

A review of the reproductive physiology of rhinoceros species in captivity

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Substantial progress in understanding the reproductive physiology of the four rhinoceros species in captivity, White rhinoceros *Ceratotherium simum*, Black rhinoceros *Diceros bicornis*, Indian or Greater one-horned rhinoceros *Rhinoceros unicornis* and Sumatran rhinoceros *Dicerorhinus sumatrensis*, has been achieved over the last two decades largely owing to advances in endocrine and ultrasound techniques, and improved methods of semen collection. Primarily through the non-invasive monitoring of hormone metabolites, scientists have studied the reproductive cycle, investigated the effects of season and potential stressors, and diagnosed pregnancy in each species. By employing the powerful tool of ultrasonography, more detailed and accurate information regarding reproductive function has been revealed. Important findings include anovulatory cycles, induced ovulation, reproductive tract pathologies, early pregnancy diagnosis and early embryonic loss. Interest in the reproductive physiology of ♂ rhinoceros has lagged behind that of ♀♀ but in recent years data on sperm and semen characteristics, reproductive glands and hormone production have been collected. While the reproductive physiology of each rhinoceros species is interesting in its own right, perhaps most fascinating is the significant inter-specific variation in reproductive traits within this taxon.

Key-words: embryo loss, ovarian function, pregnancy, pregnancy loss, reproductive cycle, reproductive endocrinology, reproductive pathology, rhinoceros, semen collection, sperm, ultrasonography

Four of the five extant rhinoceros species currently are maintained and bred in captivity but the success and challenges associated with each differ. The African Black rhinoceros *Diceros bicornis* has been bred in zoos relatively successfully. However, this species exhibits an unusual susceptibility to several diseases that result in a mortality rate approximately equal to the

proliferation rate (AZA Rhinoceros Advisory Group, 2005). Furthermore, a sex ratio skewed in favour of ♂♂ has posed another unexpected challenge to animal managers (AZA Rhinoceros Advisory Group, 2005). The breeding programme for African White rhinoceros *Ceratotherium simum* has been most successful when the rhinoceros are maintained in larger groups and enclosures, suggesting social and/or environmental factors are important to the success of this species (Fouraker & Wagener, 1996). Numerous studies have revealed that many ♀ White rhinoceros are acyclic or exhibit irregular reproductive cycles (Schwarzenberger *et al.*, 1998; Patton *et al.*, 1999; Brown *et al.*, 2001) and reproductive pathology is common (Hermes, Hildebrandt & Göritz, 2004). Even more puzzling is the failure of many F1 ♀♀ to reproduce despite exhibiting regular reproductive cyclicity and appropriate mating behaviours with ♂♂ (Swaigood *et al.*, 2006). The Indian rhinoceros *Rhinoceros unicornis* generally has reproduced well in captivity but is the least diverse genetically (Hlavacek *et al.*, 2002). This lack of genetic variation is, in part, the result of severe aggression sometimes exhibited between specific ♂–♀ pairs, which makes breeding the species challenging, especially in smaller enclosures. Similarly, the Sumatran rhinoceros *Dicerorhinus sumatrensis* is known for its aggression when ♂♂ and ♀♀ are paired, especially when the ♀ is not in oestrus, and there are no reliably detectable oestrous

behaviours exhibited by ♂♂ or ♀♀ (Roth *et al.*, 2001). The captive-breeding programme for this species had been unsuccessful until a calf was finally born in 2001 (Roth *et al.*, 2004).

In most non-domestic species, longitudinal hormone evaluations are the primary source of information regarding reproductive cyclicity, ovulation and pregnancy. Numerous hormone assays have been validated and used effectively in the rhinoceros but there are differences among species (Table 1). In general, endogenous progesterone concentrations are very useful for monitoring reproductive cyclicity and detecting pregnancy because progesterone increases during the luteal phase of the cycle, decreases during oestrus and remains elevated during pregnancy. Monitoring progesterone, or

progesterone metabolites, has proven effective across all species and it has been the most reliable hormone for studying the Black rhinoceros (Ramsay *et al.*, 1987; Brett *et al.*, 1989; Hodges & Green, 1989; Hindle *et al.*, 1992; Schwarzenberger *et al.*, 1993, 1996; Czekala & Callison, 1996; Berkeley *et al.*, 1997; Brown *et al.*, 2001; Lance *et al.*, 2001; R. W. Radcliffe *et al.*, 2001; Garnier *et al.*, 2002) and the White rhinoceros (Hodges & Green, 1989; Hindle *et al.*, 1992; R. W. Radcliffe *et al.*, 1997; Schwarzenberger *et al.*, 1998; Patton *et al.*, 1999; Brown *et al.*, 2001). Progesterone has also been monitored effectively in all biological samples (e.g. urine, faeces, serum and saliva) studied to date in the Indian rhinoceros (Kasman *et al.*, 1986; Hodges & Green, 1989; Schwarzenberger *et al.*, 2000; Gomez *et al.*, 2004; Stoops

SPECIES	SAMPLE	OESTROGEN	PROGESTERONE	ANDROGEN	LH	OTHER
AFRICAN RHINOCEROS SPECIES						
Black rhinoceros						
<i>Diceros bicornis</i>	urine	♀ ^{8,16-} ♂ ²⁻	♀ ^{2,8,9,15,16}	♂ ²⁻		
	faeces	♀ ^{1,3-}	♀ ^{1,3,5,12,19,21}	♂ ³		
	saliva	♀ ⁴	♀ ⁴			
	serum	♀ ¹	♀ ¹			
White rhinoceros						
<i>Ceratotherium simum</i>	urine	♀ ⁸	♀ ^{8,9,13,14,22}			
	faeces	♀ ³⁻	♀ ³	♂ ³		
ASIAN RHINOCEROS SPECIES						
Indian rhinoceros						
<i>Rhinoceros unicornis</i>	urine	♀ ^{10,11,23}	♀ ^{9, 10,23}		♀ ²³	
	faeces	♀ ²⁰	♀ ²⁰	♀ ²⁰		
	saliva	♀ ⁶	♀ ⁶	♀ ⁶		
Sumatran rhinoceros						
<i>Dicerorhinus sumatrensis</i>	urine	♀ ⁷				
	faeces		♀ ^{18,7}			
	serum	♀ ¹⁷⁻	♀ ^{17,18}		♀ ¹⁸	♀ ¹⁷ (PRL, Relaxin)

Table 1. Reproductive hormones monitored in the rhinoceros. A negative sign following the superscripted number denotes an attempt that failed to measure the hormone and/or changes in its concentrations associated with the reproductive state of the rhinoceros in the referenced study: ¹. Berkeley *et al.*, 1997; ². Brett *et al.*, 1989; ³. Brown *et al.*, 2001; ⁴. Czekala & Callison, 1996; ⁵. Garnier *et al.*, 2002; ⁶. Gomez *et al.*, 2004; ⁷. Heistermann *et al.*, 1998; ⁸. Hindle *et al.*, 1992; ⁹. Hodges & Green, 1989; ¹⁰. Kasman *et al.*, 1986; ¹¹. Kassam & Lasley, 1981; ¹². Lance *et al.*, 2001; ¹³. Patton *et al.*, 1999; ¹⁴. R. W. Radcliffe *et al.*, 1997; ¹⁵. R. W. Radcliffe *et al.*, 2001; ¹⁶. Ramsay *et al.*, 1987; ¹⁷. Roth *et al.*, 2004; ¹⁸. Roth *et al.*, 2001; ¹⁹. Schwarzenberger *et al.*, 1993; ²⁰. Schwarzenberger *et al.*, 2000; ²¹. Schwarzenberger *et al.*, 1996; ²². Schwarzenberger *et al.*, 1998; ²³. Stoops *et al.*, 2004.

et al., 2004) and Sumatran rhinoceros (Heistermann *et al.*, 1998; Roth *et al.*, 2001, 2004). In contrast, measuring oestrogen or oestrogen metabolites has proven challenging in all but the Indian rhinoceros. Although some studies have shown that oestrogens can be measured and do reflect changes in reproductive status in the Black rhinoceros (Hindle *et al.*, 1992; Czekala & Callison, 1996; Berkeley *et al.*, 1997), White rhinoceros (Hindle *et al.*, 1992) and Sumatran rhinoceros (Heistermann *et al.*, 1998), a similar number of studies have failed to validate the results in these species (Ramsay *et al.*, 1987; Brett *et al.*, 1989; Brown *et al.*, 2001; Roth *et al.*, 2004). Conflicting data could be a result of the specificity of antibody required for accurate assessments and/or the sensitivity of the assay owing to the low concentrations of endogenous oestrogens produced. Further complicating the effort to measure oestradiol metabolites in the faeces is the fact that oestradiol is eliminated by more than one pathway with only a portion of it excreted as faecal metabolites while another fraction is excreted through the urine (Hindle & Hodges, 1990). In contrast, regardless of assay or antibody specificity, oestrogen concentrations in the Indian rhinoceros appear to be substantial and do fluctuate to reflect accurately reproductive status and function in this species (Kassam & Lasley, 1981; Kasman *et al.*, 1986; Schwarzenberger *et al.*, 2000; Gomez *et al.*, 2004; Stoops *et al.*, 2004). Interestingly, even androgens, the precursors of oestrogen, have been shown to reflect the stage of the reproductive cycle of Indian rhinoceros (Schwarzenberger *et al.*, 2000; Gomez *et al.*, 2004).

Luteinising hormone (LH) concentrations increase near the time of ovulation in both spontaneous and induced ovulators and can serve as an accurate indicator of this event. In general, spontaneous ovulators develop a follicle(s), experience oestrus, release LH and ovulate at a set interval regardless of the presence of a ♂.

Induced ovulators develop a follicle(s), experience oestrus but only release LH and ovulate if exposed to a specific stimulus. This stimulus can vary among species but typically is linked tightly to the mating process (e.g. mechanical stimulation of the vagina or components of the seminal fluid). To date, LH concentrations have been measured and a preovulatory surge(s) detected in both the Indian rhinoceros using urine (Stoops *et al.*, 2004) and the Sumatran rhinoceros using serum (Roth *et al.*, 2001). Other protein hormones that have been evaluated in serum during pregnancy in the Sumatran rhinoceros and show potential as indicators of pending parturition include prolactin and relaxin (Roth *et al.*, 2004; Steinetz *et al.*, 2005).

REPRODUCTIVE CYCLES

No two rhinoceros species exhibit reproductive cycles of similar length (Table 2). The smallest of the species, the Sumatran rhinoceros, appears to have the shortest cycle. In one study, a ♀ Sumatran rhinoceros monitored closely by ultrasound was receptive to a ♂ every 21 days and ovulated every 21 days when mated (Roth *et al.*, 2001). In another report, a 25 day cycle was reported for this species based on faecal hormone metabolite monitoring during two cycles (Heistermann *et al.*, 1998). Because the Sumatran rhinoceros appears to be an induced ovulator, irregular cycles are common if the ♀ is not mated and could explain variations on the 21 day cycle (Roth *et al.*, 2001). Alternatively, slight differences in cycle length might naturally occur between individuals.

Most published reports for the Black rhinoceros generally agree on an average reproductive cycle length of 26 days (Schwarzenberger *et al.*, 1993; Berkeley *et al.*, 1997; Brown *et al.*, 2001; R. W. Radcliffe *et al.*, 2001; Garnier *et al.*, 2002). However, its close relative, the White rhinoceros, has proven more puzzling.

SPECIES	REPRODUCTIVE CYCLE (day)	PREOVULATORY FOLLICLE DIAMETER (mm)	ANOVLATORY, HAEMORRHAGIC LUTEINISED FOLLICLES (diameter in mm)	PREGNANCY DIAGNOSIS BY ULTRASOUND	PREGNANCY DIAGNOSIS BY PROGESTERONE (week)	EMBRYO LOSS AND ABORTIONS	PATHOLOGY
AFRICAN RHINOCEROS SPECIES							
Black rhinoceros							
<i>Diceros bicornis</i>	21–22 ¹⁰ 24 ²⁵ 26–27 ^{2,3,5,15,20,25}	49–5 ²⁰	71–7 ²⁰	27 days post-breeding ¹	9 ²⁵ 9–12 ²¹ 11 ²⁷ 12 ⁴ 13–14 ¹⁵ 20 ²	12 weeks ² 12–14 weeks ⁵ 9, 10 months ² 14 months ²⁷	hyperplasia ⁶
White rhinoceros							
<i>Ceratotherium simum</i>	31, 35 ¹⁸ 32 ¹⁰ 33, 70 ³ 35, 66 ^{7,28}	30 ¹⁸	60 ¹⁸	15 days post-ovulation ¹⁸	12–16 ²¹	< 28 days ¹⁸ < 70 days ¹⁷	endometritis ¹⁸ pyometra ¹⁷ hyperplasia ^{6,9} leiomyoma ^{9,19}
ASIAN RHINOCEROS SPECIES							
Indian rhinoceros							
<i>Rhinoceros unicornis</i>	43 ^{13,26,29} 47 ⁷ 48 ¹³	120 ²⁹	129–149 ²⁹		12 ²⁶ 16 ¹¹		leiomyoma ^{9,14}
Sumatran rhinoceros							
<i>Dicerorhinus sumatrensis</i>	21 ²³ 25 ⁸	20–25 ²³ 21, 22 ²²	30 ²³	14 days post-breeding ²³ 17 days post-breeding ²²	12 ²²	42, < 90, < 30 days ²²	cysts ²⁴ mass ^{23,24}

Table 2. Ovarian dynamics, pregnancy, pregnancy loss and pathology of four rhinoceros species. Referenced studies: ¹. Adams *et al.*, 1991; ². Berkeley *et al.*, 1997; ³. Brown *et al.*, 2001; ⁴. Czekala & Callison, 1996; ⁵. Garnier *et al.*, 2002; ⁶. Godfrey *et al.*, 1991; ⁷. Gomez *et al.*, 2004; ⁸. Heistermann *et al.*, 1998; ⁹. Hermes, Hildebrandt & Göritz, 2004; ¹⁰. Hindle *et al.*, 1992; ¹¹. Hodges & Green, 1989; ¹². Kasnan *et al.*, 1986; ¹³. Kassam & Lasley, 1981; ¹⁴. Kock & Garnier, 1993; ¹⁵. Lance *et al.*, 2001; ¹⁶. Montali & Citino, 1993; ¹⁷. Patton *et al.*, 1999; ¹⁸. R. W. Radcliffe *et al.*, 1997; ¹⁹. R. M. Radcliffe *et al.*, 2000; ²⁰. R. W. Radcliffe *et al.*, 2001; ²¹. Ramsey *et al.*, 1987; ²². Roth *et al.*, 2001; ²³. Roth *et al.*, 2004; ²⁴. Schaffer *et al.*, 1994; ²⁵. Schwarzenberger *et al.*, 1993; ²⁶. Schwarzenberger *et al.*, 2000; ²⁷. Schwarzenberger *et al.*, 1996; ²⁸. Schwarzenberger *et al.*, 1998; ²⁹. Stoops *et al.*, 2004.

Sound scientific data from studies monitoring hormone metabolites support the conclusion that White rhinoceros can exhibit reproductive cycles of two different lengths. Both 30–35 day (Hindle *et al.*, 1992; R. W. Radcliffe *et al.*, 1997; Patton *et al.*, 1999; Brown *et al.*, 2001) and 65–70 day (Schwarzenberger *et al.*, 1998; Patton *et al.*, 1999; Brown *et al.*, 2001) cycles have been reported. The difference in the two cycle lengths cannot simply be attributed to individual animal differences because several ♀♀ have exhibited both cycle lengths over a period of months (Patton *et al.*, 1999). The physiological mechanism differentiating these cycles is not yet understood but based on the incidence of pregnancy following matings, it is generally believed that the 30–35 day cycle can be fertile (R. W. Radcliffe *et al.*, 1997; Schwarzenberger *et al.*, 1998; Patton *et al.*, 1999). In contrast, pregnancy has not yet been documented following a mating associated with a 65–70 day cycle (Schwarzenberger *et al.*, 1998; Brown *et al.*, 2001). The longer cycle length in White rhinoceros appears to be more prevalent in ♀♀ >20 years of age and may be a characteristic of aging animals (Schwarzenberger *et al.*, 1998; Brown *et al.*, 2001) that have not been reproducing consistently throughout their lives.

Another cause for concern, owing to its prevalence within the White rhinoceros population in captivity, is the acyclic or 'flat liner' pattern, wherein progesterone remains at baseline concentrations for extended periods ranging from several months to years. In studies conducted on comparatively large sample sizes, close to 50% of the ♀♀ exhibited this inactive pattern (Schwarzenberger *et al.*, 1998; Patton *et al.*, 1999; Brown *et al.*, 2001). One hypothesis for cessation of reproductive activity is premature senescence resulting from oocyte depletion in animals that cycle continuously for years without becoming pregnant (Hermes, Hildebrandt & Göritz, 2004). However, not all acyclic

animals are aged (Brown *et al.*, 2001) and some encouraging information suggests that cyclicity can resume in some ♀♀ when they are introduced to new ♂♂ (Patton *et al.*, 1999). Furthermore, a recent study has shown that greater variability in corticoid concentrations and higher rates of stereotypic pacing are associated with acyclicity in this species, suggesting stressors are the culprits (Carlstead & Brown, 2005). It is likely that there is more than one explanation for acyclicity in rhinoceros. Perhaps even more puzzling is the cause of reproductive failure in F1 ♀ White rhinoceros. An interesting study provides compelling evidence favouring a post-copulatory reproductive suppression as the root cause because mating behaviours and ♂ interactions did not differ between F1 and F0 ♀♀ but far fewer copulations resulted in term pregnancies in F1 ♀♀ (Swaigood *et al.*, 2006).

The Indian rhinoceros exhibits the most variable reproductive cycles, with some as short as 36–39 days and others as long as 61–86 days (Kasman *et al.*, 1986; Gomez *et al.*, 2004; Stoops *et al.*, 2004). However, most reports agree that the mean cycle length for this species is 43–48 days (Kassam & Lasley, 1981; Kasman *et al.*, 1986; Schwarzenberger *et al.*, 2000; Gomez *et al.*, 2004; Stoops *et al.*, 2004), longer than that for the other three species in captivity. Variation in cycle length has been reported for numerous individuals and documented by both monitoring of hormone metabolites and ultrasonography, therefore it may be an inherent characteristic of this species and not necessarily indicative of aberrant reproductive function.

There do not appear to be any significant seasonal effects on reproductive function in the ♀ rhinoceros in captivity. Longitudinal studies employing hormone monitoring and/or ultrasonography clearly demonstrate that reproductive cyclicity in ♀♀ occurs throughout the year (Patton *et al.*, 1999; Schwarzenberger *et al.*, 2000; Brown *et al.*, 2001; Roth

et al., 2001; Stoops *et al.*, 2004). Rhinoceros births occur all year round and any seasonal trends in calving rates are probably a result of management preferences; for example, institutions are more likely to breed rhinoceros in nice weather.

OVARIAN DYNAMICS

By incorporating serial ultrasound examinations into rhinoceros reproductive studies, detailed characterization of normal follicular growth and ovulation, as well as the documentation of anovulatory cycles and other irregularities, have been possible (Table 2). Both ovaries of the rhinoceros are capable of developing follicles and ovulating. Typically, a single dominant follicle develops in a given cycle, alternating randomly from the left to right ovary (R. W. Radcliffe *et al.*, 1997; 2001; Stoops *et al.*, 2004). However, the Sumatran rhinoceros can develop more than one preovulatory follicle and double ovulations have been confirmed in a given cycle in this species (Roth *et al.*, 2001).

Just as cycle length differs among the four rhinoceros species in captivity, so too does the size of the preovulatory follicle (Table 2). The preovulatory follicle of the Sumatran rhinoceros is 20–25 mm in diameter; however, if not mated, the follicle in this induced ovulator sometimes continues to expand to >30 mm diameter (*c.* 80 mm in circumference) (Roth *et al.*, 2001). The White rhinoceros is a spontaneous ovulator that experiences follicular collapse after the follicle reaches *c.* 30 mm diameter (R. W. Radcliffe *et al.*, 1997). More similar to its domestic horse relative, the Black rhinoceros produces rather large preovulatory follicles that measure as much as 50 mm in diameter prior to spontaneous ovulation (R. W. Radcliffe *et al.*, 2001). Most surprising of the four rhinoceros species is the Indian rhinoceros, which produces follicles that reach 120 mm in diameter and persist at that size for several days prior to spontaneous ovulation (Stoops

et al., 2004). These are the largest follicles known to develop in any mammal studied to date.

In all four rhinoceros species in captivity, anovulatory, luteinised follicles have been documented by endocrine and ultrasound analyses (R. W. Radcliffe *et al.*, 1997, 2001; Roth *et al.*, 2001; Stoops *et al.*, 2004). This phenomenon is not surprising in the Sumatran rhinoceros, which has only been known to ovulate if mated (Roth *et al.*, 2001, 2004). However, it was a rather unexpected finding in the other three spontaneously ovulating species. Although the frequency of luteinized follicle formation in rhinoceros in captivity is unknown, its detection and prevalence in the few longitudinal serial ultrasound studies conducted suggest it is not uncommon. Anovulatory, luteinised follicle formation in the horse is attributed to the transition from the non-breeding to the breeding season (Ginther, 1995). A similar seasonal association has been suggested in the Black rhinoceros (R. W. Radcliffe *et al.*, 2001) and reduced light exposure has been considered as an associated factor in the Indian rhinoceros (Stoops *et al.*, 2004). More extensive studies with larger sample sizes will be necessary to determine if environmental factors are associated with anovulation, or if it is simply a natural, random occurrence in captive rhinoceros. Luteinised follicles produce progesterone and resemble a corpus luteum when viewed by ultrasound after they have aged, therefore they cannot be diagnosed reliably by endocrine monitoring and would not have been discovered without the use of serial ultrasound examinations.

PREGNANCY DIAGNOSIS AND LOSS

Gestation in all rhinoceros species ranges from 15 to 18 months in length (Fouraker & Wagener, 1996). Pregnancy can be diagnosed by monitoring progesterone concentrations or by ultrasonography (Table 2). For animals conditioned to allow rectal examinations, ultrasono-

graphy would be the method of choice because pregnancy can be detected with a high degree of accuracy during the first month of gestation (Adams *et al.*, 1991; R. W. Radcliffe *et al.*, 1997; Roth *et al.*, 2001, 2004). Alternatively, for less tractable animals, progesterone monitoring can be used to diagnose pregnancy 3–5 months after mating, when systemic concentrations exceed luteal levels (Ramsay *et al.*, 1987; Hodges & Green, 1989; Schwarzenberger *et al.*, 1993, 1996, 2000; Czekala & Callison, 1996; Berkeley *et al.*, 1997; Patton *et al.*, 1999; Brown *et al.*, 2001; Lance *et al.*, 2001; Roth *et al.*, 2004). Although the general reproductive physiology of the horse differs significantly from that of the rhinoceros, in terms of early embryo development, the horse is an ideal model. Intensive ultrasound monitoring of pregnancy in the Black rhinoceros (R. W. Radcliffe *et al.*, 2001), White rhinoceros (R. W. Radcliffe *et al.*, 1997) and Sumatran rhinoceros (Roth *et al.*, 2001, 2004) has confirmed that early embryo development in these species is very similar to that in the horse. Data for the Indian rhinoceros are lacking at present.

In contrast, endocrine indicators of pregnancy and gestational health in the horse cannot be extrapolated to the rhinoceros. Elevations in oestrone sulfate during gestation are used to monitor foetal health in horses (Pashen & Allen, 1979). However, oestrogens remain at low levels throughout most of gestation and, therefore, do not appear to be useful indicators of pregnancy or foetal health in the Black rhinoceros (Ramsay *et al.*, 1987), Indian rhinoceros (Kasman *et al.*, 1986; Schwarzenberger *et al.*, 2000) and Sumatran rhinoceros (Roth *et al.*, 2004). Furthermore, no chorionic gonadotrophin (CG) similar to equine CG has been identified in rhinoceros (Sherman *et al.*, 1997). Although it is possible that rhinoceros produce early-pregnancy proteins, as do most other species studied to date, such

research has not yet been carried out in this family.

Data acquired by ultrasonography have begun to confirm early pregnancy loss in rhinoceros in captivity, something that had been suspected from the endocrine data. Early embryo loss has been confirmed and reported at least once in three of the four rhinoceros species: the Black rhinoceros (Berkeley *et al.*, 1997), White rhinoceros (R. W. Radcliffe *et al.*, 1997; Patton *et al.*, 1999) and Sumatran rhinoceros (Roth *et al.*, 2001, 2004). However, several additional confirmed losses have gone unreported (N. Lung, pers. comm.; L. Kramer, pers. comm.; R. Radcliffe, pers. comm.). Similarly, although a few specific incidents of abortion or stillbirths have been detailed in papers (Schwarzenberger *et al.*, 1996; Berkeley *et al.*, 1997), others have gone unreported in the literature (C. Miller, pers. comm.; G. West, pers. comm.). The incidence appears particularly high in the Indian rhinoceros (Kock & Garnier, 1993). Although initially thought to be a problem associated with captive management, a study of wild Black rhinoceros suggests that early pregnancy loss also occurs in wild populations (Garnier *et al.*, 2002).

Early embryo loss in captive rhinoceros has been associated with endometritis (inflammation of the endometrium) (R. W. Radcliffe *et al.*, 1997) and pyometra (accumulation of pus in the uterine cavity) (Patton *et al.*, 1999). Luteal insufficiency has also been proposed, and anecdotal evidence supporting this theory exists in two studies, in which ♀ rhinoceros with a history of pregnancy loss were supplemented with a synthetic progestin and proceeded to carry their pregnancies to term (Berkeley *et al.*, 1997; Roth *et al.*, 2004). At least two additional, but unreported cases exist: one in an Indian rhinoceros (G. West, pers. comm.) and another in a Black rhinoceros (C. Miller, pers. comm.). However, actual proof of progesterone deficiency is lacking. In fact, the endogenous progesterone

terone profile for a Sumatran rhinoceros supplemented with progesterone during her first successful pregnancy was comparable to that of a later successful pregnancy during which no supplement was provided (Fig. 1). If the supplemental progestin was responsible for maintaining the first pregnancy, it may have been through some qualitative, and not quantitative, effect.

PATHOLOGY

Reproductive pathology has been reported in ♀♀ of all four rhinoceros species in captivity (Table 2) and has been implicated in reduced fertility on several occasions (R. W. Radcliffe *et al.*, 1997; Patton *et al.*, 1999; Roth *et al.*, 2001; Hermes, Hildebrandt & Göritz, 2004). Ovarian and uterine cysts are perhaps the least significant of the pathologies. In the horse cysts can be present in limited numbers and size without affecting fertility (Adams *et al.*, 1987) and their development is associated both with increased age and foaling (Chevalier-Clément, 1989). Similarly, a Sumatran rhinoceros with no initial uterine pathology developed two cysts following her first successful pregnancy. Regardless, she proceeded to produce a second-term calf following her first

post-partum conception (T. Roth, unpubl. data). However, uterine cystic hyperplasia reportedly advances with age in both mares (Chevalier-Clément, 1989) and African rhinoceros species (Godfrey *et al.*, 1991; Hermes, Hildebrandt & Göritz, 2004), eventually resulting in larger clusters of cysts within the endometrial glands that are associated with reduced fertility. A similar condition may develop in the Sumatran rhinoceros (Schaffer *et al.*, 1994). In contrast, although benign, leiomyomas (tumours) probably pose the greatest threat, both because of their prevalence and their serious effects on fertility (Montali & Citino, 1993; Hermes, Hildebrandt & Göritz, 2004). The abundance and size of these leiomyomas can fill portions of the uterus thereby blocking early embryo transport through the horns and interfering with successful implantation and placental formation. Leiomyomas can also pose a risk to animal health owing to their sometimes extensive growth compounded by the challenges associated with their removal (R. M. Radcliffe *et al.*, 2000).

The growth of uterine masses, or leiomyomas, generally occurs in aged animals, particularly those that have not been pregnant for extended periods of their life. In

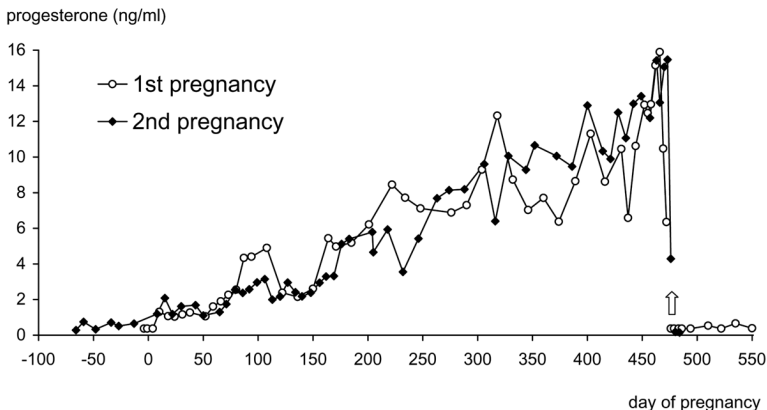


Fig. 1. Serum progesterone profiles throughout gestation in a Sumatran rhinoceros *Dicerorhinus sumatrensis* supplemented with a synthetic progestin (1st pregnancy) and without the progestin supplement (2nd pregnancy). Arrow indicates parturition.

fact, one report suggests that 80% of non-reproducing rhinoceros have leiomyomas and/or exhibit cystic hyperplasia in their reproductive tracts (Hermes, Hildebrandt & Göritz, 2004). Although it has long been presumed that these pathologies develop as a result of the repeated cyclic exposure to oestrogens that occurs when ♀♀ are maintained in the non-pregnant state, the exact aetiology is not understood. However, if true, modifications in the reproductive management of the population might prove most effective in reducing the incidence of disease. For example, breeding ♀♀ for the first time at a relatively young age and/or at regular intervals throughout life could reduce the otherwise consistent exposure of their systems to cyclic hormones. Alternatively, contraceptives that inhibit ovarian activity for months or years at a time might also delay and reduce the onset of pathologies.

MALE REPRODUCTIVE CHARACTERISTICS

The reproductive physiology of the ♂ rhinoceros has received far less attention from the scientific community than that of the ♀. Only recently has there been a report on longitudinal monitoring of androgen metabolites in ♂ Black and White rhinoceros, confirming that concentrations remain comparatively constant across the seasons (Brown *et al.*, 2001). Consistent sperm production throughout the year has also become evident through the recovery of good-quality sperm samples by electroejaculation in Indian rhinoceros (Roth *et al.*, 2005) and White rhinoceros (Hermes *et al.*, 2005). Although the database on ejaculate characteristics and sperm quality for this taxon is expanding, it must be interpreted carefully because variation in sample quality between collection attempts, even in the same individual, can be profound (Roth *et al.*, 2005). How closely collected samples mimic natural ejaculates is unknown because attempts at training ♂♂ to ejaculate into artificial vaginas have failed (Schaffer *et al.*, 1990; O'Brien &

Roth, 2000). Nevertheless, several methods of collecting semen have been useful in gaining some insight into rhinoceros ejaculate and sperm characteristics. For example, we now know that semen can be collected from ♂♂ as young as 6 years of age (White rhinoceros; Hermes *et al.*, 2005) and as old as 42 years (Indian rhinoceros; Schaffer *et al.*, 1990).

Based simply on the observation of fluid production prior to intromission and the effluent following copulation, it is apparent that the volume of rhinoceros ejaculate is substantial. However, one can only speculate what fraction is simply glandular fluid and what fraction is rich in sperm. Recent attempts at semen collection by electroejaculation have produced samples close to 100 ml or more in the Black rhinoceros, White rhinoceros and Indian rhinoceros (Table 3). Although not all fractions contributing to this 100 ml volume are spermic, total sperm production is high, averaging 3.0, 2.2 and 30×10^9 in Black rhinoceros, White rhinoceros and Indian rhinoceros, respectively. These values for samples collected by electroejaculation are quite similar to those for a natural ejaculate fraction collected from a ♀ Sumatran rhinoceros after mating (O'Brien & Roth, 2000). They are also within the range of, or slightly greater than, those recorded for other members of the order Perissodactyla, including horses and zebras (McDonnell & Love, 1990; Crump & Crump, 1994). Finally, the total number of sperm collected by recent electroejaculation attempts is not much lower than that collected during gamete rescue by flushing the vas deferens and caudal epididymis (Table 3). Therefore, it is possible that the samples are similar in total sperm number to those ejaculated naturally during mating.

If the samples collected by the several methods described in Table 3 accurately represent the characteristics of rhinoceros sperm, it appears that sperm quality may not be particularly high in these species.

SPECIES	COLLECTION METHOD	NO. ANIMALS	NO. ATTEMPTS	MEAN (RANGE)		TOTAL SPERM (× 10 ⁶)	MOTILITY (%)	STRUCTURALLY NORMAL (%)	STUDY
				AGE (years)	VOLUME (ml)				
AFRICAN RHINOCEROS SPECIES									
Black rhinoceros									
<i>Diceros bicornis</i>	E	2	2	25.5 (17-34)	58.1 (16-100)	14 (1.3-27)	40 (30-50)	29 (21-37)	1
		2	2	28 (14-42)	17.5 (1.5-20)	15 (0.2-30)	NR	NR	2
		1	2	NR	31.7 (18.8-44.6)	3 (1.4-4.4)	50 (40-60)	NR	3
	MS	1	1	40	62.2	9	NR	NR	2
	GR	4	NA	18.6 (10->35)	NA	70 (12-210)	55 (30-65)	73 (51-92)	4
White rhinoceros									
<i>Ceratotherium simum</i>	E	21	34	21.3 (6-39)	80.2	22	71	68.8	5
	MS	1	1	27	0.7	0.07	NR	NR	2
	GR	2	NA	33.5 (32-35)	NA	31 (6-56)	70 (60-80)	89	4
ASIAN RHINOCEROS SPECIES									
Indian rhinoceros									
<i>Rhinoceros unicornis</i>	E	4	11	20.5 (7.5-34)	92.4 (0.5-200)	301 (9-860)	52 (0-90)	34 (4-74)	1
		1	4	30+	0.7 (0.1-2)	2 (0.1-5)	73 (70-80)	NR	6
	MS	1	2	36.5 (31-42)	4 (0-8)	70 (0-140)	NR	NR	2
		1	19	30+	14.2 (0.2-50)	1.0 (0-27)	27.3 (0-80)	NR	6
	GR	2	NA	16 (15-17)	NA	520 (180-850)	53 (40-65)	54 (48-60)	4
Sumatran rhinoceros									
<i>Dicerorhinus sumatrensis</i>	Post-coital	1	5	>20	104	25	60	40	7

Table 3. Rhinoceros semen characteristics following collection by several methods. Sperm collected by gamete rescue (GR) that had cytoplasmic droplets but no other abnormality were considered normal. Values are means followed by ranges (when available) if more than one collection attempt is represented: NR, not reported; E, electroejaculation; MS, manual stimulation; GR, gamete rescue; ¹ Roth *et al.*, 2005; ² Schaffer *et al.*, 1998; ³ Platz *et al.*, 1979; ⁴ T. Roth *et al.*, unpubl. data; ⁵ Hermes *et al.*, 2005; ⁶ Schaffer *et al.*, 1990; ⁷ O'Brien & Roth, 2000.

However, some variations in the percentage of motile spermatozoa are probably related to the success of the collection procedure because collections by electroejaculation from the same ♂♂ on numerous occasions produced some samples with a high percentage of motile sperm and others with much lower percentages of motile sperm (Roth *et al.*, 2005). Furthermore, when sperm are rescued post-mortem motility can be significantly impacted by the cause of the donor's death, timing of rescue and storage temperature prior to rescue. However, collection procedure probably has less influence on sperm morphology, especially when sperm are collected post mortem from the vas deferens and caudal epididymis. Therefore, it does not appear uncommon for ♂♂ of the four rhinoceros species in captivity to produce comparatively high proportions (30–70%) of structurally abnormal sperm (O'Brien & Roth, 2000; Hermes *et al.*, 2005; Roth *et al.*, 2005). Regardless, many of these samples were recovered from proven breeders, indicating that the poor sperm morphology is not causing infertility.

PATHOLOGY

Reproductive pathology in the ♂ rhinoceros appears much less prevalent than that in the ♀. Sperm production has been confirmed in almost all ♂♂ examined to date (Platz *et al.*, 1979; Schaffer *et al.*, 1990, 1998; O'Brien & Roth, 2000; Hermes *et al.*, 2005; Roth *et al.*, 2005). Ultrasonography has been used to characterize the accessory sex glands of the ♂ rhinoceros and a positive association between size of glands and quality of semen has been detected but no pathological findings were reported (Hermes *et al.*, 2005). Ultrasound examinations of the testicles often reveal hyperechoic spots, representing fibrotic areas of the parenchyma, that initially were thought to be indicative of reduced fertility. However, additional research has revealed that testicular fibrosis develops naturally with

age in ♂ rhinoceros and does not affect sperm quality (Hermes *et al.*, 2005). In one case, testicular neoplasia (tumour formation) was diagnosed and the mass accounted for one third of a testicle, but the ♂ continued to produce good-quality semen (Hermes, Bryant *et al.*, 2004).

CHALLENGES FOR THE FUTURE

Despite impressive advances in our understanding of the reproductive physiology of the rhinoceros, many basic and applied scientific challenges for researchers still exist. 'Normal' reproductive cycles and ovarian function have been described for each species in captivity but several aberrant patterns also have been documented. The physiological mechanisms driving these unusual patterns, and the prevention thereof, require further research. Pregnancy can be diagnosed in all rhinoceros by a couple of methods but a non-invasive, early-pregnancy test has eluded us to date. Although the occurrence of early embryo loss has been confirmed in all four rhinoceros species, we have barely scratched the surface in terms of our understanding of its prevalence and underlying causes.

Despite the many unknowns, based on today's scientific knowledge, several modified management practices have been suggested for overcoming some reproductive hurdles in these species. For example, breeding ♀ White rhinoceros at least once before they reach their late teens could decrease the incidence of reproductive pathologies and extend the reproductive life of these animals (Hermes, Hildebrandt & Göritz, 2004). Additionally, providing Black rhinoceros with exhibits that limit exposure to humans and relocating them less frequently might increase the productive life of this species by reducing disease and mortality rates (Carlstead & Brown, 2005). Integrating serial ultrasound examinations and hormone monitoring into the management regime for Asian rhinoceros can improve breeding success and reduce risk of aggressive interactions that occur

during ill-timed introductions (Roth *et al.*, 2001; Stoops *et al.*, 2004).

With further research, additional recommendations founded on science will evolve and will offer animal managers proven methods for helping to ensure reproductive success in rhinoceros in captivity. Additionally, although beyond the scope of this paper, progress in the development of assisted reproduction technologies (i.e. hormonal induction of oestrous cycles/ovulation, artificial insemination and *in vitro* fertilization) and genetic selection/preservation (i.e. gamete rescue/cryopreservation and sperm sorting by sex) offer tremendous potential for facilitating reproduction and the genetic management of all rhinoceros species.

Perhaps the most important finding from the expanding scientific reproductive database for this taxon is the extent to which characteristics vary among species. With different reproductive-cycle lengths, hormone concentrations produced, endocrine patterns, follicular dynamics and mechanisms of ovulation, each rhinoceros species has evolved distinctively from one another. Why the reproductive mechanisms of these species differ so substantially is not fully understood. However, the fact that these differences are now known is testimony to the progress that has been made in our understanding of rhinoceros reproduction.

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