REPRODUCTIVE APPLICATIONS OF TRANSRECTAL ULTRASONOGRAPHY IN CAPTIVE AFRICAN RHINOCEROS, AND THOUGHTS ON IN SITU USE

Robin W. Radcliffe, DVM* and Steven A. Osofsky, DVM
Fossil Rim Wildlife Center, PO Box 2189, Rt. 1 Box 210, Glen Rose, TX 76043, USA

Abstract

The utilization of transrectal ultrasonography for research on and management of captive southern black (*Diceros bicornis minor*) and southern white (*Ceratotherium simum simum*) rhinoceros has proven beneficial via the elucidation of normal and abnormal reproductive function in female rhinos without the need for sedation. Examinations were facilitated by the use of a "free-stall" chute that allows the rhino to choose its own response to the process. This report highlights the types of information gained, and the potential management repercussions. Reproductive work to date in the black rhinoceros has focused on gestational monitoring, using the Aloka 500V console with a hand-held 5.0 MHz linear array transducer. Weekly exams have allowed for the documentation of: fetal heart rate, fetal mobility/orientation within the uterus, and various measurements of fetal parts. In white rhinos, a 5.0 MHz convex array transducer has proven most successful, and has been used to elucidate the estrous cycle of one white rhinoceros female, as well as to document early embryonic loss in this female. Equipment modifications to facilitate ovarian examinations in the white rhinoceros include a custom 10 foot transducer cable and an extensor. The interovulatory interval in the subject white rhinoceros averaged 33 days (n=2). A number of similarities to the horse were documented in the white rhino female. These included the formation of two ultrasonically distinct luteal structures in an approximately even ratio, the formation of anovulatory hemorrhagic follicles, the identification of intrauterine fluid collections in late diestrous as an indication of endometritis, and similar estrus behaviors. In relation to observed breeding behavior, ovulation was documented to occur within 24 hr post-breeding. Concurrent fecal hormone assays confirmed ultrasonographically identified reproductive cycle dynamics. However, delineating the precise timing of ovulation, differentiating between early pregnancy and a nonpregnant luteal phase, and identifying early embryonic loss would have proven difficult using fecal hormone assays without ultrasound. By scanning rhinos opportunistically, managers of free-ranging rhinos in a variety of contexts may obtain practical information while simultaneously enhancing understanding of the causes of infertility in captive specimens, the type of two-way information exchange the conservation community strives for.

Resumen

La utilización de la ultrasonografía transrectal para la investigación y el manejo en cautiverio del rinoceronte negro del Sur (*Diceros bicornis minor*) y del rinoceronte blanco del Sur (*Ceratotherium simum simum*) ha traído beneficios mostrando lo normal y anormal del aparato reproductivo de los rinocerontes hembras sin necesidad de sedación. Los exámenes fueron facilitados por el uso de una manga de manejo denominada "free-stall", que permite al rinoceronte elegir su propia reacción para el proceso. Este reporte destaca los tipos de información obtenida y las potenciales repercusiones de su manejo. El trabajo reproductivo hecho en el rinoceronte negro se ha basado en el control de la gestación utilizando un aparato de consola Aloka 500V con un trasductor manual lineal de 5 MHz.

Exámenes semanales permitieron la documentación de los siguientes parámetros: frecuencia cardíaca fetal, movilidad y orientación fetal en el útero, y la medida de partes fetales. En rinocerontes blancos fue más exitosa la utilización del transductor convexo de 5 MHz, el mismo que se ha utilizado para poner en evidencia el ciclo estral en un rinoceronte blanco hembra, así como para documentar una pérdida embrionaria temprana en esta hembra. Las modificaciones al equipo para facilitar el examen de los ovarios en el rinoceronte blanco incluyeron el empleo de 3 metros de cable para el transductor y un extensor. El intervalo interovulatorio en el rinoceronte blanco fue de 33 días (n= 2). Se documentaron algunas similitudes encontradas en el caballo y en el rinoceronte blanco. Estas similitudes incluyen la formación de dos estructuras lúteas ultrasónicamente distintas en proporción similar, la formación de folículos hemorrágicos anovulatorios, la identificación del fluido intra-uterino recolectado en el di-estro tardío como un indicador de endometritis y un comportamiento estral similar. En relación con la conducta observada, se concluyó que la ovulación ocurre dentro de las 24 hrs. después de la cópula. Los ensayos de una hormona fecal confirmaron la dinámica del ciclo reproductivo identificado por el ultrasonido. Sin embargo, la determinación del tiempo preciso de la ovulación, diferenciación entre preñez temprana y fase lútea de no gestación, como la identificación de pérdidas embrionarias tempranas han sido complicadas utilizando las pruebas de hormona fecal sin el auxilio del ultrasonido. Al examinar rinocerontes oportunísticamente, los manejadores de rinocerontes silvestres en una amplia variedad de contextos pueden obtener información práctica mientras simultaneamente se incrementa el conocimiento de las causas de infertilidad en especímenes en cautiverio. Este es el tipo de intercambio de información mútua por el que la comunidad conservacionista se esfuerza.

Introduction

Ultrasonography is a tool being applied in captive management to resolve some of the basic mysteries surrounding rhinoceros reproduction. Managed breeding decisions can finally be based on objective reproductive assessments of individual animals instead of conjecture. This technology can be taken into the field setting where it could provide valuable information about the reproductive functioning of wild rhinos as well. At its most basic level, opportunistic ultrasonography at the time of capture could provide insights into the effects of the translocation process on embryo/fetal viability during different stages of gestation. For an animal producing one offspring at a time with a long inter birth interval, this information could prove valuable as rhinos are by necessity becoming more painstakingly managed in parks, reserves, conservancies, sanctuaries, and intensive protection zones throughout Africa.

The following case studies highlight the application of this tool in the captive setting, the types of information gained, and the potential management repercussions.

Black Rhinos

Reproductive work to date in the southern black rhinoceros (*Diceros bicornis minor*) at the Fossil Rim Wildlife Center has focused on gestational monitoring. In black rhinos, the Aloka 500V console used with a hand-held 5.0 MHz linear array transducer has proven most successful.

Case Study I

An approximately 12-yr-old female southern black rhinoceros was captured in Zimbabwe, held in a boma for several months, and then transferred to the Fossil Rim Wildlife Center in Texas, U.S.A. in April of 1992. Upon arrival at Fossil Rim, this female was aggressive in nature and remained apprehensive in the presence of humans. Starting in January of 1995, a full-time caretaker began intensive conditioning of the rhino to allow hands-on examinations with the hopes of eventually performing transrectal ultrasound evaluations without sedation. The conditioning process involved exposing the female to long hours of human contact along with visual, tactile, and auditory stimuli, including the intermittent playing of a radio to add background noise to her normal environment.

The positive conditioning process began with food such as apples and sweet potatoes as a reward for tolerating the proximity of people. This soon expanded to the application of human touch on different areas of the rhino's body at the time of feeding. Over a period of several months, the rhino began to trust her human caretakers enough to facilitate twice daily examination and treatment of a potentially serious hoof crack. The conditioning process was facilitated by the use of a "free-stall" chute that was designed to allow the rhino to choose its own response to the process.[5] The rhino was never restrained physically or chemically for the purposes of conditioning, examination, or treatment. Starting in July of 1995, the female was exposed to daily rectal examination in the chute without chemical restraint. Within 2 wk the application of transrectal ultrasound was successful, again without sedation. The fetal ultrasonographic images obtained correlated well with a breeding date approximately 11 mo earlier.

Case Study II

Another female southern black rhinoceros, 5 yr of age, is part of ongoing research designed to document normal fetal dynamics throughout gestation in this species. This female also had a history of a foot problem that was being treated in the free-stall chute. Separation from the male was necessary in order to facilitate daily therapy. An ultrasound exam at 57 days post-breeding confirmed an early pregnancy, thus making separation of the pair for medical management more feasible. The continuation of weekly exams over time has allowed for the documentation of: fetal heart rate, fetal mobility/orientation within the uterus, and various measurements of fetal parts. As sufficient data is collected to document and chart fetal dimensions such as eye diameter or skull length over time as has been done in the horse[3], gestational age charts can be developed for the rhino. Ultrasonic monitoring of the fetus also proved beneficial following a Type-I hypersensitivity reaction this rhino cow exhibited in response to routine leptospirosis vaccination. Despite the severe effects noted (serosanguinous fluid exuded from various skin sites, fever), the fetus appeared unaffected based on ultrasonographic visualization.

The management implications of this work are obvious regarding captive rhinoceros propagation. The conditioning process not only allowed for the transrectal ultrasound examinations, but enabled successful treatment and monitoring of medical problems in previously intractable rhinos. In the first case, a decision to postpone immobilization of the black rhino female for more aggressive treatment of the hoof crack was based partly on ultrasonographic confirmation of late-term pregnancy. Furthermore, documentation of the stage of pregnancy facilitated dietary modifications to match the nutritional demands of late gestation. A healthy calf was born in December, 1995 to the older cow,

and the birth of the younger cow's first calf is anticipated in January - February, 1997. Foot problems have resolved in both females.

White Rhinos

The potential applications of transrectal ultrasound in large nondomestic animals have recently been recognized.[1,4] This technology has been used to elucidate the estrous cycle of one of Fossil Rim's southern white rhinoceros (*Ceratotherium simum simum*) females, as well as to document early embryonic loss in one female. The early embryonic loss is believed to have been caused by a uterine infection and, like endometritis in the horse, was characterized by intrauterine fluid collections in late diestrous.

In white rhinos, the Aloka 500V console used with a 5.0 MHz convex array transducer has proven most successful. Equipment modifications to facilitate ovarian examinations in the white rhinoceros include a custom 10 foot transducer cable and an extensor. The extensor, formed of PVC pipe reshaped via thermal manipulation, is required in order to consistently image the left ovary, which is beyond the operator's unassisted reach in an adult white rhino.

The rhinoceros belongs to the order Perissodactyla or 'odd-toed' ungulates which includes the horse and tapir. The equine species was used as a model for interpretation and evaluation of ultrasonographic information in this study[2, 3] and this comparative approach has proven essential to a greater understanding of rhinoceros reproductive biology. The following is an outline of important reproductive events documented to date through utilization of transrectal ultrasound in white rhinos in the captive setting:

1) The interovulatory interval in the subject white rhinoceros averaged 33 days (n=2).

2) A number of similarities to the horse were documented in the white rhino female. These included the formation of two ultrasonically distinct luteal structures in an approximately even ratio, the formation of anovulatory hemorrhagic follicles, the identification of intrauterine fluid collections in late diestrous as an indication of endometritis, and similar estrus behaviors.

3) Maternal recognition of pregnancy appeared to occur both times there was early embryonic loss on or before day 28, with subsequent persistence of the luteal phase for 10-11 wk. This suggests that maternal recognition of pregnancy in the white rhinoceros occurs prior to 28 days of gestation.

4) In relation to observed breeding behavior, ovulation was documented to occur within 24 hr post-breeding. Obtaining this information is a requisite step prior to the application of advanced reproductive techniques such as artificial insemination in the rhinoceros.

5) Concurrent fecal hormone assays confirmed ultrasonographically identified reproductive cycle dynamics (Czekala, personal communication), and this type of correlation helps validate both methodologies. Without ultrasound, however, delineating the precise timing of ovulation, differentiating between early pregnancy and a nonpregnant luteal phase, and identifying early embryonic loss would prove difficult. Estrous cycle lengths of approximately 10 wk, identified

via fecal EIA alone, have been reported elsewhere as the expected normal in this species,[6] and may be inaccurate based on the findings in this study. In situ and ex situ managers should, if at all practical, monitor rhinos with noninvasive (and less expensive) fecal assays. It is suggested, however, that ultrasound "spot-checks" could yield additional, complementary information.

In Situ Use

The potential applications of this work in rhino range states remain open to debate. The ability to determine pregnancy status could have management repercussions regarding how an immobilized female rhino would be handled during and following capture/translocation. The stresses associated with immobilization, transport, and boma confinement can result in abortion in a wide variety of species, including rhinos; the detection of an embryo/fetus could potentially change the course of action regarding boma management or translocation, for example. Data collection from field scanning of females could provide managers with a measure of a rhino population's reproductive health. This would facilitate sound management decisions, differentiating between populations which could sustain translocation of individuals to other areas and those populations requiring more intensive conservation efforts. Fertility problems are certainly bound to be more prevalent in captive situations than in the wild; information gleaned from wild animals could help zoos tease apart environmental, social, as well as nutritional factors that may be contributing to reproductive failure in captivity.

Detecting cyclicity and the corresponding stage of an estrous cycle of a female rhino on one ultrasound exam would be difficult, but this has been done in the equine species based on size and echogenicity of the corpus luteum.[3] Since both the rhinoceros and the horse, as perissodactylids, share a common evolutionary history, it seems reasonable to look for similarities in their reproductive biology as part of ongoing research efforts.

Thoughts for the Future

The utilization of transrectal ultrasonography for research and for management of captive black and white rhinoceros has proven beneficial via the elucidation of normal and abnormal reproductive function in female rhinos without the need for sedation. This technology can be taken into the field setting where it could provide valuable information about the reproductive functioning of wild rhinos as well. The authors are not suggesting that wild rhinos be subjected to immobilization simply to be scanned ultrasonographically. This would be an inappropriate use of financial and technological resources in most contexts. It may, however, be worth integrating a 10-15 min scanning procedure into some capture and translocation protocols already in place for a variety of reasons. By scanning rhinos opportunistically, managers of free-ranging rhinos in a variety of contexts may obtain practical information while simultaneously enhancing understanding of the causes of infertility in captive specimens, the type of two-way information exchange the conservation community strives for. It may be worth considering the selective application of this tool as an adjunct when questions regarding fecundity and fertility arise in free-ranging populations that are, by default, requiring more and more intensive management in the face of a plethora of human-related pressures. In short, the selective application of transrectal ultrasonography could help shape management decisions that underlie the maintenance of healthy conservation units both in situ and ex situ.

ACKNOWLEDGMENTS

The authors would like to thank the Fossil Rim Wildlife Center, the International Rhino Foundation, and ABC/Kane Productions for supporting this project. We would also like to acknowledge Chuck Boland of Aloka for generous contributions of ultrasound equipment.

LITERATURE CITED

1. Adams, G.P., E.D. Plotka, C.S. Asa and O.J. Ginther. 1991. Feasibility of characterizing reproductive events in large nondomestic species by transrectal ultrasonic imaging. Zoo Biol. 10: 247-259.
2. Ginther, O.J. 1992. Reproductive Biology of the Mare, Basic and Applied Aspects, 2nd ed. Equiservices Publishing, Cross Plains, Wisconsin.
3. Ginther, O.J. 1995. Ultrasonic Imaging and Animal Reproduction: Horse, Book 2. Equiservices Publishing, Cross Plains, Wisconsin. Pp. 198-246.
4. Hildebrandt, T.B., and F. Goritz. 1995. Ultrasonography as a tool in propagation of zoo animals. Proc. Am. Assoc. Zoo Vet. / Wildl. Dis. Assoc. / Am. Assoc. Wildl. Vet., East Lansing, Michigan: Pp. 356-364.
5. Radcliffe, R.W., S.A. Osofsky, and A.I. Eyres. 1995. Design and applications of a "free-stall" chute for passive restraint of the non-sedated white rhinoceros (*Ceratotherium simum simum*). Proc. Am. Assoc. Zoo Vet. / Wildl. Dis. Assoc. / Am. Assoc. Wildl. Vet., East Lansing, Michigan: Pp. 430-434.
6. Walzer, C., and F. Schwarzenberger. 1995. Estrous cycle induction in a white rhinoceros (*Ceratotherium simum simum*) and concomitant EIA fecal progestagen analysis. Proc. Am. Assoc. Zoo Vet. / Wildl. Dis. Assoc. / Am. Assoc. Wildl. Vet., East Lansing, Michigan: Pp. 365-368.