



Parturition in white rhinoceros

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ABSTRACT

In captive rhinoceros stillbirth and perinatal death are recorded at a rate of 6–17% in the various species. At the same time there is a substantial lack of knowledge on rhinoceros parturition. Yet, predicting parameters for birth and progress of parturition are fundamental for the recognition of dystocia and perinatal problems. Therefore, we here intended to pay close attention to the Achilles heel of the 1.5–2.5 year reproduction cycle in rhinoceros, the parturition. For the prediction of parturition we recorded timelines for pre-birth udder development, genital swelling, milk production, behavioral unrest, and decrease of serum progesterone concentration and the gestational length in 19 white rhinoceros. First, second and third labour stage, foetal presentation and events in perinatal period were recorded to describe normal parturition and establish a guideline for better birth management in rhinoceros. Udder development and genital swelling were observed 3 and 2 weeks prior birth, respectively. Milk production was observed to start up to 3 weeks prior birth and increased significantly in the last week with most significant increase one day before parturition to 50.6 ± 45.4 mL ($p < 0.006$). Serum progesterone concentration started to decrease 7 days prior parturition and more significantly 48 h before parturition. While behavioral unrest and first stage labour was not observed reliably in all females the break of foetal waters and thus the start of second stage labour was unmistakably observed. Second stage labour, when foetal membranes had ruptured until the foetus was born, took $1:50 \pm 0:20$ h:min. Eighty-four percent of fetuses were born in anterior presentation ($n = 16/19$) and the final expulsion took <25 min suggesting that this is the normal presentation in white rhinoceros. In the less frequent posterior presentation final expulsion took up to 47 min. Overall, 95% of calves were born alive. Calves were standing and nursing in $0:55 \pm 0:12$ min and $3:32 \pm 0:53$ h:min, respectively. In 10.5% of births ($n = 2/19$) in anterior presentation perinatal complications occurred. Stillbirth occurred once (5.3% $n = 1/19$) when the foetus was born in posterior presentation. The recorded gestational length was 506 ± 2 d. Delivering live offspring is of key importance to establish a new generation and secure long-term survival of a species. Various pre-birth changes, significant decrease of serum progesterone 48 h prior birth, different labour stages, foetal presentation and perinatal events described here add substantial knowledge on the understanding of normal rhinoceros parturition and may help diagnose dystocia and perinatal complications.

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1. Introduction

The investment of slow reproducing species like the rhinoceros into generating one offspring is tremendous. The reproduction cycle in rhinoceros spans over 4–6 weeks of oestrous cycle, 16 months gestation and 1–6 months lactation summing up to ≥ 1.5 –2.5 years, one of the longest reproduction cycle in terrestrial

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mammals [1,2]. Delivering a live and healthy calf is of key importance to ensure that the resources the female invested over 2 years are harvested and not lost 'last minute'. Birth and perinatal period represent the eye of the needle of the reproductive cycle to establish a new generation and to secure long term survival of the species.

Before parturition, many other factors have negative impact on reproduction and self-sustainability of rhinoceros populations in captivity and in the wild. Anoestrous, reproductive pathologies, lack of breeding or diseases are amongst the causes for a lack of reproduction or slow population growth in captivity [3–9]. When a female rhinoceros has managed to avoid all those reproduction obstacles: she has a normal oestrous, has no diseases of the genital tract preventing conception, and has established a stable pregnancy, dystocia and stillbirth remain a substantial risk even if the female and the foetus are healthy up until the beginning of parturition.

In captive rhinoceros breeding programs stillbirth and perinatal death is documented in the studbooks by records on the day of death occurring either at birth or during 1st week post-partum. In recent years, stillbirth and perinatal death in captive populations was 6% ($n = 3/50$; 2000–2015), 17% ($n = 39/226$; 2002–2019) and 17% ($n = 28/164$; 2000–2014) for black, white and greater one-horned rhinoceros, respectively [10–12]. Despite one out of six births in greater one-horned and white rhinoceros resulting in stillbirth or perinatal death there are only few anecdotal reports on dystocia and stillbirth [13,14]. Further, there are no records on infectious causes for stillbirth or perinatal death in captive rhinoceroses suggesting that prolonged parturition or non-infectious perinatal events might be reason for a perinatal death rate of 6–17% in captive rhinoceros. At the same time there are many obstacles to better manage parturition in captive rhinoceros. Firstly, data on gestational length in rhinoceros species ranges between 2 and 8 weeks in black, white and greater one-horned rhinoceros [13,15–17]. Secondly, as flight animals, rhinoceros parturition predominately occurs at night. Thus, 2–8 weeks range in gestational length and the onset of parturition occurring at 'off-duty' times, the birth of rhinoceros calves remains unnoticed in many facilities until the morning after, too late to react in case of dystocia or misdirected, harmful maternal instincts [13]. The long and tight uterine cervix in rhinoceros in combination with one or multiple factors in captivity such as advanced age at first birth, lack of physical fitness, maternal overweight, high foetal overdevelopment may cause dystocia, perinatal asphyxia and subsequent perinatal death. However, current knowledge on predicting parameters for the onset of parturition in white rhinoceros or rhinoceroses in general is limited to two cases reporting the approximate increase of estrogens 1 month prior birth, the decrease of fecal progestagens 17 days before birth and visible changes of the developing mammary gland 10 days prior birth [18,19]. Further, the lack of knowledge on the duration of different labour stages in rhinoceros makes it difficult to assess parturition progress, to diagnose dystocia in time or to take decisions on veterinary or husbandry interventions when parturition is stalled. Yet, detailed knowledge on labour stages and progress of parturition is critical to prevent prolonged perinatal asphyxia and ensure the survival of the calf and/or the female [13].

Similar to captive wildlife species, animal welfare and ethical concerns demand for a reduction of losses due to dystocia or neonatal death in domestic species. A reliable prediction of parturition to improve dam and offspring survival has become of increasing importance in horses and cattle [20]. In horses and cattle gestational age can be estimated based on fetal morphometry. A tool useful during early pregnancy, but non-practical in late gestation due to fetal size and not suitable for reliable prediction of parturition [20]. In rhinoceros, fetal morphometry has been

reported in single cases of different species [21–23]. Yet, maternal age, parity, individual and species variations of fetal growth and limited data limits this tool in rhinoceros to a rough gestational age estimate but does not provide accurate prediction of parturition. In cattle and horses, the observation of multiple clinical signs such as mammary gland development and secretion, relaxation of pelvic ligaments, behavioral changes, and decreased body temperature have proven as reliable for parturition prediction. Specifically, the concentration of electrolytes in the mares pre-colostrum, the increase in calcium and decrease of pH, predicts parturition with good accuracy [20]. Prediction of parturition based on hormones is very effective in cattle, where a decrease in progesterone concentration indicates calving within 24 h [24]. Yet in horses, the decrease of progestin prior birth is not suitable to predict parturition [25]. In the mare, individual increase of cortisol concentration before foaling predicts parturition better [20].

Given the stillbirth rates in captive populations and a substantial lack of knowledge on rhinoceros obstetrics we intended in this study to pay close attention to the 'physical' bottleneck and Achilles heel of the reproductive cycle, the parturition, to better build, maintain or restore captive populations. In a larger study group we wanted to establish reliable predictors for the onset and progress of parturition in rhinoceros. For this, we firstly wanted to record timelines for pre-birth udder development, genital swelling, milk production and behavioral unrest. In addition, decrease of serum progesterone concentration was thought to provide an estimate for imminent onset of parturition. Further we attempted to record different labour stages during parturition as a roadmap for a physiological birth and guideline for veterinary birth management in rhinoceros.

2. Methods

2.1. Ethics statement

This study was conducted on captive white rhinoceros (*Ceratotherium simum simum*) listed as near threatened by the IUCN. It was approved by IACUC animal ethics committee of the Leibniz Institute for Zoo and Wildlife Research (permit number: 2014-11-01) and carried out in accordance with the German National Protection of Animals Act (last revision 15th July 2009).

2.2. Animals

This study included 19 pregnant, mostly primiparous (58%, 11/19) Southern white rhinoceros housed at six European facilities. Elevated faecal or serum progesterone concentration >5 months after mating/insemination had indicated pregnancy [3,22]. Known dates of mating in 15 females and birth were used to retrospectively calculate the exact gestational length. The diet during pregnancy consisted of hay and browse supplemented with minerals and vitamins. Pellet concentrates of <0.5 kg were fed for training purposes only. For parturition females had access to two adjacent calving stalls that could be separated by a sliding door in case of perinatal intervention.

2.3. Animal management

The start of visible development of the udder and swelling of the perineal genital area were subjectively recorded in the weeks prior to parturition. In order to allow daily palpation of the udder and milking, the females were conditioned to stand in a chute or in parallel to the bars. Palpation of the udder was established as part of the female's daily routine in order to determine the onset and amount of pre-colostrum prior parturition. When pre-colostrum

secretion started, the samples were collected into sterile vials. The volume was recorded and the sample frozen at -20°C in case colostrum substitution would become necessary. Night vision cameras in the calving stall facilitated real time, remote monitoring of animal behaviour and the observation of the labour stages and perinatal events.

2.4. Progesterone monitoring

Six weeks prior to the estimated day of birth weekly blood samples were taken from the ear vein or plantar foot vein plexus (Fig. 1). When progesterone values declined $<30\text{ ng/ml}$ the frequency of blood sampling was increased to every other day. When the progesterone further declined $<20\text{ ng/ml}$ blood was taken daily until birth. The step-by-step increase of blood sampling frequency towards birth accounted for challenging circumstances to take daily blood from a white rhinoceros over longer periods of time in addition to behaviourally exceptional times in the last weeks of pregnancy. Serum samples were analyzed on site or frozen and couriered to a local, commercial laboratory for progesterone analysis using a previously validated equine progesterone assay profiles. This non-standardized method for hormone analysis was chosen to facilitate a quick turnover time of hormone results and to adapt to field conditions in logistically remote locations. Because of different laboratories used for analysis, we cannot provide specific hormone assay details.

2.5. Definition of labour stages

To describe and evaluate the progress of rhinoceros parturition several time intervals were recorded by night vision cameras to determine onset and progress of different labour stages. First stage labour was defined as the interval from the onset of constant unrest with frequent urination and defecation attempts and lack of lateral or sternal recumbency longer than 10 min until the rupture of foetal membranes. Second stage labour was defined as the interval from the rupture of foetal membranes and break of water until the calf was born on the ground. Third stage labour was defined as the interval from birth until the placenta had passed. Other data points recorded during parturition were foetal orientation and intervals from birth until the calf stood and nursed during the perinatal period.



Fig. 1. Daily blood sampling from the ear vein in a conscious female using half-closed sliding door between indoor stables as provisional chute.

2.6. Statistical analysis

Values are reported as mean \pm SEM. Where adequate the data was analyzed using paired *t*-test with a two-tail *p* value. Differences were considered significant when $P < 0.05$.

3. Results

3.1. Predictors of rhinoceros parturition

The gestational length in white rhinoceros calculated retrospectively was $506 \pm 2\text{d}$. Pre-birth udder development and genital swelling started about 3 and 1.8 weeks prior birth, respectively (Table 1). Towards birth, behavioral unrest was observed about 2 days prior birth (Table 1). Yet, behavioral unrest preceding the day of parturition was not observed in 32% ($n = 6$) of the females. First stage labour, when the long and complex rhinoceros cervix is dilated, was observed starting $6:11 \pm 0:51\text{ h:min}$ prior birth ($n = 14/19$; Table 1). Yet again, first stage labour was not observed in 26% ($n = 5$) of the females.

In those females in which the udder was accessible for manipulation ($n = 10$), small amounts of pre-colostrum were secreted starting as early as 3–2 weeks prior birth ($1.9 \pm 0.6\text{ mL}$; range 0.2–7 mL). One week prior parturition milk secretion increased significantly ($14.9 \pm 4.3\text{ mL}$; range: 1–75 mL; $p < 0.03$, Fig. 2) compared to the weeks before and again significant 24 h prior to birth ($50.6 \pm 16.0\text{ mL}$; range: 15–150 mL; $p < 0.006$).

Progesterone concentration was $30.3 \pm 3.5\text{ ng/mL}$ up until one week prior parturition before it started to decrease during last seven days of pregnancy (Fig. 3). On days 7–5 prior parturition progesterone concentration decreased to $21.3 \pm 1.6\text{ ng/mL}$. On days 4 and 3 before parturition mean progesterone concentration further decreased to 17.3 ± 2.3 and $13.2 \pm 1.2\text{ ng/mL}$, respectively (Fig. 3). After this steady but non-significant decline between days -7 and -3 progesterone concentration dropped significantly by 30%, 45% and 39% when compared with the value of the previous day to 9.3 ± 0.6 , 5.1 ± 0.5 and $3.1 \pm 0.7\text{ ng/mL}$ 48 h, 24 h and on the day of parturition, respectively ($p < 0.004$, $p < 0.0001$, $p < 0.04$; Fig. 3). The daily decline of the serum progesterone concentration allowed for prediction of parturition in the week preceding parturition and more accurately when the concentration dropped significantly 48 h prior birth.

3.2. Rhinoceros parturition

While behavioral unrest or first stage labour was not observed in all females, the rupture of foetal membranes represented a landmark which was observed unmistakably in all but one female $01:50 \pm 0:20\text{ h:min}$ prior birth (Table 1). The rupture of the foetal membranes and passage of foetal fluids indicated that first stage labour, either noticed or unnoticed, had dilated the long and complex cervix of the rhinoceros in the preceding hours (Fig. 4). The foetus had entered the cervix and the pelvic birth canal causing the foetal membranes to burst. When the foetal membranes ruptured clear water passed from the genital opening similar to urination. Yet, unlike during urination foetal waters were not actively excreted. They ran passively from the genital which resulted in wetting of the inner hind legs and feet. The wetting of the inner leg and feet was distinct from urination where urine is actively passed and does not stain the inner legs (Fig. 4). So even when the moment of rupture was not observed ($n = 1$), the fact that it had happened was noted.

During second stage labour the calf fully entered the pelvic birth canal and was finally pushed out by bearing-down pains. During this stage females had visible abdominal contractions, flattened

Table 1
Gestational length and timelines for pre-birth changes, labour stages and perinatal events in white rhinoceros.

Parameter	N	Mean	SEM	Min	Max	remark
Gestation length (d)	15	506	2	496	525	mating not observed n = 4
Udder swelling (weeks)	17	3,0	0,3	1	4	not noted n = 2
Genital swelling (weeks)	15	1,8	0,2	0,3	4,0	not observed n = 4
Behavioural unrest (days)	13	2,2	0,4	0,5	6,0	not observed n = 6
Parturition:						
1st stage labour (h:min)	14	06:11:21	00:51:37	01:11:00	11:11:00	not observed n = 5
2nd stage labour:						
Break of fetal membranes (h:min)						
combined	18	01:50:07	00:20:55	00:29:00	05:23:00	not observed n = 1
anterior presentation	15	01:57:00	00:23:54	00:29:00	05:23:00	not observed n = 1
posterior presentation	3	01:36:40	00:38:21	00:52:00	02:53:00	stillborn: n = 1
Foetal membranes visible prior birth (hr:min)						
combined	19	00:14:41	00:02:49	00:01:00	00:47:00	
anterior presentation	16	00:11:37	00:01:34	00:01:00	00:24:00	
posterior presentation	3	00:31:00	00:14:03	00:03:00	00:47:00	stillborn: n = 1
Total time of birth from onset of labour to birth (h:min)						
combined	14	07:38:34	01:04:26	00:29:00	12:28:00	not observed n = 5
anterior presentation	12	06:56:40	01:07:53	00:29:00	12:28:00	not observed n = 4
posterior presentation	2	11:50:00	00:03:00	11:47:00	11:53:00	not observed n = 1, stillborn: n = 1
3rd stage labour: placenta passed (hr:min)	17	03:02:28	00:44:14	00:01:00	04:35:00	not observed n = 2
Perinatal period:						
Calf standing after birth (hr:min)	17	00:55:18	00:12:53	00:15:00	01:15:00	not included stillborn, perinatal conditions n = 2
Calf suckling after birth (hr:min)	16	03:32:12	00:53:03	00:50:00	14:45:00	stillborn, perinatal conditions n = 3

ears, elevated respiratory rate >16 breaths/min, and prolonged horizontal or curled position of the tail. Second stage labour was further divided into two distinguished intervals. The first interval from the break of foetal waters until parts of the foetal membranes or the foetus became visible in the genital opening. The second interval, the final expulsion of the foetus, from the first appearance of foetal membranes until the calf was born on the ground (Fig. 4). This final expulsion was characterized by very strong labour activity, horizontal tail position, prolonged abdominal pressing in standing or recumbent position. Overall the final expulsion of the foetus, regardless of its presentation took on average 14 ± 2 min, with a range of 1–47 min (Table 1). In the 84% of births (n = 16/19)

the foetus was born in anterior presentation in <25 min (Fig. 4). In 56% of births in anterior presentation (n = 9/16) the final expulsion of the foetus took ≤ 10 min. Foetuses born in anterior presentation were all alive (n = 16/16). In posterior presentation (16%; n = 3/19) the final expulsion of a live foetus took as quick as 3 min or as long as 47 min. One stillborn recorded in this study was born in posterior presentation (n = 1/19) to a primiparous female, 43 min after foetal membranes had become visible. This stillborn did not enter the rigor mortis. Infectious causes for stillbirth were not determined upon post mortem examination.

During third stage labour the placenta was passed spontaneously ~3 h after birth (Table 1).

3.3. Perinatal period

Birth monitoring in this study resulted in 95% live offspring (n = 18/19). Healthy calves (84%; n = 16/19) stood on their feet 55 ± 12 min after birth (Table 1). Two calves with perinatal condition struggled with motoric difficulties and failed to stand up within this time period. Separation of the female and its offspring to mobilize and treat the calf for perinatal asphyxia and paresis was attempted in both incidences. In the first, the calf was separated within 90 min after recognition of motoric difficulties. Physical mobilization of the calf's legs, oxygen supplementation and glucocorticoids resolved the paresis of the front legs. As a result the calf stood within 30 min after the intervention, was released back to the female and started to nurse 13 h post-partum. In the second case, the calf was not separated after recognition of motoric difficulties. Anxiety of the female increased over 4 h after birth so that a separation of the newborn from the female could not be achieved in time to prevent perinatal death.

Calves nursed $3:32 \pm 0:53$ h:min (n = 16/19) after birth (Table 1). The time the calf required to nurse depended on 1) its own abilities to search for the udder and 2) on the females



Fig. 2. Milking of rhinoceros udder 3 ante partum produces a yellow-turbid, pre-birth secrete. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

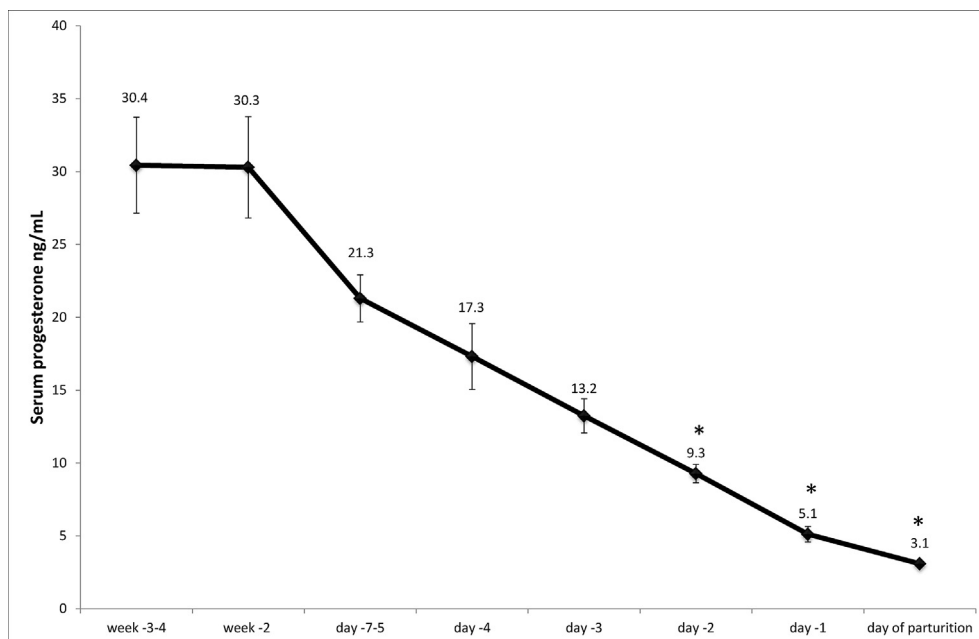


Fig. 3. Mean serum progesterone concentration before parturition in white rhinoceros. *values are significantly lower than those of the previous day.

experience and willingness to present her teats. The two longest periods from birth until the calf first suckled were 13:00 h:min and 14:45 h:min, respectively. Both records represented two behavioral extremes. In the first, it took the primiparous female 13 h to overcome her anxiety towards the newborn and to allow the calf to suckle. In the second, it took the newborn almost 15 h to find the teats of the experienced, relaxed pluriparous female. In comparison, one calf that was separated shortly after birth and treated for perinatal asphyxia nursed 12:45 h:min after birth within the maximum range recorded for healthy calves.

One calf was separated and bottle-fed after it had struggled with the primiparous female to access the udder for over 4 h. This primiparous female had shown anxiety and aggressive behavior towards the steadily walking newborn facing it head on, not letting it pass her head, and repeatedly lifting the calf high up towards the ceiling and sending it flying through the stall. The risk of injury to

the newborn seemed considerable so that this calf was separated from the female and bottle-fed. Despite this separation in the perinatal period, the calf was successfully re-socialized with the primiparous female in the outdoor enclosure 4 weeks after birth.

4. Discussion

This is the first, detailed report on parturition and perinatal period in a large group of white rhinoceros. Firstly, we describe timelines for a variety of pre-birth changes in rhinoceros that in sum allow a solid prediction of parturition. Secondly, we describe the chronology of events during parturition and the perinatal period. Thus the data allows for better concerted birth preparations, obstetrical care and shortens the period for staff to be on-call duty. Furthermore, our insight into the physiology of rhinoceros parturition provides a better basis to recognize dystocia and take knowledge-based, quick, clinical decisions for immediate interventions during parturition or the perinatal period.

While the estrous cycle length is precisely described by endocrine data for all rhinoceros species within a range of just a few days, reports on gestational length lack such precision ranging between 2 and 8 weeks [15–18]. This large range of gestational length in rhinoceros impeded so far a more narrow prediction of parturition and hampered efforts for intensive birth management specifically during off-duty hours. The gestational length of $506 \pm 2d$ determined in this study is one of multiple predictors which allows for a narrower estimate on the day of parturition in white rhinoceros.

Reported timelines for udder development, genital swelling, milk production and behavioral unrest prior birth provided further predictors for the onset of parturition in white rhinoceros. Yet these easy to obtain predictors were difficult to measure in absolute and comparable values. E.g. recorded volume of pre-colostrum production depended on factors such as daily accessibility of the udder, time spent to milk the sample or the parity of the female. Behavioral unrest, a parameter very subjective to the observer, was difficult to quantify across various staff and institutions. In some rhinoceros behavioral unrest was not observed at all in the days



Fig. 4. Foetal membranes have ruptured and foetal waters have run passively down from the vagina wetting the inner legs and feet. This is distinct from urination that is active and does not stain the inner legs. Foetal membranes are visible marking the full entry of the foetus into pelvis and its final expulsion. Upright orientation of the whitish toe nails are visible beneath the foetal membranes indicating anterior presentation of the foetus.

preceding parturition. In conclusion, pre-birth changes observed in the udder, outer genital or the behavior provided valuable, but estimates at best for the onset of parturition.

Different from these approximate predictors, serum progesterone concentration prior parturition represented a parameter with absolute values that was comparable between animals. The decrease of serum progesterone prior birth has extensively been reported in human and a diversity of domestic and wildlife species like the dog, cat, cattle, elephant or killer whale [20,26,27]. Similar to horses [26], serum progesterone in white rhinoceros started to decrease one week prior birth and more significantly 2 days before calving. Since the decline of serum progesterone concentration is independent of the female's age, parity and of visible or non-visible behavioral changes we conclude that this decline is an accurate predictor of parturition during the last week of pregnancy in white rhinoceros and more specifically 48 h prior birth. In elephants and killer whales a marked decrease in serum progesterone has also been used as predictor of parturition to impose a better birth and perinatal management [28,29]. In reverse, our results suggest that dystocia should be suspected in a female rhinoceros when serum progesterone concentration persists at < 5 ng/mL for > 24 h without progress towards second stage labour. In one female, progesterone concentration persisted at < 5 ng/mL for > 48 h, transrectal palpation and ultrasound in standing sedation provided information on fetal vital signs, permitted a temporary pain relief and fetal reposition. Eventually, the female gave birth to a live calf 24 h after sedation and 72 h after serum progesterone had dropped < 5 ng/mL. Yet, other parturition predicting parameters used in cattle and horses such as increasing Ca and decreasing pH in the precolostrum, increase in serum cortisol or increase of heart rate ≤ 3 h before allantochorion rupture [20] might be interesting to assess for their relevance in the prediction of rhinoceros parturition.

In this study different labour stages during normal parturition were first recorded in detail for a rhinoceros species. While first stage labour was not observed in all females, the start of second stage labour was clearly identified in all females by the passage of foetal waters. Eighty-four percent of foetuses in this study were born in anterior presentation within 25 min from when foetal membranes first became visible suggesting that this is the normal presentation in white rhinoceros. Birth complications in anterior presentation (10.5%) were observed only in the postnatal period. In the less frequent posterior presentation final expulsion of the foetus took up to 47 min. Five percent of births in posterior presentation resulted in stillbirth suggesting that posterior position possibly increases the risk of foetal asphyxia. In general, the reported timelines for the progress of labour stages provide a roadmap for physiological parturition in rhinoceros. Knowledge on different timelines for anterior and posterior presentation during the final expulsion is most valuable for the assessment of labour progress during the most critical period of birth and for the decision to intervene during dystocia and prevent foetal asphyxia.

Stillbirth and perinatal death was 10.5% ($n = 2/19$) in this study compared to 17% ($n = 39/226$) in the European captive white rhinoceros population 2002–2019 [12]. In other wildlife species, stillbirth is also reported at seemingly high rates. In captive great apes; chimpanzees, gorillas and orangutans, species kept under intensive husbandry and veterinary care, stillbirth rate is reported at 12% [30]. In elephants, stillbirth rate ranges from 3 to 4%, in semi-captive populations and 8% to 25%, in western captive facilities [31–33]. In comparison, in the horse, the intensively-cared, closest domestic relative to rhinoceros, dystocia occurs in just 4% of the births with abnormalities of foetal posture as the most common cause [34]. In the mare, a delay in foal delivery during the final stage of labour is associated with a significant increase in foal mortality. Foal morbidity and mortality is increased when important

timelines from birth to standing or birth to nursing are prolonged [34]. Similar to these findings in horses, prolonged times to stand up after birth in two newborn rhinos was successfully resolved only in one calf in which the condition was immediately attended. Similar to our study, in well-managed and intensively cared captive Asian elephant facilities stillbirth rate was improved from 26% to 7% [35]. How extensive knowledge on parturition, intensive monitoring, and advanced medical care tremendously reduces stillbirth is demonstrated in human obstetrics where foetal and perinatal mortality is reported at a rate $< 0.3\%$ [36].

In captive rhinoceros stillbirth and perinatal death is due to non-infectious causes. While in greater one-horned rhinoceros hereditary malformations have been reported [14], in black, white and in the majority of greater one-horned rhinoceros stillbirth seems to result from unnoticed dystocia and subsequent perinatal asphyxia [13]. Long deprivation of oxygen to a foetus during birth due to umbilical cord compression, damage, rupture or premature detachment of the placenta causes physical harm to the neonate's brain, poor responsiveness and muscle tone and may manifest in the inability to establish and sustain spontaneous respiration upon birth. In domestic animals, perinatal asphyxia, regardless of its etiology, is the main cause of new-born mortality of non-infectious origin. It accounts for 10–20% of mortality in piglet, 19.5% in foals, 17–30% in puppies, 2.5–8.6% in calves and 20% in lambs [37]. In human perinatal asphyxia occurs in 0.2–1% of newborn [38]. One stillborn and one perinatal death reported in this study account for 11% calf mortality due to perinatal asphyxia. Poor muscle tone and inability to stand up in a third neonatal rhino also suggested perinatal asphyxia. Here, immediate intervention resolved the leg paresis and ensured the calf's survival.

Dystocia has rarely been recognized in rhinoceros and its causes remain speculative [13,14]. In general, dystocia results from abnormalities of uterine contractions or maternal expulsive forces, the foetus viability, position, size, or presentation or the pelvis or soft tissues [39]. Clinically, dystocia covers the failure to progress or arrest of cervical dilatation, foetopelvic disproportion, prolonged active phase, secondary arrest of cervical dilatation, and foetal malposition ultimately resulting in perinatal asphyxia. Possible maternal causes of dystocia in rhinoceros are 1) physiologic uterine inertia due to exhaustion of uterine muscles, 2) psychological uterine inertia: as in the mare and the elephant which can cease labour efforts at will because of pain, stress or any other environmental disturbances [40], 3) hypocalcaemia stalling progress of second stage labour, 4) lack of physical fitness and excessive bodyweight. In cattle, it was shown that elevated food intake and less exercise in winter resulted in increased blood and nutrient flow to the uterus, gestation length, birth weight and the incidence of dystocia [41,42]. In human, obese women are at increased risk for delayed labour onset and slow labour progress that often results in unplanned caesarians [43]. Reason enough to assume over condition in captive rhinoceros as a risk for dystocia and to consider restricted diets during pregnancy as prophylaxis against over condition and foetal overdevelopment. While intra-uterine infectious diseases have not yet been reported in rhinoceroses 1) overdevelopment of the foetus, 2) its presentation during birth, 3) malposition or 4) foetal malformation [14] are factors to consider as foetal causes for dystocia in rhinoceros.

For those delivering obstetrical care, it might be useful to determine the circumstances under which stillbirth occurred to further improve on their birth management. Amongst many factors to consider it is important to diagnose when the foetus had died: in-utero or shortly before birth. When the foetus dies in-utero, it enters the rigor mortis shortly after. Due to the body's rigidity during the rigor mortis it cannot pass through the birth canal. Passage of the foetus becomes feasible again only after rigidity of

the muscles has resolved 48 h later. The stillborn in our study did not enter rigor mortis after birth, thus had died in-utero. A foetus that dies of asphyxia during second stage labour shortly before being born enters the rigor mortis after birth. Therefore, knowledge on the time of death is of clinical relevance. If foetal death in utero is diagnosed, birth management should be sustained for 48 h until spontaneous birth can occur again when the rigor mortis has resolved (unpublished data).

CRedit authorship contribution statement

Robert Hermes: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - review & editing, Visualization, Project administration. **Frank Göritz:** Supervision, Resources. **Miriam Wiesner:** Investigation, Methodology, Resources, Visualization, Writing - review & editing. **Nicole Richter:** Investigation, Methodology, Resources, Visualization, Writing - review & editing. **Baptiste Mulot:** Investigation, Methodology, Resources, Visualization, Writing - review & editing. **Vanessa Alerte:** Investigation, Methodology, Resources, Visualization, Writing - review & editing. **Sarah Smith:** Investigation, Methodology, Resources, Visualization, Writing - review & editing. **Tim Bouts:** Resources, Investigation. **Thomas B. Hildebrandt:** Supervision, Resources.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.theriogenology.2020.06.035>.

References

- Hermes R, Goeritz F, Streich WJ, Hildebrandt TB. Assisted reproduction in female rhinoceros and elephants – current status and future perspective. *Reprod Domest Anim* 2007;42:33–44.
- Hermes R, Hildebrandt TB. Rhinoceros theriogenology. In: Miller RE, Fowler ME, editors. *Fowler's zoo and wildlife medicine current therapy*, vol. 7. Elsevier Saunders; 2012. p. 546–61.
- Schwarzenberger F, Walzer C, Tomasova K, Vahala J, Meister J, Goodrowe KL, et al. Faecal progesterone metabolite analysis for non-invasive monitoring of reproductive function in the white rhinoceroses (*Ceratotherium simum*). *Anim Reprod Sci* 1998;53:173–90.
- Patton ML, Swaisgood RR, Czekala NM, White AM, Fetter GA, Montagne JP, et al. Reproductive cycle length and pregnancy in the southern white rhinoceroses (*Ceratotherium simum simum*) as determined by faecal progesterone analysis and observations of mating behavior. *Zoo Biol* 1999;18:111–27.
- Hermes R, Hildebrandt TB, Göritz F. Reproductive problems directly attributable to long-term captivity—asymmetric reproductive aging. *Anim Reprod Sci* 2004;82–83:49–60.
- Hermes R, Hildebrandt TB, Walzer C, Göritz F, Patton ML, Silinski S, Wibbelt G, Tomasova K, Schwarzenberger F. The effect of long non-reproductive periods in the genital health in captive female white rhinoceroses. *Theriogenology* 2006;65:1492–515.
- Hermes R, Göritz F, Saragusty J, Stoops MA, Hildebrandt TB. Reproductive tract tumours: the scourge of woman reproduction ails Indian rhinoceroses. *PLoS One* 2014;9(3):e92595.
- Edwards KL, Shultz S, Pilgrim M, Walker SL. Irregular ovarian activity, body condition and behavioural differences are associated with reproductive success in female eastern black rhinoceros (*Diceros bicornis michaeli*). *Gen Comp Endocrinol* 2015;214:186–94.
- Walker SL, Dunham AE, Pilgrim M, Okita-Ouma B. Low birth rates and reproductive skew limit the viability of Europe's captive eastern black rhinoceros, *Diceros bicornis michaeli*. *Biodivers Conserv* 2015;24:2831–52.
- Bindle R, Pilgrim M. Eastern black rhino EEP annual studbook report 2015. Chester, UK: Chester Zoo; 2015.
- Houwald F. Greater one-horned rhinoceros (*Rhinoceros unicornis*) international studbook 2014. Basel, Switzerland: Zoo Basel; 2014.
- Versteeg L. European studbook white rhinoceros. Safaripark Beekse Bergen. Netherlands: Beekse Bergen; 2019.
- Molnar V, Sos E, Mezösi L, Jakab CS, Rigo D, Garamvölgyi R, Petrási ZS, Bogner P, Hildebrandt T, Göritz F, Walzer C, Hermes R. A 'seemingly' normal Parturition and a stillbirth of a Southern white rhinoceros (*Ceratotherium simum simum*). Proceedings European association of zoo- and wildlife veterinarians (EAZWV) 6th scientific meeting, 24–28.5.2006, Budapest, Hungary. pp 81–84.
- Schaftenaar W, Fernandes T, Fritsch G, Frey R, Szentiks CA, Wegner RD, Hildebrandt TB, Hermes R. Dystocia and fetotomy associated with cerebral aplasia in a greater one-horned rhinoceros (*Rhinoceros unicornis*). *Reprod Domest Anim* 2011;46. <https://doi.org/10.1111/j.1439-0531.2010.01610.x>. e97–e10.
- Pilgrim M, Bindle R. EAZA best practice guidelines black rhinoceros (*Diceros bicornis*). Chester, UK: Chester Zoo; 2013. 2015.
- Versteeg L. EAZA Best Practice Guidelines for the white rhinoceros (*Ceratotherium simum*). Netherlands: Safaripark Beekse Bergen, Beekse Bergen; 2018.
- Houwald F. EAZA best practice guidelines greater one-horned rhinoceros (*Rhinoceros unicornis*). Basel, Switzerland: Basel Zoo; 2015.
- Bowers S, Gandy S, Paul K, Woods L, D'Angelo D, Horton C, Willard S. The relationship between vaginal electrical impedance and hormone profiles during pregnancy and parturition of a white rhinoceros (*Ceratotherium simum simum*). *J Zoo Wildl Med* 2005;36:451–6.
- Sos E, Molnar V, Hermes R, Schwarzenberger F, Mezösi L, Kacsokovic I, Hildebrandt T, Walzer C, Göritz F, Silinski S. European association of zoo- and wildlife veterinarians (EAZWV) 6th scientific meeting, 24–28.5.2006, Budapest, Hungary. pp75–80.
- Nagel C, Aurich J, Aurich C. Prediction of the onset of parturition in horses and cattle. *Theriogenology* 2020;150:308–12.
- Radcliffe RW, Eyres AL, Patton ML, Czekala NM, Emslie RH. Ultrasonographic characterization of ovarian events and fetal gestational parameters in two southern black rhinoceros (*Diceros bicornis minor*) and correlation to fecal progesterone. *Theriogenology* 2001;55:1033–49.
- Hildebrandt TB, Hermes R, Walzer C, Sós E, Molnar V, Mezösi L, Schnorrenberger A, Silinski S, Streich J, Schwarzenberger F, Göritz F. Artificial insemination in the anoestrous and the postpartum white rhinoceros using GnRH analogue to induce ovulation. *Theriogenology* 2007;67:1473–84.
- Hermes R, Göritz F, Saragusty J, Sós E, Molnar V, Reid CE, Schwarzenberger F, Hildebrandt TB. First successful artificial insemination with frozen-thawed semen in rhinoceros. *Theriogenology* 2009;71:393–9.
- Streyl D, Sauter-Louis C, Braunert A, Lange D, Weber F, Zerbe H. Establishment of a standard operating procedure for predicting the time of calving in cattle. *J Vet Sci* 2011;12:177e85.
- Holtan DW, Houghton E, Silver M, Fowden AL, Ousey J, Rossdale PD. Plasma progesterone in the mare, fetus and newborn foal. *J Reprod Fertil Suppl* 1991;44:517e28.
- Conley AJ. Review of the reproductive endocrinology of the pregnant and parturient mare. *Theriogenology* 2016;86:355–65.
- Michel E, Sporri M, Ohlerth S, Reichler IM. Prediction of parturition date in the bitch and queen. *Reprod Domest Anim* 2011;46:926–32.
- Robeck TR, Steinman KJ, O'Brien JK. Characterization and longitudinal monitoring of serum progesterone and androgens during normal pregnancy in the killer whale (*Orcinus orca*). *Gen Comp Endocrinol* 2016;236:83–97.
- Brown JL. Reproductive endocrine monitoring of elephants: an essential tool for assisting captive management. *Zoo Biol* 2000;19:347–67.
- Saiyed SC, Liubicich RC, Fidino M, Ross SR. Stillbirth rates across three ape species in accredited American zoos. *Am J Primatol* 2018;80:e22870. <https://doi.org/10.1002/ajp.22870>.
- Taylor VJ, Poole TB. Captive breeding and infant mortality in Asian elephants: a comparison between twenty western zoos and three eastern elephant centers. *Zoo Biol* 1998;17:311–32.
- Dale RHL. Birth statistics for African (*Loxodonta africana*) and Asian (*Elephas maximus*) elephants in human care: history and implications for elephant welfare. *Zoo Biol* 2010;29:87–103.
- Mar KU, Lahdenperä M, Lummaa V. Causes and correlates of calf mortality in captive Asian elephants (*Elephas maximus*). *PLoS One* 2012;7(3):e32335.
- McCue PM, Ferris RA. Parturition, dystocia and foal survival: a retrospective study of 1047 births. *Equine Vet J* 2012;44:22–5.
- Kiso W, Wiedner E, Isaza R, Lindsay W, Aria J, Jacobson G, Jacobson K, Schmitt D. Reproductive parameters and birth statistics for a herd of Asian elephants (*Elephas maximus*) in North America over a 20-year period. *J Zoo Wildl Med* 2017;48:987–96.
- MacDorman MF, Gregory ECW. Fetal and perinatal mortality: United States, 2013. *Natl Vital Stat Rep* 2015;64:1–23.
- Sanchez-Salcedo J, Bonilla-Jaime H, Gonzalez-Lozano M, Hernandez-Arteaga S, Greenwell-Bear V, Vega-Manriquez X, et al. Therapeutics of neonatal asphyxia in production animals: a review. *Vet Med* 2019;64:191–203.
- Truwit CL, Barkovich AJ. Brain damage from perinatal asphyxia: correlation of MR findings with gestational age. *Am J Neuroradiol* 1990;11:1087–96.
- ACOG. Dystocia and augmentation of labor. *ACOG Bull* 49. *Obstet Gynecol* 2003;102(6):1445–54.
- Hermes R, Saragusty J, Schaftenaar W, Göritz F, Schmitt DL, Hildebrandt TB. Obstetrics in elephants. *Theriogenology* 2008;70:131–44.

- [41] Colburn DJ, Deutscher GH, Nielsen MK, Adams DC. Effects of sire, dam traits, calf traits, and environment on dystocia and subsequent reproduction of two-year-old heifers. *J Anim Sci* 1997;75:1452–60.
- [42] Johanson JM, Berger PJ. Birth weight as a predictor of calving ease and perinatal mortality in Holstein cattle. *J Dairy Sci* 2003;86:3745–55.
- [43] Carlson NS, Hernandez TL, Hurt KJ. Parturition dysfunction in obesity: time to target the pathobiology. *Reprod Biol Endocrinol* 2015;13:135. <https://doi.org/10.1186/s12958-015-0129-6>.