of postmortem samples

The time and method of collection, transportation and preservation of tissues are the important factors for successful biobanking and outcome of the samples intended for preservation as described in details in the manual on 'Biological sample collection and preservation for genetic, reproductive and disease analyses'. (http://cza.nic.in/uploads/documents/publications/english/Fina%20A5%20Manual%20(1)%20(1).pdf).

Briefly, the following steps should be followed for collection of biological samples:

1.Biological samples from dead animals should be collected as early as possible after the death of animal to avoid tissue damage associated with postmortem changes. It is always better to place the whole carcass in low temperature areas such as cold room (4 ^{O}C) as quickly as possible.

2. All the samples should be collected under aseptic conditions to avoid contamination during culture. The tissue samples should be excised with sterile tissue forceps and surgical blade or scissors.

3. Cleaning of the sample site especially for skin tissue is one of the most important factors for sterile cultures. The sampling site should be cleared off hair and washed with disinfectant, and finally sterilized with 70% alcohol before excision of the tissue. Multiple samples are preferred to increase the success rate of cell culture establishment. Ear is the easiest place to collect skin tissue but cartilage in the ear limits the attachment of skin explants to the culture dish/flask. The flank region or lower abdomen or base of the tail is the other choices for collection of skin tissue. After excision, skin tissue sample should be washed 3-4 times in sterile PBS, once in 70% alcohol (30 sec) and again 3-4 times in PBS before transferring into a test tube.

4. The containers or tubes that carry tissue should be labelled with the following information: Date and place of the collection, species, animal Id/name, age, sex, cause and time of the death.

5. Tissues should be preserved in appropriate medium immediately after collection (Table 7). Phosphate buffered saline (PBS), DMEM are commonly

used media to preserve the tissue samples. PBS is available as tablets (1 tablet for 200 ml) and it is very easy to prepare. Normal saline also can be used for preservation of tissues. All the media should be supplemented with antibiotics (Gentamycin solution 0.1 mg/ml).

6. If medium is not readily available, especially in field conditions, tissues (skin or ovaries) should be placed in sterile polythene zips lock bags and kept in a cool place. Tissues should be kept in refrigerator before sending to laboratory. During transportation, the tissues should be placed in a cool box that is maintained at 4-8^{0} C.

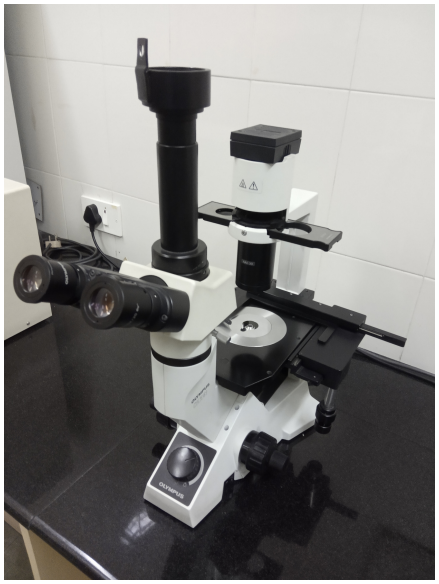
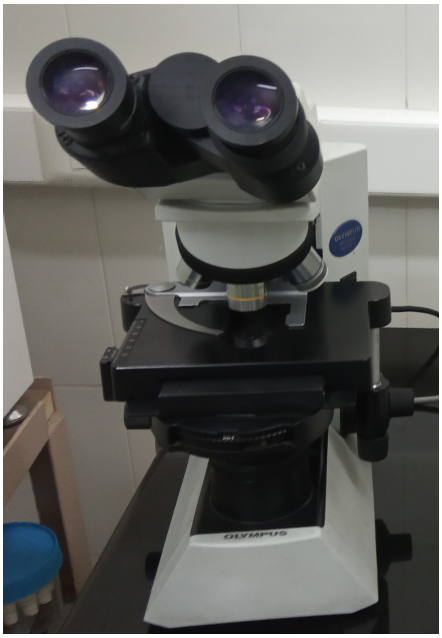
7. Always collect the samples in duplicate, and each tissue sample should be placed in separate vials.

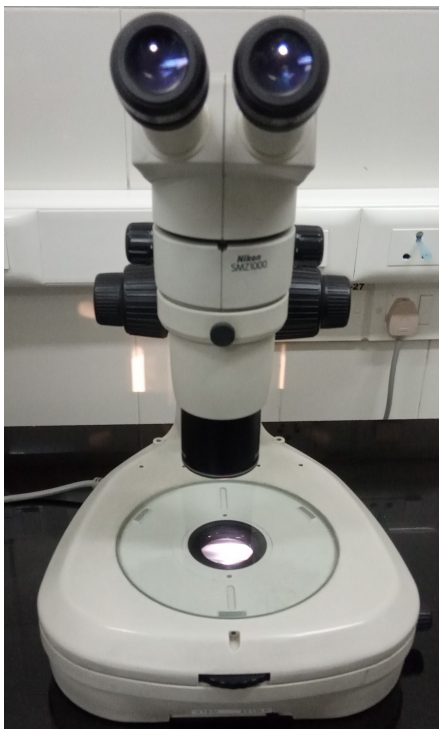
8. Label the samples with details.

9. Tissues can be also obtained from the live animals by excision of the hanging tissues from the injuries caused by accidents. A small piece of skin can be excised from ear, neck, flank or lower abdomen by sterile scalpel blade, scissors or biopsy needle under anaesthesia.

Table 8: Basic equipments required for establishment of biobanks

Name of equipment	Required number	Location	Purpose
Biosafety cabinet - Class II 	2	Culture room & anteroom for sample handling	To handle and process the biological samples for various purposes: collection, culture and cryopreservation of gametes or tissues and DNA/RNA isolation, cell culture etc., to protect to handlers and environment from the known or unknown pathogens (viruses, bacteria, fungi) associated with the biological samples, to provide contamination-free environment for processing of biological samples

Phase contrast inverted microscope with camera 	1	Culture room	For observing the growth of the cells, oocytes and embryos
Upright microscope with phase contrast 	1	Culture room	To estimate the viability, motility and concentration of the spermatozoa as well as count the cells before and after cryopreservation

Stereo zoom microscope 	1	Culture room	For isolation and handling of the oocytes and embryos, tissue dissection
CO_2 incubator 	1	Culture room	To develop cells from the tissues collected post-mortem and for maturation of the rescued gametes and culturing of the cells and embryos
Refrigerator 	1	Sample handling/ processing room	For storing chemicals, enzymes, media and reagents for culture and preservation of cells, gametes and tissues. Refrigerator is also useful for storing DNA/RNA and tissues for short time preservation

Water bath 	1	Sample handling/ processing room	For warming of the reagents prior to preparation of solutions and for thawing and warming of the cells, oocytes, embryos, spermatozoa and tissues
Refrigerated centrifuge 	1	Culture room	To separate the cells and spermatozoa prior to preservation
Consumables 			Consumables such as media, reagents, chemicals, plastic ware, culture dishes, micropipettes etc. are necessary to run the facility.

Forms for submitting biological materials at the National Wildlife Genetic Resource Bank

SAMPLE DEPOSIT FORM

Date: ----------

1. ***Details of the Depositor:***

   Agency: Government/Private:

   Name and Address of the Organization/Institute:

   Authority holding the Animal/Plant (Govt/Private):

2. ***Details of the Donor:***

Class:	*Order:*
Family:	*Genus:*
Species:	*Species:*
Common /local name:	*Subspecies:*
Location:	*Name of the organism:*
House Name:	*Identification No:*
Studbook No:	*Date of Birth:*
Accession no. if present:	*Age:*
Type of Collection: *(captive born/ wild)*	*Sex:*

3. ***Details of the Biological Sample(s)***

Date of the Sample(s) Collected	*Collected from (live or post-mortem)*
Name of the sample(s):	
Name of the preservative (if added):	*Cause of Death: If sample is collected post-mortem*
Purpose of the sample:	
Comments if any:	

I/We_____ **on behalf of** _____**am/are submitting the abovementioned biological samples to National Wildlife Genetic Resource Bank (NWGRB) at CCMB-LaCONES for biobanking purpose. I/We authorize NWGRB to biobank the materials and their derivatives in NWGRB for research on wildlife conservation in India only.**

Signature of the Authorized Official
With Designation

----For official use----

Date:

Curator NWGRB, CSIR-CCMB

National Wildlife Genetic Resource Bank *(NWGRB) ID:____*

Class:	*Order:*
Family:	*Genus:*
Species:	*Subspecies:*
Common /local name:	*Authority holding the animal/plant (Govt/Private):*
Location:	*Name of the Animal:*
House Name:	*Identification No:*
Studbook No:	*Date of Birth:*
Accession no if present:	*Age:*
Type of Collection: (captive born/ wild caught)	*Sex:*

Sample Information:

Sample Date	*Type of sample*	*Tissue piece preservation*	*Freezing Date*	*Passage No*	*Contami-nation*	*Location*				
						80ºC/-20ºC	*LN_2Tank No/-*	*Rack No*	*Box No*	*No Vials*

Shipping Information

Date	*Recipient*	*Sample type*	*Purpose*	*Comments*
	Name:			
	Institution:			

Date:

Curator NWGRB
CSIR-CCMB

REQUISITION FORM

Requisition form for tissue/cells/DNA/RNA/semen from
National Wildlife Genetic Resource Bank **(NWGRB).**

1. Name of the Applicant:
2. Designation:
3. Name of the Organization/Institute:
4. Address of the Organization/Institute:
5. Name of the material and species requested from NWGRB:
6. Quantity of the material:
7. Purpose of the sample: (please provide the information in 100-150 words).

Declaration form duly filled in enclosed.

Signature of the applicant
Date:

Signature
Head of the Institute
with Seal and Address
Date:

----For official use----

Curator NWGRB
CSIR-CCMB

DETAILS FOR CCMB-LaCONES

(to be made available to users)

1. ***Details of the Depositor:***

   Agency: Government/Private:

   Name and Address of the Organization/Institute:

   Authority holding the Animal/Plant (Govt/Private):

2. ***Details of the Donor:***

Class:	*Order:*
Family:	*Genus:*
Species:	*Subspecies:*
Common /local name:	
Location:	*Name of the organism:*
House Name:	*Identification No:*
Studbook No:	*Date of Birth:*
Accession no if present:	*Age:*
Type of Collection: *(captive born/ wild caught)*	*Sex:*

3. ***Details of the Biological Sample(s)***

Date of the Sample(s) Collected	*Collected from(live or post-mortem)*
Name of the sample(s):	
Name of the preservative (if added):	*Cause of Death: If sample is collected post-mortem*
Purpose of the sample:	
Comments if any:	

Date:

Curator NWGRB
CSIR-CCMB

DECLARATION FORM FOR APPLICANT

Declaration by the Applicant(s) and Head of the Organization/Institute

I/We_____, Dept._____, Institute/Organization_____am/are declaring that the bio-specimen samples of the following -

S.No	Name of the Sample	Name of the Species	NWGRB ID.

Received from the National Wildlife Genetic Resource Bank (NWGRB), LaCONES, CSIR-CCMB will be used exclusively for bona fide research purpose on wildlife conservation in India, and not for any commercial purpose.

I/We abide by the provision of the Wildlife Protection Act (1972) and Biodiversity Act (2003) for access/use to the abovementioned samples.

I/We agree to abide by the terms and conditions laid out for accessing the abovementioned samples.

Signature of the Applicant
Date:

Signature
Head of the Institute
with Seal and Address
Date:

MEMORANDUM OF UNDERSTANDING

Between
Central Zoo Authority, Government of india
and
CSIR-CCMB, HYDERABAD
and
Participating zoo_____

Central Zoo Authority (CZA) is a statutory body under the Ministry of Environment, Forest and Climatic Change, Government of India. CZA coordinates to implement national policies related to zoo management, health of zoo animals, conservation breeding programs and research on zoo animals.

Laboratory for the Conservation of Endangered Species (LaCONES) is a dedicated national laboratory of CSIR-Centre for Cellular & Molecular Biology (CSIR-CCMB), Hyderabad for wildlife research and conservation in India. For the last two decades, LaCONES has made significant contributions in wildlife research and conservation. LaCONES houses National Wildlife Genetic Resource Bank (NWGRB) .

Participating zoo: _____

This Memorandum of Understanding is entered on day of _____2019 at CGO Complex, Central Zoo Authority, Government of India (hereinafter referred to as "CZA"), between Laboratory for the Conservation of Endangered species [LaCONES], CSIR-Centre for Cellular and Molecular Biology (CSIR-CCMB) (hereinafter referred to as "CCMB-LaCONES"), Hyderabad represented by NAME _____Director of CCMB and the NAME, _____Member Secretary, CZA Government of India AND name and designation of the authorized officer_____ [PCCF/Director], address of the PARTICIPATING ZOO _____for biobanking of wildlife genetic resources for strengthening of conservation breeding programs by submitting biological samples to National Wildlife Genetic Resource bank at CCMB-LaCONES.

The CZA, PARTICIPATING ZOO_____and CCMB-LaCONES have agreed on the importance of bio-banking of wildlife and the infrastructure available at CCMB-LaCONES, Hyderabad and welcomed mutual cooperation for biobanking of Wildlife Genetic Resource Bank.

Now, therefore, the CZA, PARTICIPATING ZOO_____& CCMB-LaCONES wish to formalize the understanding as follows:

1.CCMB-LaCONES will provide technical support/inputs to 1. Establish/develop methods for collection and transportation of samples, 2. Maintenance of the preserved samples, 3. Train veterinarians, zoo biologists for collection and transportation of the samples. 4. Release e-newsletter on bio banking of wildlife genetic resources.

2.CCMB-LaCONES will provide technical support to establish required facilities to keep the dead animals and biological samples in zoos, National Wildlife Sanctuaries or Zoological Parks before shifting to NWGRB.

3.CZA will provide financial support to establish cold rooms in the participating zoo, training programs and e-newsletter.

4.CZA will facilitate to participate the institutes for strengthening NWGRB at CCMB-LaCONES.

5.Participating Institutes/zoos will contribute biological material of their wild animals to NWGRB at CCMB-LaCONES by collecting postmortem samples as well as samples from live animals as and when the animals tranquilized.

6.Participating Institutes/Zoos will follow the guideline made by the CCMB-LaCONES for collection and transportation of the samples.

7.Participating Zoo will have access to their stored samples always. They will also have access to assisted reproductive technologies to be used for breeding programs of animals in their collection with the help of CCMB-LaCONES.

8.Utilization of bio banking samples: 1. The samples preserved in NWGRB at CCMB-LaCONES will be accessible to CZA and participating zoo. 2. The samples preserved in NWGRB at CCMB-LaCONES will be permitted to use for wildlife research purposes only.

9.Sample accessions will be shared with CZA and Participating Zoo.

10.Access to samples will be made to third party member after requisition form (Annexure1) is submitted with necessary support documents and approval form from the concerned zoo and CZA is obtained.

For CZA, Government of India **For Participating Zoo**

For CCMB-LACONES

REFERENCES

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