Semen Collection from Rhinoceros During Standing Sedation

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Assisted reproductive technologies can facilitate genetic management of *ex situ* wildlife populations but rely on access to sperm samples. Sperm collection via electroejaculation (EEJ) and urethral catheterization (UC) is established for black (*Diceros bicornis*), greater one-horned (GOH; *Rhinoceros unicornis*), and southern white rhinoceros (*Ceratotherium simum*), but rely on anesthesia which can be risky and costly. Our main objective is to develop and optimize a protocol for sperm collection from rhinos that does not require general anesthesia, via UC during standing sedation. A procedure, which to our knowledge, has not been reported in any species. The success of anesthesia-based UC sperm collection appears to be promoted by alpha-2 agonists, which may stimulate epididymal contractions aiding the movement of sperm into the urethra. Alpha-2 agonists, such as medetomidine, are a commonly used sedative for rhinos. As a secondary objective, we also aimed to assess the use of intramuscular administration of oxytocin, a peptide hormone demonstrated to promote sperm collection in some species. Thus far, two trials of standing sedation UC were conducted with one white and one GOH rhino. For both procedures, oxytocin (20 IU) was administered alongside the sedatives, and once sedation was achieved, a rectal ultrasound was conducted to visualize sex accessory glands. The males were trained for rectal palpation leading up to the procedure and did not display any signs of discomfort during the exam. For both, the penis was fully extended following ultrasound exam and a sterile catheter was slowly inserted into the urethra. A highly concentrated (1.25×10^9 sperm/mL) and highly motile (~90%) sample was collected from the white rhino. The GOH rhino did not provide a spermic sample. There are several possible reasons to explain why the outcomes differed, ranging from physiological variations between individuals or species to procedural differences. To address the second objective, oxytocin was administered (20 IU) to four GOH males opportunistically during veterinary procedures and serum samples were collected at 0, 15, 30, 45, and 60 min post injection. Serum samples were used to validate an enzyme immunoassay for measuring oxytocin in rhinoceros' serum (Oxytocin Kit; Arbor Assays). Parallelism was confirmed between serially diluted pooled sample and the standard curve. Inter- and intra- assay coefficients of variation were maintained at < 15%, except for time 0 samples which fell below the detection limit of the assay. Results revealed oxytocin concentrations ~2X – 5X above basal were detectable at 15 min and remained high for the full 60 min. This study is ongoing; additional collections and efforts to optimize techniques will aid in the development of a protocol that may reduce risk and cost of sperm collection procedures and allow for collection of genetic material from a wider range of individuals.