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### **Semen Collection in *Diceros bicornis***

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#### **Aim**

The aim of the project was to condition Mwaniki to produce regular semen samples, in response to a modified artificial vagina, and to evaluate the quality of the samples on production.

#### **Introduction**

*Diceros bicornis* is on the verge of extinction. 65 000 roamed wild in Africa in 1970. Today estimates are as low as 2 500. Western Plains Zoo, Australia, is involved in one of the largest captive black rhinoceros breeding programs in the world. The aim is to reintroduce their offspring back into the wild when poaching has been effectively eliminated. The zoo currently has seven females, and five males. Two of the males have reached puberty, which occurs at approximately 7 to 8 years of age.

#### **Materials, Methods and Results**

Mwaniki, a 16 year old Kenyan black rhino, is of a different subspecies to the females involved in the breeding program. His semen is therefore destined for genome banking. Initially, Mwaniki had to be comfortable with his surroundings, and being shut into a chute for half an hour at a time. The design of the chute at Western Plains Zoo is illustrated in the first photo. It consisted of sliding gates at either end, which were secured with bolts. When rectalling was attempted, two horizontal bars were slid through rings, and secured. The gate was then opened to allow access to Mwaniki. As part of his daily routine Mwaniki was encouraged to walk through the chute. This progressed to feeding him tasty treats, of bananas, apples, sweet potatoes, and browse (*Casuarina stricta*) while shut in, and gradually conditioning him to accept the presence of humans close to him.

In the conditioning of a rhino for regular semen collection, manual stimulation is the proven method, on a fully conscious, 'tractable' rhino. With a rhino it is not practical for an oestrus female to be used as a teaser. *Diceros bicornis* are solitary, territorial animals, and will only tolerate company if the female is fully in oestrus. At this time they can be especially difficult to manage. In previous trials faecal material from an oestrus female did not show any improvement in the success rate. *Diceros bicornis* have acute olfactory and auditory senses, though vision is limited. Predicting potential distractions was therefore quite a challenge!

The chute connected Mwaniki's night yard to Taronga's night yard. In order to allow Mwaniki

to turn around to re-enter his own night yard, he was allowed into Taronga's night yard. Taronga is the 48 year old 'retired' female Kenyan black rhinoceros. On entering Taronga's yard, Mwaniki exhibited a flehmen response and scent marking. This occurred where Taronga urinated, defaecated and at her water trough. Mwaniki re-entered the chute in his own time, which ranged from 5 minutes to 40 minutes. The response was always better on the way into Taronga's yard. Mwaniki often became impatient, agitated and eager to re-enter his own territory on the return through the chute.

### **1. Electro-ejaculation**

Performed on a conscious rhino (if there was any sign of irritation the procedure was terminated), this requires specialist equipment and an experienced operator. This option was not available at the zoo.

### **2. Artificial vagina**

Previously artificial vaginas have been too heavy, requiring an operator on either side just to hold it, and caused abrasions of the lateral flaps of the penis. The artificial vagina especially modified for Mwaniki was a light, easy to apply, latex lined, air filled model. It applied a modulated pressure to his penis. The sample was to be collected into a hand held sterile container. The artificial vagina was warmed to approximately 37°C by wrapping it around a bottle filled with hot water. Once an erection was sustained, the AV was wrapped around the erect penis, secured with Velcro, and a pressure of 10 kPa applied. On one occasion modulation was achieved, but not for any significant length of time. Initially the noise of the air being modulated, the sound of the Velcro being undone, and the weight of the larger cuff appeared to distract Mwaniki. The fact that there was often only one operator to massage also meant that with the application of the cuff, and the cessation of massaging, there was often a decrease in the size of erection. These were all surmountable obstacles, but the conditioning was unachievable in the time available.

### **3. Rectal massage**

The natural response to defaecation in a rhino involves kicking out the hind legs to ensure a good spread of the faeces. This immediately created a problem with rectals. Care had to be taken to avoid the powerful kick, and there was also concern that Mwaniki did not injure himself on one of the horizontal bars of the chute. Initially Mwaniki was gradually conditioned to accept a presence behind him, then the noise of the horizontal bars sliding in, and finally the rectals. Massage of the prostate was attempted with penile let down of 1cm to 2cm, on the most successful occasion. Previous studies suggest that when an ejaculate is collected regularly, rectal massage can enhance the quality and quantity of the sample. The fact that Mwaniki is conditioned for rectal massage holds interesting prospects for the future.

### **4. Penile massage**

Once a full erection had been produced, ejaculation took place within a few minutes of massaging the distal portion of the penis. The ejaculate was collected into a sterile container, preheated in hot water to about 35°C. This was the method by which the two samples were collected...

## **Semen Sample**

The ejaculate was produced in squirts of fluid, all of the same consistency.

| Sample | Volume | Consistency | Colour |
|--------|--------|-------------|--------|
| 1      | 2mls   | Thick       | White  |
| 2      | 12mls  | Thick       | Yellow |

The sperm were difficult to visualise with unfamiliar equipment and no comparative slides. A different microscope allowed better visualisation. A great deal of "Brownian motion" motility was seen. On dilution there was at least 50% progressive motility of the particles seen in the sample. Only a few sperm could be definitively identified on a higher power. However the colour, consistency, and motility of the sample were highly suggestive of sperm rich samples. The second sample had considerable movement. The colour was suggestive of urine contamination, but prostate fluid can appear yellow. A measurement of pH would have helped determine which was the cause of the colour. This was not done at the time, as the sample was believed to have been contaminated.

## **Conclusion**

The project presented an interesting challenge. Conditioning in itself is time consuming, but the results when it has been achieved are well worth it. The majority of distractions have been identified, and any unavoidable stimuli integrated into the conditioning. The samples collected proved that Mwaniki is now at a point where regular sample collections are a reality.

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## **Author Notes**

Susan Inkster is a veterinary student in the Class of 1997 at the Glasgow Veterinary School in Scotland. She won second place in the prestigious "Governor's and Mitchell of Cranstonhill Prize" in November 1996 for this talk "Semen Collection in the Black Rhino".

## References

Also check out , SOS RHINO, a multimedia site dedicated to rhino education.

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