Conditioning a Southern Black Rhino (*Diceros bicornis minor*) For Semen Collection

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The question is often asked, "How long did it take to condition that rhino for that behavior?" A seemingly simple answer is complicated by the fact that no one behavior is conditioned by itself. All previous conditioning contributes to future conditioning. This paper will explain the semen collection conditioning at Fossil Rim Wildlife Center in the context of other conditioning that contributed to its success.

Fossil Rim Wildlife Center has been conditioning rhinos since 1987 when the first white rhinos arrived. These initial animals were from zoos where they had been housed in pairs and had never bred. The goal was to place these non-breeders in a ten acre yard as a multi-female herd including two bulls that were in visual, auditory and olfactory proximity and hope that the examples set by places like the San Diego Wild Animal Park would translate to Texas and lots of rhino babies would be born.

The primary conditioning was simply to train the white rhinos to go to the yard in the morning and back to the barn in the evening. The large yard has a small feed area (24.4 m x 30.5 m [80 feet x 100 feet]) where the animals are fed. If the ambient temperature is 4.5C (40F) degrees or colder the rhinos are moved to the barn which is 182.9 m (600 feet) from the yard down a 3 meter (10 foot) wide pipe lane. This is accomplished by opening and closing splitting gates that allow the rhinos to move in the desired direction, but does not allow them to go the wrong direction. The rhinos soon learned this and the splitting gates were no longer used except to train new arrivals, calves, and on occasions when the rhinos were not cooperating (e.g. bad weather, distractions, etc.)

Working with non-sedated rhinos is facilitated by the use of chutes. Fossil Rim uses two different types of chutes—the 'closed chute' and the 'free stall chute'. The closed chutes have gates in the front and rear, forcing the rhino to remain until the door is opened. The free-stall chute is open in the back and allows the rhino to come and go as it pleases. The inclusion of chutes within the animals' daily routine made chute conditioning easier (2,3,17). These chutes (one in the lane between the barn and the yard, another in the barn, and several in the yards) have been used for blood collection, urinary tract infection treatment, hoof work, serial ultrasonography, and basic skin and foot care. The conditioning was half complete prior to the chute construction because the animals were already moving through the area. The only challenge then was desensitizing the rhinos to being in the chute and to having people near them (12).

With the initial training in place, all future training was shaped fairly easily. The conditioning for the black rhinos was similar in its initial steps to the conditioning of the white rhinos with some exceptions. The first work was to bring the animals to the fence

where they would be more tractable. Most of the white rhinos had already been in captivity and were used to tactile rewards—rubbing on the face, under the back legs, or on the ears--so getting them to the fence was simple. Once at the fence the bridge from saying 'good' to the tactile reward was instant. The black rhinos, however, had come directly from Zimbabwe and weren't nearly as comfortable with people as the whites. The work with them required a considerable amount of time and several additional training steps.

Fossil Rim Wildlife Center acquired black rhinos in the spring of 1992 and utilized many of the training techniques already proven successful with the white rhinos. Unfortunately, participation in a feed palatability study early in 1993 that involved blood collections and measurements of the animals, required quarterly chemical immobilizations. The bull was not tractable, the female was not much better, and after multiple chemical immobilizations they became worse. In the fall of 1994, the bull was crate trained for a move to Australia and this was the only real training that was done with this animal. The inability to work closely with these animals because of time restrictions contributed to the need for the rhino internship.

Beginning in 1995, Fossil Rim Wildlife Center began the Rhino Research Intern Program. This position provided a full time person who worked solely with the black rhinos. Each internship lasts about four months, and among other things, each intern is responsible for a project that they will choose based on their interests and the needs of Fossil Rim. The first intern's project was clear: "to make the remaining female, a somewhat wild animal, tractable to collect blood, do foot work, and ultimately perform transrectal ultrasonography." Future projects focused on conditioning work because of its importance in the reduction of stress levels in the animals (6,7), but also included browse studies, cow/calf relationships, crate training, and studying the effects of separation of a calf from the mother for management purposes. One of the great benefits of this program is that each intern is trained on the specific conditioning protocol, assuring consistency in the training of the rhinos. This has proven very important in the rapidity and reliability of rhino conditioning. Each person has different mannerisms and the rhinos react differently to them, but the training schedule and methods remain consistent.

At the inception of the conditioning program, there was only the one rhino, the original female from Africa named Sinampande (studbook # 0466). She came to the fence for apples and alfalfa and could be moved from pen to pen in this manner, but she was not tractable for hands on care. From January 1995 until July 1995 she was the sole subject for training by the intern. In July 1995, another female, Coco (studbook #0462) and a male, Gota Gota (studbook #0401) came in from a ranch in south Texas. There were several health concerns with them that required immediate conditioning for blood collections, and more importantly, chute conditioning so that we could do foot work. Gota Gota had several hoof cracks, and Coco had a hole in the sole of her foot about the diameter of a quarter and approximately 3 centimeters deep.

From these beginnings, the training program was expanded specifically for semen collection from Gota Gota. A number of observations led to this decision. He had already successfully bred with Coco and produced two offspring with a third pregnancy

due in July of 2001. It was determined by the Rhino Taxon Advisory Group (TAG) that this pairing should not produce anymore offspring. Following two unsuccessful and aggressive introductions between Gota Gota and Sinampande, a third introduction was attempted utilizing hormone therapy. It was determined that her fecal progestin levels were always much higher than Coco's, and that this might have contributed to the fighting (9). There was less aggression during this introduction, but there was still no breeding despite coordinating the introduction with the most likely time frame for estrus based on ultrasound. Fossil Rim had already done work documenting the female reproductive cycle with fecal analysis and ultrasound and hoped that if we could collect semen, we could perform artificial insemination (AI) (9,10).

In the spring of 1997, the intern had an interest in artificial insemination and began conditioning Gota Gota for semen collection. He researched what had already been attempted in this field and based his conditioning on what had been successful. Digital manipulation was tried back in 1967 (18) with some positive results, and Columbus Zoo had conditioned and collected Clyde, a hand-raised male black rhino from 1979 to 1982 (12). Dr. Nan Schaffer and her extensive work in the male reproductive field was a constant source of information as well (14, 15, 16, 17). The proceedings from the 1991 Rhino Conference in San Diego, several articles in the Animal Keeper's Forum, and many of the papers and posters that Dr. Nan Schaffer produced were the foundation of our training program. We were fortunate to already have a somewhat tractable black rhino bull, good conditioning protocol, and excellent chutes. We had also done a very brief conditioning regiment on one of the white rhino males that had been worked with at Milwaukee County Zoo for years by Dr. Schaffer. With his previous conditioning we were able to move quickly with the schedule and see the results of an already trained bull back in 1990--even though we never successfully collected semen from him.

Gota Gota was already comfortable in the free stall chute. He had been coaxed to the fence with apples, sweet potatoes, browse--sumac or willow, or COB (corn, oats, barley), then led into the chute until he would stay in for as long as we chose, usually 10-15 minute sessions (1). Once comfortable in the chute, we would pile the fruit, COB, or browse in front of him and begin to desensitize him from the head to the tail, touching him until he no longer reacted. This took several weeks with one person working with him, and then several more weeks to allow multiple people to be present during the sessions. On occasion Gota Gota was better with multiple people than he had been with a single technician. The second person could offer the fruit more consistently, adapt to the changes Gota Gota made and keep his attention longer, thereby keeping him in the chute. As with most rhino work, it is important to have the animal comfortable with as many people as will be necessary for the procedure. When blood is collected it takes a minimum of three people, and there are usually four or five. The semen collection work currently requires two people, but we hope to expand it to several so that he can be worked for longer periods of time. It is too tiring for one person to work him for the duration of the session.

Cleaning the penis with warm water and cotton.

After he was desensitized to the people present and to the touching of other parts of his body, conditioning began for the actual semen collection. It was important to gain Gota Gota's trust in the chute before going to this stage. During the first months, time was spent trying to determine which techniques would work best to attain the goals. Planning to do everything without sedation meant we had to spend time getting the conditioning to elicit the best responses quickly. We began with penile massage and have expanded since then, but it was this beginning that took us to where we currently are. After just a few weeks Gota Gota would unsheathe when he entered the chute, sometimes attaining a full erection even before being massaged.

The next step was to actually work with the erect penis. The chute was modified to allow better access to the penis. Originally the pipes were horizontal, spaced with only 15 centimeters (6 inches) between them. One rung was removed from a 90 centimeter (3 foot) section to allow total access to the penis. Warm water and cotton were brought to the chute in an ice chest to clean the penis after unsheathing. KY Lubricant was used judiciously and the manipulation was performed for as long as the bull would stand in the chute, or until he was no longer erect. After the session, the penis would again be cleaned with the warm water. Using these methods, we were able to collect minimal samples, but none that were considered true ejaculates, or that were worth saving (2-5 cc's at most per sample). There were some attempts made to utilize urine and feces from females in heat as additional enticement but were unsuccessful at the time.

In the fall of 1997 we were forced to chemically immobilize Gota Gota for a large quantity blood collection for a transfusion to a sick animal. This set us way back in our training. Each negative occurrence would require extra training time with positive reinforcement to get back to where we were before the negative event. Also the other animals took a priority over our semen work and we didn't get back to working with him until the fall of 1999, and even then it was not as high a priority as it had been in 1997. Finally, in the summer of 2000, we began in earnest again with his conditioning and re-educated ourselves on what had happened in semen collection work throughout the world since 1997. Again, the intern put in time conditioning Gota Gota to the chute and to the tactile aspect of semen collection. He was called into the chute three times per day for training of about 10-15 minutes per session, based partly on what others had done in their training programs (1,7,12). He did well with a single technician but with multiple people he was nervous and wouldn't stand for any conditioning. It was decided to make the first attempt in the chute, but if he remained nervous, to move the conditioning to the back fence line where he traditionally seemed more comfortable. The main focus of the semen collection now occurs at the fence line. Gota Gota will stay parallel to the fence for as long as the session lasts and is released from training--as opposed to finishing the training because he has wandered away. Another benefit has been that with the comfort zone at the fence, he will allow multiple people near him, and will usually get an erection immediately. He has also allowed blood collections and hoof work in the same area without problems.

When he was again working well, we attempted to use an artificial vagina (Colorado A/V, distributed by Har-Vet) designed for use on the horse. The A/V uses warm water and pressure to simulate the female. Different temperatures and pressures that are based on the weight of water in the A/V were used to determine the most effective combination. Unfortunately, Fossil Rim faced the same shortfalls other institutions using A/V's had experienced (15); they are heavy, about 11.4 kg (25 pounds) and awkward to use. Also, since the A/V must be underneath the animal, it is difficult to remove quickly if the animal becomes uncomfortable in the chute and backs out, or moves away from the fence and is out of reach.

Application of the A/V.

There are some other options available that haven't been tried yet, but are likely substitutes. Susan Inkster utilized an A/V similar in structure to a blood pressure cuff (4) With some minor modifications this may be a good option. Fossil Rim Wildlife Center is also experimenting with air splints designed to immobilize fractured bones in humans (Moore Medical Corp. 389 John Downey Dr. New Britain, CT). These are inflatable, vinyl bladders that are designed to immobilize different fracture sites. The best size has been the 'hand/wrist' splint which weighs less than one pound including the latex lining. The latex lining is inserted as in the horse A/V and the splint can be inflated to the desired pressure. This will be a very lightweight and portable option that should work as well as the commercially available A/V for pressure, but without the option of warming it with water.

There is another possibility for better success in collecting—the use of phantoms. A phantom is a life size model designed to simulate the female. There is no such phantom designed as an artificial mount for rhinos, but one could be built. These are widely used for horses, and Gota Gota is very receptive when the female is in heat. If the phantom were to be built where the closest the bull could get to the female was by mounting the phantom with the A/V built into the phantom, a technician could monitor the A/V and determine that it was placed properly. The bull could then mount the phantom, ejaculate into the A/V, and the semen could be collected. Coco always backs up to the fence when she is in heat, so the use of the phantom may have potential at Fossil Rim.

Some other types of collection have been tried (successfully and unsuccessfully) in the rhino (and the horse). Dr. Terri Roth has been collecting semen post coitally from the female Sumatran rhino (8). This is a great, non-invasive option that Fossil Rim will definitely try on our white rhinos, and possibly our blacks. Unfortunately, it won't work for Gota Gota while he is at Fossil Rim since he won't breed here again. Administering Oral Imipramine followed by Intravenous Xylazine has been used in equine research with mixed results (5). There are a couple of concerns at Fossil Rim about this. First, the study showed that results in horses were inconsistent and that Imipramine has been associated with hemolysis in some horses, which would obviously be a negative side effect considering the already high occurrence of hemolytic events in the black rhino. Also, it showed that other aspects were still necessary (e.g. teasing with a mare). We feel that we can get ejaculates without immobilization or electroejaculation, and are hesitant to give other drugs (due to possible adverse reactions) especially with such inconsistent results and potential risks.

The irony of all this is that Fossil Rim Wildlife Center has not successfully collected semen from any of the rhinos. The conditioning protocol here is consistent and effective. We have serially collected blood and done ultrasound exams for seven years, and feel confident that semen collection is around the corner, but right now it is all still just academic--and experimental.

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