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Use of assisted reproductive technology for breeding of captive pheasants and waterfowl

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Species Survival Commission (SSC) of IUCN.

- Present and previous work
- Problems of breeding small populations
- Genetic Resource Banking (GRB)
  - Semen collection
  - Semen analysis
  - Semen processing
  - Cryopreservation of semen
  - Artificial Insemination
- Summary

Present work

Emus as a model to investigate :

1. Extending shelf life of **liquid semen** beyond 6 h without

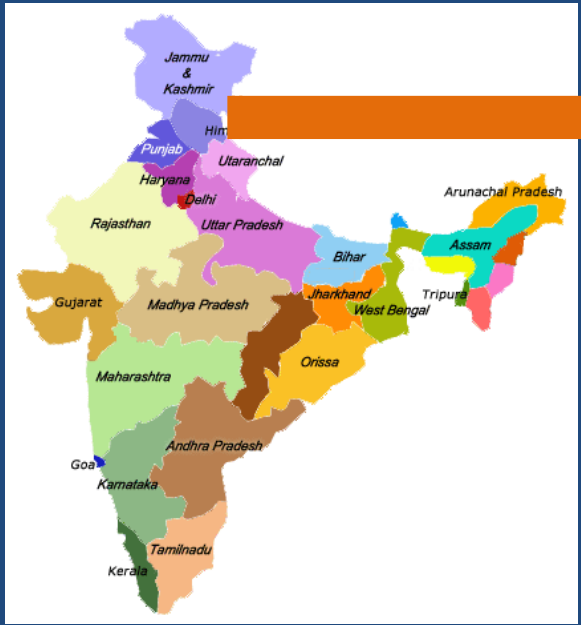
A new diluent for Emus that has increased shelf life of emu semen to 48 h in Emus.

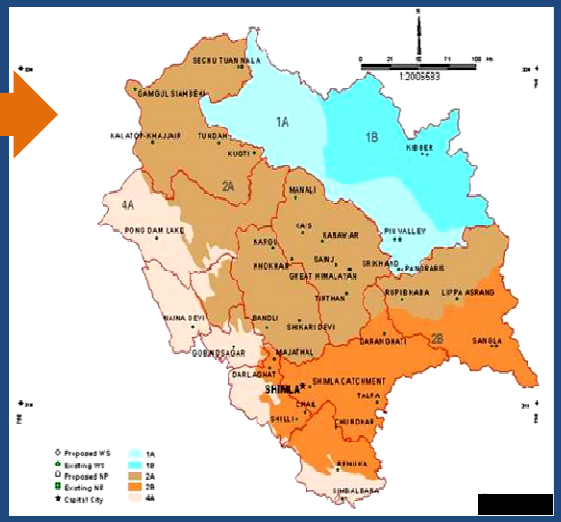
Semen diluent - Pigeons, Cranes and Tragopans - 24 to 30 h

2. Improving quality of **frozen thawed semen**.

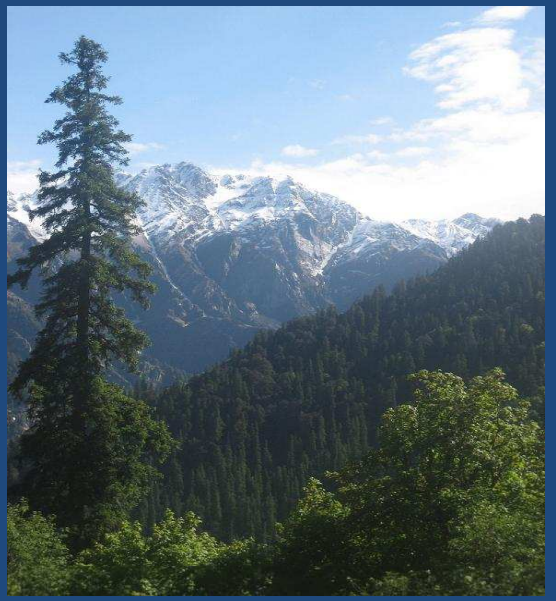
- **Develop a freezing protocol for field conditions**
- **Increased the sperm viability from 27 % to 65 and motility and 13% to 60% – Emus**
- **Pigeons – 70% viability and 55% motility**
- **Cranes – 55 – 60% viability and 50% motility**

Some of the previous work





Himachal Pradesh



Western Tragopan
(Tragopan melanocephalus)

Cheer pheasant
(Catreus wallichi)

Himalayan Monal pheasant
(Lophophorus impeyanus)

CLINICAL & NECROPSY FINDINGS IN CAPTIVE PHEASANTS OF HIMACHAL PRADESH, INDIA

Presented by S. Sood
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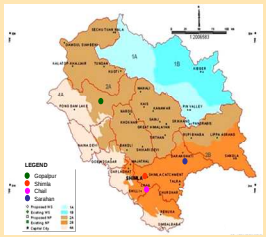
INTRODUCTION

The state of Himachal Pradesh is a hot spot for free ranging and captive pheasants. Various mortality causes have been observed in last ten years amongst different captive pheasants of the state. Young chicks and mature pheasants have been found to die each year with a range of diseases like Mycotoxicosis, Capture myopathy and Coccidiosis. Aflatoxicosis symptoms have been observed in Red Jungle Fowl and Cheer chicks at Himalayan Bird Park, Shimla and Chail Pheasantry, respectively. Incidence of foreign bodies have been found in digestive tract of Monal and Western Tragopan at Sarahan Pheasantry. Mild to heavy infestation of *Ascariasis* and *Capillariasis* have been observed in Red Jungle fowl, Kaleej, Monal and Western Tragopan. Infertile eggs and early embryonic mortality have been observed in Monal and Western Tragopan. Incidence of cannibalism, predation, fighting within groups and fractures are other contributing mortality factors reported by the staff.

OBJECTIVES

- ❑ To devise a strategy to minimize mortality rate.
- ❑ To contribute in better management for future conservation breeding programmes envisaged by Wildlife Wing of Himachal Pradesh.

STUDY SITES

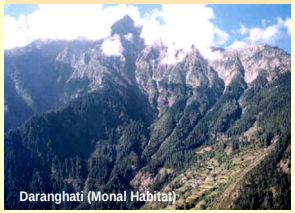


The state of Himachal Pradesh is situated south of Jammu and Kashmir, north-east of Punjab, north-west of Haryana and Uttaranchal and west of Tibet, between latitude 30° 22' 40'' N and 33° 12' 40'' N and longitude 75° 45' 55'' E and 79° 04' 20'' E with an altitude ranging from 350 m (low valleys) to 6,975 m (snow covered mountains) above mean sea level. Geographically the state is located in the North Western Humid Himalayan Region (NWHHR) comprising Jammu & Kashmir, Himachal Pradesh and Uttaranchal.

MATERIAL AND METHODS

This study was based on

- Post mortem records available for last ten years with the H.P. Forest Department
- Physical examinations
- Health screening of birds
- Necropsy examinations
- Personal communications with staff and veterinarians associated with providing treatment to the pheasantries


Daranghati (Monal Habitat)

OBSERVATIONS

EGG RELATED PROBLEMS	CHICK RELATED PROBLEMS	ADULT RELATED PROBLEMS
Infertile eggs	Mycotoxicosis	Capture myopathy
Thin shelled eggs	Coccidiosis	Foreign body ingestion
Embryonic Mortality	Predation	Beak deformities
Predation by rats, snakes & yellow throated marten	Hypothermia	Injuries
Breaking of eggs as a result of laying from perches	Low survivability	Egg binding
	Low body weight gain	Cannibalism
		Endoparasites

INTERPRETATIONS

Regular monitoring of Breeding Stock should be done with respect to:

- ❑ Eggs and Birds:
  - Clutch size, Egg conformations and morphometry.
  - Proper nesting environment.
  - Ensuring fertility of eggs and compatibility among birds.
  - Contamination of eggs by any bacteria, virus or fungi.
  - Hatchability of eggs.
  - Detection of early symptoms.
  - Disease surveillance and evaluation of microbial and parasitic load.
  - Nutritional disorders affecting fertility status of birds.
  - Low founder stock.
  - No proper maintenance of laying or chick records.
- ❑ Management Problems
  - Analysis of feed composition for providing birds with a balanced diet.
  - In addition feed should be supplemented with vitamins and minerals to avoid deficiency problems and increase breeding status of captive pheasants.
  - Access to sunlight to avoid dampness in pens.
  - Regular changing of top soil to break direct life cycle of parasites.
  - Peckable food for maintaining proper beak length.
  - Provision of predator free environment.

FUTURE ACTIONS

- ❑ Increasing clutch size and fertility :
  - Proper training to staff for maintenance of laying and chick records
  - Explore possibilities of increasing the existing breeding stock either by way of introduction from wild or importing semen for artificial insemination in birds.
- ❑ Construction of properly designed pheasantries:
  - To avoid loss of breeding birds through predation
  - To provide stress free environment.
  - Regular Biochemical and molecular analysis for early detection of early diseases.
  - Role of predators in transmission of diseases.

References:
Avian Medicine and Surgery; by Robert Altman, Susan L. Clubb, Gerry M. Dorrestein and Katherine Quesenberry, Published by W.B. Saunders Company page 950-953

Helminths , arthropods and protozoa of Domesticated Animals, 7th Edition by E.J. L. Soulsby, Published by ELBS and Bailliere Tindall – London, page 655

1. **Tragopan pheasantry** - Peers & Reimst, Belgium
2. **Grouse and Blood pheasant** - Messelbroek , Belgium
3. **Crane breeding center** - Wisbroek , Holland
4. **Olmense Zoo** - Olmen, Belgium
5. **Dutch Research Institute for Birds and Exotic Animals (NOIVBD)** - Veldhoven, Holland
6. **Papegaaienpark** – Veldhoven, Holland
7. **Rotterdam Zoo** –WPA Belenux Conference, Holland

Present status for importing of pheasants or their semen and eggs

Australian Government Department of the Environment, Water, Heritage and the Arts (formerly the Department of the Environment and Heritage) (DEWHA)

In order to import reproductive material from exotic birds the bird species in question has to appear on the "List of Specimens taken to be Suitable for Live Import"

Australian Quarantine and Inspection Service (AQIS).

Protocol and regulations for screening the reproductive material (frozen semen and eggs)

- Bird semen is currently not permitted entry into Australia.

- Import conditions have not been developed for the importation of any fertile eggs other than : domestic poultry, domestic turkeys, or domestic ducks.

- Current quarantine facilities are booked for tentatively 10 years.
is the agency responsible for risk assessment of new commodities. A risk assessment [by Biosecurity Australia (BA)] is needed before import conditions can be developed.

- Support from Australian industry for the import of peafowl, pheasant or other fertile eggs will be helpful in getting the issue on to the BA work program for import risk analyses.

- Likely to take a period of a few years to start this process and then some more to complete.

Problems in Breeding of Small populations

Abundant

Many Reasons

Small captive populations

B Neck

Limited genetic diversity

- ❖Establishing compatible pairs,
- ❖ Inappropriate sexual imprinting,
- ❖Aggression,
- ❖Subordination,
- ❖Confinement induced stress,
- ❖Anatomical disability (e.g. rehabilitated crippled wild birds),
- ❖ Poor sperm concentration , and
- ❖International/national regulations on the movement of live birds hinders the exchange of genetic material between CBPs.

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loss

- ➢ Sper
- ➢ AI a
- ➢ Esta

preserving variation in valuable genetic stock

Managed **Demographic** and **Genetic principles**

Genetic resource banking

Habitat protection

Genetic resource banking

Bottle Neck

Short term

Long term

Liquid semen

Frozen semen

Artificial insemination (AI)

- **High proportion of genetic diversity to be preserved indefinitely**
- GRB permits the reproductive lifespan of individuals to extend beyond their actual lifespan, and thus increases the 'effective' size of a small population.
- increasing security against demographic catastrophes
- reducing disease transfer risk
- increasing gene flow
- extending generation times
- maximising genetic diversity
- **cost-effectiveness**
- minimising inbreeding
- providing a repository of genetic material for species not yet endangered that may be threatened in the

PROBLEM

Semen banking is only effective for species whose reproductive physiology is well characterized

Fresh semen characteristics of various species of birds [mean; (range) where data available (nd – no data)

Species	Volume (µl)	Concentration (x 10^6/ml)	pH	Reference
Chicken (*Gallus domesticus*)	*(500-800)*	*3,500*	*7.3*	*Gee, 1995a*
Du				
Bu (*M*				
Sar				
Ost Ho (*Ch*				
Ale (*Br*				
Peregrine (*Falco peregrinus*)	*94*	47.4	nd	Parks *et al, 1986*
American kestrel (*Falco sparverius*)	*12*	*31*	*nd*	*Bird and Laguẽ, 1977*
Golden eagle (*Aquila chrysaetos*)	*24*	*183*	*nd*	*Knowles-Brown & Wishart, 2001*
Magellanic penguin (*Spheniscus magellanicus*)	*35.6*	*608*	*7.4*	*O'Brien et al, 1999*
Northern pintail (*Anas acuta*)	*66*	*5.7*	*8.5*	*Penfold et al, 2001*

Semen collection in non-domestic birds :

- ❑ Cooperative
- ❑ Massage and
- ❑ Electroejaculation

Fresh Semen Quality Characteristics and Evaluation

Th

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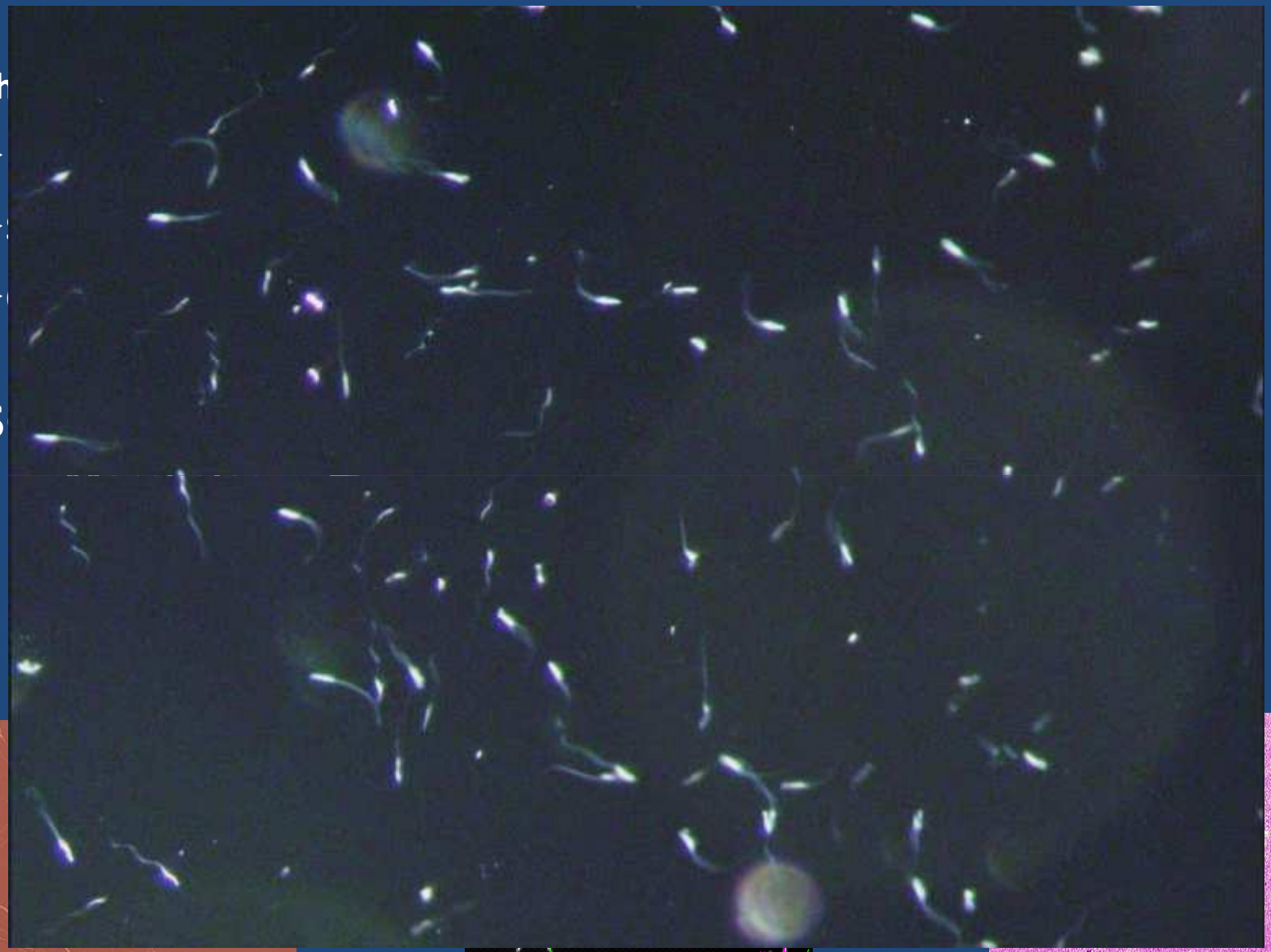
Ass



General Aspects of Semen Cryopreservation

Cooling spermatozoa that are bathed in a medium containing a cryoprotectant

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Down to –196°C, the temperature of liquid nitrogen

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All cellular metabolic processes are ‘frozen’ and the spermatozoa can be stored indefinitely.

Summary of cryopreservation techniques used in non-domesticated avian species (mean±SEM or range; nd – no data; n=no. eggs).

Species	Freezing technique	Freezing rate	Thawing method	Fresh motility (%)	Thawed motility (%)	Fertility (%)	Hatchability (%)
Budgerigar (*Melopsittacus undulatus*)	*Modified glycerolated BWW solution*[1]	*-5°C/min from +5°C to-70°C*	*Rapid (37°C) and dialysis*	*92*	*45*	*67*	*33*
American kestrel (*Falco sparverius*)	*Glycerol 13.6%*	*-6°C/min*	*Slow (3-5°C)*	*40-60*	*32-48*	*8.3 (n=24)*	*4.15*
American kestrel (*Falco sparverius*)	*DMA 12.3%*	*-6°C/min*	*Slow (5°C)*	*64±2.8*	*13±1.6*	*30*	*nd*
American kestrel (*Falco sparverius*)	*Glycerol 10.2%*	*-6°C/min in nitrogen vapour*	*Slow (5°C) in nitrogen vapour*	*57±2*	*41±2.1*	*12*	*nd*
American kestrel (*Falco sparverius*)	*DMSO 6-10%*	*-1°C/min from +5°C to –20°C,* -50°C/min from –20 to –80°C and -160°C/min from –80 to –196 °C	Slow (5°C)	77±9	57±12	35-57	11-29
Peregrine falcon (*Falco peregrinus*)	*Glycerol 1.48M*	*-6°C/min to -180°C, and dialysis*	*Slow (4°C)*	*70 (60-85)*	*54*	*33*	*33*
Golden eagle (*Aquila chrysaetos*) *Wishart, 2001*	*DMA 6%*	*Mr Frosty in - 15°C freezer for 1-2 hours and rapid immersion in liquid nitrogen*	*Rapid (37°C)*	*39±4*	*11±8*	*100 (n=5)*	*80*
Sandhill crane (*Grus canadensis*)	*DMSO 6%*	*-1°C/min from, +5°C to –20°C* -50°C/min from –20 to –80°C and 160°C/min from –80 to –196 °C	Slow (2°C)	80	45	25 (n=4)	nd
Domestic fowl (*Gallus domesticus*)	*DMA 6%*	*Diluted semen held at –12°C for 20 minutes and then dropped in liquid nitrogen at –196°C*	*Rapid (60°C)*	*Nd*	*nd*	*95 (n=1500)*	*nd*
Northern pintail (*Anas acuta*)	*DMSO 4%*	*-1°C/min from +5°C to –20°C, 60°C/min from –20 to –70°C and then dropped in liquid nitrogen at –196°C*	*Slow (20 min) at 4°C*		*57.4±6.1*	*32±8.3*	*0*
Houbara bustard (*Chlamydotis undulata*)	*DMA 8%*	*Pellets formed directly in liquid nitrogen*	*Slow (5°C)*	*60*	*15*	*100 (n=6)*	*50*

Various problems are associated with the technique of cryopreservation and these relate to:

1) Choice of suitable diluents;
2) Methods to prerefrigerate the semen before it is frozen;
3) Choice of cryoprotectant to reduce cell damage;
4) Choice of freezing temperature;
5) Choice of suitable storage vessel for refrigeration and freezing;
6) Thawing procedure; and
7) Separation of cryoprotectant from the seminal material before fertilization;
8) The AI technique.

Need for AI/germplasm conservation in Captive populations

Houbara bustards
(Chlamydotis undulata)

Gyrfalcon
(Falco rusticolus)

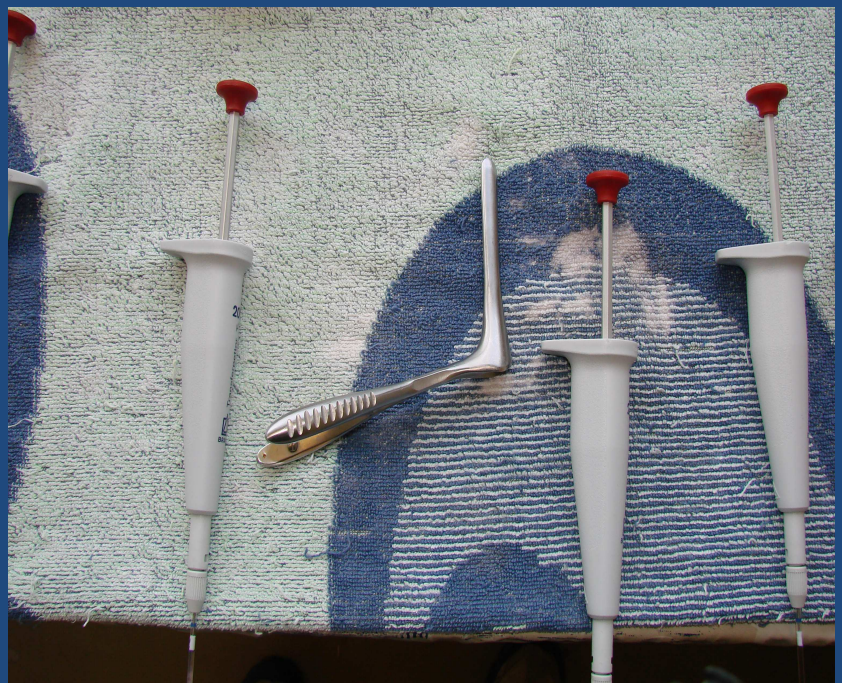
Western Tragopan
(Tragopan melanocephalus)

Red Jungle Fowl
(Gallus gallus)

Prairie Chicken
(Tympanuchus cupido)

Blood Pheasant
(*Ithaginis cruentus*)

Steps in assisted breeding of pheasants

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Buddy
DIGITAL EGG MONITOR
Buddy
AVITRONICS - ENGLAND - (44) 01872-262777

A young American kestrel *Falco sparverius,* 'Nitro', the first bird of prey to be conceived by the use of frozen-thawed spermatozoa.

(Brock, M. K., Bird, D. M. & Ansah, G. A. 1984. Cryogenic preservation of spermatozoa of the American kestrel. *International Zoo Yearbook, 23, **67-71.)***

Video of Semen collection, Treatment and AI in pheasants

Semen Cryopreservation for Genome Resource Banking and

One of the problems of cryopreservation of semen from non-domesticated birds has been the need to use sophisticated laboratory equipment under laboratory conditions and time consuming methodologies.

Additionally, it is not inconceivable that such methods could be adapted for use by field biologists to collect samples from wild birds and the implications of this for the management of wildlife are both profound and exciting.

Video about Assisted Hatching

Summary

Small populations for diverse reasons have limited genetic diversity

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Needs to be preserved at its maximum

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It proves costly to preserve genetic diversity by maintaining adequate founders in captivity

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Short and long term semen preservation of germplasm of founder stock

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Protocols and diluents can be available for conservation of genetic resources under filed conditions.

↓

Possibility of importing semen across national and international borders

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Supplemented with Artificial insemination and other assisted breeding techniques

THANKS !