Greater one-horned rhinoceros (Rhinoceros unicornis) operant conditioning for artificial insemination program: a "recipe" for success

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Introduction:

The Cincinnati Zoo and Botanical Garden (CZBG) first displayed a greater-one horned rhinoceros (Rhinoceros unicornis) on loan in the spring/summer of 1877 (Ehrlinger, page 19). As a consequence of its popularity, a permanent display was created in 1923 (Ehrlinger, page 58). As the number of greater one-horned rhinos in captivity grew, so did their reputation for intractability (Kasman, 1986). In addition, this species presented challenges when introduced for breeding due to the severe aggression which could escalate quickly and result in severe injury (Guldenschuh, 2002). However, this species has reproduced well in captivity, but is deficient in genetic variation due to the lack of representation of behaviorally incompatible individuals (Hlavackek, 2006; Roth, 2005 & 2006). To offset these skewed genetics, we needed to increase our knowledge of greater one-horned rhinoceros reproduction to enable us to develop an artificial insemination (AI) program to improve the diversity of the population while ensuring the safety of the individuals.

To help offset this genetic dilemma, Dr. Monica Stoops, a Reproductive Physiologist at the Center for Conservation and Research of Endangered Wildlife (CREW), collaborated with a group of dedicated rhino keepers to apply their knowledge of operant conditioning practices and animal behavior expertise to enable her to apply scientific principles and technology in order to enhance reproductive efforts for this species.

Training:

The first phase of this research project was to condition the CZBG two greater one-horned rhinoceros to routine restraint and transrectal ultrasonography procedures. All training was done using operant conditioning principles based on positive reinforcement. As with any training program, the animals dictated the rate of progression, but during the entire process the keeper staff focused on reinforcing calm attitude and demeanor from each rhino. From the beginning, everyone involved made a concerted effort at effective communication which enabled us to maintain consistency throughout the process and have fewer occurrences of regression and/or breakdowns in behavior (Millwood, unk).

We were fortunate that the chute system needed for the procedure is one that the animals must pass through regularly for shifting purposes and the animals were already acclimated to the area. We were thus able to refine our training and focus on

confinement within the chute and the movement and sounds associated with it. For instance, any ultrasonography or artificial insemination procedure would require movement of a rear panel located within the chute into place behind the animal. By gradually exposing the animals to these new stimuli of panel movement and sound, we desensitized them to this vital piece of equipment since it must be in place so the procedures could be performed in a safe manner (Ramirez, page 137). The next step in our progression was to get them accustomed to a person being in position behind them, and progressing to tactile contact and tail manipulation. Desensitization to the sound and movement of the equipment cart (carrying the ultrasonography equipment and supplies) was done while the animal was in the chute and they were rewarded, as before, for calm attitude and demeanor. The final phase was the desensitization to the rectal insertion of finger, then hand, then arm, and finally, arm with hand holding the probe itself. Due to the large body mass of this species, we needed to ensure acceptance of the probe "extender" that is utilized to lengthen the reach of the examiner.

With all of these steps successfully conditioned, routine ultrasounds were conducted on each female three times per week. The solid foundation of positive reinforcement for compliance with these procedures enabled the reproductive physiologist to record and document subtle changes in ovarian structures while the keeper safely positioned and handled the animal during the procedure.

Due to the influence of anesthetics on the estrous cycle, we began to investigate the feasibility of conditioning the females to artificial insemination without immobilization. Initial attempts at desensitization to tactile contact of the vulva and vestibule was met with limited success. While training is thought of as a creative endeavor, the unique behavioral peculiarities of this species mandated that we think outside the box (Ramirez, page 66). We found that neither female was responsive to any vulvular contact or manipulation except when she was in the "follicular" phase of her cycle and under the influence of endogeous estrogen production. After discussing these cyclic characteristics, the project team decided not to do any desensitization training until the females entered this portion of the cycle. During this phase we would coordinate the conditioning sessions between keepers and the reproductive physiologist. For vulvular desensitization, we thoroughly cleaned the perineal area and used a sterilized insemination rod (Figure 1) with non-spermicidal lubricant. The progress was slow, but the dedication of all team members resulted in the accomplishment of this integral task.

Figure 1. Hand-held insemination rod with syringe attached (rod and syringe are sterilized for use in procedure)

The training, via successive approximation, for the insemination rod proceeded slowly due to this species' reproductive anatomy. In this species, as you enter the

vagina, the tract goes up and over the pelvic girdle before reaching the cartilaginous rings and folds of the cervix. In addition, we wanted to inseminate in the same horn in which the maturing follicle was present. Eventually we were able to condition one of the females to insertion of an arm into the reproductive tract with the insemination rod grasped within the palm of the hand. The second female, however, still requires the endoscope (Olympus CLV-10; colonoscope – Figure 2 and 3) for the insemination procedure.

Figure 2. Olympus CLV-10 machine without endoscope connected

Figure 3. endoscope used for insemination

To date, we have successfully performed the intrauterine insemination technique in both greater one-horned rhinoceros females.

In addition to the chute, ultrasonography, and artificial insemination training done with this species, we also conditioned animals for routine venipuncture. Venipuncture is truly a twelve-letter word when associated with the temperamental greater one-horned rhinoceros since they have proven to be a difficult species to condition for this procedure. However, I don't believe any of us involved felt it would be such an arduous task.

The precision of our training was critical since the same chute system is used for both ultrasonography and venipuncture procedures. We had to be cognizant of our actions as to not jeopardize the positive foundation already established for ultrasonography. After some discussion, it was decided that we would try and circumvent the (perceived) negative association with venipuncture by switching the direction the animal was facing for each procedure. Each animal would continue the ultrasound procedures three times per week facing one direction, and switched to face the opposite direction on venipuncture training day. We feel this simple, yet effective, technique enabled us to continue routine ultrasonography without incident, no matter how the animal(s) reacted during venipuncture.

In addition, at CZBG, all training is initially done by the keeper staff; but then we have to incorporate veterinary technicians since they are the only personnel permitted to perform the actual venipuncture. As with most rhinoceros species, venipuncture sites were on the medial aspect of the legs and the training steps have been documented and used previously with different rhinoceros species. Initially animals must be desensitized to tactile contact, then progress to utilizing nolvasan and/or alcohol solution on gauze pads and rubbing the venipuncture site on the legs, and finally begin desensitization to the venipuncture itself. To maximize flexibility for our

procedure, we have chosen to desensitize different sites for venipuncture. The medial surface of the front leg below the knee facilitates venipuncture of the cephalic vein (Figure 4). The medial surface of the front leg below the pastern enables venipuncture of the distal metacarpal vein (Figure 5). And finally, the venipuncture site on the medial surface of the rear leg for the medial sapheneous vein (Figure 6).

Figure 4. Venipuncture site on front leg to facilitate withdraw from cephalic vein

Figure 5. Illustrating front leg venipuncture of distal metacarpal vein

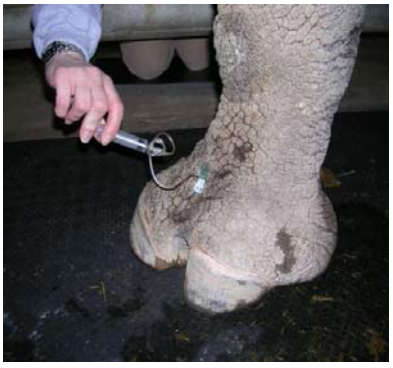

Figure 6. View of cleaning rear leg site to enable venipuncture of medial sapheneous vein

We currently use a 23 gauge x 1 inch butterfly catheter and syringe for our procedures since use of larger gauge needles have been unsuccessful. The actual venipuncture procedure itself was initially met with great resistance and a lack of cooperation from the rhinos. However, with persistence and patience, we shaped the desired positioning, posture and demeanor we wanted. Desensitization to needle re-

direction was done in the same manner as the venipuncture itself. Additionally, we found that implementing a two-keeper system for initial training for the venipuncture itself and the needle re-direction was beneficial since this action resulted in a break in the animals' concentration and an unwillingness to cooperate. Prior to venipuncture, one trainer would be responsible for conditioning and shaping desired behavior while second trainer remained quiet, but in position near the animal. We found that once venipuncture was accomplished, the animal would be unwilling to cooperate with first trainer, so the trainers would shift roles. This was somewhat confusing at first, but eventually it enabled us to maintain consistent focus despite the animals' initial negative response to the venipuncture. Since we have made it over this mental hurdle, only one keeper is required for a venipuncture procedure. And routine blood collection, and subsequent analysis, provides yet another avenue to assess and evaluate their health.

Reproductive data:

As this program evolved, and our knowledge of this species increased, we found several unique features of their reproduction. The estrous cycle is approximately 45 days in length, but can range from 35 – 60 days (Roth, 1999; Stoops, 2004 & 2005). Throughout her cycle, the female can grow multiple follicles until one reaches approximately 6 cm in size. At this point in time the follicle will become "dominant" and increase in size while the others regress (Stoops, 2004). As estrogen rises above baseline values it acts upon the uterine environment until the maximum follicle size of 10 – 12 cm is reached (Stoops, 2004). Since the follicle can be maintained for up to a week prior to signs of behavioral estrus, the astute observations of the keepers are critical to determining when the female is in estrus. Generally behavioral estrus signs occur at day 12 of the "follicular phase" and may be manifested by loss of appetite, increased pacing activity, "whistling" vocalizations, "squirting" urination patterns and vulvular "winking" but the intensity can vary between individuals. The positive foundation of reinforcement for ultrasonography has enabled us to increase the frequency and alter the time of the procedure throughout the day and/or evening to track ovarian changes. However, Dr. Stoops relies on the keepers' ability to pick up on the subtle nuances in behaviors as the indicator that ovulation is approaching and an increase in monitoring may be required. All of these factors (including evaluation of urinary hormone concentrations) are critical to the establishment of the AI time-line.

Semen from rhinoceros species have been successfully electroejaculated and cryopreserved using a standard hoofstock semen freezing protocol (Roth, 2001; Stoops, 2006). Spermatozoa are evaluated for evidence of morphological defects, total sperm number and motility prior to storage in subzero temperatures (Blanchard, pages 155-157). The aforementioned spermatozoal characteristics are then re-evaluated after thawing to determine if there was any damage as a result of cryopreservation. We have found that post-thaw sperm motility has averaged 55% and mean total motile sperm per inseminate was 620 million (Stoops, 2007). In the greater one-horned rhinoceros, the females are inseminated with 8 ml of semen at both 72 and 48 hours prior to ovulation (Stoops, 2007). At the conclusion of each insemination Dr. Stoops verifies the semen deposition site and subsequent transrectal ultrasounds will confirm ovulation of the follicle. Pregnancy can be

determined by the presence of the embryonic vesicle via transrectal ultrasonography at 18 days post ovulation (Stoops, 2007; Stoops, pers. communication).

In addition to the innovative AI procedure, we had the opportunity to be involved with an early fetal sexing study. This study is designed to determine fetal gender through the detection of Y-chromosome specific genes in maternal serum (Stoops, 2008). Our successful venipuncture training procedures enabled for routine sampling throughout one female's gestation and confirmed the calf's gender as female. The final results of this study will be published in a peer-reviewed journal once the appropriate data set is complete (Stoops, 2008). But, we were pleased to be able to participate and contribute to this innovative project.

Future goals:

The artificial insemination program has been a success in several ways. First and foremost, we can attest to the fact that scientific principles, coupled with keeper expertise, is truly a "recipe for success". We have successfully performed the intrauterine insemination numerous times and have documented vesicle implantation, embryo development and the subsequent formation and growth of a fetus. We have made our data and findings public and shared information with colleagues in an effort to improve knowledge and management of this species.

We will continue with the vaginal desensitization in an effort to have calm attitude and demeanor on a more consistent basis. One female has already displayed more stability and rarely wavers during a procedure. The second female has a more spirited temperament and can fluctuate dramatically as to her preferences during an AI procedure. In addition, we want to be able to use the insemination rod on both females, and thus, eliminate the need for the endoscope equipment for AI.

We have made great strides in our venipuncture practice, but there is still room for improvement. We do have deviations in our stable posture and positioning, which, as a result, makes successful venipuncture difficult. But, with persistence, the familiarity with each site will improve and so will our consistency with needle location to ensure repeatable results.

In the end, the team has benefited from the close comradery, reveled in the accomplishments, and supported each other during our darkest hour. We have accomplished the lofty goal of not only proving that AI can be done, but that it can become a valuable tool for reproduction of the greater one-horned rhinoceros, and we have taken steps to ensure this knowledge is shared with our colleagues (Shaffstall, 2007).

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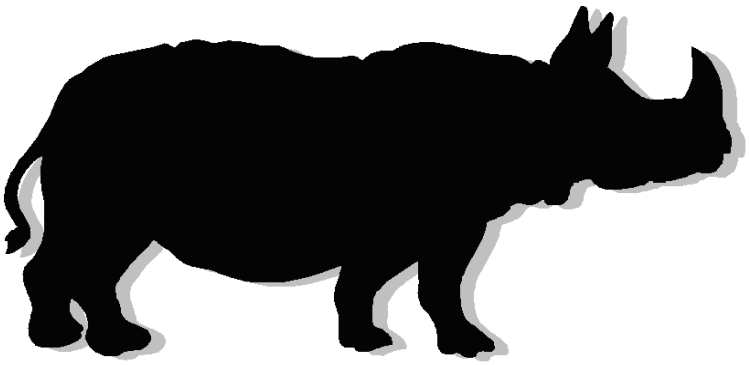

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