PREGNANCY MONITORING AND A PARTURITION EMERGENCY PLAN OF A SOUTHERN WHITE RHINOCEROS *(Ceratotherium simum simum)*

E. SÓS[1], V. MOLNÁR [1], R. HERMES [2], F. SCHWARZENBERGER[3], L. MEZŐS[1], I. KACSKOVIC[4], T. HILDEBRAND[2], C. WALZER[3], F. GÖRITZ[2] and S.SILINSKI[4]

AFFILIATION:

1. Budapest Zoo and Botanical Garden, Állatkerti krt 6-12, H-1146 Budapest, Hungary;
2. Institute for Zoo and Wildlife Research, Berlin
3. University of Veterinary Medicine, Biochemistry Institute, Vienna
4. Department of Physiology and Biochemistry, Faculty of Veterinary Science, Szent István University, Budapest
5. Zoo Münster

Introduction

The Southern white rhino (*Ceratotherium simum simum*) EEP is currently facing the problem of ageing. There are only relatively few individuals that are able to breed naturally, thus the captive population is far from self-sustaining. The prolonged exposure to endogenous sex steroids induces asymmetric reproductive ageing (HERMES et al., 2005; HERMES et al, in press). This is one important reason, why the assisted reproduction techniques have a strong justification nowadays.

The Budapest Zoo keeps 1,1 Southern white rhinos since 1983. Due to the brother–sister relationship there was no chance for natural breeding, therefore colleagues from the Institute for Zoo and Wildlife Research, Berlin made an evaluation of the reproductive organs of the animals in September, 2001. This assessment revealed that the sperm of the male was suboptimal and the female (Lulu) had already pathologic alterations in her reproductive tract. After several examinations, β-carotene treatment and an unsuccessful artificial insemination (AI) attempt in 2003, a successful AI took place on 6 April, 2004.

In order to monitor the pregnancy and to prevent any kind of "accidents" during the birth process a holistic approach and an emergency plan was created. According to our knowledge similar emergency plans were created for elephant births, formerly, although there are several excellent sources providing more general aspects of this topic (Nelson, 1978, BLAKESLEE AND ZUBA, 2002). This paper will go through the different elements of this emergency plan, which had the following goals:

1. Monitoring the pregnancy, forecasting the exact timing of the birth
2. Modification of the enclosure for the needs of the monitoring, the calving process and the maternal care
3. Preparation for unexpected events, such as dystocia, maternal aggression, hand-raising, etc.

General ideas of the emergency plan

The fact that the Budapest Zoo have never had rhino babies in its 140 years long history made clear, that an inexperienced team must collect as much information as possible from other sources. The plan was the product of a team work which was led by the veterinarians. This plan determined the number of individuals who were involved in the project, decided the exact roles of each participant, and put up deadlines for each tasks. We must conclude that some parts of our parturition emergency plan are based on the special situation at the Budapest Zoo (enclosure shape and size, character of our female, etc.), but other parts are general and could be used in any other zoo situation as well.

1. ***Monitoring the pregnancy, forecasting the exact timing of the birth***

Detection of the successful AI and monitoring of the pregnancy was mainly done by endocrinological methods. Well before the pregnancy we collected faecal samples two times a week which were analyzed by the Biochemistry Institute, University of Veterinary Medicine, Vienna

Fig. 1: The chute-like part of the Rhino enclosure at the Budapest Zoo, designed for regular blood collection.

By the help of the medical training we were able to habituate both of our animals for the collection of blood from the ear vein without any kind of sedation. For this purpose the outside enclosure was modified, some rocks and the concrete wall formed a "primitive" kind of, back-opened chute (Fig. 1.). While the animal was fed from the front, the veterinarians could collect blood from the top, which system provided protection both for the keeper and also the veterinarian.

Apart from the hormone values we wanted to visualize the pregnancy during the second trimester, when the fetus was not too big yet, but the most risky first trimester was over. Ultrasonography is a proper tool for pregnancy detection in wild animals (Hildebrandt et al, 2000). Therefore, on 22 August, 2004 we used a 3D ultrasonography during a standing sedation intervention and made an image of the fetus (Holden, 2005) (Fig. 2.).

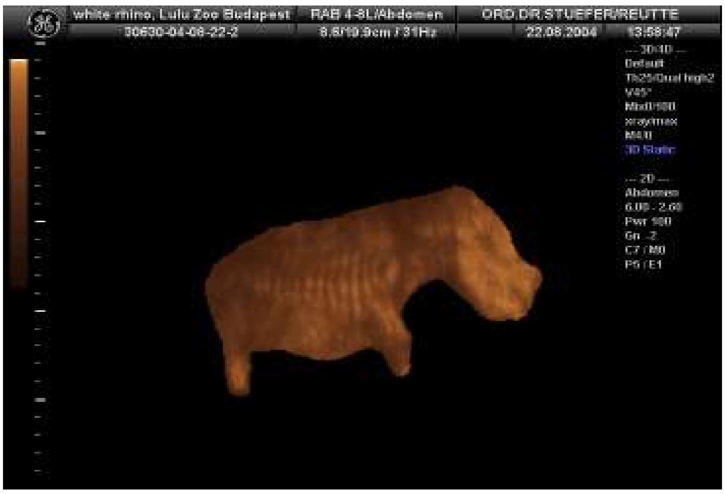

Fig. 2: The 3D ultrasound picture of the fetus on 22 August 2004

These methods were good enough to get information whether the pregnancy still persists, but gave less knowledge about the timing of the calving process. As the pregnancy of the Southern white rhino is estimated to be 16-18 months we wanted to have a better prediction and get a more precise date for the possible birth. Therefore, we trained our female to tolerate transcutaneous ultrasound examinations without any kind of sedation.

The animal was standing in the specially modified part of the outside enclosure and we examined the mammary gland and the abdominal cavity on a weekly basis. Actually, the habituation took a very short time, as the animal enjoyed the transcutaneous examination of the mammary gland. Unfortunately, the portable ultrasound machine (Logiqbook, 4 MHz probe) was not convenient for the examination of the uterus, due to the thickness of the abdominal wall and the long distance from the probe. The examinations took place on a weekly basis during the pregnancy, apart from the last days, when we carried out it daily. During the ultrasound sessions photographs of the mammary gland were taken (manuscript in preparation)

The visible changes of the mammary gland started 10 days before the calving process. The diameter of the blood vessels became wider exactly a week before this event, and the gradual development took place rapidly.

2. Modification of the enclosure for the needs of the monitoring, the calving process and the maternal care

The needs of the monitoring were discussed formerly. In this situation the only “risky” part was the ultrasound examination, not because of the bad nature of the animal, but because of the unexpected events, when the animal felt unsafe or uncomfortable. The two examining veterinarians fixed the escaping routes in advance and also agreed who was responsible for which device or part of the instruments. During the transcutaneous examinations we also touched the anogenital region in order to habituate the animal for a possible intervention during dystocia.

The rhino facility of the Budapest Zoo does not meet the current standards of desirable enclosures and will be reconstructed within 2 years. Nevertheless, we had to cope with this situation. We wanted to avoid the sole usage of the small inside boxes, but at the same time our goal was to reduce the size of the relatively spacious outside enclosure, in order to gain access for any possible intervention, if needed. Therefore, an extra covered stable was attached to the rhino house in order to avoid the possibility to push the rhino baby against the wall or fence by his/her mother after birth. Meanwhile, a separate room was created for possible hand-raising of the calf, another room was furnished for the night watches which started when the pregnancy was 15.5 months old and all the equipment for the veterinarian intervention in the case of a dystocia was transferred to the rhino house. As we wanted to record every possible moment a 24-hours recording camera system was set up, consisting 7 cameras. The records were saved for 3 days, after this period automatic erasing took place.

There was no former information about rhino dystocia, but there were several claims about maternal aggression which was a major threat in the case of an inexperienced, middle-aged, nulliparous female.

3. Preparation for unexpected events, such as dystocia, maternal aggression, hand-raising, etc.

Dystocia was a possible, but unlikely threat. Due to the lack of such an event in the literature we prepared all the equipment, but we thought that such a case should probably require at least a standing sedation situation. In order to avoid this stage we paid an extra attention for the nutrition of the animal. Extra fruit was given and following the increase of the calcium intake the calcium level of the food was decreased weeks before the expected birth.

Maternal aggression was our main fear due to the experience of other zoos in Europe and the United States. This is a factor, when the keepers/vets have a very limited time and possibility to interact. For this reason we prepared goalkeeper gloves and hooks in order to grab or hold the baby. The female was put together with goats in order to have experience with small animals around her. Moreover, the head keeper trained the animal to obey for certain commands, but we knew that this would probably not work in a critical situation. We refused to give tranquillizers for the female due to the fact of possible paradox reaction or unknown effect of the calving process.

The preparation for a possible hand-raising was an important element of this part. We collected the successful recipes from other zoos, literature (Nelson, 1978; Blakeslee and Zuba, 2002) and all the equipment was prepared.

One of the key questions was to gain the colostrum as the placenta of the rhino is epitheliochorial, so all the defending immunoglobulins must be given by the colostrum during the first 24 hours due to the closure time effect.

Another important element was that if the mother does not accept her baby we might have a chance to milk the animal since she liked the touching of the mammary gland. When the birth progress started we even milked the mother slightly in order to raise her oxytocine level and cause higher milk production.

We prepared a second scenario as well, when we do not have a co-operative mother and we have a rhino baby, requiring hand-raising. Since there is a lack of information on hand raising members of the Rhinocerotidae family, we must have relied on veterinary reference material available for the domestic equine neonate as both animals are members of the order Perissodactyla. The immune system of newborn foals is not sufficiently developed despite the fact that equine fetuses are capable of immune reactions to antigenic stimuli (Naylor, 1979). Compared with adult animals, immune reactions of foals are slower and weaker in terms of antibody production (Tizard, 2004). Therefore, newborn foals can quickly succumb to infections, which pose only minor problems in adult animals. Their ability to resist to the common infections depends on passive intake of maternal antibodies. The epitheliochorial placenta of equids, in which the fetal and the maternal circulations are markedly separated from each other, makes transplacental transfer of immunoglobulins impossible. Only the absorbed colostral antibodies provide the antibody protection in newborn foals that are therefore a significant factor influencing their morbidity and mortality (McGuire et al., 1977). Blood serum IgG concentrations exceed 8 g/l in most foals aged 24 to 48 hours. Finding of IgG concentrations from 4 to 8 g/l is interpreted as a partial and that below 4 g/l as a total failure of passive antibody transfer (FPT). FPT affects 10 to 18 % of newborn foals and has thus become the most frequently occurring cause of neonatal septic infections (Erhard et al., 2001). Such infections can be prevented by administration of homologous immunoglobulin preparations to these animals provided that immunoglobulin concentrations in 24 to 48-hour-old foals are known. Thus, simple solution of the FPT problem consists in gammaglobulin assessment in all newborns and preventive application of equine serum preparations containing equine immunoglobulins to FPT-affected ones (Tyler-McGowan, 1997).
In the parturition emergency plan we established a rapid method that analyzes the serum IgG level. Furthermore, we worked out a plan if the rhinoceros calf would be identified as FPT, administration of intravenous immunoglobulin preparation would be instituted immediately. The immunoglobulin preparation has been made from ponies at the Budapest Zoo in order to have identical environment that ensures antibodies against the identical pathogens.

The materials and methods of the immunoglobulin preparation
Zinc-sulphate turbidity reaction (ZST) – Briefly, turbidity induced by addition of zinc-sulphate was measured using the following modification of the McEva's method (McEwan et al., 1970): 25 µl of the tested serum was mixed with 1.7 ml of 0.7 mM zinc sulphate, pH 5.8. The mixture was shaken and left to stand at room temperature for 2 hours. Light absorption due to turbidity was measured photometrically at 590 nm. Blood serum mixed with PBS at the same ratio was used as the blank. The immunoglobulin levels of the tested sera were derived from a calibration curve plotted on the basis of turbidity values corresponding to eight and four dilutions of horse and rhinoceros sera, respectively, with fetal calf serum. Horse and fetal calf sera were obtained from commercial supply (Gibco BRL), while rhinoceros serum was prepared from blood samples drawn from the ear vein of the rhinoceros cow.
Immunoglobulin preparation – Around 5 l blood was drained from 10 healthy ponies at the Budapest Zoo. The blood was let to coagulate for 3 hours at room temperature and then at 4ºC, overnight. Subsequently, 2.5 liters of serum has been retrieved by centrifugation at 3500 g, 4ºC, 30 minutes. We then used this serum to make an immunoglobulin enriched preparation by filtration of it through a 100 kDa Millipore Pellicon 2 tangential flow filtration device (Millipore). Finally, it was sterilized by 0.22 µm filtration (Millipore) and frozen at -20ºC.

Results and discussion of the method
Zinc sulphate turbidity reaction – As there are no reagents that would specifically recognize rhinoceros IgG and could be used in ELISA to accurately analyze rhinoceros serum IgG level, we applied a quick and reliable semiquantitative test – the zinc-sulphate turbidity reaction – that has been described as a useful tool to detect FPT in horses (Sedlinska et al., 2005). This method precipitates IgG in a non-antigen specific way and the reaction can be

analyzed by photometric reading. To obtain a calibration curve for this assay, we prepared nine dilutions of horse and four dilutions of rhinoceros sera mixing them with fetal calf serum as the latter does not contain immunoglobulins, and measured their turbidity after the zinc-sulphate reaction. As it can be seen on Fig. 3, the test was reliable and the rhinoceros serum contained slightly higher IgG compared to the horse serum. As a conclusion this method is appropriate to analyze the newborn rhinoceros serum IgG level and to decide whether the newborn animal is affected by FPT or not.

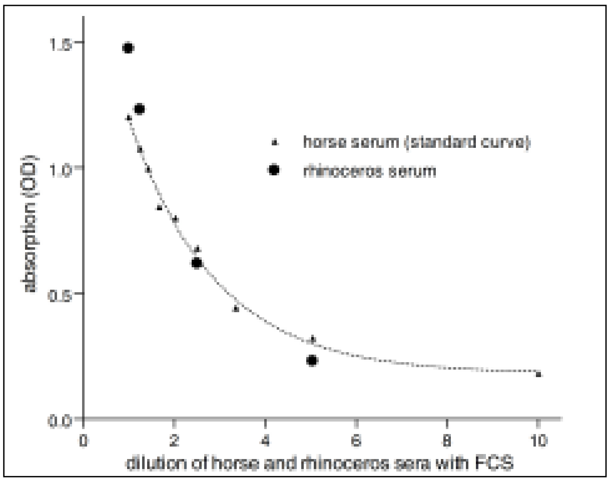

Fig.3:Photometric reading of zinc sulphate turbidity analyzing rhinoceros serum IgG content. As a control horse serum was used generating the standard curve.

Immunoglobulin preparation – in order to substitute the colostral immunoglobulins in the newborn rhinoceros identified as FPT, we prepared a pony immunoglobulin enriched preparation that can be used for intravenous application. As the donor ponies are kept at the Budapest Zoo, they live in the same environment as the rhinoceroses and thus have identical microbial environment. This ensures the presence of those antibodies which neutralize those pathogens that endanger the newborn rhinoceros. As the expected weight of the newborn rhinoceros is about 60 kilograms it would require around 46-115 grams IgG, in case of complete FPT.
This amount was estimated due to the fact that an equine foal is not considered FPT if the IgG level in the plasma is higher than 8-10 mg/ml (Erhard et al., 2001) and that the antibody in plasma may be expected to comprise ~20–50% of antibody in the body (Lobo et al., 2004). Suppose that the newborn rhinoceros has ~4.2 liters of blood (which is equal to ~2.4 liters of plasma), the blood IgG content is about 24 grams and hence the total IgG in the body is around 48-120 grams. This amount of IgG can be purified from ~ 2.4-6 liters of pony plasma supposed its IgG concentration is ~20 mg/ml. As this amount of plasma contains not only IgG but also large amount of oncotically active smaller molecules (e.g. albumin), the infusion of this amount of plasma in short time would generate an extraordinary high hydrostatic pressure in the vascular system and would be fatal for the newborn animal. Thus, we filtered the pony plasma through a 100 kDa filtering system to exclude these small but rather active molecules. After this step the liquid preparation become relatively thick and contained the original amount of IgG, based on the ZST test we performed. Nevertheless, it seemed an appropriate solution to substitute the maternal immune transport that would be mediated by the colostrum.

Conclusion

Despite our efforts our female white rhinoceros, Lulu was not able to give birth for a live calf. Nevertheless, we think that such an emergency plan is a useful method to prepare for unexpected events in zoo situations.

Acknowledgements

We thank for József Sipos (CEVA–PHYLAXIA) for his help with the serum preparation. This study was supported by National Research Foundation of Hungary (OTKA 049015).

References

1. Blakeslee T and Zuba J (2002): Rhinoceros. In Hand rearing wild and domestic mammals. L. J. Gage, ed. Iowa State Press, Ames. 236-243.
2. Erhard M, Luft C, Remler H and Stangassinger M (2001): Assessment of colostral transfer and systemic availability of immunoglobulin G in new-born foals using a newly developed enzyme-linked immunosorbent assay (ELISA) system. J Anim Physiol Anim Nutr (Berl) 85:164-173.
3. Hermes R, Göritz F, Walzer C, Sós E, Tomasova K, Molnár V, Mezősi L, Schwarzenberger F and Hildebrandt TB (2005): Assisted reproduction and artificial insemination in rhinoceroses. In Molnár V, Sós E (eds): Vadállatok szaporodásbiológiája, állatkerti tenyészprogramok 6.
4. Hermes R, Hildebrandt TB, Walzer C., Göritz F, Patton ML, Silinski S, Anderson MJ, Reid CE, Wibbelt G, Tomasova K and Schwarzenberger F (in press): The effect of long non-reproductive periods on the genital health in captive female white rhinoceroses (Ceratotherium simum simum, C.s. cottoni). Theriogenology, article in press.
5. Hildebrandt TB, Hermes R, Jewgenow K and Göritz F (2000): Ultrasonography as an important tool for the development and application of reproductive technologies in non-domestic species. Theriogenology 53(1):73-84.Lobo ED, Hansen RJ, Balthasar JP (2004): Antibody pharmacokinetics and pharmacodynamics. J Pharm Sci 93:2645-2668.
6. Holden C (2005 - ed.): Random samples - A rhino first. Science 306: 44.
7. Mcewan AD, Fisher EW, Selman IE and Penhale WJ (1970): A turbidity test for the estimation of immune globulin levels in neonatal calf serum. Clin Chim Acta 27:155-163.
8. Mcguire TC, Crawford TB, Hallowell AL and Macomber LE (1977): Failure of colostral immunoglobulin transfer as an explanation for most infections and deaths of neonatal foals. J Am Vet Med Assoc 170:1302-1304. Naylor JM (1979): Colostral immunity in the calf and the foal. Vet Clin North Am Large Anim Pract 1:331-361.
9. Nelson L. 1978. Rhinocerotidae. In Zoo and wild animal medicine. M. E. Fowler, ed. Saunders, Philadelphia. 763-768.
10. Sedlinska M, Krejci J and Viskocil M (2005): Evaluation of Field Methods for Determining Immunoglobulins in Sucking Foals. Acta Vet Brno 74:50-58.
11. Tizard I (2004): Immunity in the fetus and newborn animals. In Veterinary Immunology: an introduction, 6th ed. W.B. Saunders Company.
12. Tyler-Mcgowan CM, Hodgson JL and Hodgson DR (1997): Failure of passive transfer in foals: incidence and outcome on four studs in New South Wales. Aust Vet J 75:56-59.

drsos@zoobudapest.com