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RHINO REPRODUCTIVE PHYSIOLOGY - WHAT WE KNOW TODAY AND WHAT WE NEED TO KNOW TOMORROW FOR ENSURING THE LONG-TERM STABILITY OF OUR CAPTIVE BREEDING PROGRAMS

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Considerable progress has been achieved through reproductive studies in all four captive rhinoceros species. Research results have revealed interesting variation among species within this taxon and also have provided information that will facilitate the management of captive populations. Much of the current reproductive database has resulted from studies that relied upon sample collections and/or behavioral observations conducted by keepers working directly with the animals. For example, keepers have collected fecal, urine, saliva and blood samples for hormone analyses which have been used by researchers for characterizing the reproductive cycles and determining pregnancy status in rhinos of each captive species. Observations and data from behavioral check-sheets recorded by keepers have been instrumental in determining estrous behaviors, breeding activity and inter-animal relationships. For more intensive studies, such as those requiring ultrasound evaluations and blood collection, animals have been conditioned to enter a chute or move near the side of the enclosure and stand still for the procedure. This type of conditioning also was either conducted by the keepers or, at least, involved their significant participation. The diligent efforts of numerous keepers are reflected in our current knowledge of rhino reproduction.

Blood collection once was thought necessary for evaluating hormone levels in animals, however, we now know that animals excrete hormones in slightly modified forms (metabolites) into the urine and feces. The discovery of this noninvasive method of determining hormone levels opened the door to tremendous opportunities in studying nondomestic species. Noninvasive fecal hormone metabolite monitoring has been the primary method used for characterizing rhino reproductive cycles, and resulting data have provided a foundation of basic knowledge upon which we now can build by employing additional research tools like ultrasonography. Already, ultrasonography has proven useful for identifying reproductive characteristics that might otherwise have remained undetected.

The African black rhinoceros (*Diceros bicornis*) has been the most prolific and best studied of the captive rhinos. Most female black rhinos are exhibiting reproductive activity. Their reproductive cycles average about 25 days, however, variable cycle lengths are common, with approximately 50% of cycles <20 or >30 days. Although reproductive success has been relatively high, there are several animals that appear to be healthy and reproductively active but continue to breed without becoming pregnant. Identifying the cause of this apparent infertility is the primary challenge ahead for black rhino reproductive research. However, the greatest threat to the captive black rhino population is their unusual susceptibility to several uncommon diseases.

Fecal sample collection in the African white rhinoceros (*Ceratotherium simum*) has been difficult due to the common social group management style adopted by many institutions and, thus, the inability to identify fecal samples from particular individuals. Several potential fecal markers (cake decorating dye, sunflower seeds, biodegradable barrier tape, art glitter) were tested by rhino keepers at the San Antonio Zoo, but none proved both safe and effective for long-term use. Regardless of the challenge, keepers at numerous institutions have diligently collected samples, providing enough material for researchers to obtain a significant data-set on reproductive activity in the captive population.

Fecal progesterone data for the southern white rhinoceros, has been more difficult to interpret than that of its close relative, the black rhino. Approximately 50% of captive female white rhinos appear acyclic, showing no reproductive behavior and no change in progesterone levels. The remaining female white rhinos can be categorized as exhibiting one of three different types of reproductive patterns: 1) 60-70 day cycles; 2) 30-35 day cycles; or 3) a mixture of long (60-70 day) and short (30-35 day) cycles. Several females with 70 day cycles are breeding without producing calves. These long cycles are characterized by an extended luteal (nonreceptive) phase identified by prolonged elevated progesterone, and fertility is questionable since no pregnancies have been documented in animals exhibiting long cycles exclusively. Determining the causes of both acyclicity and extended cycles are research priorities for the southern white rhinoceros. Additionally, early pregnancy loss and uterine pathology have been documented by ultrasound in a f...

further examinations of additional animals are necessary to determine their prevalence and potential association with infertility.

The reproductive cycle of the Indian rhinoceros *(Rhinoceros unicornis)* has been characterize by both behavioral observations and urinary hormone metabolite monitoring. The reproductive cycl appears to vary among individuals ranging from 39 to 64 days in length and also can vary between cycles within an individual. In this rhino species, significant increases in urinary estrogen metabolit concentrations are associated with estrous behavior and breeding. Recent research using serial ultrasound examinations in a young, regularly cycling female has revealed the development of extremely large follicles (>10 cm diameter) on the ovaries several days before ovulation which might explain the high levels of estrogen produced by this species. Captive breeding of the Indian rhino h been relatively successful, however, aggressive interactions between some male-female pairings, ev during the female's estrus, have interfered with breeding success on several occasions. These behavioral incompatibilities between individuals of specific pairs limit our ability to genetically manage the captive population. The development of artificial insemination may provide a useful method for overcoming this hurdle in the Indian rhino captive breeding program.

The other Asian rhinoceros in captivity, the Sumatran rhino (*Dicerorhinus sumatrensis*), has been studied intensively during the last few years. In the last century, captive breeding efforts with this species have been unsuccessful due to our inability to detect estrous behavior combined with aggressive interactions between pairs when animals are introduced during the female's nonreceptive period. Long-term serial ultrasound examinations, serum hormone analyses and behavioral observations have revealed that the Sumatran rhinoceros experiences a 21 day reproductive cycle and is an induced ovulator, meaning she ovulates only if she breeds. This characteristic has not been reported for any other species within the perissodactyla family. Early pregnancy loss has been detected in one animal on three occasions, and uterine pathology has been reported in several other animals. The reason for this uterine pathology is unknown and warrants investigation. Similarly, th cause of early pregnancy loss is a mystery and determining why it is occurring and how to overcome it will be research priorities as efforts to produce offspring in the captive Sumatran rhino continue.

Future reproductive research in rhinos often will require more invasive methods. For example, ultrasound examinations are recommended for animals that breed repeatedly without producing calves to determine if they are undergoing early embryo loss, or simply never becoming pregnant. Ultrasound also will be important for identifying reproductive tract pathology that might explain reduced fertility (or infertility) in some animals. Several institutions already have conditioned several rhinos of each species to walk into a chute and to allow rectal ultrasound examinations and/or blood sample collections. Rhino keepers typically play an integral role in the process. The benefits c conditioning rhinos for simple procedures are far-reaching. Not only will it facilitate research efforts but it also will help veterinarians care for the animals. For example, if an animal becomes ill or injures itself, veterinarians will be able to examine the individual closely, and diagnose and treat problems without forced contact or anesthesia which could further stress an already compromised animal.

There are many ways in which our knowledge of rhino reproduction can be exploited to benefit animal managers. Perhaps one of the most obvious benefits is the ability to detect pregnancy early in gestation. Pregnancy can be diagnosed by ultrasound as early as 16 days after breeding. A single examination could be used prior to introducing a male-female pair for another breeding, and this might alleviate the efforts and concerns involved with pairing a pregnant animal with a male. It also will provide managers with a relatively accurate conception date so that preparation for parturitio is timely. Concerns about the potential loss of pregnancy later in gestation could be alleviated by an ultrasound examination confirming a fetal heartbeat. Alternatively, to detect and monitor pregnancy serial blood, fecal or urine samples collected over several weeks can be analyzed for progesterone or progestin metabolites. Sustained elevated progesterone levels serve as a relatively accurate indicatio of pregnancy.

In addition to diagnosing pregnancy, ultrasound examinations can be used to detect reproductive tract abnormalities causing infertility. This information is important for both the institution managing the animal and for the Species Survival Plan which might make a recommendation to transfer the rhino to a different facility in hopes of a successful breeding with a genetically valuable male. Furthermore, if a reversible or treatable condition is identified, steps can taken to improve the condition so that reproductive success might be achieved by the female.

Serial fecal, urine or serum collection also could prove beneficial when a pair of rhinos exhibits no reproductive activity over a long period of time. These samples can be analyzed for hormone metabolites to determine if the female is exhibiting reproductive activity. Once acyclicity is ruled out as the cause of reproductive apathy, other factors could be considered and the introduction of a new male or transfer of the female to a new male might be warranted.

In summary, great strides have been made in characterizing reproductive activity in all captive rhinoceros species, and this progress would not have been possible without the help of rhino keepers who have played an integral role in sample collections, behavioral observations and animal conditioning. We now know that the reproductive cycle of each rhino species differs, ranging from 21 days in the Sumatran rhino to 70 days in some white rhinos. Similarly, ovarian activity differs among species. For example, preovulatory follicles in the Sumatran rhino are ~20-25 mm in diameter and breeding induces ovulation. In contrast, preovulatory follicles in the Indian rhino grow to >10 cm in diameter and spontaneously ovulate. With a clearer understanding of each species' reproductive physiology, and future challenges defined, it is time to move forward with research that will help us overcome those obstacles currently limiting the long-term stability of captive breeding programs. Challenges for the future include understanding the reasons for: 1) repeated copulations without pregnancy; 2) early pregnancy loss; 3) uterine pathology; 4) extended luteal phase cycles and acyclicity in the white rhino; and 5) aggressive interactions between Indian and Sumatran rhino pairs introduced for breeding. Similar to previous reproductive research, the success of these studies will depend upon continued cooperation and assistance from rhino keepers at institutions throughout the US. As a result, the rhinos, researchers and animal care staff all should benefit from what is learned.

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