Semen Collection from Rhinoceros During Standing Sedation

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Introduction

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*)[1-3] but rely on anesthesia which can be risky and costly. Our main objective is to develop and optimize a protocol for sperm collection from rhinoceros 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 [4]. Alpha-2 agonists, such as medetomidine, are a commonly used sedative for rhinoceros. 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. To do so, an oxytocin enzyme immunoassay was validated to monitor oxytocin in serum post exogenous administration for GOH rhinoceros.

Methods

Urethral Catheterization

Thus far, two trials of standing sedation UC were conducted in one white (Figure 1.A, C, D) and one GOH (Figure 1.B) rhinoceros. Oxytocin (20 IU) was administered alongside the sedatives which included medetomidine. Once a twilight-like sedation was achieved, a rectal ultrasound was conducted to visualize sex accessory glands via ultrasonography (convex probe). The males were trained for rectal palpation leading up to the procedure and did not display any signs of discomfort. For both, the penis was fully extended following ultrasound exam. A modified snake-hook was used to move penis towards the researchers without having to reach between bars. The distal end of the penis was rinsed with sterile PBS and dabbed dry with gauze. A sterile catheter (7 fr.) was lubricated and slowly inserted into the urethra until slight pressure indicating narrowing of the lumen associated with proximity to the prostate was felt by the practitioner. Proximity to the prostate was confirmed by rectal ultrasonography for the first catheter placed. Slight negative pressure was created using a 3 mL syringe and pulling back to ~0.5mL. Catheter was left in place for ~1 min. Pressure was relieved and catheter removed. Contents of catheter were emptied into 15 ml conical tube and stored insulated until assessment and cryopreservation.IN total, three catheters were placed per male. Sperm concentration, motility, viability, and progression were assessed, and samples were cryopreserved as previously described [5].

Oxytocin Enzyme Immunoassay

Oxytocin (20IU) was administered to four GOH males opportunistically during veterinary anesthetic procedures and serum samples were collected at 0, 15, 30, 45, and 60 min post-injection. The serum samples were used to validate an Oxytocin EIA Kit (Arbor Assays) by confirming parallelism between serially diluted pooled samples and the standard curve, confirming appropriate recovery of spiked –in oxytocin, and demonstrating biological relevance of patterns. A repeated measures general linear model was used to assess difference among time points (SPSS, v.29, IBM).

Figure 1: Photographs of semen collection via urethral catheterization from rhinoceros under standing sedation. A) Rhinoceros is sedate and penis fully extended. B) rectal ultrasound to visualize accessory sex glands, C) catheter insertion, D) transfer of collected sample from catheter to conical tube.

Results

Urethral Catheterization

- Neither male displayed signs of discomfort during placement of the catheter.
- Ultrasonography was successfully used to confirm appropriate placement of the catheter tip proximal to the prostate (Figure 1).
- A highly concentrated (1.25 x 10^9 sperm/mL) and highly motile sample (~90%) was collected from the white rhinoceros and cryopreserved.
- The GOH male did not provide a spermic sample.

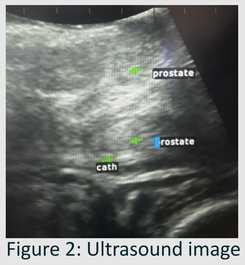

Figure 2: Ultrasound image depicting catheter tip placement proximal to prostate.

Oxytocin Enzyme Immunoassay

- 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.
- Oxytocin concentrations measured ~2-5X above basal at 15 min and remained high for the full 60 min (Figure 3).

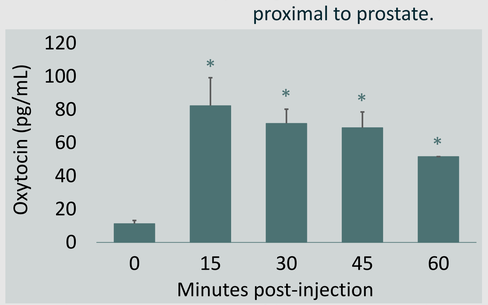

Figure 3: Average GOH rhino serum oxytocin concentration at 0, 15, 30, 45, and 60 min post intramuscular injection of 20IU exogenous oxytocin.

Conclusions

- Preliminary findings are promising indicating urethral catheterization during standing sedation is a feasible method for semen collection from rhinoceros.
- There are several possible reasons to explain the differing outcomes between the two males , ranging from physiological variations between individuals or species, to health status to procedural differences. Additional trials will aid in determining cause.
- The increase in oxytocin concentrations above basal at 15 min indicates intramuscular administration is an appropriate technique to achieve circulation of exogenous oxytocin and allow a physiological impact within the time limits of the collection.
- The validation of the oxytocin EIA will aid in further assessment of the pharmacokinetics of oxytocin in male rhinoceros.
- This study is ongoing; additional collections and efforts to optimize techniques are needed before final conclusions can be made about the efficacy of this newly developed semen collection technique.

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Citations

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