

Progress in sperm sorting and cryopreservation technologies for modifying population sex ratio and preserving genetic diversity in the rhinoceros and elephant

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AZA TAG/SSP-endorsed sperm sorting and cryopreservation research has been conducted in zoological rhinos and elephants by our group over the past decade. Our long-term goal is to establish a cooperative cryobank of sex pre-selected sperm for use in artificial insemination toward managing socially cohesive and genetically diverse elephant and rhino populations in conjunction with natural breeding efforts. Optimized methodologies for sperm sex-sorting and cryopreservation have been established in all studied species (white rhino: *Ceratotherium simum*, black rhino: *Diceros bicornis*, Indian rhino: *Rhinoceros unicornis*, Asian elephant: *Elephas maximus*, African elephant: *Loxodonta africana*) but inconsistent ejaculate quality has been the greatest challenge to establishing banked insemination doses. Electroejaculation of anesthetized males remains the optimal semen collection method for rhinos. For elephants, semen continues to be collected using transrectal massage of standing males but the majority of males produce poor quality ejaculates, with a considerable proportion of sperm exhibiting characteristics of aged cells (poor motility and DNA integrity). To date, sexed sperm banking is underway in all of the aforementioned species except the African elephant, from which high quality ejaculates have been difficult to access. Post-thaw sexed sperm quality has been more extensively studied in rhino than elephant species, but overall in vitro longevity data (computer assisted sperm analysis, DNA integrity, membrane and acrosome integrity, mitochondrial activity) indicate that sex-sorted sperm need to be inseminated into the uterus within 6 h prior to ovulation. This short insemination time-frame relative to ovulation necessitates an extensive understanding of female reproductive physiology, especially knowledge of temporal relationships among luteinizing hormone (LH) surge initiation, LH surge peak and ovulation. Because growing evidence indicates ovulation induction methods are needed to address anovulatory cycles that are exhibited by many rhinos of all three species, research is also needed to establish endocrine markers of follicular/oocyte maturity in association with trans-rectal ovarian ultrasound monitoring. For female elephants which typically ovulate spontaneously, serum LH profiles using a thrice-daily collection schedule during the three day ovulation window (the determination of which is based on the timing of the prior anovulatory LH surge) may be acceptable for timing inseminations with sex-sorted sperm. Pachyderm female reproductive research and sex-sorted sperm banking efforts remain ongoing and insemination trials with sex-sorted sperm are planned to start in the Indian rhino and Asian elephant during 2017.

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