Implementation of assisted reproductive technologies in Javan Banteng (*Bos javanicus javanicus*)

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Free-ranging Javan Banteng

- Indonesian islands of Java and Bali
- <4,000 free ranging animals remaining in population
  - Declining in all areas
- IUCN Red List: Endangered
- AZA-endorsed WAZA Global Species Management Program

Previous Reproductive Research in Banteng

- Documentation of natural estrus cycle in *ex-situ* banteng (Asa et al., 1993)
- Conventional AI
  - successful at The Wilds and Western Plains Zoo (Johnson et al., 2002)
- In-vitro fertilization (without embryo transfer)
  - Successful (Barnes et al., 1988)
- Embryo flushing and embryo transfer from banteng into domestic cow recipients
  - produced two calves (Wiesner et al., 1984)
  - Inter-species transfer is not ideal due to uteroplacental differences between the two species, and lack of behavioral learning
- Nuclear transfer (cloning) of banteng genetics to domestic cow oocytes and recipients (see photo)
  - Lack of pre and postnatal viability likely due to issues from uteroplacental differences (Sansinena et al., 2005)

Ex-Situ Banteng at The Wilds

- Small herd of 9 Javan banteng
  - 6 females, 3 males
- Age ranges from 1-18 years old
- Managed on large pasture in summer, and small paddock with indoor access in winter
- Historically easy to manually restrain with low stress

Project Goals

- Establish protocols and assess efficacy of assisted reproductive technologies in Javan banteng
- Coordination with South-East Zoo Alliance for Reproduction and Conservation (SEZARC)
- Ultimate goal:
  - Utilize techniques established here in free-ranging banteng populations
  - Allow for selected gamete and embryo acquisition from wild banteng for use in *ex-situ* banteng populations

Assisted Reproductive Technologies

- Large scale project aiming to determine efficacy of:
  - deep intra-uterine artificial insemination
  - Embryo flushing and embryo transfer
  - Oocyte aspiration, In-vitro fertilization, and embryo transfer
  - Use of sex-sorted spermatozoa in above techniques

Current Progress

- Assessing efficacy of induced estrus synchronization protocol in banteng by hormonal analysis
  - Documented successful pregnancy after estrus synchronization and conventional artificial insemination
    - Semen provided by St. Louis Zoo

- Determining appropriate protocols for sex-sorting banteng spermatozoa
- Assessing efficacy of conventional artificial insemination

Next Steps:

- In **May**:
  - Perform super-stimulation, conventional artificial insemination, and embryo flushing to determine efficacy
  - Assessing efficacy of induced super-stimulation protocol in banteng by hormonal analysis

Next Steps:

- In **October**:
  - Perform super-stimulation, conventional artificial insemination, embryo flushing, and embryo transfer after recipient cows' estrus cycles synchronized

Institutional Benefits of Project

- Allows for more selective reproductive management
- Decreases chance of male calves when space is limited
- Eliminates cost and stress of live animal transport for breeding purposes
- Allows use of valuable deceased bull sperm
- Allows preservation of currently housed banteng genetics

International Benefits of Project

- Use of techniques in global *ex-situ* banteng populations
- Enhance genetics of insularized and isolated herds of banteng in range countries
- Allows acquisition of free-ranging banteng germplasm for use in *ex-situ* populations
  - Improving *ex-situ* banteng genetic diversity to represent that of wild populations

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Questions?