International e-Conference
on
RECENT ADVANCES IN REPRODUCTIVE TECHNOLOGIES IN WILDLIFE CONSERVATION

Schedule and abstracts

About the Conference

As part of Advancements in Veterinary Sciences for Wildlife Conservation (ADVETCON) conference series, we at the Laboratory for the Conservation of Endangered Species (LaCONES), CSIR-Centre for Cellular and Molecular Biology, Hyderabad, extend a warm welcome and cordially invite you all to take part in the International e-conference on "Recent Advances In Reproductive Technologies In Wildlife Conservation" from 11th to 13th of November 2021. The aim of this conference is to highlight the current status of reproductive technologies in wildlife conservation and management. Due to anthropocene, multiple species have come under severe threat of extinction. The efforts to prevent the extinction of these species require intensive conservation methods. Modern reproductive technologies can contribute better conservation and management of these endangered species, both in captive and free ranging. The conference is organised for students, wildlife biologists, veterinarians, conservationists, scientists, forest and zoo mangers for understanding the current status of reproductive technologies in wildlife conservation.

The Laboratory for the Conservation of Endangered Species (LaCONES), is a part of the national laboratory of CSIR-Centre for Cellular & Molecular Biology (CCMB), dedicated to wildlife research and conservation in India. For the last two decades, LaCONES has made significant contributions in wildlife research such as wildlife forensics, assisted reproduction, reproductive and stress physiology, conservation breeding, evolutionary and population genetics and has also established Wildlife Genetic Resource Bank (http://e-portal.ccmb.res.in/lacones/).

Themes of the Conference

This conference aims to bring together eminent scientists and experts working in conservation breeding and reproductive technologies. The major themes of the conference are -

1. Conservation breeding of endangered species
2. Reproductive technologies in conservation and management
3. Cryopreservation and genome resource banking

International e-Conference on

Recent Advances In Reproductive Technologies In Wildlife Conservation

Schedule

Timings - Indian Standard Time

Inaugural session		
Time:	**Moderators: S.Manu and Vinay Teja**	
17.30-17.35	**Opening remarks**	**Karthikeyan Vasudevan** Scientist In- charge, LaCONES, CCMB
17.35-17.40	**Inaugural Address**	**Sonali Ghosh** DIG of Forests, Central Zoo Authority, New Delhi
17.40-17.45	**Inaugural Address**	**Vinay Kumar Nandikoori** Director, CCMB
17.45-17.55	**Chief guest Address**	**Sekhar Mande** Secretary, DSIR and Director General, Council of Scientific & Industrial Research, Govt. of India
18.00-18.05	**Vote of thanks**	**G. Umapathy,** Convener of ADVETCON, 2021
Day 1 Nov 11, 2021 Session no. 1 **Moderator: G.Umapathy**		
.18.10-19.00	**Plenary and inaugural speaker**	**Janine Brown** Smithsonian Conservation Biology Institute USA Topic: *Hormone monitoring as an important tool to aid breeding management of wildlife species*
Day 2 Nov 12, 2021 Session no. 2 **Moderators: Sambasiva Rao and Vinod Kumar**		
9.00 - 9.45	**Invited speaker**	**Chatchote Thitaram** Center of Elephant and Wildlife Health, Chiang Mai University, Thailand Topic: *Welfare of captive elephants in Thailand: implication for reproductive technology*
9.45 - 10.30	**Invited speaker**	**Edward Narayan** Faculty of Science The University of Queensland, Australia Topic: *Non-invasive monitoring of stress physiology during management and breeding of amphibians*

Day 2 Nov 12, 2021 Session no. 3 Moderators: Mihir Trivedi and Gopi krishnan		
10.40 - 11.25	**Invited speaker**	**Vibhu Prakash** Bombay Natural History Society Topic*: The Conservation Breeding Programme of three critically endangered Gyps species in India*
11.25 - 12.10	**Invited speaker**	**Parag Jyoti Deka** Durrell Wildlife Conservation Trust, Pygmy Hog Conservation Programme, Assam Topic: *How we breed pygmy hog in captivity?*
12-10 - 12.55	**Invited speaker**	**Gowri Mallapur** Central Zoo Authority of India Topic: *Husbandry implication for reproductive success in reptiles and ART*
Day 2 Nov 12, 2021 Session no. 4 Moderators: G.Umapathy and Anusha Kiran		
14.00 - 14.45	**Invited speaker**	**Andre Ganswindt** Mammal Research Institute University of Pretoria, South Africa Topic: *Non-invasive endocrine monitoring for assessing reproductive function in wildlife for conservation and management*
14.45 - 15.30	**Invited speaker**	**Joseph Saragusty** Faculty of Veterinary Medicine, University of Teramo, Italy Topic: *Reproductive biotechnologies to save the functionally extinct northern white rhinoceros*
15.30 - 16.15	**Invited speaker**	**Isabel Callealta** ECO lifes (Wildlife reproduction and ultrasonography), Spain Topic: *Reproductive management and assisted reproduction techniques in lions*

Day 2 Nov 12, 2021 - Session no. 5
16.15 -17.40 Short presentations
Moderators: Sambasiva Rao and Manisha Ray
10 mins each

1. *Reproductive Ultrasonography in zoo and wildlife* ***- Mozammel Hoque****, ICAR-Indian Veterinary Research Institute, Izatnagar.*
2. *Ultrasonographic studies on reproductive organs in captive chelonians -* ***Divya Ganesh****, Karnataka Veterinary, Ani. & Fisheries Sci. Univ. Bidar, Karnataka*
3. *Comparisons of two approaches (midline and lateral incisions) for vasectomy in leopards (Panthera pardus fusca)-* ***Nikita Salian****, Karnataka Veterinary, Ani. & Fisheries Sci. Univ. Bidar, Karnataka*
4. *A method for storage, preservation and transportation of anuran urine samples using filter paper for hormone analysis-* ***Vinod Kumar****, LaCONES, CCMB,*

Day 2 Nov 12, 2021 Session no. 6
Moderators: G.Umapathy and Aamer Khan

18.00-18.45	**Invited speaker**	**Budhan Pukazhenthi**, Smithsonian Conservation Biology Institute, USA Topic: *Status of assisted reproductive technologies for endangered species: opportunities and challenges*
18.45-19.15	**Invited speaker**	**Ursula Bechert,** SpayVac for Wildlife, USA Topic: *SpayVac®-pZP applications in wildlife population fertility control*
19.15-20.00	**Invited speaker**	**Douglas C. Eckery** USDA APHIS WS National Wildlife Research Center,USA Topic: *Development of Fertility Control Tools for Wildlife Management*

Day 3 Nov 13, 2021 Session no. 7 **Moderators: Sambasiva Rao and Gopi Krishnan**		
9.30-10.15	**Invited speaker**	**Satish K Gupta** Basic Medical Sciences Division, Indian Council of Medical Research, New Delhi- Topic: Immuno-contraception: an emerging option for wildlife population management
Day 3 Nov 13, 2021 Session no. 8 **10.15 -12.15 Short presentations** **Moderators: Sambasiva Rao and Vinod Kumar** **10 mins each**		
1. *Dreadful Delta Variant COVID19 in Asiatic Lions (Panthera leo persica)- **K.Sridhar**, Arignar Anna Zoological Park, Chennai* 2. *Nutritional Secondary Hyperparathyroidism (NSH) in a Royal Bengal a Tiger Cub-a case report **- Ramesh Tiwary**, Bihar Animal Sciences University, Patna* 3. *Reproductive and stress physiology of wild hangul Cervus hanglu in Kashmir Himalaya- Tanusree Srivastva, LaCONES, CSIR-CCMB* 4. *Determination of estrous phases and behavioural changes in captive Asian elephants.- **Madhvee Dhairykar**, School of Wildlife Forensic and Health, NDVSU, Jabalpur (M.P.).* 5. *Predictors of Adrenal and Gonadal Hormones in Relation to Biological and Management Factors Among Captive Red Pandas in Indian Zoos- **Aamer Khan**- LaCONES, CCMB* 6. *Captive Breeding of Critically Endangered Cotton-top tamarin Saguinus oedipus (Linnaeus, 1758) at the Sardar Patel Zoological Park, Gujarat, India **- Ravikumar Patel**, Sardar Patel Zoological Park, Gujarat, India*		
Day 3 Nov 13, 2021 Session no. 9 **Moderators: Sambasiva Rao and Aamer Khan**		
12.15 – 13.00	**Invited speaker**	**Naresh Selokar** Animal Biotechnology Centre ICAR-National Dairy Research Institute, Karnal, India Topic: Buffalo cloning technology for conservation of endangered bovine species
Day 3 Nov 13, 2021 Session no. 10 **Time 16.00-17.30** **Panel discussion on "Role of policy, habitat management, zoo, conservation breeding and reproductive technologies in conservation of endangered animals in India"** **Moderator: Mihir Trivedi and Aamer Khan**		
Prof. Sukumar, CES, IISc **Dr. B.P. Ravi, Karnataka Zoo Authority** **Dr. Goutam Narayan, Pygmy hog breeding centre, Assam** **Dr. Sonali Ghosh, Central Zoo Authority** **Dr. G.Umapathy, LaCONES, CSIR-CCMB**		
Concluding remarks: Dr.Sambasiva Rao, Convener, ADVETCON 2021.		

ABSTACTS OF INVITED SPEAKERS

Janine L. Brown

Smithsonian Conservation Biology Institute, USA

Hormone monitoring as an important tool to aid breeding management of wildlife species

Janine L. Brown
Smithsonian Conservation Biology Institute, Center for Species Survival, Front Royal, Virginia 22630, USA, email: brownjan@si.edu

As more species reach vulnerable or endangered status, captive facilities are increasingly being tasked with sustaining genetically healthy populations in case of catastrophic extinctions. Unfortunately, many of those populations are not self-sustaining. It is clear now that the ability to track reproductive activity via hormones is key to developing successful ex situ breeding programs, through both natural and assisted reproductive means. In our 30+ years of hormone monitoring activities, we have observed a diversity of reproductive mechanisms among taxa. For elephants in particular, a major focus in our laboratory, hormone monitoring and other biomarker analyses of blood, urine, saliva, and feces has been critical to understanding basic species biology, but also how factors in the captive environment impact, health, reproduction and welfare. As an example, two decades of studies on ovarian acyclicity led to discoveries of how important sociality and environmental enrichment and diversity are to pituitary-ovarian function. To take advantage of the latest reproductive technologies, the ability to monitor hormones is absolutely essential. In elephants, the double luteinizing hormone surge now is used routinely to time artificial insemination, as well as natural breeding. Understanding reproductive mechanisms is vital to effective management, particularly insurance populations in captivity, and to that end, long-term biological databases are key to understanding how intrinsic and extrinsic factors affect reproductive function at individual and population levels.

Chatchote Thitaram

Center of Elephant and Wildlife Health,
Chiang Mai University, Thailand

Welfare of captive elephants in Thailand: implication for reproductive technology

Chatchote Thitaram[1,2], Pakkanut Bansiddhi [1,2], Jaruwan Khonmee [1,3], Janine L. Brown [1,4]
1-Center of Elephant and Wildlife Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand; 2-Department of Companion Animals and Wildlife Clinics, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand; 3-Department of Veterinary Bioscience, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand; 4-Center for Species Survival, Smithsonian Conservation Biology Institute, Front Royal, VA 22630, USA. *Correspondence: chatchote.thitaram@cmu.ac.th ; Tel.: (+66)53-948015

There are more than 3,500 captive elephants in Thailand used in tourist and legal logging industries. The welfare of these elephants has been a topic of intense debate among the public, scientists and stakeholders, as they are exposed to a range of working conditions and management practices. Given that stress and poor welfare can negatively impact reproduction, it is important to identify best practices for managing elephants to ensure population sustainability. Here we describe welfare assessment tools we have used to study how captive management impacts the biology of Asian elephants in range countries. Results indicate that captive facilities could improve the welfare of elephants through better management practices that take into account physiological and psychological needs of elephants, including social and nutritional requirements, providing good health care, and maintaining adequate facilities. Elephant facilities must adjust their management to prevent elephants being overweight or obese; for example, by reducing the amount of supplementary diets with high energy food stuffs and dividing food into several meals. To reduce foot problems, avoid letting elephants stand or walk on hard surfaces and control the walking distance so it is not too long. Preventing wounds requires appropriate use of control tools (e.g., ankus, chains) and providing a sleeping ground with softer surfaces. Improving management to reduce stress is key; such as, allowing the elephants to stay in a natural area, ensuring they exercise; namely, walking or having social interaction with one another, and controlling the workloads so they are not excessive. The results should be developed into scientifically based standards or guidelines for the development of better elephant management in the tourism business and effort to create healthy, self-sustaining populations in the future. Part of that effort will require developing reproductive technologies for captive propagation, but possibly also for control of elephants in overpopulated areas in the wild. There are numerous challenges to breeding elephants due to the large body size, and length of the reproductive cycle and gestation periods. Here we should consider and balance the advances in reproductive technology, with conservation and welfare of our gentle giants.

Edward Narayan

Faculty of Science
The University of Queensland,
Australia

Non-invasive monitoring of stress physiology during management and breeding of amphibians

Dr Edward Narayan, Senior Lecturer, School of Agriculture and Food Sciences
Affiliate Senior Research Fellow, Queensland Alliance for Agriculture and Food Innovation (QAAFI),
School of Agriculture and Food Sciences, Faculty of Science
The University of Queensland, Email : e.narayan@uq.edu.au

Amphibian stress endocrine system is responsive both to acute and chronic factors associated with the captive environment. Stress responses should be closely monitored in amphibian breeding programs because the stress endocrine system interacts with reproductive axis and influences breeding performance. Chronic stress can also impact on the successful integration of amphibians into conservation breeding programs. Minimally invasive hormone monitoring using glucocorticoid hormone (corticosterone), such as urine-sampling, buccal swabs, and water-borne methods provide easy and reliable means of tapping into the stress responses of amphibians. These hormone monitoring tools can be used to monitor stress responses in contexts such as social groupings, transportation, capture, handling, and artificial breeding stimulations. Thus long-term baseline hormonal data could be used to gauge the success of conservation breeding programs (encompassing both captive breeding and release) such as improvements of genetic stock and inter-institutional evaluation of species' performance and fitness in relation to health, welfare, and breeding success.

Vibhu Prakash

Bombay Natural History Society
India

The Conservation Breeding Programme of three critically endangered Gyps species in India

Vibhu Prakash, Dy. Director
Bombay Natural History Society
v.mathur@bnhs.org

Populations of three resident Gyps species of vultures, the Oriental White-backed vulture Gyps bengalensis, the Long-billed vulture Gyps indicus and the Slender-billed vulture Gyps tenuirostris have declined by over 99% since the 1990s. The overall cause of declines was identified as a veterinary drug called diclofenac.

The Conservation Breeding of three critically endangered Gyps species of vultures was initiated in 2004 as an insurance against extinction. Eight vulture conservation breeding centres (VCBC) have been established in the country of which four are being managed by Bombay Natural History Society in collaboration with state forest departments. So far, 234 Oriental White-backed, 190 Long–billed and 65 Slender-billed vultures have hatched and fledged at the four VCBCs. At Pinjore centre, artificial incubation, double clutching and chick swapping are carried out to increase their productivity.

The first clutch egg is removed within 10-15 days of laying and is incubated artificially. The birds lay a second clutch usually within 3 weeks. The eggs in incubators hatch around 55 days. First clutch nestlings are hand reared for ten days. Thereafter they are given back to the parents. The second clutch egg is removed and hatched artificially and chick is hand reared. So far, 329 nestlings have been hatched by artificial incubation.

Parag Jyoti Deka

Durrell Wildlife Conservation Trust, Pygmy Hog Conservation Programme, Assam

How we breed pygmy hog in captivity?

Parag Jyoti Deka

Durrell Wildlife Conservation Trust, Pygmy Hog Conservation Programme, Indira Nagar, Basistha, Guwahati, Assam 781029, India, parag.deka@durrell.org
Aaranyak, Threatened Species Recovery Programme, Tayab Ali Bye Lane, Bishnu Rabha Path, Beltola, Guwahati, Assam 781028, India

Pygmy hog conservation programme (PHCP) is a population recovery programme for Pygmy Hog (Porcula *salvania*) which involved conservation breeding, sub-Himalayan grassland habitat restoration and reintroduction. Founding a captive breeding population with a small number of funder was a challenge for PHCP for long term management of a population in captivity and preparing for reintroduction. However, proper studbook management and breeding planning involving modern methods and tools helped PHCP to follow the goal. Population management methods and tool like studbook software SPARKS (Single Population Analysis and Records Keeping System) was used to maintain the captive pygmy hog population in captivity. At the same time, Population Management / Analysis Software PM2000/PMx provided tools for genetic and demographic analyses and management of pedigreed and breeding planning captive populations. Recent studies confirm that these scientific approaches of conservation breeding of PHCP aided in the retention of the genetic diversity in the captive bred pygmy hog. Till the date PHCP breed 187 litters (722 births) at the two breeding facilities in Assam, 142 hogs released into the wild and maintain: 80+ hogs in captivity.

Gowri Mallapur

Veterinary Consultant, Central Zoo Authority

New Delhi

Reptiles- health, welfare and reproduction

Gowri Mallapur- Veterinary Consultant, Central Zoo Authority, New Delhi

vets-cza@govcontractor.in

The class Reptilia encompasses thousands of species globally, grouped as chelonians, crocodilians, squamates, and the Rhynchocephalia. Since their emergence 310–320 million years ago, reptiles have evolved to be one of the most adaptive and remarkable groups of vertebrate animals on earth. They can be found in diverse niches ranging from the arid desert to the tropical, they can be arboreal or fossorial in habit. The diversity of the species within this class can make work challenging.Along with species diversity comes the diversity in reproductive physiologies and behaviors, with adaptive characteristics such as parthenogenesis and development of venom glands for prey immobilization.

Reptile health management is complex and dependent on understanding of the species complexities and ecological niches that are occupied. Infectious diseases have caused a decline in captive and wild populations of reptiles. Contributing factors for the decline, and their increased susceptibility to infectious diseases, include environmental pressures, such as loss or decline of habitat, exposure to pollutants, and translocation into new habitats. Emerging disease trends like snake fungal disease, and monitoring the same in wild populations as added a dimension to the contemporary medicine practice. In captivity, reptiles are heavily dependent on appropriate husbandry practices to ensure health, both physiological and psychological. Reptiles have adopted a vast number of reproductive strategies. Many of the life-history features in which reptiles differ from endothermic vertebrates—such as their small offspring sizes, large litter sizes, and infrequent reproduction—are direct consequences of ectothermy. Ectothermy confers immense flexibility, enabling a dynamic matching of life-history traits to local circumstances. This flexibility has generated massive spatial and temporal variation in life-history traits via phenotypic plasticity as well as adaptation. Assisted reproduction techniques and the value of gene banking is coming to the fore with species with high declines in reptiles and amphibians.

Andre Ganswindt

Mammal Research Institute
University of Pretoria, South Africa

Non-invasive endocrine monitoring for assessing reproductive function in wildlife for conservation and management

A. Ganswindt[1]

[1] Mammal Research Institute, Department of Zoology and Entomology, Faculty of Natural and Agricultural Sciences, University of Pretoria, South Africa; andre.ganswindt@up.ac.za

Although the level of intactness of native fauna seems key for the stability of ecosystem diversity, an increasing amount of wildlife has to be managed in a continuously decreasing and often altered environment due to the progressing expansion and global resource utilization by man. In this regard, the ability to monitor reproductive potential and success greatly facilitate attempts to manage wildlife and often supports programs for population growth of threatened species. Although hormones for monitoring reproductive function can be quantified in various biological matrices, non-invasive sample collection have gained popularity over the past decades, as a more practical and safer approach, especially when monitoring intractable wildlife species, and also to comply with aspects of animal well-being, which is increasingly recognised as a crucial component when managing wildlife.

By showcasing a series of studies focussing on female reproductive profiling as well as describing the physical and behavioural characteristics and endocrine correlates associated with male sexual activity, this presentation will highlight the importance of monitoring reproductive function as a valuable tool to better understand species-specific reproductive processes and thereby assisting with management and welfare decisions for wildlife.

Joseph Saragusty

Faculty of Veterinary Medicine,
University of Teramo, Italy

Reproductive biotechnologies to save the functionally extinct northern white rhinoceros

Joseph Saragusty

Laboratory of Embryology, Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy
E-mail: jsaragusty@unite.it

Almost 25% of the animal species face the risk of extinction. A variety of stresses, mostly anthropogenic, and the lack of sufficient resources and political support mean that many will become extinct in the coming years. Regrettably, *in situ* efforts often seem insufficient to halt this dire situation. Reproductive biotechnology has advanced over the past several decades, slowly trickling into the field of wildlife conservation. However, with a few exceptions, their great promise has not been fulfilled, and their use in wildlife has been mostly anecdotal. The northern white rhinoceros is an iconic animal on the brink of extinction (functionally already extinct). I will discuss the attempts to save it from extinction through the range of (potentially) available reproductive biotechnologies. These span two general assisted reproductive fields, one based on natural gametes and the other on somatic cells. While initially separate, the two converge and overlap. Joint efforts of experts representing a range of disciplines and supported by governments, international organizations, stakeholders, and the general public could push these efforts forward at a pace that would match the urgency of the action needed.

Isabel Callealta

ECO lifes
(Wildlife reproduction and ultrasonography)
Spain

Reproductive management and assisted reproduction techniques in lions

Dr Isabel Callealta, ECOlifes (wildlife reproduction and ultrasonography), Spain. Tel. +34 622817246 / Email: isabelcallealta@ecolifes.es

In the last two decades, the global lion population has declined by around 40%. The International Union for the Conservation of Nature currently lists this large felid (i.e. Panthera leo) as Vulnerable, although most subpopulations all over its geographical range meet the criteria for Endangered.

Proper breeding management is essential to keep and improve healthy wildlife populations, both in situ and ex situ. Therefore, in addition to traditional measures, such as monitoring of threatened species, relocation of individuals, and exchange of breeders, assisted reproduction technology (ART) has been advocated as a new tool in wildlife breeding and conservation. The application of advanced techniques such as gamete cryopreservation, artificial insemination, in vitro fertilization, or embryo transfer could provide a faster diversification and redistribution of genetics, a reduction of disease transmission, and the independence from translocation of animals for breeding purposes. Although these benefits would enhance our efforts in wildlife conservation and breeding, to date, relatively little has been researched into this field and ART success in non-domestic felids remains, overall, lower than 25%.

In this talk, we will review the current methods that can be used to monitor the reproductive physiology of lions and the actual applicability of assisted reproduction techniques into this species conservation.

Budhan Pukazhenthi

Smithsonian Conservation Biology Institute
USA

Status of assisted reproductive technologies for endangered species: opportunities and challenges

Budhan Pukazhenthi, Smithsonian Conservation Biology Institute, Front Royal, VA, USA

The International Union for Conservation of Nature and Natural Resources recently reported that twenty eight percent of all species assessed to date are threatened with extinction. On-going conservation efforts focus on protecting the habitat, safeguarding animals from poaching, and maintaining self-sustaining insurance populations in human care (ex situ; zoos and conservation breeding centers). However, not all animals in human care reproduce naturally and often require to be transported to other facilities to promote genetic management. Assisted reproductive technologies including gamete and embryo preservation, artificial insemination, embryo transfer, and most recently, cloning offer alternate approaches to increase reproductive output, maintain/improve gene diversity, and achieve population sustainability. To date, several reproductive technologies have resulted in the production of live offspring in rare and endangered species. This presentation will review the (i) advantages and limitations of various assisted reproductive technologies for wildlife conservation, (ii) highlight the importance of integrating animal management (infrastructure, animal care, and veterinary medicine) and research to ensure success, and (iii) identify future research priorities.

Ursula Bechert

SpayVac for Wildlife, USA

SpayVac®-pZP applications in wildlife population fertility control

Ursula Bechert, Research and Development,SpayVac for Wildlife, Inc.
Email: ursula@spayvac.com

As wildlife populations become increasingly confined to smaller ranges due to the expanding human footprint, they can become locally overabundant, resulting in conflicts and negative effects on other species. Population management methods offer band-aid solutions to this problem. Immunocontraception has the greatest potential to regulate wildlife population numbers; however, vaccines must have multi-year efficacy to be both technically feasible and cost-effective. Immunocontraception based on porcine zona pellucida (PZP) antigens is highly tissue-specific, targeting the ova, and blocking sperm binding through antibody occupation of ZP receptors on the ova. SpayVac-pZP® achieves multi-year efficacy with a single dose, without boosters, because the antigens are encapsulated within liposomes (multi-layered, submicroscopic vesicles), which gradually release PZP glycoproteins to antigen-presenting cells over an extended period of time. This presentation will review results from SpayVac trials in seals, deer, elephants, and horses. Three trials in horses helped us learn more about the relationship between antibody titers and contraceptive efficacy, as well as potential mechanisms of action (e.g., preferential stimulation of IgG4/7 antibody isotypes). Practical and logistical field application challenges are site-specific based on terrain and other variables, and employing one or more fertility control tools in concert with removals offers the best potential for success.

Douglas C. Eckery

USDA APHIS WS
National Wildlife Research Center, USA

Development of Fertility Control Tools for Wildlife Management

Douglas C. Eckery

USDA APHIS WS National Wildlife Research Center
4101 LaPorte Avenue, Fort Collins, CO, USA
douglas.c.eckery@usda.gov

Effective management of wildlife and pest species is becoming increasingly necessary throughout the world. The use of fertility control as a tool to aid in wildlife management strategies is considered to have numerous benefits and has attracted substantial attention. The greatest benefits from the use of wildlife fertility control will be realized when it is used in conjunction with other tools in integrated management programs. In the United States, two different contraceptive vaccines have been registered for use in wildlife. Both vaccines have been shown to be effective in suppressing fertility in individual animals of a number of species; and have also been used successfully for the management of isolated populations of deer, horses and goats. While the use of these vaccines and other methods of fertility control has provided important evidence in support of this approach for wildlife management, it has also highlighted the need for improved methods that cause long-term infertility or permanent sterility, and the need for more effective methods of delivery. The challenges associated with the use of fertility control are not only technical in nature, but also involve regulatory, social, political and cultural aspects.

Satish K Gupta

Basic Medical Sciences Division,
Indian Council of Medical Research,
New Delhi, India

Immunocontraception: an emerging option for wildlife population management

Satish Kumar Gupta

Emeritus Scientist, Basic Medical Sciences Division, Indian Council of Medical Research, V. Ramalingaswami Bhawan, Ansari Nagar, New Delhi-110 029, India
Email skgupta.nii53@gmail.com

The uncontrolled increase in population of free-ranging animal species such as elephants in Africa, kangaroos in Australia, feral horses & white-tailed deer in USA, and street dogs, nilgai, monkeys, wild boars & elephants in India is leading to an increase in human-animal conflicts. To minimize human-animal conflicts for habitation and their respective welfare, it is imperative to develop strategies for wildlife population management. Several national authorities involved in wildlife population management advocate immunocontraception as an alternate to shooting, surgical sterilization and translocation. Contraceptive vaccines based on native porcine zona pellucida (PZP) glycoproteins have been used successfully to manage population of feral horses, white-tailed deer, and free-ranging African elephants. Currently, three PZP-based commercial vaccines (Spayvac®, ZonaStat-H, and PZP-22) are available. Further, gonadotropin releasing hormone (GnRH)-based contraceptive vaccines have been used to inhibit fertility in various animal species such as wild horses, white-tailed deer, pigs, and cats etc. Till date at least six GnRH-based commercial vaccines (GonaConTM, Improvac®, EquityTM, Bopriva®, Improvest®, and Repro-BLOCTM) are available. The data pertaining to the efficacy of a novel recombinant fusion protein encompassing canine ZP3 fragment and two copies of GnRH with appropriate spacers and promiscuous T cell epitopes to inhibit fertility in female beagle dogs will be presented. It will be desirable to develop novel vaccine delivery platforms, incorporating more potent but safe adjuvants to achieve long term infertility by administering single injection of contraceptive vaccine.

Naresh Selokar

Animal Biotechnology Centre
ICAR-National Dairy Research Institute,
Karnal, India

Buffalo cloning technology for conservation of endangered bovine species

Naresh L Selokar[1], Dharmendra Kumar[2], Manoj Kumar Singh[1], Rakesh Sharma[2], Jerome A[2], Pradeep Kumar[2], Prem Singh Yadav[2]
[1]ICAR-National Dairy Research Institute, GT Road, Karnal-132001, Haryana
[2]ICAR-Central Institute of Research on Buffaloes, Sirsa Road, Hisar-125001, Haryana
Presenting author: Dr. NL Selokar (Naresh.selokar@icar.gov.in)

India has made its way in the field of somatic cell nuclear transfer (SCNT) by producing several clones of buffalo (seven cloned copies from a single breeding bull). In addition to the multiplication of elite buffalo germplasm, buffalo cloning can also be used to conserve endangered species, both domestic and wild bovines. For the successful cloning of any species, there are basic biological necessities such as donor nuclei, a large number of oocytes, and suitable surrogate females to carry pregnancies. In endangered species, the lack of availability of oocytes and recipient females precludes the use of SCNT technology effectively. This problem can be resolved to some extent by using the recipient oocytes and females of closely related domestic animal species. This alternative way of animal cloning is termed interspecies somatic cell nuclear technology (iSCNT). iSCNT involves a transfer of a nucleus or cell from one species into the cytoplasm of an enucleated oocyte from another species. For example, transfer of wild buffalo somatic cells into enucleated oocytes of domestic buffalo. Keeping this in mind, we initiated iSCNT using buffalo oocytes and reported the production of interspecies blastocyst stage embryos using buffalo cytoplasts and differentiated somatic cells from cattle and goat; however, rat embryos were arrested at 16-cell stages (Selokar et al., 2011). Later, we employed iSCNT to clone a wild female buffalo available at Udanti Wildlife Sanctuary, Chhattisgarh (Priya et al., 2014, Saini et al., 2015). We also attempted iSCNT of horses; however, horse iSCNT embryos could not develop to the transferable quality blastocyst stage (unpublished data). In 2017, we produced the Assamese buffalo in the field at an organised dairy farm (Selokar et al., 2019). This cloned buffalo has several holdings: a) India's first cloned calf of Assamese buffalo, earlier only Murrah breed of buffalo was cloned; b) a small tail tissue biopsy of Assamese buffalo was airlifted from the College of Veterinary Science, Guwahati, which is 2000 km away from our cloning laboratory, to establish the cultures of donor cells. It proves that distant and remote animals can be cloned; c) a murrah buffalo was served as a surrogate mother to carry the pregnancy of the Assamese cloned embryo. At present, the cloned Assamese buffalo bull is producing quality semen, and his semen qualifies all semen attributes including CASA indices. We also performed IVF-based fertility of this cloned bull, which is found normal (unpublished data). These preliminary experiments encourage us to continue more interspecies cloning attempts using somatic cells of endangered bovines and try to find out which species nuclei would reprogramme best using buffalo oocytes and remain optimistic to make it possible to clone the endangered bovine species

ABSTRACTS OF SHORT PRESENTATIONS

Reproductive Ultrasonongraphy in Zoo and Wildlife

Mozammel Hoque, Principal Scientist & Head Division of Surgery, ICAR-Indian Veterinary Research Institute, Izatnagar-243122 (UP)

Ultrasonography has a great potential to be used as a reproductive technology in wildlife conservation. Details of ovarian dynamics, pregnancy detection, expected date of delivery (EDD) or laying term, fetal development, sex determination, ovum pick-up (OPU) technology for embryo transfer are well documented in domestic animals. On the same direction, a lot of work on reproductive ultrasonography is reported in zoo and wild animals. Ultrasound is used to document changes in reproductive tract morphology throughout the estrous cycle in elephants, rhinoceros, monkeys, tortoises, hen and lowland anoa. Reports of pregnancy diagnosis in a large number of zoo and wild animals (amphibians, reptiles and mammals including elephant, rhinoceros and deer) with transrectal or transabdominal ultrasound are available. Early embryonic death can be detected ultrasonographically. It is possible to assess fetal cardiac activity, volume of embryonic fluid and status of placenta during mid-to-late pregnancy. Ultrasonography has been very useful for screening of monkey's uterus before sterilization under animal birth control (ABC) program. Transcloacal and trans-intestinal sonography have been employed to image the yolk of ovary in birds or reptiles and based on the extent of calcification, a prediction regarding the laying term is possible. Many monomorphic species exhibit no, or slight phenotypic differences between sexes that are more evident in captivity. These difficulties have been overcome by trans-cloacal or trans- intestinal scanning of gonads using miniature transducer.

Ultrasonographic Studies on Reproductive Organs in Captive Chelonians

Divya Ganesh, Ravi Raidurg, Madan KV, C. Ansar Kamran, Kavita Rani B and Naveen Kumar S

Department of Wildlife, Veterinary College Hebbal, Bengaluru, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka,
Sri Chamarajendra Zoological Gardens, Mysuru, Karnataka

Chelonians are some of the most endangered vertebrate groups, and are at a high risk of extinction because of climate change, illegal wildlife trade and hunting for their meat and shells. To improve breeding in captivity and to aid in conservation, aid of reproductive technologies like ultrasound are employed. It can help assess reproductive health, monitor reproductive cycling, and determine the best measures for breeding. Ultrasonographic examinations were done on 17 clinical cases presented to "Zoo hospital" of Sri Chamarajendra Zoological Gardens, Mysuru with the history of anorexia, off-feed and lethargy. The species studied included the 11 Indian Black Pond Terrapin (Melanochelys trijuga), 02 Indian Star Tortoise (Geochelone elegans), 02 Indian Flapshell Turtle (Lissemys punctata), and 02 Red-eared Slider (Trachemys scripta elegans). Of the four species studied, the first three are native to India and no previous database for ultrasound has been established. he turtles were positioned in ventral recumbence and ultrasound examination of the reproductive organs was done using a high-frequency (10-12 MHz) microconvex probe through a "Pre-femoral acoustic window". The follicles were visualised as rounded structures with anechoic lumen and hyperechoic walls of different sizes in 11 chelonians. Around 4-6 follicles were visualised in the Indian Black Pond Terrapins, 2-4 follicles in the Indian Star Tortoise, 4-8 in the Indian Flapshell Turtle, and 3-6 follicles in the rRed-eared sSliders. The testicles were difficult to visualize ultrasonographically and appeared as roughly round to oval hypoechoic to hyperechoic parenchyma closely associated with the kidneys in 5 chelonians. The shell of the turtle and the convex shape of the transducer limited visualization of the deeper structures. Testis could not be visualized in 01 male Indian Black Pond Terrapin.

Ultrasonography can be used to determine the gender of chelonians who display no sexual dimorphism and in smaller-sized chelonians (about 1kg) which are difficult to sex.

Comparisons of Two Approaches (Midline and Lateral Incisions) for Vasectomy in Leopards (Panthera pardus fusca)

Nikita Nishit Salian[1]., Ravi Raidurg[1]., Sujay CS[2]., Vanishree Hiremath[3]., Patel Suresh Revanna[1]., Manjunatha SS[1] and Naveen Kumar S[1],

[1]Department of Wildlife, Veterinary College Hebbal, Bengaluru, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka, [2]Tiger and Lion Safari, Tyavarekoppa, Shivamogga, Karnataka, [3]Sri Atal Bihari Vajpayee Zoological Gardens, Kamakapura, Hospete. Karnataka

Zoos and rescue centers in the country have witnessed an overpopulation of Indian leopards (Panthera pardus fusca) in the last decade due to healthy breeding. To overcome crowding, vasectomy in captive animals is performed for population control, maintain pedigreed animals and to prevent inbreeding. Nine male leopards presented for vasectomy from Tiger and Lion Safari, Shivamogga and Sri Atal Bihari Vajpayee Zoological Gardens, Kamakapura, Hospete. Vasectomy was performed through two approaches bilateral lateral incisions (n=4) and midline incision (n=5). The animals were sedated with xylazine and ketamine combinations (2mg/kg and 5mg/kg respectively) in four cases and dexmedetomidine and ketamine combinations (0.025mg/kg and 5mg/kg respectively) in the remaining five cases intra-muscularly. The vas deferens was then palpated as a piece of cooked spaghetti at the neck of the scrotum on the anterior aspect by three finger palpation technique and was fixed between the thumb and middle finger. Through a key-hole incision the vas deferens was exteriorized, ligated at two places with polyglycolic acid suture size 1-0 and resected. Fascia and skin incision was sutured with sub dermal sutures (polyglycolic acid suture size 1-0) and sealed with skin glue (methyl methacrylate). Post operatively, no wound dressing was done on the surgical wounds. Both approaches (unilateral midline incision and bilateral lateral incisions) were found to be successful and effective with respect to accessibility of vas deferens and ease of operation, however, the midline approach proved to be of less time consuming and minimally invasive as the need for second incision was avoided.

A method for storage, preservation and transportation of anuran urine samples using filter paper for hormone analysis

Vinod Kumar, Govindhaswamy Umapathy, Laboratory for the Conservation of Endangered Species (LaCONES), Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India

Urine sample provides crucial insights into the physiological status of captive and free-ranging amphibians. However, storage and preservation of urine samples in field conditions is always a difficult task due to limited facilities. The present study aimed to standardize and validate a field method for short term storage and preserve of anuran urine samples using Whatman filter papers. About 30 anuran urine samples were collected and pooled to experiment with short term storage on the filter papers. The filter papers were stored for ten weeks, with six replicates each week at room temperature. Hormone metabolites were extracted from filter papers at one-week interval. To examine the efficacy of storage conditions, urinary-based enzyme immunoassays were used to measure the reproductive (progesterone and testosterone) hormone metabolites. High-Performance Liquid Chromatography was performed and revealed the presence of immunoreactive progesterone and testosterone metabolites in the urine samples. No significant difference was observed in the hormone metabolites concentration between samples stored in filter paper at room temperature and control samples stored in -20 C for the same period. Further, Whatman filter paper grade 50 was found to be more consistent and stable for storage and preservation of anuran urine samples compared to Whatman filter paper grade 3. No significant difference between the overall weekly (Day0-Week10) hormone concentrations over at room temperature was observed. The results indicate that anuran urine could be stored, preserved and transported at ambient conditions without significant changes and loss in steroid hormone concentration in field conditions in the absence of refrigeration. The finding of this study would facilitate endocrine monitoring of anurans in remote areas where limited logistics on the sample collection and preservation.

Dreadful Delta Variant COVID19 in Asiatic Lions (Panthera leo persica)

Sridhar Kanniappan1*, Aparna Gopinadhan1, Chirukandoth Sreekumar2, Jeyaraja Kirubakaran2, Sridhar Ramasamy2, Baranidharan GR2, Moulidharan Manickam1, Palanivelrajan Muthusamy2, Sandeep Bhatia3, Karigalan Mathesh4, Karthikeyan Vasudhevan5, Vasantha Kumari Dhayanithi6, Silambarasan Ramu1, Abelraj Thaya Sekhar1

1. Arignar Anna Zoological Park, Chennai-600048
2. Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai – 600051
3. National Institute of High Security Animal Diseases (NIHSAD), Bhopal – 462021
4. Indian Veterinary and Research Institute (IVRI), Bareilly – 243122
5. CSIR – Centre for Cellular and Molecular Biology (CCMB) – 500 007
6. Advanced Institute for Wildlife Conservation (AIWC) – 600048.

The Asiatic Lions (*Panthera leo persica*) at Arignar Anna Zoological Park, developed respiratory signs in May 2021. On screening the nasal swabs, rectal swabs and fecal samples of 13 lions, genome of SARS CoV- 2 was detected by Reverse Transcription Polymerase Chain Reaction (RT-PCR) in 10 lions and genome sequencing revealed Pangolin lineage B.1.617.2 of Delta variant, a variant of concern. Of the 10 positive lions, two were asymptomatic and other eight lions had mild to severe respiratory disease, of which two succumbed. Four lions suffered an acute kidney injury and were treated with fluids and sildenafil citrate. The clinical signs observed include loss of appetite, pyrexia, chain of cough, wheezing, sneezing, nasal discharge, tachypnea and vomiting. The animals were treated symptomatically with a course of antibiotics, dexamethasone, enoxaparin and vitamin supplements, until clinical signs disappeared. Nebulization with salbutamol and budesonide and high flow oxygen were provided for whose animals which were severely affected. All the affected lions with continuous monitoring and treatment recovered completely after 40 days of incidence.

Nutritional Secondary Hyperparathyroidism (NSH) in a Royal Bengal a Tiger Cub-a case report

Ramesh Tiwary[1] Kaushal Kumar[2], Ajeet kumar[3], Pankaj Kumar[4], Archana Kumari[1], G. D. Singh[1], S. B. Singh[5], R. K. Pandey[5], Bibha Choubey[6] and Nand kishore[7]
Bihar Veterinary Colollege, Bihar Animal Sciences University, Patna
[1]Dept. of Surgery and Radiology, [2]Dept. of Pathology, [3]Dept of Biochemistry, [4]Dept. of Microbiology, [5]Patna Zoo vets, [6]Ph.D. Scholar, Patna University, [7]Patna Zoo Director

Nutritional secondary hyperparathyroidism (NSH) is a metabolic disease most commonly affecting the young puppies or Cubs having inadequate availability of proper ratio of calcium and phosphorus in their diet. The present case report is nutritional secondary hyperparathyroidism in royal Bengal female tiger cub in captive condition. An eighty days old female cub was reported with hind limb ataxia, abnormal gait, reluctant to move and depressed condition. The cub was separated from her mother for clinical examination. The reflexes were normal but suspected for fracture in left femur and suggested for radiographic examination. The lateral radiograph showed simple midshaft diaphyseal fracture of left femur. The animal was sedated with 18.75 mg tramadol (50mg/ml) and 1.25 mg diazepam (5mg/ml) injection intravenously and induced with 20 mg propofol (10mg/ml) and Robert Jones bandage was applied to stabilize the fracture fragment. Animal well responded with calcium and phosphorus supplement plus vitamin D injection 3 lakh IU (IM), at weekly intervals as post operative care. The biochemical estimation of Ca and P values were 8.6 mg/dl and 6.4 mg/dl respectively. But again, once the activity of animals increases there were multiple bone fracture noticed in the cub. Right femur proximal metaphysic, left tibia mid shaft old fracture, right humerus proximal physis. The overall bone density decreased and typical paper-thin cortex with opaque line in right distal humerus and proximal tibia with angulations (Folding fracture) indicates pathological fracture which is pathognomonic radiographic sign of NSH. The necropsy finding of thin bony cortex, parathyroid gland, liver, kidney and lungs also correlates with the clinical findings. In conclusion NSH is a common problem of young cubs, where number of litters' size is more, proper nutritional supplements of the cubs may help in successful captive rearing of new born cubs.

Reproductive and stress physiology in wild hangul *Cervus hanglu* in Kashmir Himalaya

Tanushree Srivastava, Laboratory for the Conservation of Endangered Species (LaCONES), Centre for Cellular and Molecular Biology Hyderabad - 500048 email: tanz.d.cool@gmail.com

Reproduction in mountainous ecosystems is extremely challenging given the marked seasonal fluctuations in environmental conditions and thus resource availability. Ungulates inhabiting these ecosystems adopt different strategies to cope with these seasonal fluctuations and maximize their reproductive success. We therefore aimed at understanding the patterns and underlying factors of reproductive and stress physiology in the Critically Endangered hangul Cervus hanglu. We conducted this study in Dachigam National Park in Kashmir Himalaya between September 2019 and August 2020. We measured concentrations of reproductive (progesterone) and stress (glucocorticoid) hormone metabolites in fresh fecal samples of hangul collected during monthly monitoring of 11 permanent trails marked in the study area. We recorded low and high mean monthly progesterone values between October and December representing estrous and luteal phases, respectively whereas continuously rising values between January and March probably indicated gestation in the population. Higher values of fecal glucocorticoid metabolites (FGM) between October and December most probably indicated rutting related stress in males, whereas that in May should be the parturition related stress in females. Also, the lower values of FGM in winter suggest that harsh weather conditions do not cause stress in the species. Moreover, majority of the sampling trails followed similar patterns thus indicating a synchronized reproduction in the population. The knowledge on timing and extent of various reproductive phases can aid in identifying and keeping the habitats free from disturbances, especially during the critical times of the year.

Determination of estrous phases and behavioural changes in captive Asian elephants (*Elephas maximus*)

Madhvee Dhairykar[1], Shilpa Sahu[2], K.P. Singh[1], Nidhi Rajput[1] and Shobha Jawre[1]
[1]School of Wildlife Forensic and Health, NDVSU, Jabalpur (M.P.)-482001
[2]Department of Veterinary Obstratics and gynecology, NDVSU, Jabalpur (M.P.)-482001
Corresponding author – madhvidhairykar@gmail.com

The estrous cycle was monitored by analysis of vaginal cytology and by observing the estrous behaviour in three (one adult and two sub-adults) female captive Asian elephants (Elephas maximus) of different tiger reserves of Madhya Pradesh. The cytological analysis was done by using direct Giemsa staining of the vaginal smear. Reproductive history was noted. Out of three female sub-adults were given birth one time, while adult female was calved three times. The sampling was done about four weeks and behavioural changes were also observed. Changes in the vaginal smear of several mammalian species have been observed during the estrous cycle which reflects ovarian events most accurately. Consequently, in the present study, cornified, superficial (anuclear and nuclear) cells, parabasal and intermediate cells were identified as small circular shape with poor cytoplasm. While, changes in the behaviour were also observed in the form of estrous walk, tail flicking and investigative behaviour. The results suggested that increase in the number of superficial cells, parabasal and intermediate cells, indicate the changes from proestrus to estrus (follicular phase) and from metestrus to diestrus (luteal phase), respectively. The vaginal smears indicated the different stages of estrous cycle along with changes observed in behaviour. Estrous phases indicated the reproductive status of animal and it is also helpful in reproductive success of elephants.

Predictors of Adrenal and Gonadal Hormones in Relation to Biological and Management Factors Among Captive Red Pandas in Indian Zoos

Aamer Sohel Khan[1], Janine Brown[2], Vinod Kumar[3], Govindaswamy Umapathy[3], and Nagarajan Baskaran[1],*,
[1]Dept. of Zoology and Wildlife Biology, A.V.C College (Autonomous), Mannampandal, Mayiladuthurai - 609305, Tamil Nadu, India; khannaamirsohel@gmail.com, [2]Smithsonian Conservation Biology Institute, Center for Species Survival, 1500 Remount Road Royal, VA 22630; BrownJan@si.edu [3]Laboratory for the Conservation of Endangered Species, CSIR-Center for Cellular and Molecular Biology, Uppal Road, Hapsiguda – 500007, Hyderabad, Telangana, India; vinod@ccmb.res.in, guma@ccmb.res.in

Animals in human care are affected by stressors that can ultimately reduce fitness. When reproduction is affected, endangered species' conservation programmes can be severely compromised. Thus, understanding factors related to stress and reproduction, and measures of related hormones, are important to ensure captive breeding success. Red pandas are endangered and populations in the wild are threatened with extinction. Global captive breeding programmes have been launched to conserve the species with one goal being to support reintroduction. However, there is little informaiton on how stressors impact reproductive aspects of the species biology. This study measured fecal glucocorticoid (fGCM), fecal progestagen (fPM) and fecal androgen (fAM) metabolite concentrations in 12 female and 8 male red pandas (Ailurus fulgens fulgens) at three zoos in northeastern India to determine predictors of adrenal and gonadal steroids and the influence of fGCM on reproduction. Results indicated that fGCM concentrations were higher in males than females, and positively correlated with number of visitors, while negatively related to frequency of feedings and enclosure area. Sex, visitor number, frequency of feeding, and enclosure area explained 67% of the variation in fGCM concentrations in the study population. Concentrations of fPM were positively associated with tree density in the enclosure, explaining 47% of the variation among females. For fAM, positive associations were found with frequency of feeding, but concentrations were negatively related to age and number of visitors; these three covariates explained 45% of the variation in fAM concentration among males. Comparison of fGCM with fPM showed a negative trend, indicating increasing adrenal hormones may decrease reproductive function among female red pandas. A relationship between fGCM and fAM was not observed in males. Results thus suggest that zoo management should consider increasing feeding frequency, providing larger enclosures with more trees, and regulating visitor numbers to reduce stress and increase reproductive fitness among this species of red pandas.

Captive Breeding of Critically Endangered Cotton-top tamarin (Saguinuso edipus) at the Sardar Patel Zoological Park, Gujarat, India

Ravikumar Patel, Ram Ratan Nala, Hiren J. Patel, Nitesh Chaudhari, Jahanvi Chitariya,
Sardar Patel Zoological Park, Kevadiya colony, Gujarat 393151, India.
e-mail: ravikpatel8980@gmail.com

Cotton-top Tamarin (CTT) (Saguinusoedipus (Linnaeus, 1758), one of the smallest primates' is a Critically Endangered Species according to IUCN. The species is threatened as a result of habitat destruction and wildlife trade. We report the successful breeding of CTT in captivity from India. Here, in the newly established Sardar Patel Zoological Park, Kevadiya, we have a pair of CTT. After the mating and the gestation period of 168 days, twins were born. The estrous cycle of the CTT is 15 days and sexual maturity is reached at 18 months in females and 24 months in males. After birth, the twins spend their time on the male's back as he provides them protection, caring, introducing feed and making them familiar within the cage situation, while the female carries infants only while feeding them. In between 42-48 days, both infants started moving on their own and feeding on solid food. During the parental care, the male loses 23% of body weight, and it is also reported in other studies that after the infants grow, the male again starts to gain weight, which is also the same in our case. The weight gain of infants after birth is 40 g to 601g within nine months (270 days), the birth weight was around 40g and they have crossed the weight of their parents (560-590g). We also note down 18 behaviours (15 active and 3 passive) during the breeding period. Our report on the CTT is the first of its kind in an Indian environment zoo. The present observations on CTT will be useful for maintenance of captive populations.