THE ROLE OF REPRODUCTIVE RESEARCH AND TECHNOLOGY IN FACILITATING CAPTIVE BREEDING PROGRAMS FOR THE RHINOCEROS

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The rhino taxon comprises a group of related species each with distinct reproductive characteristics and facing different reproductive challenges in captivity. Reproductive research and technology could facilitate the captive breeding programs for all species and, in several cases, they already have. However, based on the reproductive biology, behavior and associated problems of the four captive rhino species, the most logical approach for helping to conserve each of them differs.

Of the reproductive technologies available for use in the rhino taxon, hormone monitoring has been the most broadly employed both in efforts to understand the reproductive biology of these species and to facilitate management strategies. Fortunately, many animals can be conditioned to allow blood collection, and it is becoming common practice at many institutions for veterinary and keeper staff to develop routine blood collection protocols. Although somewhat species dependent, it is possible to collect blood from the foreleg, ear and tail of the rhinoceros. However, most endocrine data has been collected through noninvasive (fecal and urine) hormone metabolite monitoring. The responsibility for accurately collecting, labeling and sending these samples to the scientists for analyses lies primarily with the daily keeper staff. Progesterone, estrogen, testosterone and corticosterone metabolites all can be measured in rhino urine or fecal samples (Kasman & Lasley, 1981; Kasman et al., 1986; Hodges & Green, 1989; Hindle & Hodges, 1990; Hindle et al., 1992; Schwarzenberger et al., 1993; Schwarzenberger et al., 1996; Berkeley et al., 1997; Radcliffe et al., 1997; Garneira et al., 1998; Heistermann et al., 1998; Roth et al., 1998; Schwarzenberger et al., 1998; Patton et al., 1999; Roth & Brown, 1999; Brown et al., 2001; Roth et al., 2001). Thus, noninvasive hormone monitoring provides one method for evaluating ovarian activity, sexual maturity, pregnancy and adrenal function as a potential indicator of stress.

Ultrasound technology is becoming another valuable reproductive tool for the rhinoceros, in part, because animals can be conditioned to allow the procedure (Schaffer et al., 1994; 1998; Radcliffe et al., 1997; Radcliffe, 1998; Roth & Brown, 1999; Roth et al., 2001). Therefore, ultrasound examinations can be conducted at the specific times and frequencies necessary to produce accurate data on reproductive tract dynamics. The role of keepers in conditioning animals for these procedures is critical to the long-term success of research projects. Once the animals are conditioned to tolerate ultrasonography, researchers continue to rely on the animal staff to handle the rhinos during exams. Ultrasonography already has been used to directly monitor ovarian activity throughout the reproductive cycle in white (Radcliffe et al., 1997), Sumatran (Roth et al., 2001) and Indian (Roth & O'Brien, 2000) rhinoceros and has been used to detect uterine pathology (Schaffer et al., 1994; Radcliffe et al., 1997; Radcliffe 1998; Roth et al., 2001) and early pregnancy loss (Radcliffe et al., 1997; Radcliffe, 1998; Roth et al., 2001). In males, ultrasonography has been used to evaluate reproductive tracts and to monitor changes associated with artificially stimulated ejaculation (Schaffer et al., 1994; 1998; Hermes et al., 2000).

The potential benefits of developing assisted reproduction for rhinoceros were recognized years ago, but progress has been slow. For artificial insemination (AI) or in vitro fertilization (IVF), semen is required, and semen collection in rhinoceros has proven difficult. Early efforts employed a manual penile and/or rectal massage technique that resulted in some success with a few animals (Schaffer & Beehler, 1988; Schaffer et al., 1990; Schaffer et al., 1991), but could require up to 3 years of animal conditioning. In other cases, manual massage yielded seminal fluid but no true ejaculate (O'Brien & Roth, 2000). The general problem with the penile massage method is that conditioning the animals can be extremely time consuming and some rhinos never do ejaculate in response to it. However, it is a technique that several keepers have started using in attempts to check their bull's fertility and/or to cryopreserve his gametes.

Although electroejaculation can be successful (Platz et al., 1979; Schaffer et al., 1990; 1998), it has been somewhat unreliable. However, recent attempts with rectal probes that have been modified to better fit the rhino's anatomy have shown promise both in our laboratory and by others (Hermes et al., 2000). Opportunistic methods that have proven successful for obtaining rhino spermatozoa include post-coital semen collection from the female (Roth et al., 2001) and epididymal sperm rescue post-mortem (Williams et al., 1995; O'Brien & Roth, 2000). Initially, rhino spermatozoa appeared challenging to freeze (Platz et al., 1979; Schaffer & Beehler, 1988; Williams et al., 1995), but spermatozoa from both a Sumatran and black rhinoceros now have been cryopreserved successfully using a standard hoof stock semen freezing protocol (O'Brien & Roth, 2000). This same protocol recently proved equally effective for cryopreserving epididymal spermatozoa from two white rhinoceros.

Recently, AI was attempted in the white rhinoceros using cold stored semen collected by electroejaculation (Hermes et al., 2000). The procedure was conducted on anesthesized females pre-treated with exogenous hormones (progesterone and hCG). Endoscopic and ultrasonographic visualization indicated the insemination procedure was successful, but no sustained pregnancies have yet been confirmed following these attempts. The use of exogenous hormones to induce or synchronize estrus in the rhinoceros is, in itself, still very experimental (Patton et al., 1998). However, the regimen employed for the AI trial holds promise as it does appear to induce ovulation (Walzer & Schwarzenberger, 1995; Hermes et al., 2000).

Rhino IVF has been attempted opportunistically on a few occasions in conjunction with gamete rescue efforts, but no embryos have been produced (Godfrey et al., 1990). There are no reports of embryo collection and transfer attempts in any rhino species and, due to the complicated and invasive procedures required for these ARTs, they may not become research priorities for the rhinoceros any time soon. However, several of the reproductive tools described above have immediate application to resolving problems that currently hinder rhino captive breeding programs.

The African black rhinoceros (*Diceros bicornis*) has been the most prolific of the captive rhinoceros. Most female black rhinoceros exhibit reproductive cycles that average 25 d in length but range from 20-30 d (Hindle et al., 1992; Schwarzenberger et al., 1993; Schwarzenberger et al., 1996; Berkeley et al., 1997; Roth & Brown, 1999; Brown et al., 2001). With reproductive success relatively high, this species is not likely to require high-tech reproductive approaches to improve captive propagation. However, to facilitate efficient management of the species, endocrine and ultrasound monitoring should be employed to detect pregnancy, pregnancy loss and pathology, especially in those few individuals that breed repeatedly without producing offspring (Roth & Brown, 1999; Brown et al., 2001).

In contrast to the black rhinoceros, the southern white rhinoceros (*Ceratotherium simum simum*) has not reproduced well in captivity. Endocrine monitoring studies indicate that approximately 50% of captive female white rhinoceros are acyclic, whereas the remaining females are exhibiting either 5 wk, 10 wk or a mixture of 5 and 10 wk cycles (Hindle & Hodges, 1990; Hindle et al., 1992; Radcliffe et al., 1997;1998; Roth et al., 1998; Schwarzenberger et al., 1998; Patton et al., 1999; Roth & Brown, 1999; Brown et al., 2001). The 10 wk cycles are characterized by an extended luteal phase, and data suggest these cycles are infertile (Roth et al., 1998; Patton et al., 1999; Brown et al., 2001). Determining the causes of both acyclicity and extended cycles are research priorities for the southern white rhinoceros and probably will require studies that combine hormone monitoring and serial ultrasound examinations. Ultrasonography already has allowed the detection of early pregnancy loss and uterine pathology in the white rhinoceros (Radcliffe et al., 1997; Radcliffe, 1998) and may hold the key to understanding reduced fertility in this species. In the interim, exogenous hormone administration in conjunction with AI may provide a means of producing pregnancies in otherwise acyclic animals (Hermes et al., 2000).

The reproductive cycle of the Indian rhinoceros (*Rhinoceros unicornis*) varies among and within individuals but averages 43-48 d in length (Kasman and Lasley, 1981; Kasman et al., 1986; Roth & O'Brien, 2000). A long-term, serial ultrasound study on one animal revealed the development of 10-12 cm pre-ovulatory follicles that persisted approximately 10 d before spontaneously ovulating (Roth & O'Brien, 2000). Although reproduction generally has been good, aggressive behavior exhibited by males towards seemingly estrual females has limited our ability to genetically manage the captive Indian rhino population, and the sire gene pool is largely restricted to males of a particular founder line (Foose & Reece, 1998). Therefore, the justification for developing AI to genetically manage rhinos may be strongest for this species.

In the last century, captive breeding efforts with the Sumatran rhinoceros *(Dicerorhinus sumatrensis*) have failed, largely due to a lack of knowledge about their reproductive biology and aggressive interactions between pairs introduced for mating (Khan et al., 1999). However, in a recent study, a female monitored intensively by ultrasound and endocrine analyses was found to be an induced ovulator with a 21 d reproductive cycle (Roth et al., 2001). This information has greatly facilitated a natural breeding strategy that involves timed introductions based on progesterone concentrations and follicle size. Furthermore, ultrasound has been used to detect early pregnancy, pregnancy loss and uterine pathology in the Sumatran rhinoceros (Schaffer et al., 1994; Roth et al., 2001). Due to the limited number of animals in captivity (n=15), AI someday may be required, and spermatozoa has been cryopreserved for that eventual purpose (O'Brien & Roth, 2000). But, for now, reproductive technology is most valuable in this species as a tool for facilitating natural breeding and for detecting and monitoring resulting pregnancies.

Reproductive research already has contributed significantly to our understanding of rhino reproductive physiology and the challenges we face in trying to breed these species in captivity. Species specific characteristics are diverse, as are the problems facing each species. Additional research and the application of reproductive technology will facilitate rhino propagation efforts, but there is no universal approach that can be applied to all species. Instead, priorities must be customized to address the particular set of circumstances surrounding each species. Decisions on how and when to use reproductive techniques must reflect logical and practical considerations. Only then, will reproductive research and technology truly be working for conservation.

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SELECTED REFERENCES

1. Berkeley EV, Kirkpatrick JF, Schaffer NE, Bryant WM, Threlfall WR. Serum and fecal steroid analysis of ovulation, pregnancy, and parturition in the black rhinoceros (*Diceros bicornis*). Zoo Biol 1997;16:121-132.
2. Brown J, Bellem A, Fouraker M, Wildt D, Roth T. Comparative analysis of gonadal and adrenal activity in male and female black and white rhinoceros in North America by non-invasive endocrine monitoring. Zoo Biol 2001;(submitted).
3. Czekala NM, Callison L. Pregnancy diagnosis in the black rhinoceros (*Diceros bicornis*) by salivary hormone analysis. Zoo Biol 1996;15:37-44.
4. Foose TJ, Reece RW. American Zoological and Aquarium Association Species Survival Plan Rhinoceros Masterplan. 1998:22.
5. Garniera JN, Breen DL, Pickard AR, Shaw HJ, Holt WV. Non-invasive diagnosis of pregnancy in wild black rhinoceros (*Diceros bicornis minor*) by faecal steroid analysis. Reprod Fertil Dev 1998;10:451-458.
6. Godfrey RW, Pope CE, Dresser BL, Bavister BD, Andrews JC, Olsen JH. An attempt to superovulate a southern white rhinoceros (*Ceratotherium simum simum*). Theriogenology 1990;33:231.
7. Heistermann M, Agil M, Hodges JK. Metabolism and excretion of oestradiol-17ß and progesterone in the Sumatran rhinoceros (*Dicerorhinus sumatrensis*). Anim Reprod Sci 1998;53:157-172.
8. Hermes R, Hildebrandt TB, Göritz F, Schwarzenberger F, Walzer C, Göltenboth R, Achnorrenberg A. Artificial insemination in the white rhinoceros (*Ceratotherium simum simum*). Proc Am Assoc Zoo & Aquar 2000; (in press).
9. Hindle JE, Hodges JK. Metabolism of oestradiol-17ß and progesterone in the white rhinoceros (*Ceratotherium simum simum*). J Reprod Fertil 1990;90:571-580.
10. Hindle JE, Mostl E, Hodges JK. Measurement of urinary oestrogens and 20-dihydroxyprogesterone during the ovarian cycle of black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceros. J Reprod Fertil 1992;94:237-249.
11. Hodges JK, Green DI. The development of an enzyme-immunoassay for urinary pregnanediol-3-glucuronide and its application to reproductive assessment in exotic mammals. J Zool London 1989;219:89-99.
12. Kasman AAH, Lasley BL. Estrogen excretory patterns in the Indian rhinoceros (*Rhinoceros unicornis*) determined by simplified urinary analysis. Am J Vet Res 1981;42:251-255.
13. Kasman LH, Ramsay EC, Lasley BL. Urinary steroid evaluations to monitor ovarian function in exotic ungulates: III. Estrone sulfate and pregnanediol-3-glucuronide excretion in the Indian rhinoceros (*Rhinoceros unicornis*). Zoo Biol 1986;5:355-361.
14. Khan MKM, Roth TL, Foose TJ. In situ and ex situ efforts to save the Sumatran rhinoceros (*Dicerorhinus sumatrensis*), In: Roth TL, Swanson WF, and Blattman LK (eds.), Proceedings of the 7th World Conference on Breeding Endangered Species. Cincinnati Zoo & Botanical Garden;1999:163-174.

15. O'Brien, JK, Roth TL. Post-coital sperm recovery and cryopreservation in the Sumatran rhinoceros (*Dicerorhinus sumatrensis*) and application to gamete rescue in the African black rhinoceros (*Diceros bicornis*). J Reprod Fertil 2000;118:263-271.
16. Patton ML, Czekala N, Schaffer N, Lance V. Hormonal manipulations of rhinoceros. Proceedings of a Workshop on Problems Associated with the Low Rate of Reproduction Among Captive-born Female Southern White Rhinoceros (*Ceratotherium simum simum*). Zoological Society of San Diego;1998:16-21.
17. Patton ML, Swaisgood RR, Czekala NM, White AM, Fetter GA, Montagne JP, Rieches RG, and Lance VA. Reproductive cycle length and pregnancy in the southern white rhinoceros (*Ceratotherium simum simum*) as determined by fecal pregnane analysis and observations of mating behavior. Zoo Biol 1999;16:445-456.
18. Platz C, Seager SWJ, Bush M. Collection and analysis of semen from a black rhinoceros. J Am Vet Med Assoc 1979;175:1002-1004.
19. Radcliffe RW. Hypothesis on infertility in captive white rhinoceros (*Ceratotherium simum*): Thoughts on normal and abnormal biology. Proceedings of a Workshop on Problems Associated with the Low Rate of Reproduction Among Captive-born Female Southern White Rhinoceros (*Ceratotherium simum simum*). Zoological Society of San Diego;1998:22-27.
20. Radcliffe RW, Czekala NM, Osofsky SA. Combined serial ultrasonography and fecal progestin analysis for reproductive evaluation of the female white rhinoceros (*Ceratotherium simum simum*): Preliminary results. Zoo Biol 1997;16:445-456.
21. Roth TL, Brown JL. Is there any rhyme or reason to rhino reproduction? - A summary of reproductive characteristics, species-specificities and challenges for the future. Proc Am Assoc Zoo Vet 1999:97-99.
22. Roth TL, O'Brien JK. Ultrasonographic characterization of unique follicular dynamics and associated estrogen production in an Indian rhinoceros (*Rhinoceros unicornis*). Biol Reprod 2000;(Suppl. 1) 62:290.
23. Roth TL, O'Brien JK, McRae MA, Bellem AC, Romo SJ, Kroll JL, Brown JL. Ultrasound and endocrine evaluation of ovarian cyclicity and early pregnancy in the Sumatran rhinoceros (*Dicerorhinus sumatrensis*). Reproduction 2001;121:139-149.
24. Roth T, Patton L, Brown J, Czekala N, Swaisgood R, Lance, V. The North American experience: female hormonal cycles in southern white rhinoceros. Proceedings of a Workshop on Problems Associated with the Low Rate of Reproduction Among Captive-born Female Southern White Rhinoceros (*Ceratotherium simum simum*). Zoological Society of San Diego;1998:6-9.
25. Schaffer NE, Beehler B. Overview of procedures and results of semen collection from ambulatory rhinoceroses. Proc Am Assoc Zool Parks & Aquar 1988:273-279.
26. Schaffer NE, Beehler B Jeyendran RS, Balke B. Methods of semen collection in an ambulatory greater one-horned rhinoceros (*Rhinoceros unicornis*). Zoo Biol 1990;9:211-221.
27. Schaffer N, Bryant W, Agnew D, Meehan T, Beehler B. Ultrasonographic monitoring of artificially stimulated ejaculation in three rhinoceros species (*Ceratotherium simum, Diceros bicornis, Rhinoceros unicornis*). J Zoo Wildl Med 1998;29:386-393.
28. Schaffer NE, Jeyendran RS, Beehler B. Reproductive procedures and restraint for rhinoceroses, In: Ryder OA (ed.), Proceedings of an International Conference: Rhinoceros Biology and Conservation. Zoological Society of San Diego;1993:153-158.

29. Schaffer NE, Zainal-Zahari Z, Jainudeen MR, Jeyendran RS. 1994. Ultrasonography of the reproductive anatomy in the Sumatran rhinoceros (*Dicerorhinus sumatrensis*). J Zoo Wildl Med 1994;25:337-348.
30. Schwarzenberger F, Francke R, Goltenboth R. Concentrations of faecal immunoreactive progestagen metabolites during the oestrous cycle and pregnancy in the black rhinoceros (*Diceros bicornis michaeli*). J Reprod Fertil 1993;98:285-291.
31. Schwarzenberger F, Goodrowe KL, Zima J, Straub G, Lynch M. Faecal progesterone metabolite analysis for noninvasive monitoring of reproductive function in the white rhinoceros (*Ceratotherium simum*). Anim Reprod Sci 1998;53:173-190.
32. Schwarzenberger F, Tomasova K, Holeckova D, Matern B, Mostl E. Measurement of fecal steroids in the black rhinoceros (*Diceros bicornis*) using group-specific enzyme immunoassays for 20-oxo-pregnanes. Zoo Biol 1996;15:159-171.
33. Walzer C, Schwarzenberger F. Estrous cycle induction in a white rhinoceros (*Ceratotherium simum simum*) and concomitant EIA fecal progestagen analysis. Proc Am Assoc Zoo Vet 1995;365-368.
34. Williams KR, Dyche WK, Brinders J, Molteno F, van der Lanken M, Armstrong DL, Simmons LC. Longevity in vitro and glycerol toxicity of epididymal sperm recovered from a white rhinoceros (*Ceratotherium simum*). Theriogenology 1995;43:353.
35. Young E. Semen extraction by manipulative technique in the Black rhinoceros. Int. Zoo Yearbook 1967;7:166-167.