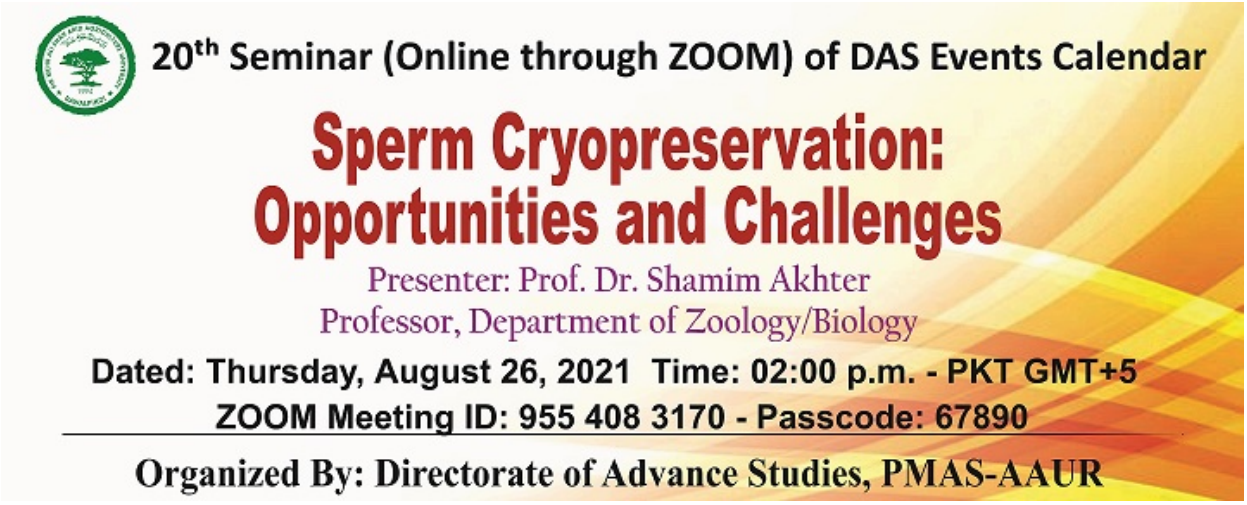
DIRECTORATE OF ADVANCED STUDIES

EVENT CATALOGUE

2021

20TH SEMINAR OF DAS EVENTS CALENDAR – 2021

SPERM CRYOPRESERVATION: OPPORTUNITIES AND CHALLENGES



ACTIVITIES

TOPIC COVERS:

2

INTRODUCTION

- Cryo-frost
- Cryopreservation-preservation in frozen state
- Sperm Cryopreservation
  - Semen
  - sperm

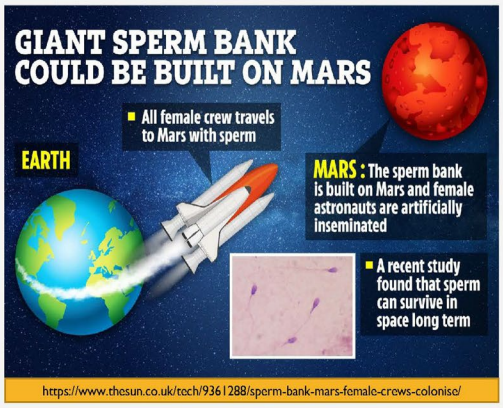
3

BRIEF HISTORY

- Snow cooling
- Glycerol cryoprotectant
- The earliest offspring produced from cryopreserved spermatozoa were:
  - **Bull** (1951)
  - **Human** (1953)
  - **Pig, Horse** (1957)
  - **Ram** (1967)
- Sperm cryobanks: cattle 1060s, human 1970s, avian species 1990s
- Most popular is cryopreserved semen for AI in farm animals and human (Hezavehei, M. 2018)

HUMAN PERSPECTIVE

- Management and preservation of male fertility
- Pre-Cytotoxic treatments reserve (chemotherapy, radiotherapy)
- Testicular sperm reserve for **Azoospermic Patients**
- A back-up sperm source for **Assisted Reproduction**
- Autologous and donor **Sperm Banking** for infertility
- Sperm banking for **Space Crew**



Live birth with sperm cryopreserved for 21 years prior to cancer treatment: Case report

https://doi.org/10.1093/humrep/deh249

LIVESTOCK

- Artificial insemination for **Genetic Improvement** in dairy cattle
- Semen from genetically superior sires-
  - **Cryopreserved**
  - **Breed thousands of females in bull's lifetime**
- Transport/exchange of valuable germplasm
- **Sexed Semen Banking**
- AI in buffalo not as successful-
  - **More cryodamages**

https://fairfaxcryobank.com/sperm-banking-storing-your-own-specimens

COMPANION ANIMALS

- Pedigree dog breeds in Britain suffering from a diminished gene pool
- Health problems after years of inbreeding
- The solution is
  - Naturally breeding using unknown dogs from the market
  - Use gene pool from the past
- Pedigree dog breeders are turning to artificial insemination
- AI uses cryopreserved semen
- Pedigree dog breeders earning from one dose equal to a dog (Queiro 2015)

https://cn.wikipedia.org/wiki/File:Pug_600.jpg

WILDLIFE CONSERVATION

Sperm cryopreservation for:

- ex situ conservation strategies
  - In vitro Fertilization
  - Semen Sexing
  - Genome Resource Banking
  - Somatic Cell Nuclear Transfer (cloning)

https://www.fairplanet.org/developing-stories/saving-rhinoceros/saving-the-genetic-variety-of-the-species-collection-and-preservation-of-dna-sperm-and-ovules/

CONT...

- Northern white rhinos: The audacious plan that could save a species (https://www.bbc.com/news/world-africa-52228181)

Precious sperm

- Cryopreserved after death of male Rhino

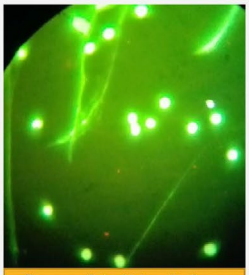
https://ichef.bbci.co.uk/news/976/cpsprodpb/2283/production/_111753880_najinrightfatugettyimages-934988458.jpg

Sudan the rhino is dead. But his sperm could save the species | Helen Pilcher | The Guardian

FISHERIES

- Hatchery Production of Fish Seed
- Inbreeding and Genetic drift
- Cryopreserved sperm: Transport / exchange of male germplasm between hatcheries
- Control inbreeding and genetic drift-genetic improvement
- Overcome non-synchronous maturation of male & female
- Simplification of brood stock management
- Availability of gametes throughout the year
- Manage work load in the busiest breeding season

Conservation of Species at risk (Kowalski et al., 2014).

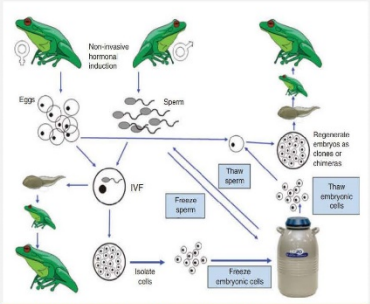


Fluorescent Stained *Labeo rohita* Sperm

https://www.aquaculturealliance.org/advocate/probiotics-benefit-three-stages-of-juvenile-rohu

AMPHIBIANS

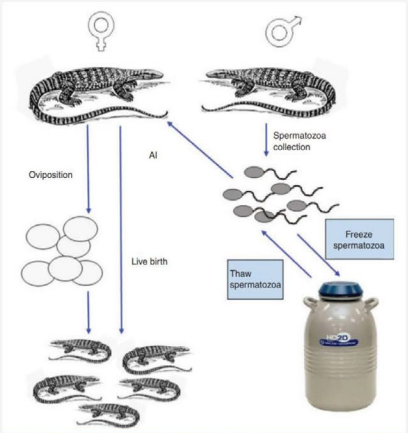
- The Amphibian Ark was established as a zoo based organization to support amphibian CBPs.
- Out of the 570 amphibian species requiring CBPs, 500 species or 90% need the support of gene banks including cryopreserved spermatozoa
- Anuran spermatozoa cryopreserved - successful fertilization late-1990´s
- Cryopreserved spermatozoa from Anurans (frogs and toads) has resulted in reproducing pairs
- ¨Proof of Concept Projects¨ for the use of cryopreserved spermatozoa in conservation programs are required (Browne, R. K., et al., 2019)



A realistic configuration of a functional amphibian genome resource bank based on existing technological understanding indicating that spermatozoa and isolated embryonic cells may be stored and used to regenerate live offspring and/or restore wild-type genotypes, but recognises existing technical blocks to the cryopreservation of mature oocyte and intact embryo (DOI: 10.1071/RD15466).

REPTILES

- Reptiles; most neglected group.
- Around 25% of reptilian species are threatened with extinction globally (Böhm et al., 2013).
- Testudines and Crocodilia around 50% threatened with extinction globally.
- Few successful sperm cryopreservation protocols developed .
- Lower post-thaw recovery rates (Clulow and Clulow, 2016).
- Lizards, an appropriate group for the development of sperm cryopreservation protocol (Young et al., 2017).



A realistic configuration of a functional reptile genome resource bank (GRB) based on the likely achievement of reptilian AI with cryopreserved spermatozoa, but the current lack of resource availability and support to pursue the development of other assisted reproductive technologies, such as IVF or ovarian and testicular tissue cryopreservation. Such a GRB for threatened reptiles would at least allow the retention and restoration of lost genetic diversity to genetically compromised wild populations (DOI: 10.1071/RD15466).

AVIANS

https://ebird.org/species/redjun

- Fowl spermatozoa Cryopreservation (Polge, 1951)
- *Ex situ* management of avian genetic resources in context of decreasing genetic diversity
- Avian germplasm cryobank in North America, The Netherlands and France
- The future avenues
  1. Predictors of males suitability for semen freezing
  2. Standardization of semen freezing technique in other species
  3. Development of avian cryobanks (Blesbois, 2017)

THE TECHNIQUE

Semen collection

↓

Evaluation (Volume, motility and concentration)

↓

Holding time (15 min at 37°C)

↓

Extension of semen with *tris*-citric acid extender (concentration of viable spermatozoa 50x106/ml)

↓

Cooling from 37°C to 4°C (in 2 hr)

↓

Equilibration at 4°C (for 4 h)

↓

Filling in 0.5 ml straw (at 4°C)

↓

Freezing on liquid nitrogen vapours

↓

Plunged of straws in liquid nitrogen (-196°C)

↓

Thawing at 37°C for 30 sec

Schematic Presentation of The Cryopreservation Process of Buffalo Semen

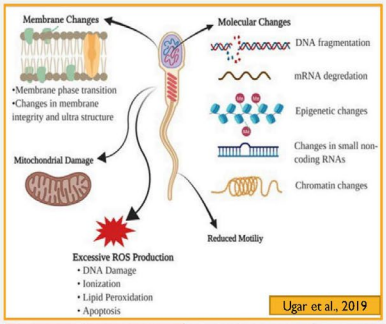
SEMEN / MILT COLLECTION:
(BIRD & FISH)

SEMEN COLLECTION:
(BUBALINE & BOVINE)

SEMEN EVALUATION: SPERM MOTILITY

CHALLENGES

- Semen Collection from non domestic species
- Sperm Cryodamages
  - Osmotic/thermal shock
  - Reactive Oxygen Species
  - Membrane changes
  - Molecular Challenges



Hypo-osmotic Swelling Test Showing Osmotic Shock

SPECIFIC CHALLENGES OF FISH, AMPHIBIAN, REPTILES

- Currently at research level
- Cryopreservation protocols are not standardized
- Commercialization extremely limited
- **Critical factors**
- Period of time between collection of sperm and freezing
- Equilibration time
- Longevity of sperm viability after thawing
- In vivo fertility test

RESEARH ACTIVITIES

- Species Specific Protocols
  - Media
  - Collection process/frequency
  - Processing (dilution, freezing, packaging)
  - Freezing (Slow, rapid), Programmable
  - Thawing (Temperature, duration)
  - Storage (vials, loops, straws, ampules0
  - Evaluation techniques
- Bacterial control
- Fertility trials

ETHICAL ISSUES

- Anonymity and non-anonymity of sperm doners
- Risks of unintentional consanguinity
- Use of frozen sperm posthumously (UK, Israel)
- Posthumous use of sperm is not permitted in Pakistan (religious/legal issues)
- **Use of electro ejaculators (Some mammalian species)**
- **Multigenerational inbreeding in all non-human species**

FINANCIAL ISSUES

- **Scarce reports on cryobanking costs**
- Fixed costs (building) and maintenance costs.
- Cryostorage rooms
- Security equipment
- Cryogenic tanks
- Equipment for collection, analysis and transportation of semen (artificial vagina, photometer, microscope, refrigerator, slide warmer, water bath)
- Maintenance costs; liquid nitrogen, labour costs.

A BETTER UNDERSTANDING OF SPERM CRYOPRESERVATION WILL ENABLE US TO IMPROVE

- Fertility management
- Success of assisted reproductive techniques like IVF and ICSI
- Genetic improvement of farm animals through artificial insemination
- Production of desired sex of farm/companion animals
- Conservation of endangered species

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