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CAN THE PROBLEMS ASSOCIATED WITH THE LOW REPRODUCTIVE RATE IN CAPTIVE WHITE RHINOCEROSES (*CERATHOTERIUM SIMUM*) BE SOLVED WITHIN THE NEXT 5 YEARS?

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Introduction

The reproductive rate of the two subspecies of the white rhinoceros (*Ceratotherium simum*), the northern (*C. s. cottoni*) and the southern (*C.s. simum*), in captivity is very low. As a consequence the populations are not self sustaining. This is in contrast to wild southern white rhinoceroses which are increasing at an annual rate of 8-9 % (OWEN-SMITH, 1988). The captive populations of both subspecies are in a demographic crisis. As of December 1996, over 50% of captive southern white rhinoceroses were >20 years of age, and the reproductive rates of founders and first and second generation offspring were as low as 30%, 8% and 0%, respectively (OCHS, 1995).

These reproductive problems have prompted the IRF (International Rhino Foundation) to sponsor a 'Workshop on Problems Associated With the Low Rate of Reproduction Among Captive-born Female Southern White Rhinoceros (*Ceratotherium simum simum*) 29-31 Oct. 1998, San Diego, CA, USA'. Problems defined at this workshop and the consequently generated suggestions for management (ANONYMOUS, 1998; SSP recommendations compiled by ROTH, 1998) of white rhinoceroses will be summarized and discussed in this paper.

Facts about the reproductive physiology of white rhinoceroses in captivity

Profound information on the oestrous cycles of white rhinoceroses is now available (RADCLIFFE et al., 1997; ROTH et al., 1998; SCHWARZENBERGER et al., 1998; PATTON et al., 1999). Although it still is unclear whether the normative cycle length is 35 or 70 days, our recently published results on luteal activity and the oestrous cycle length have been fully confirmed by two independent working groups in the USA (San Diego: PATTON et al., 1999 and Cincinnati/Front Royal: ROTH et al., 1998).

In summary our study revealed that two thirds of white rhinoceroses (n=21) of both subspecies had erratic or missing luteal activity, whereas variable cycles of 4-10 weeks in length were evident in six females, and regular oestrous cycles 10 weeks in length were found in two animals. As reproductive patterns were highly variable among and within individual animals, rhinoceroses were classified into four major categories on the basis of oestrous cycle length and luteal phase 20-oxo-P concentrations: 1) regular oestrous cycles of 10 weeks duration and >800 ng/g (n = 2 animals); 2) oestrous cycles between 4-10 weeks and 250-750 ng/g (n = 6); 3) no apparent cycle regularity, but luteal activity (20-oxo-P of 100-200 ng/g; n = 6); 4) no apparent luteal activity (20-oxo-P of <100 ng/g; n = 7). Wild caught and F1 animals were equally distributed in these 4 categories and age of the animals had no influence on this categorisation (SCHWARZENBERGER et al., 1998).

Although not definitely confirmed, the arguments used by most researchers at the San Diego conference to define 35 days as the normative cycle length are derived from the following studies. RADCLIFFE et al. (1997) observed 2 pregnancies (which resulted in resorption) after two non-conceptive cycles of 31-35 days in length and ROTH et al. (1998) have seen a pregnancy in an animal that conceived after three cycles about 30 days in length. In contrast, fertile breeding after 70 days cycles still has to be demonstrated and the tendency at the San Diego workshop was to consider the 70 days as extended luteal phase cycles. However, there is still the argument of the consistency of cycles found in category 1 animals of our study (SCHWARZENBERGER et al., 1998). Whatever the normative cycle length will finally be, faecal steroid analysis from animals at the San Diego WAP has shown that 2 of the regularly breeding females conceived after a lactational anoestrus period without exhibiting any luteal cycles. Therefore the main problem is not the changing cycle length, but the missing or erratic luteal activity in over 50% of the captive rhinoceros population.

Suggestions to solve the reproductive problems

Reproductive problems defined at the San Diego workshop include 1) acyclicity and varying cycle length, 2) mating failure 3) conception failure and 4) pregnancy failure (ANONYMOUS, 1998; ROTH, 1998). Mating failure: Beside lack of mating behaviour from unexperienced animals, in many cases this is attributable to acyclicity or to silent oestrous. Pregnancy failure (early embryonic death) has been observed on ultrasound in 2 animals (2 times in the same female; RADCLIFFE et al., 1998). Causes for conception failure are only speculative and the statement that it should primarily occur in females exhibiting 70 days cycles (ROTH, 1998) is unconfirmed; this could only be investigated with serial ultrasonographic examinations. According to observed matings in our results, conception and pregnancy failure is equally distributed over animals with 35 and 70 days cycles.
To elucidate on these above mentioned problems, the following suggestions should be applied in combination wherever possible!

1. Continue faecal progestagen analysis

In light of the results on missing luteal activity and on varying oestrous cycle length the great need to continue faecal reproductive monitoring is obvious. Reliable reproductive monitoring is a helpful tool prior to clinical examination under sedation or anaesthesia, and/or prior to the administration of treatment regimes. It will also help to evaluate effects of management measures.
Measuring faecal progestagens in samples collected at a frequency of 2-3 samples/week is adequate for diagnosing luteal function. It is suggested to collect samples for extended periods (4-6 months), as the cycle length within the same animal is varying and as oestrous cycles can suddenly start in previous anestrous females. Furthermore faecal progestagen analysis allows pregnancy diagnosis during the first third of gestation (SCHWARZENBERGER et al., 1998; PATTON et al., 1999).

2. Change social structures by moving animals between zoos

Breeding of white rhinoceroses in captivity was first recorded in 1967 in Pretoria and in 1971 and 1972 in Europe and North America, respectively (RIECHES, 1998). Similar to endocrinological data, behavioural and questionnaire data did not reveal any conclusive explanation for the reproductive failure of the F1 generation (SWAISGOOD et al., 1998). Reproduction in captivity has traditionally been most successful in institutions with larger enclosures and subsequently larger group sizes (>2 animals) and in animals which are constantly kept together. This, however, is in contrast to the wild where adult females are usually only accompanied by a single offspring, whereas bulls typically are territorial and solitary. If a bull remains with a cow for more than a day, it is assumed the cow is coming into oestrus and the average group size of rhinoceroses in South Africa is in the range of n = 2.1-2.3 animals (OWEN-SMITH, 1988).
In light of reports on captive white rhino reproduction the importance of a proven breeding male is obvious, i.e. the same breeding male sired 17 calves in Hodenhagen (BÖER and HAMZA, 1996) and one single male sired over 60 calves in San Diego WAP (KILLMAR, 1997). These males sired most of their offspring with the same females, and also in institutions with a good record of breeding, not all females at breeding age are fertile (i.e. San Diego, PATTON et al., 1999). Since many white rhinos are kept together in a non-breeding situation since they were brought into captivity in the early 1970, an estab-

lished sibling relationship or mate choice problems in these animals result in a silent oestrous situation. However, currently there is no conclusive explanation for missing ovarian cycles available.
Comparison with cheetahs suggest that the movement of animals and change of social structures could significantly contribute to solve the problems of mating failure. Therefore management recommendations made at several EEP meetings (TOMASOVA, 1998) and at the San Diego meeting were to change the social environment by moving animals between institutions and by experimenting with multi-male groups (ANONYMOUS, 1998; ROTH, 1998).
Movement of males has already resulted in breeding of previously inactive males, i.e. the 29 years old male Balthasar sired an offspring after been transferred from a pair situation in Antwerp to Hilvarenbeek. This male had experienced a typical white rhino management history; he arrived in Antwerp in 1974 together with a female at about 5 years of age and from then on was kept in a pair situation. Five years later he sired a baby born in 1981, but no reproduction occurred since then. Balthasar was 26 years old and 15 years after the last reproductive activity he was moved, socialised with a group of 7 females and started breeding (TOMASOVA, 1998). Other movements of males occurred and are planned in the EEP and SSP.
We also strongly encourage movement of females to break up sibling relationship, but also to test if movement has an influence on luteal activity and the reproductive cycle. To test this hypothesis faecal sample collection for reproductive monitoring should start at least 3 months before and should continue until 9 month after the animal transfer. In addition, ultrasonographic assessment of the reproductive status should be included, as this would provide the opportunity to eleminate reproductively unsound individuals prior to translocation. First results of two movements of females (Salzburg to Usti nad Labem and Berlin Friedrichsfelde to Salzburg) will be available at the time of this meeting.

3. Reproductive assessment by transrectal ultrasound (in combination with endoscopy)

To date, rhinos in general are rarely investigated by ultrasonography (HILDEBRANDT et al., 1995 and 1999; RADCLIFFE et al., 1997; SCHAFFER, 1998). This could change in the near future since ultrasonographic assessment of the reproductive status of zoo animals has become a routine technique (HILDEBRANDT and GÖRITZ, 1998). This has proved to be an efficient tool for reproductive health assessment for selecting potential breeders, i.e. in elephants and it would provide the opportunity to eliminate reproductively unsound individuals prior to translocation. Furthermore, serial ultrasound examinations in white rhinoceroses after training are possible without immobilisation (RADCLIFFE et al., 1997). Serial examinations would be best to elucidate reasons for reproductive failure. However, if training is not feasible even single ultrasound examinations of immobilised animals will be invaluable contributions to our understanding of reproductive failure in white rhinos.
There was a strong tendency by some researchers at the IRF workshop to attribute acyclicity, conception failure and pregnancy failure solely to uterine pathology, like endometrial cysts, uterine infections and pyometra. However, these pathologic findings have not been confirmed in a larger number of animals to date. The tendency of a significant occurrence of uterine pathology is very much based on the finding of uterine fluid accumulation in one older female by RADCLIFFE et al. (1997) who have described two non-conceptive cycles (31-35 days) and two conceptive periods (70 days) which ended in early embryonic death and resorption after day 28. Pregnancy failure was attributed to suspected endometritis defined by intrauterine fluid accumulation in late dioestrous. A similar case was investigated in a 14 year old female at the San Diego WAP during the course of the IRF workshop (RADCLIFFE and SCHAFFER, 1998; personal comm.). SCHAFFER (1998) presented post mortem (n=6) findings and clinical examinations of alive (n=2; above mentioned 14 year old female of San Diego WAP is included) white rhinos and described normal uterus and ovary in 3 animals. Few uterine cysts were described in 2 animals, whereas the causes of the 'abnormal' uterus in the 3 remaining animals was not further defined. Thus, if the few cysts are a borderline to pathology, the incidence of uterine pathology is not as prominent as suspected at the San Diego meeting.

In addition, ultrasonographic assessment (20 min.) and endoscopic examination (2hours) of the reproductive tract of the white rhino Baby (Studbook # 361) under anaesthesia (Immobilon® and Detomidine - Naltrexone and Atipamezole; about 2.5 hours) at Salzburg zoo in Dec. 1998 revealed no uterine pathology in this 28 years old animal. Her ovaries appeared to be inactive (HERMES, pers. comm.). After the San Diego meeting, the finding of a healthy, normal uterus was not anticipated. The oestrous cycle of Baby has been continously examined in the past 7 years by faecal steroid analysis. Baby had no luteal activity in the period of 1991-1996, except 2 therapeutically induced ovulations and luteal phases in 1994 and 1995 (SCHWARZENBERGER et al., 1998). To our surprise, luteal activity in Baby jump started in Jan 1997 (category 2 according to our classification) and continued throughout 1997.
Complete passage of the cervix of the animal Baby by a catheter with special equipment under endoscopic and ultrasonographic visualisation was possible. Full passage of the cervix of this animal was to our knowledge the first documented case in a living rhinoceros. This, although, due to description of the rhinoceros cervix had been previously considered impossible. Gross anatomy of the cervix of rhinoceroses was described as firm, its lumen has a very tortuous path with 3-5 large folds of fibrous tissue and crypts between each fold of tissue (GODFREY et al., 1991). Uterine flushing with 250 ml Lotagen (4%) solution was performed and Baby exhibited strong oestrous behaviour 5 days after this treatment.

4. Ovulation induction by hormones in cases of missing or erratic luteal activity

In addition to oestrous cycle monitoring, faecal steroid analysis is an important tool prior to, as well as during and after the application of hormonal treatments. Results will help to determine the timing of application, to find a useful drug combination and to evaluate the efficacy of hormonal treatment. A summary of published and unpublished hormonal treatments in female white rhinos (PATTON et al., 1998) revealed that our combination of orally fed chlormadinone acetate (fed on two occasions for 35 and 45 days, respectively) followed by a subsequent hCG injection was the only successful regime in ovulation induction (SCHWARZENBERGER et al., 1998). Several other combinations of PGF2a and Regumate® followed by GnRH, hCG or FSH induced follicular activity in some cases but failed to induce ovulation. However, since treatments were also applied to animals scheduled to be euthanized and since faecal steroids have not been monitored in all cases, efficacy could not be completely evaluated. Possible reasons for failures could be that Regumate® is not effective in causing a rebound effect or that the duration of application, which in all cases was less than 3 weeks, was too short. As revealed by post treatment faecal progestagen analysis, the timing of Regumate® application was wrong in two females from Arnheim zoo (LUTTEN, personal comm.). Regumate® was fed during periods of luteal activity and the subsequent hCG application could not induce follicular growth and ovulation, as no rebound effect was produced.

5. Artificial Insemination (AI)

Successful AI in rhinos in general has yet to be developed yet and it could become an important management tool. However, significantly more research in this area will be necessary to simply solve the basic requirements, like semen collection and preservation, exact timing of ovulation or to develop a reliable regime for ovulation induction. On a very positive note, under endoscopic and ultrasonographic visualisation full passage of the cervix of the animal Baby from Salzburg zoo was possible with a special catheter. Other basic requirements like long term non-invasive reproductive monitoring through faecal steroid analysis are routine techniques. In the future, this should be combined with ultrasonographic monitoring in selected animals.
Time of ovulation in white rhinos seems to occur 7-9 days before a substantial rise in faecal progestagens (RADCLIFFE et al., 1997) and as the length of the follicular phase is about 12 to 15 days (SCHWARZENBERGER et al., 1998) it occurs about 3 to 7 days after faecal progestagens dropped to baseline levels. Semen collection has been successfully performed in male white rhinoceroses by

manual massage of the internal sexual accessory glands (SCHAFFER et al., 1995 and 1999) and, again on a positive note, the 2 white rhinoceros bulls from Salzburg zoo might get used ot this procedure in the near future (WALZER, personal. commun.)

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Summary

Can the problems associated with low reproductive rate in captive white rhinoceroses (Cerathoterium simum) be solved within the next 5 years?

The reproductive rate of the white rhinoceros in captivity is very low and >50% of animals are >20 years of age. At a recent IRF (International Rhino Foundation) workshop reproductive problems have been defined as 1) acyclicity and variable oestrous cycle length of 35 or 70 days, 2) mating failure due to acyclicity, or silent oestrus due to sibling relationship/mate choice problems and 3) conception – pregnancy failure due to presumed uterine pathology. Although reasons for any of the above problems could not be identified definitively, possible solutions are available. Serial faecal reproductive monitoring will help to evaluate effects of management measures. The most promising recommendation is to transfer animals between zoos in order to break up sibling relationships/overcome mate choice problems. The additional use of diagnostic ultrasound for reproductive assessment is strongly encouraged. Serial ultrasound examinations would be best, however, even single ultrasound exams will provide invaluable contributions to the understanding of white rhino reproductive failure. Other options are to work on protocols for ovulation induction in animals with missing or erratic luteal activity and to continue research into the development of artificial insemination.

Zusammenfassung

Können die Probleme der niedrigen Fortpflanzungsaktivität von Breitmaulnashörnern (Cerathoterium simum) in Menschenobhut innerhalb der nächsten 5 Jahre gelöst werden?

Die Fortpflanzungsrate von Breitmaulnashörnern in Menschenobhut ist sehr niedrig, und über 50% der Tiere sind bereits über 20 Jahre alt. Im Oktober 1998 wurden bei einem vom IRF (International Rhino Foundation) gesponsorten Workshop folgende Probleme definiert: 1) Fehlende Sexualzyklen oder variierende Zykluslängen von 35 oder 70 Tagen; 2) Ungenügende Deckaktivität, verursacht durch fehlende Sexualzyklen oder stille Brunst infolge von Problemen mit 'Geschwister ähnlichen Beziehungen' und 'mate choice' in den Tiergruppen, 3) Ausbleibende Konzeption - Embryonaltod durch vermutete Uteropathien. Obwohl die Ursachen der genannten Probleme ungeklärt sind, bestehen mögliche Lösungsansätze. Eine wichtige Grundlage für die Evaluierung dieser Vorschläge ist die Weiterführung endokrinologischer Untersuchungen von Kotproben. Am erfolgversprechendsten scheint ein Tiertausch zwischen Zoos, um die Probleme mit 'Geschwister ähnlichen Beziehungen' und 'mate choice' zu lösen. Zusätzlich wird die Anwendung transrektaler Ultraschalluntersuchungen für die Diagnose des Reproduktionsstatus empfohlen. Obwohl wiederholte Untersuchungen an unsedierten Tieren am Besten wären, sind auch Einzeluntersuchungen in Narkose diagnostisch von unschätzbarem Wert. Andere Optionen sind die Weiterentwicklung von Therapien für die Ovulationsinduktion oder weitere Forschungen für die Entwicklung der künstlichen Besamung.

References

ANONYMOUS (1998): Round table disscussion and protocol synthesis: Meeting minutes. Recorder Angela White. San Diego, CA, USA. Proc. of the workshop on problems associated with the low rate of reproduction among captive-born female Southern white rhinoceros (Ceratotherium simum simum), 6 pages.

BÖER, M., und H. HAMZA (1996): Haltung und Zucht des Breitmaulnashorns (Ceratotherium s. simum) im Serengeti Safaripark Hodenhagen. Zool. Garten N.F. 66, 349-364.

GODFREY, R.W., POPE, C.E., DRESSER, B.L., and J.H. OLSEN (1991): Gross anatomy of the reproductive tract of female black (Diceros bicornis) and white rhinoceros (Ceratotherium simum simum). Zoo. Biol. 10, 165-175.

HILDEBRANDT, T.B., und F. GÖRITZ (1995): Graviditätskontrolle beim Breitmaulnashorn mit Hilfe der transrektalen Adaptersonographie. Ultrasch. Med. 16, 68.

HILDEBRANDT, T.B., and F. GÖRITZ (1998): Ultrasonography as a tool in propagation of zoo animals. M.E. Fowler and E. Miller (Eds.), Zoo and wild animal medicine, Current therapy, 4th ed. Philadelphia, USA: W.B. Saunders Company. pp. 41-54.

HILDEBRANDT, T.B., GROBLER, D., HERMES, R., QUANDT, S., RAATH, C., and F. Göritz (1999): Pregnancy diagnosis in African rhinoceroses by transrectal ultrasonography under field conditions. Ultrasch. Med., in press

KILLMAR, L.E. (1997): Captive rhinoceros reproduction at the zoological society of San Diego: a conservation success story. Zool. Garten N.F. 67, 53-60.

OCHS, A. (1995): International studbook for the white rhinoceroses (Ceratotherium s. simum / cottoni). In: R. Göltenboth and A. Ochs (Eds.), International studbook for African rhinoceroses (Diceros bicornis/Ceratotherium simum), No. 6. Berlin, Germany: Zoologischer Garten Berlin, pp. 212-217.

OWEN-SMITH, R.N. (1988): Megaherbivores: the influence of very large body size on ecology. Cambridge, UK: Cambridge University Press.

PATTON, M.L., CZEKALA, N., SCHAFFER, N., and V. LANCE (1998): Hormonal manipulations of rhinoceros. San Diego, CA, USA. Proc. of the workshop on problems associated with the low rate of reproduction among captive-born female Southern white rhinoceros (Ceratotherium simum simum), 16-21.

PATTON, M.L., SWAISGOOD, R.R., CZEKALA, N., WHITE, A.M., FETTER, G.A., MONTAGNE, J.P., and V. LANCE (1999): Reproductive cycle length and pregnancy in the southern white rhinceros (Ceratotherium simum simum) as determined by fecal pregnane analysis. Zoo Biol., in press

RADCLIFFE, R.W., CZEKALA, N.M., and S.A. OSOFSKY (1997): Combined serial ultrasonography and fecal progestin analysis for reproductive evaluation of the female white rhinoceros (Ceratotherium simum simum): Preliminary results. Zoo Biol. 16, 445-456.

RICHES, R. (1998): Concerns with breeding F1 white rhinoceros in zoological institutions. San Diego, CA, USA. Proc. of the workshop on problems associated with the low rate of reproduction among captive-born female Southern white rhinoceros (Ceratotherium simum simum), 4-5.

ROTH, T. (1998): SSP Recommendations from the southern white rhinoceros workshop. (These were written from the round table disscussion and protocol synthesis as stated in the 'Anonymous citation' of this report. 12 pages.

ROTH, T., PATTON, L., BROWN, J., CZEKALA, N., SWAISGOOD, R., and V. LANCE (1998): The North American experience: Female hormonal cycles in Southern white rhinoceros. San Diego, CA, USA. Proc. of the workshop on problems associated with the low rate of reproduction among captive-born female Southern white rhinoceros (Ceratotherium simum simum), 6-9.

SCHAFFER, N.E. (1998): Reproductive pathology in the southern white rhinoceros. San Diego, CA, USA. Proc. of the workshop on problems associated with the low rate of reproduction among captive-born female Southern white rhinoceros (Ceratotherium simum simum), 4 pages.

SCHAFFER, N.E., and R.S. JEYENDRAN (1995): Manual massage of reproductive organs to obtain evidence of spermatogenesis in the rhinoceros. In: Annu. Conf. Soc. Therio., 319.

SCHAFFER, N.E., BRYANT, W.M., AGNEW, D., MEEHAN, T., and B. BEEHLER (1998): Ultrasonographic monitoring of artificially stimulated ejaculation in three rhinoceros species. J. Zoo Wildl. Med. 29, in press

SCHWARZENBERGER, F., WALZER, C., TOMASOVA, K., VAHALA, J., MEISTER, J., GOODROWE, K.L., ZIMA, J., STRAUSS, G., and M. LYNCH (1998): Faecal progesterone metabolite analysis for non-invasive monitoring of reproductive function in the white rhinoceros (Ceratotherium simum). Anim. Reprod. Sci. 53, 173-190.

SWAISGOOD R.R., DICKMAN, D., WHITE, A., HANDRUS, E., and J.P. MONTAGNE (1998): An evaluation of potential behavioral mechanisms of reproductive failure in captive-born Southern white rhinoceros. San Diego, CA, USA. Proc. of the workshop on problems associated with the low rate of reproduction among captive-born female Southern white rhinoceros (Ceratotherium simum simum), 5 pages.

TOMASOVA K. (1998): Letter of the White Rhino EEP Coordinator to The White Rhino EEP Representatives; Dvur Kralove, Dec 1998.

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