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

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Artificial insemination (AI), in vitro fertilization (IVF) and embryo transfer have the potential of facilitating the genetic management of most endangered species. However, the time and resources necessary to develop consistently successful protocols for all endangered species are not available, and in some cases, conservation could be better served through less heroic, but nonetheless important, contributions from reproductive physiologists.

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, noninvasive (fecal and urine) hormone monitoring has been the most broadly employed both in efforts to understand the reproductive biology of these species and to facilitate management strategies. 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. 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 AI or 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), bu 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). 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 (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).
Recently, artificial insemination 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 artificial insemination 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 days in length but range from 20-30 days (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 week 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 artificial insemination 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 days in length (Kasman & 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 days 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 artificial insemination to genetically manage captive rhino populations 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 day 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=16), artificial insemination 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.

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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