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The effects of animal transfers on the reproductive success of female white rhinoceroses (*Ceratotherium simum simum*) kept in European zoos

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1 Introduction

As listed in the European and International White Rhinoceros Studbooks, southern white rhinoceroses are being kept in zoos in Europe for about 60 years. In the 1970s, there was a great wave of imports from South Africa. Within a decade, however, it became apparent that the captive rhinoceroses hardly breed (Ogden, 2011; van den Houten, 2013; Versteege, 2018a, 2015, 2012a, 2012b, 2009). The low breeding propensity of captive white rhinoceroses is in strong juxtaposition to the growing numbers in South Africa (Eltringham, 1990). For many years, more white rhinoceroses were imported from the wild than were living in zoos. The breeding programmes seemed to be doomed (Swaisgood et al., 2006). It is only recently that the captive population seems to stabilize (Versteege, 2018a). Although the southern white rhinoceros is now the most widely represented rhinoceros species in the world, numbering around 10,000, it is still considered near threatened and decreasing (Emslie, 2020). The animals remain under severe threat from poaching and the growing market for illegally traded rhinoceros horns. Without protection, the rhinoceros species would most likely slip into the IUCN's red list category "vulnerable" within the next five years, despite its current high numbers. Therefore, it is important to establish a self-sustaining captive population (Versteege, 2018a). The low rate of reproduction in the captive population created an urgency to identify the causes for the lack of breeding activity of captive southern white rhinoceroses. Males are not expected to be the primary cause of reproductive difficulties (Versteege, 2018a).

Knowledge about the reproductive physiology of female rhinoceros has been advanced primarily through three techniques. Hormone analyses provided the first information about the activity of the ovaries and corpus luteum and enabled numerous insightful studies on oestrus cycles and pregnancies (Berkeley et al., 1997; Kassam and Lasley, 1981; Schwarzenberger and Brown, 2013; Stoops et al., 2014). In parallel with the development of non-invasive faecal hormone analysis, techniques to examine the reproductive tract of rhinoceroses with transrectal ultrasound were developed (Adams et al., 1991; Hermes et al., 2006, 2009a; Schwarzenberger et al., 1996, 1997, 1998). The third important tool developed together by researchers, veterinarians and zoo staff are animal training methods that allowed blood drawing, ultrasound (transrectal or abdominal) or even artificial insemination (AI) with standing sedation (Hermes et al., 2009a, 2009b; Hildebrandt et al., 2007; Roth et al., 2018).

1.1 Hormone analysis

The gold standard for non-invasive fertility testing has long been the determination of faecal progesterone metabolites in faecal samples. Urine or saliva samples were rarely employed. Recently, blood samples have been used more and more frequently (Schwarzenberger and Brown, 2013).

These methods made it possible to clearly identify the cyclic status of southern white rhinoceroses (Brown et al., 2001; Patton et al., 1999; Schwarzenberger et al., 1998). Faecal steroid hormone metabolite analysis detects concentrations of cross-reactive 5α- and 5β-reduced pregnane metabolites containing a 20-oxo group. Faecal progesterone mirrors both luteal and placental function and significantly correlates to plasma progesterone with a delay of approximately 2 days (Schwarzenberger et al., 1997, 1996). Through its examination, corpus luteum activity, gestation or miscarriages, seasonality and hormonal therapies can be revealed or studied (Schwarzenberger et al., 1996). This made it possible to study and define the oestrus cycle of southern white rhinoceros in more detail.

Ovarian activity in the female white rhinoceros can be divided into four major groups based on their cycle length and 20-oxo-pregnane (20-oxo-P) levels in the luteal phase (Schwarzenberger et al., 1998):

1) oestrous regularity with cycles of 10 weeks and 20-oxo-P concentrations of >800 ng/g faeces.

2) oestrous cycles of 35 or 70 days duration and luteal phase 20-oxo-P concentrations between 250 and 750 ng/g faeces.

3) no obvious regularity but persistent corpus luteum activity with 20-oxo-P values between 100 - 200 ng/g faeces.

4) no obvious corpus luteum activity with 20-oxo-P values below 100 ng/g faeces.

Rhinoceroses in groups 1 and 2 ovulate on a regular basis, in contrast to two thirds of the animals in groups 3 and 4, who likely do not ovulate at all. Females in groups 3 and 4 may have cystic structures that either are or are not luteinised (Schwarzenberger et al., 1998). Presence of cystic ovarian structures were confirmed by rectal ultrasonography and it was shown that this condition leads to pathological changes and infertility (Hermes et al., 2006).

1.2 Ultrasound-diagnostics

Ultrasonographic assessment of ovarian activity has simplified breeding and artificial insemination methods (Adams et al., 1991; Hermes et al., 2009a, 2009b; Hildebrandt et al., 2007; Radcliffe et al., 1997; Roth et al., 2018). A problem known from hormonal monitoring in white rhinoceros females is acyclicity. Initially, it was thought that acyclic rhinoceroses did not show any follicular activity. However, ultrasound examinations have shown that new follicles are continually forming in the ovaries, but they do not reach the pre-ovulatory size. (Hermes et al., 2012). Nevertheless, ultrasound examination is not only important to determine the status of the oestrus cycle, but also to detect possible pathologies in the reproductive tract. It has been proven that white rhinoceroses that do not breed for a long time show progressive reproductive abnormalities after a while. The most common are cystic endometrial hyperplasia, leiomyomas of the cervix, uterus and ovaries, adenomas, para-ovular cysts and hydromucometra. The occurrence of such diseases is significantly lower in breeding animals. The development of genital tract diseases and ovarian dysfunction are age associated complications of prolonged non-reproductive periods but can be prevented by a minimum of one pregnancy. This infertile cycle, along with inactive ovaries, is thought to be a major cause for the low breeding success rate in captivity (Hermes et al., 2006). This is why any rhinoceros cow that does not breed for unknown reasons, should undergo a hormonal examination and an ultrasound scan to exclude reproductive pathologies (Versteege, 2018a).

1.3 Artificial reproductive techniques

Artificial reproduction techniques (ART) have gained importance to support successful ex situ conservation, population management and preservation of genomic diversity in captive southern white rhinoceroses (Reid et al., 2012). There are published AI protocols that have been used effectively in white rhinoceroses, but unlike the results of AI in elephants, with a very low success rate (Schwarzenberger and Brown, 2013). A protocol has been established for the induction of ovulation and this is used for the termination of AIs in white rhinoceroses (Hermes et al., 2012). In 2007, results of the first successful AI performed in the white rhinoceros with fresh sperm was published. Results of the first AI using frozen-thawed sperm was published in 2009 (Hildebrandt et al., 2007; Hermes et al., 2009b, 2009a) . The use of frozen semen facilitates the maintenance of genetic diversity, as animals living in distant zoos can be fertilized without transports. In addition, the animals' reproductive time can be prolonged past their biological time of life. (Hermes et al., 2009b).

1.4 Social environment

Wild female southern white rhinoceroses seldom roam by themselves. They usually live in groups of two, dam and calf (for up to three years), or two animals of the same age and sex. Herds as big as six animals are not uncommon either. Their territories intersect with multiple territories of males. These are normally solitary and only re-join females in heat. Males usually have territories of one to three km^2, while females roam 6-20 km^2, (Versteege, 2018b). Previous studies have found that it is feasible to breed rhinoceroses in great numbers in a game-ranched environment. Here it is possible for the animals to live out their normal social conduct. Under these circumstances, the breeding successes of zoos were surpassed just as much as those of rhinoceroses in the wild (Ververs et al., 2017).

1.5 Aims of this study

Since subadult females leave their mother in the wild, the EEP considers it important to separate them from the maternal herd in captivity as well. Measures recommended to increase breeding success are transferring males or females from one herd to another or isolating males temporarily from the female group for a couple of months. It is suspected, but not yet proven that such alterations in herd structure result in conceptions, however, not among all females, highlighting the importance of partner preference in the white southern rhinoceroses. It is too soon to say if the transfers performed stimulate the subadult females to breed on their own or avoid them turning into flat liners. But the present findings are promising (Schwarzenberger and Brown, 2013). The aim of this study was to use a combination of studbook and hormone data to determine what effects such transfers actually have on the rhinoceroses.

2 Material and methods

To determine whether transferring white rhinoceroses (male or female) to different zoos can improve breeding outcomes in captivity, we conducted a retrospective study using the European White Rhinoceros Studbook 2009, 2011, 2014, 2017 (Versteege, 2018a, 2015, 2012b, 2009), the International White Rhinoceros Studbook 12th (Ogden, 2011) and 13th (van den Houten, 2013) edition. Furthermore, we analysed the hormone data collected by Dr. Franz Schwarzenberger during reproductive monitoring of captive white rhinoceros over a period of 30 years (1991 – 2021). Hormone analysis was conducted at a laboratory of the University of Veterinary Medicine, Vienna, using established methods (Schwarzenberger et al., 1998, 1996). The data was updated with information from the websites “Rhinos of the World” (https://worldrhino.com/white-rhinos-european-zoo/) and “Rhinos in Europe” (https://rhinos-in-europe.net).

The European and International Studbooks contain tabular listings of white rhinoceros participating in international, and specifically important for this study, in the European EAZA Ex-situ breeding program (EEP) (Figure 1).

Stud#	Sex	Birth Date	Sire	Dam	Location	Date	LocalID	Event	Name	Transponder
COPENHAGE - Copenhagen Zoo, Frederiksberg, Region Hovedstaden, Denmark										
1361	M	~ Jun 1996	WILD	WILD	S.AFRICA	~ Jun 1996	NONE	Capture	OSCAR	0001C542D37
					BANDHOLM	24 Oct 2000	CESI01	Transfer		
					COPENHAGE	21 Jun 2012	CER004	Transfer		
1542	F	~Jul 1996ñ6m	WILD	WILD	S.AFRICA	~ 2004	NONE	Capture	MINNA	0006691523
					COPENHAGE	12 Sep 2006	CER002	Transfer		
2067	F	16 Nov 2011	1233	1338	BORAS	16 Nov 2011	EN0009	Birth	Zuri	968000010081367
					COPENHAGE	14 Oct 2014	CER006	Transfer		
T80	M	16 Oct 2017	1361	1542	COPENHAGE	16 Oct 2017	CER008	Birth		
Totals: 2.2.0 (4)										

Figure 1: extract of the European White Rhino Studbook 2017 (Versteege 2018a) with Copenhagen Zoo as an example

To get a better representation of the reproductive impact that the transfer of white rhinoceroses could have, we created two tables based on the tabular listings in the studbooks: one for female (Table 1) and one for male (Table 2) white rhinoceroses. We extended studbook listings by adding the generation, number of offspring and stud number of offspring. Since male rhinoceroses earliest begin breeding at the age of three, and it usually takes 2 months to introduce a new bull to the female group in a zoo (Versteege 2018a), we highlighted the zoos of residence where the male was older than three years and the stay was longer than two months. To exclude males that live in bachelor groups without female contact, such transfers were not taken into consideration.

Table 1: extract of table containing female white rhinoceroses participating in the EEP with example Copenhagen Zoo

Stud#	Name	Zoo	Birth	Location of Birth	f	Sire	Dam	Death	#Offspring	Offspring
140	MINNA	COPENHAGE - Copenhagen Zoo, Frederiksberg, Region Hovedstaden, Denmark	1965	WILD	0	WILD	WILD	22.04.2006	4	231, 382, 572, 1127
141	MAXA	COPENHAGE - Copenhagen Zoo, Frederiksberg, Region Hovedstaden, Denmark	1965	WILD	0	WILD	WILD	04.01.1993	3	232, 571, 1126
1542	MINNA	COPENHAGE - Copenhagen Zoo, Frederiksberg, Region Hovedstaden, Denmark	01.07.1996	WILD - S. AFRICA	0	WILD	WILD	-	3	1543 (S.Africa), T25, 2255, 2861
2067	ZURI	COPENHAGE - Copenhagen Zoo, Frederiksberg, Region Hovedstaden, Denmark	16.11.2011	CAPTIVITY - BORAS	3	1233	1338	-	0	-

f = generation

f0 = wild born

f1 = born in captivity, wild born parents

f2 = born in captivity, one wild born parent, one parent born in captivity

f3 = born in captivity, both parents born in captivity

#Offspring = number of offspring

Table 2: extract of table containing male white rhinoceroses participating in the EEP with example Copenhagen Zoo

Stud#	Name	Zoo	Birth	Location of Birth	Sire	Dam	Death	Transfers	Stays
93	FERDINAND	COPENHAGE - Copenhagen Zoo, Frederiksberg, Region Hovedstaden, Denmark	1962	WILD	WILD	WILD	27.08.2008	4	UMFOLOZI, **WHISPNADE** (05.08.1970 - 18.05.1972), **SOEST** (18.05.1972 - 23.06.1972), **COPENHAGE** (23.06.1972 - 27.08.2008)
1361	OSCAR	COPENHAGE - Copenhagen Zoo, Frederiksberg, Region Hovedstaden, Denmark	06.1996	WILD - S. AFRICA	WILD	WILD	29.08.2019	2	S. AFRICA, **BANDHOLM** (24.10.2000 - 21.06.2012), **COPENHAGE** (21.06.2012 - 29.08.2019)
2861	MOLOTO	COPENHAGE - Copenhagen Zoo, Frederiksberg, Region Hovedstaden, Denmark	15.04.2021	CAPTIVITY - COPENHAGE	1361	1542	-	0	COPENHAGE

Using Table 1 and 2, we determined the periods during which certain males and females were in the same zoo at the same time and thus had the possibility to interact (Figure 2). Rhinoceroses from the ZSL Whipsnade Zoo and the Serengeti Park Hodenhagen were excluded from analysis because both zoos had good breeding results without animals being transferred to stimulate breeding, and from these two zoos, only very few hormone data were available.

Copenhagen Zoo

- 139: from 31.08.1971 to 25.06.1972
- 93: from 23.06.1972 to 27.08.2008
- 1540: from 12.09.2006 to 12.06.2012
- 1361: from 21.06.2012 to 29.08.2019

Figure 2: extract of male zoo timeline with example Copenhagen Zoo

In order to select the female rhinoceroses that met the criteria of our study, individual „résumés“ were created (Figure 3), using Table 1, Figure 2, the studbooks, as well as the previously mentioned internet sites as data sources. The criteria for our study were that a female that had not previously bred or had not bred in a long time either had been relocated to a new zoo or was exposed to a new male. Hormone data were available from 1991 onwards, and therefore we analysed data from the last 30 years. We limited the age range to four to 25 years, as the youngest dam at first reproduction was four years old and the oldest dam at first reproduction was 28 years old, followed by the second oldest dam at 25 years (Ogden, 2011). The females not meeting our criteria were sorted out.

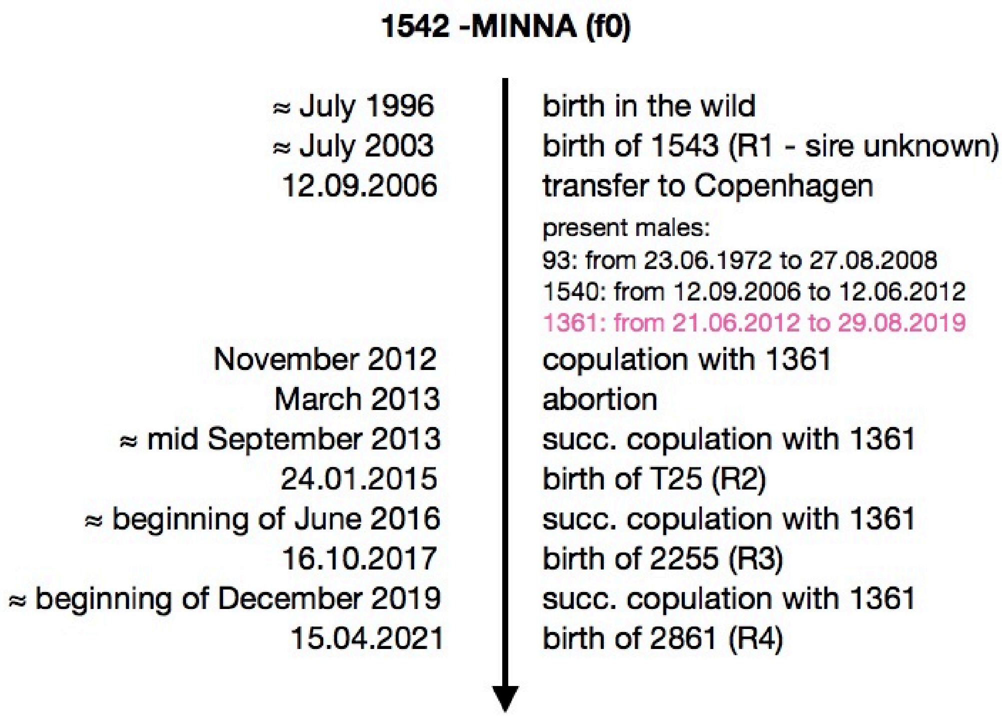

Figure 3: résumé of a female white rhinoceros with example Minna (#1542)

Further examination of these résumés consisted of determining whether a transfer (male or female) resulted in pregnancy and, if so, how many days elapsed between the transfer and the birth of a new calf. The findings were summarised in a new table (Table 3).

Table 3: extract of the table summarizing effects trough transfer (male or female)

Stud#	positive effect through female transfer	positive effect through new male	# of days between transfer/new male & birth (days)	f
361	no	-	-	0
362	no	-	-	0
458	-	no	-	0
504	no	-	-	1
643	no	no	-	1
649	no	-	-	1
651	no	no	-	1
652	-	no	-	1
653	-	no	-	1
767	-	yes	1124	1
773	no	-	-	1
812	no	no	-	1
835	-	no	-	1
856	-	yes	660	1
859	no	no	-	1
868	-	no		2
907	no	no	-	1
931	yes	-	805	1
949	-	no	-	1

Positive effect = pregnancy

f = generation

f0 = wild born

f1 = born in captivity, wild born parents

f2 = born in captivity, one wild born parent, one parent born in captivity

f3 = born in captivity, both parents born in captivity

Using Table 3, the percentages of pregnancies resulting from female or male transfers were calculated. This was repeated separately for generations f0 and f1-3 to determine if there were differences in breeding success between wild-born and captive-born white rhinoceroses.
In addition, bar charts were created showing the number of pregnancies in relation to the length of time until they occurred after the respective transfers.
The studbook and transfer data were then compared with the hormone diagrams (Figure 7 - 12). Analysis and evaluation were carried out as described in the publication “Faecal progesterone metabolite analysis for non-invasive monitoring of reproductive function in the white rhinoceros Ceratotherium simum” (Schwarzenberger et al. 1998). If a previously anoestrus or cyclically irregular female rhinoceros had an oestrus cycle at the latest three years after transfer or the addition of a new bull, this was considered positive. The results were summarized in a table similar to Table 3 and also presented in a bar chart.

3 Results

In total, we analysed n = 740 (344 males and 396 females) rhinoceroses. Of these, 85 females met our criteria. Some of these 85 individuals were part of transfer events (female or male) more than once, which is why the total number of transfers comes to 105 (65 male and 40 female transfers). A successful transfer is one that resulted in the birth of a calf, an unsuccessful transfer is one that did not. Thus, 26.2% of the male transfers (n = 17 out 65) and 30.0% of the female transfers (n = 12 out of 40) resulted in births.

Table 4: evaluation of the effects of transfers on breeding resulting in the birth of a calf

	successful	unsuccessful	total
male transfers (n = 65)	17	48	65
percentage (%)	26,2	73,8	100.0
female transfers (n = 40)	12	28	40
percentage (%)	30.0	70.0	100.0

Table 5: male transfers resulting in the birth of a calf

Stud Number (Name)	Zoo	Arrival of new male	Birthdate of calf	Number of days between arrival of male and birth of calf
230 (Freya)	Arnhem	532 (Dale): on the 20.10.1998	19.03.2002	1246
767 (Noelle)	La Palmyr	796 (Christian): on the 19.02.2008	19.03.2011	1124
856 (Sula)	Marwell	828 (Hannu): on the 18.04.1997	08.02.1999	660
1307 (Satara)	Beauval	1048 (Smoske): on the 07.06.2006	17.01.2008	589
1338 (Zinzi)	Boras	1233 (Bhasela): on the 11.05.2007	16.11.2011	1650
1339 (Merula)	Boras	1233 (Bhasela): on the 11.05.2007	27.01.2009	627
1457 (Emily)	Colchester	1360 (Otto): on the 27.11.2009	13.04.2013	1233
1542* (Minna)	Copenhagen	1361(Oscar): on the 21.06.2012	24.01.2015	947
1556 (Sakile)	Nyíregyháza	1540 (Curt): on the 12.06.2012	12.10.2016	1583
1625 (Chris)	Augsburg	1526 (Bantu): on the 29.04.2014	18.02.2016	660

1626 (Kibibi)	Augsburg	1526 (Bantu): on the 29.04.2014	06.02.2016	648
	Augsburg	1526 (Bantu): on the 22.02.2017**	07.10.2021	1689
1627 (Jasira)	Dortmund	1581 (Amari): on the 25.06.2011	23.09.2014	1186
1659 (Shakina)	Dortmund	1581 (Amari): on the 25.06.2011	21.04.2014	1031
2073 (Yoruba)	Coulange	2075 (Benny): on the 02.02.2012	02.07.2016	1612
2074 (Hekaw)	Coulange	2075 (Benny): on the 02.02.2012	01.12.2014	1033
2101 (Madiba)	Cambron	1424 (Joby): on the 15.03.2017	25.11.2019	985

* Within 5 months of the arrival of the male Oscar (#1361), female Minna (#1542) was mated twice, and conception occurred 143 days after arrival of the bull. However, the resulting pregnancy ended in abortion after only 4.5 months (Figure 8). The successful mating and pregnancy that took place after this abortion (Figure 9) then resulted in the 947 days between arrival of the bull and the successful birth listed in this table.

** Bantu (#1526) was transferred to Cambron on the 18.11.2015 and came back to Augsburg on the 22.02.2017 after being gone for over a year. This is why this transfer qualifies as a new male transfer.

The number of days between the arrival of a new bull and a resulting birth are shown in (Fig. 4). Almost a third of the births (n= 5 out of 17) occurred at 500 - 700 days after the transfer. This means that copulation took place shortly after the transfer (gestation period = 500 days). The remaining births took place between 900 and 1300 or 1500 and 1700 days, i.e., successful copulation took place one to just over three years after the arrival of the new male.

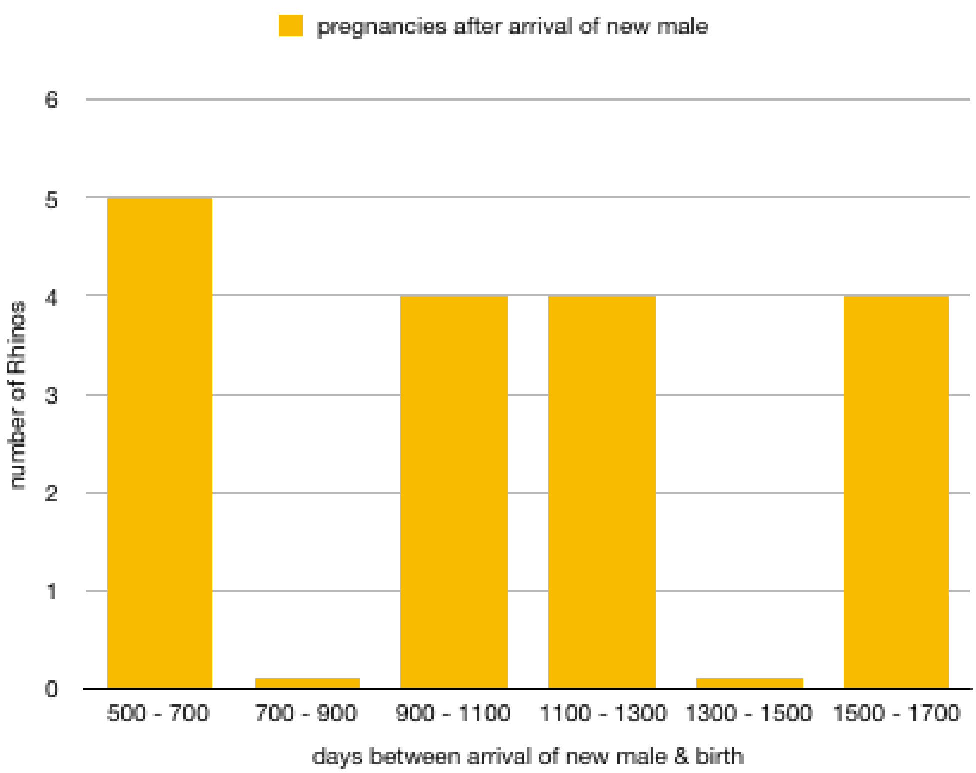

Figure 4: time frame of births after the arrival of a new male

Table 6: female transfers resulting in the birth of a calf

Stud Number (Name)	Transfer	Birthdate of calf	Number of days between transfer and birth of calf
931 (Diuna/Dyini)	04.09.1997: from Pretoria to Poznan	18.11.1999	805
1047 (Makoubu)	24.04.2009: from Whipsnade to Hilvarenbeek	02.04.2011	708
1083 (Tandamanse)	02.09.2003: from Jerusalem to Ramat Gan	23.09.2007	1482
1425 (Beth)	12.05.2016: from Kessingland to Lisieux	03.12.2018	935
1444 (Manzi)	01.07.2014: from Coulange to So Lakes	25.12.2016	908
	05.2018: from So Lakes to Lisieux	01.06.2021	1130
1460 (Tala)	04.07.2014: from So Lakes to Coulange	05.03.2017	975
1463 (Mafunyane)	13.03.2013: from Montpellier to Beauval	18.11.2016	1346
1474 (Jane)	05.10.2011: from Blairdrummond to Munster	23.05.2013	596
1480 (Izala)	05.11.2013: from Kolmarden to Arnhem	25.01.2016	811

1501 (Zola)	31.03.2011: from Givskud to Cabarceno	26.09.2013	910
1578 (Hildegard/Marcita)	12.12.2016: from Osnabrück to Erfurt	29.12.2018	747

After successful female transfers (n=12), 66.7% of them (n = 8) gave birth after 700 - 1100 days. Copulation in these cases took place almost one to two years after transfer.

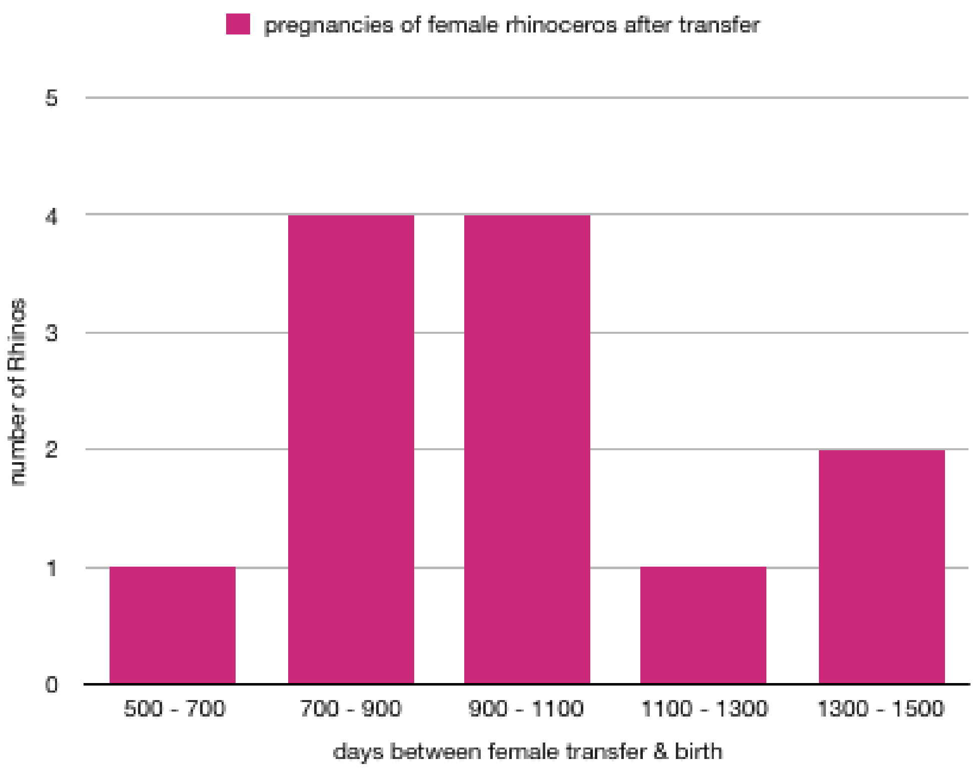

Figure 5: time frame of births after female transfer

In comparison, male transfers seem to succeed earlier than female transfers (see Fig. 6). However, female transfers had an overall higher success rate than male transfers (see Tab. 4).

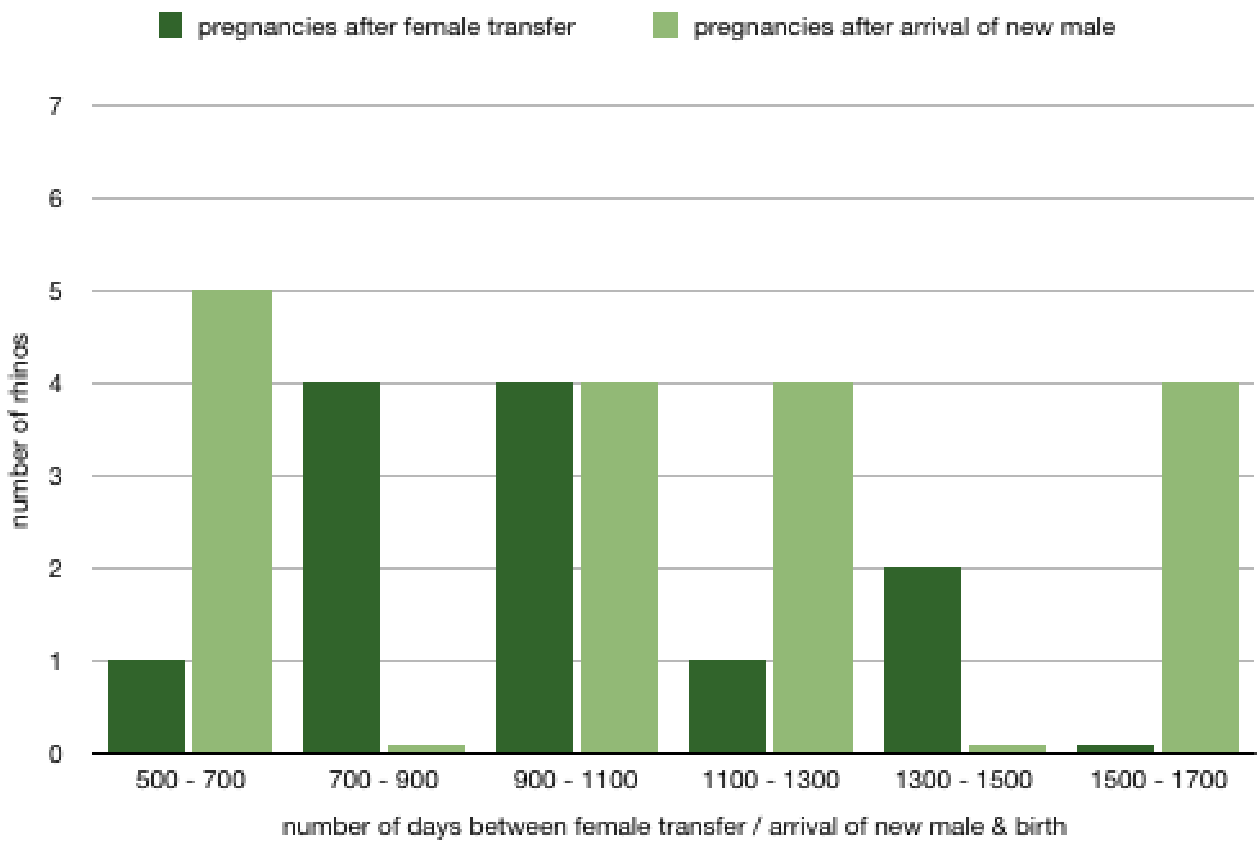

Figure 6: comparison of time frames until pregnancies between female and male transfers

We also calculated whether the different generations (wild born/captive born) have an impact on the effects of transfers (Tab. 5 and 6). For female transfers, 28.6% of wild born females (n = 6 out of 21) were successful, compared to 31.6% of captive born females (n = 6 out of 19). The transfer of a new male to a zoo with wild-born females resulted in a 28.9% success rate (n = 11 out of 38) and 22.2% in captive-born females (n = 6 out of 27).

Table 7: generational success in female transfers

	successful	unsuccessful	total
f0 (n = 21)	6	15	21
percentage (%)	28.6	71.4	100
f1-3 (n = 19)	6	13	19
percentage (%)	31.6	68.4	100

Table 8: generational success in male transfers

	successful	unsuccessful	total
f0 (n = 38)	11	27	38
percentage (%)	28.9	71.1	100
f1-3 (n = 27)	6	21	27
percentage (%)	22.2	77.8	100

Lastly, the hormonal effects were considered (Tab. 7). Successful means that a female rhinoceros developed at least one cycle after a transfer or the arrival of a new bull. Results from females Minna (#1542) and Karen (#1543) serve as an example for the hormonal analysis done in this study. They were at Copenhagen Zoo where a new bull, Oscar (#1361), arrived on the 21.06.2012. Within two weeks of the arrival Minna showed signs of oestrus. The first reported mating took place mid-September, the second one in November. She had an abortion end of March 2013 and gave birth to a healthy calf on the in January 2015, 947 days after Oscars arrival.

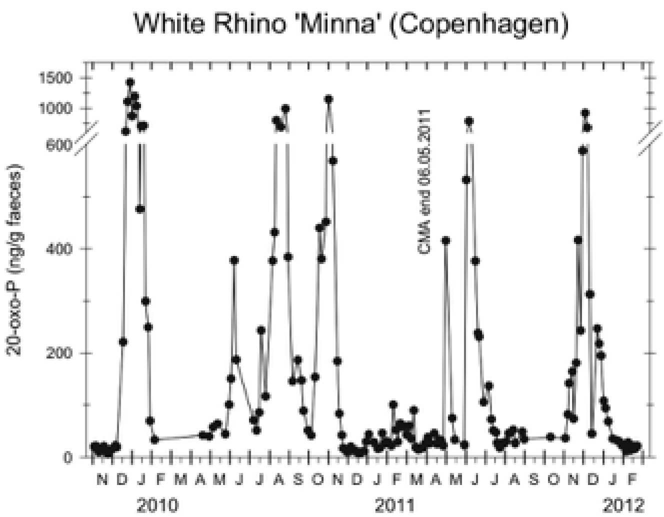

Figure 7: Hormone profile of Minna 2010 – 2012

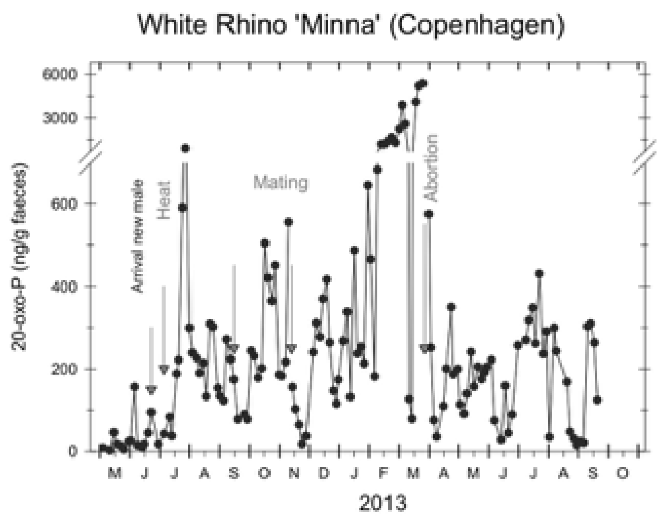

Figure 8: Hormone profile of Minna 2013

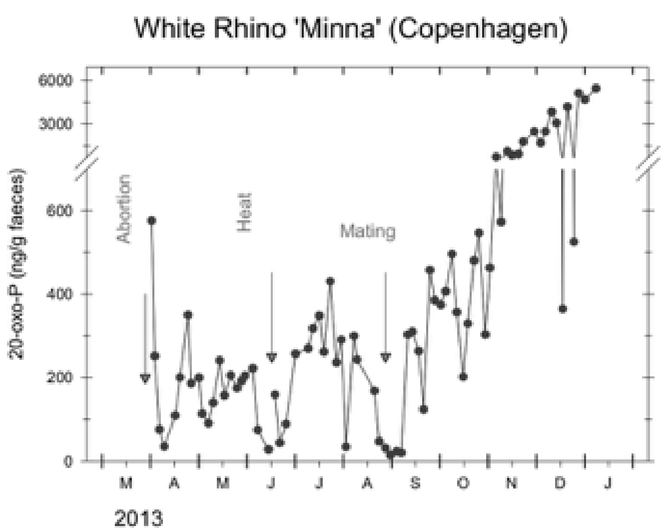

Figure 9: Hormone profile of Minna 2013

Karen was acyclic before Oscar's arrival, but already had a luteal phase shortly before the arrival of the new bull. In the following two years Karen was mated several times, however none of the matings resulted in a pregnancy.

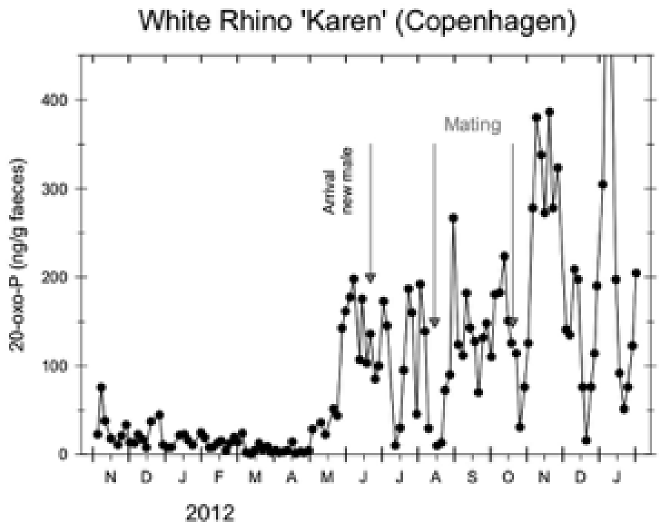

Figure 10: Hormone profile of Karen 2012

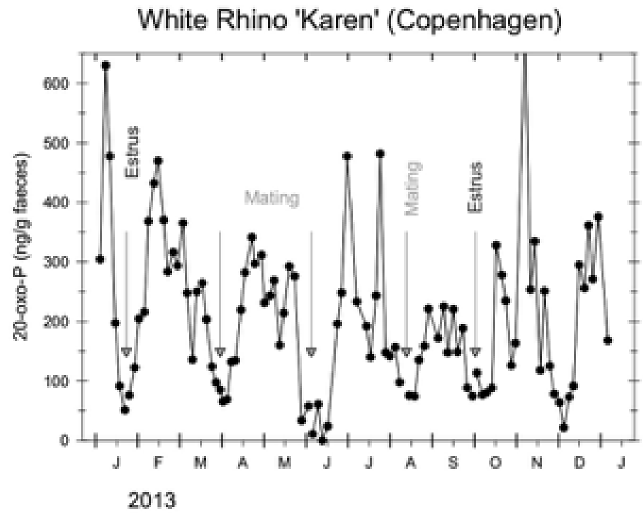

Figure 11: Hormone profile of Karen 2013

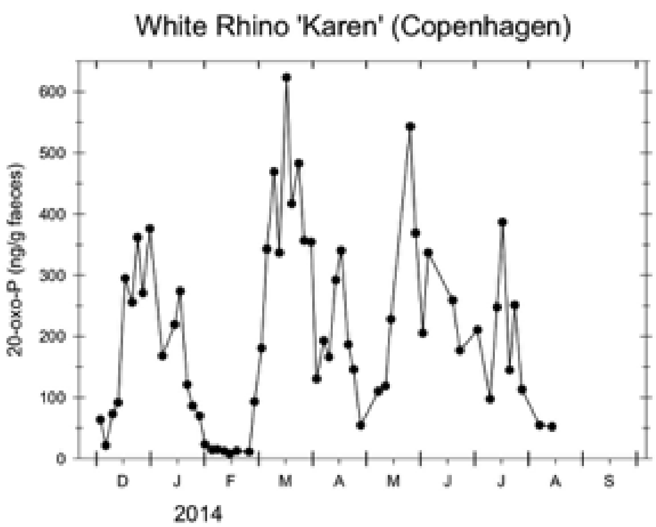

Figure 12: Hormone profile of Karen 2014

Table 9: hormone data analysis (extract)

Stud Number (Name)	positive effect through female transfer	positive effect through new male
…		
1410 (Lucy)	no	-
1444 (Manzi)	yes	yes
1457 (Emily)	-	yes
1460 (Tala)	yes	-
1474 (Jane)	yes	-
1501 (Zola)	yes	-
1542 (Minna)	-	yes
1543 (Karen)	-	yes
1556 (Sakile)	-	yes
1575 (Tamu)	-	yes
1576 (Cera)	-	yes
1578 (Hildegard)	yes	-
1596 (Kara)	no	-
…		

The figures of the hormonal analysis are clearly more positive than those of the breeding successes. 84% of the females with available data had hormonal responses to male transfers (n = 21 out of 25) even if these did not result in pregnancies. For female transfers it was 50% (n = 8 out of 16).

Table 10: hormonal successes through transfers

	successful	unsuccessful	total
male transfers (n=25)	21	4	25
percentage (%)	84.0	16.0	100.0
female transfers (n=16)	8	8	16
percentage (%)	50.0	50.0	100.0

4 Discussion

This study generated accurate figures on the effects of transfers and creates a working ground for future research. It is vital to have properly organised studbook data for the ongoing management of captive populations (Reid et al., 2012). Results of this study show that transfers lead to births in 26.2% (male transfers) and 30.0% (female transfers) of cases. Unfortunately, these figures are lower than hoped for. The success rate of female rhinoceros transfers was slightly lower for founder animals (28.6%) than for captive-born offspring (31.6%). For male transfers, the founder animals had a 6.7% higher success rate than captive-born females. However, because of the low number of successful transfers, it is not possible to make a final statement whether there is a significant difference between captive born and wild born rhinoceroses and vice versa.
In 2003 Schwarzenberger et al., suggested several approaches to overcome the reproductive problems in the captive white rhinoceros population. Hormone monitoring, transfer of animals into new breeding situations, clinical examinations of reproductive soundness and the development of assisted reproductive techniques were the cornerstones of these recommendations. In order to promote breeding, much hope was given to the transfer of animals between zoos. Now, about 20 years later, we see that only about 25-30% of the transferred animals have actually given birth to offspring in the new zoos. This is not the expected result, but it is at least a possibility to stimulate breeding. In addition, animal transfers are an important factor in the management of genetic variability (Versteege, 2018a). Possibly, in order to resemble more closely the situation in the wild (Eltringham, 1990), a further transfer should be considered in case of lack of success, so that animals are transferred two, or even three times. As the past has shown, a wait-and-see position without transfers does not lead to success and may even promote the development of reproductive tract pathologies (Hermes et al., 2006).
Two thirds of parturitions following female transfers (n=12) occurred after 700 - 1100 days. This means, conceptions did not occur until one to two years after transfer. Two animals gave birth after 1300 - 1500 days, i.e., the successful copulation was two to three years after transfer. In male transfers, almost one third of births occurred as early as 500-700 days after transfer, thus conception occurred shortly after arrival in the new facility. About half of parturitions (47.1%) occurred 900-1300 days after transfer, indicating that successful copulations took place one to two-and-a-half years after arrival. In 23.5% of the cases the births took place after 1500-1700

days, the conceptions consequently almost three to three-and-a-half years after transfer. These numbers show it is worth to have some patience after transfers.

The positive hormonal effects are far more prominent with 84% for male and 50% for female transfers. Nevertheless, the success rates of transfers are rather modest. It is unclear why pregnancy does not occur in all of these cases. Further research is needed on the acyclicity and unwillingness of white rhinoceroses to breed. In addition, the effects of new bulls seem to be very specific to the individual. For instance, the transfer of the male "Lekuruh" (#1574) to the "Parc Zoologique de La Barben" stopped the cycle of the residing female rhinoceros "Bela" (#1684), which subsequently became acyclic. Thus, negative impacts in the context of transfers seem possible, although they are rarer than the positive impacts or no impacts at all.

The transfer of southern white rhinoceroses between different zoos is not sufficient to solve the reproductive difficulties of rhinoceroses in captivity. An additional tool is iatrogenic oestrus induction. The synthetic progestin chlormadinone acetates in combination with hCG or deslorelin has been very useful for inducing oestrus in flatliners or females with a persistent corpus luteum, and in using induced ovulation for artificial insemination (Hermes et al., 2012).

This study cannot give an answer to why females do not breed after transfers even if there was a positive hormonal effect, as it is limited to studbook and hormone data only. Effects of e.g., herd management, social environment or enclosure size could not be taken into consideration. Living out species-typical social and reproductive patterns is one of the biggest hurdles and is difficult to ensure in zoos (Swaisgood et al., 2006). Rhinoceroses may show spatial distress and hierarchical repression of conception when there is not enough space (Metrione et al., 2007). Another obstacle in captive rhinoceros management is feeding, which is suspected to affect fertility. Supplementary feeding, for example increased the conception rates in game ranched rhinoceroses in South Africa (Ververs et al., 2017). Whereas phytoestrogens, present e.g., in clover hay or soy and alfalfa-based pellets, may have negative effects on the reproductive health of white rhinoceroses, because they can engage and activate oestrogen receptors. Standard feeding protocols in North American zoos may subject rhinoceroses to considerable isoflavonoid exposure over the course of their lives (Tubbs et al., 2012). Nutritional breeding suppression can lead to adaptability in a marginal habitat, but it is harmful if the duration of the exposition is extended (Patisaul, 2012). Whether and to what extent phytoestrogens affect reproduction of white rhinoceroses in the EEP has not been studied yet.

5 Conclusion

Southern white rhinoceroses are an endangered species at the present time. In the wild mainly due to poaching, in captivity due to low breeding success. The reasons for poor breeding in captivity can be manifold and are still not clear. There are several attempts at a solution, but the perfect solution does not yet exist. The transfers examined in this study result in a birth in a quarter to a third of the cases, with conceptions taking place a few months to up to three-and-a-half years after transfer. Positive hormonal responses are by far more numerous (50 – 84% of all transfers). Although there are slight differences in transfer success rates between wild-born and captive-born rhinoceroses, these figures are not significant due to the small numbers of cases.

Transfers are an important tool of stimulating breeding but are by no means sufficient on their own. Sonographic and hormonal examinations must be continued to rule out pathologies in the reproductive tract. Breeding support measures, such as ovulation induction, to overcome the frequently occurring acyclicity should also be pursued.

The insufficient reproduction of southern white rhinoceroses in zoos needs to be studied further if a stable or growing population is to be achieved. Outer circumstances like feeding and size of enclosure may play a role. The data compiled in this work could be useful for subsequent studies.

6 Summary

Southern white rhinoceroses, although their numbers have increased, are still an endangered species due to continued poaching. Zoos should maintain a stable or, at best, even growing population in order to be able to support the wild population in the given situation. Although white rhinoceroses have been kept in Europe since the 1960s, breeding success has been low. The exact reasons for this multifactorial and complex syndrome are not fully understood. Social composition, diet and available space all seem to affect reproductive success. Female rhinoceroses that have never bred, or have not bred for a very long time, have a greatly increased risk of developing reproductive tract pathologies. In many cases, this leads to infertility already occurring at a young age. Due to medical progress in recent years, it is now possible to closely monitor the reproductive activity of female white rhinoceroses. Animals that have not yet produced offspring, despite having reached breeding age should be examined sonographically, as well as via non-invasive hormone monitoring. If the animals are physiologically healthy, transfers, among other measures, are a possibility of stimulating reproduction. This means that either the females themselves are transferred to other zoos, or males are integrated into new groups. High expectations were given to these transfers. The aim of this study was to provide concrete figures on the success of the transfers. We found that births within a maximum of four years occurred after 26.2% of male and 30.0% of female transfers. Through endocrine analysis positive hormonal responses to transfers were identified in 84% of resident females, after arrival of a new male and in 50% of transferred females. It is unclear why pregnancy does not occur more frequently in these cases.

7 Zusammenfassung

Südliche Breitmaulnashörner sind, auch wenn ihre Zahlen gestiegen sind, durch die andauernde Wilderei immer noch eine gefährdete Spezies. Zoos sollen eine stabile oder bestenfalls sogar wachsende Population aufrechterhalten, um die Populationen in freier Wildbahn im gegebenen Fall unterstützen zu können. Obwohl südliche Breitmaulnashörner schon seit den 60er Jahren in Europa gehalten werden, sind die Zuchterfolge mangelhaft. Die genauen Gründe hierfür sind nicht ausreichend bekannt, allerdings scheint es ein multifaktorielles Geschehen zu sein. So haben soziale Zusammensetzung, Ernährung, sowie Platzangebot Auswirkungen auf den Zuchtwillen. Nashornweibchen die noch nie, oder über sehr lange Zeit nicht mehr gezüchtet haben, haben ein stark erhöhtes Risiko an Pathologien des Reproduktionstraktes zu erkranken. Sie werden dadurch in vielen Fällen frühzeitig unfruchtbar. Durch den medizinischen Fortschritt der letzten Jahre ist es heutzutage möglich die reproduktive Aktivität der Nashörner engmaschig zu überwachen. Tiere, die trotz erreichtem Zuchtalter noch keine Nachkommen haben, sollten sonografisch, sowie über nicht-invasive Hormonproben untersucht werden. Sind die Tiere physiologisch gesund sind unter anderem Transfers ein Mittel den Zuchtwillen anzukurbeln. So werden entweder die Weibchen selbst in andere Zoos transferiert, oder aber Männchen in neue Gruppen integriert. Von diesen Transfers wurde sich viel erhofft. Das Ziel dieser Studie war es konkrete Zahlen über Erfolge der Transfers zu liefern. Unsere Ergebnisse zeigen, dass es nach 26,2% der männlichen Transfers und 30,0% der weiblichen Transfers innerhalb von maximal vier Jahren zu Geburten kam. Durch die zusätzliche Analyse von Hormondaten konnte festgestellt werden, dass es in 84% der weiblichen Tiere nach Ankunft eines neuen Männchens bzw. in 50% der transferierten weiblichen Tiere positive hormonelle Reaktionen gab. Wieso es in diesen Fällen trotzdem nicht öfter zu einer Trächtigkeit kommt, ist nicht geklärt.

8 List of tables

9 List of figures

10 References

Adams, G.P., Plotka, E.D., Asa, C.S., Ginther, O.J., 1991. Feasibility of characterizing reproductive events in large nondomestic species by transrectal ultrasonic imaging. Zoo Biology 10 (3), 247-259. https://doi.org/10.1002/zoo.1430100308

Berkeley, E.V., Kirkpatrick, J.F., Schaffer, N.E., Bryant, W.M., Threlfall, W.R., 1997. Serum and fecal steroid analysis of ovulation, pregnancy, and parturition in the black rhinoceros (Diceros bicornis). Zoo Biology 16 (2), 121-132.

Brown JL, Bellem AC, Fouraker M, Wildt DE, Roth TL (2001) Comparative analysis of gonadal and adrenal activity in the black and white rhinoceros in north America by noninvasive endocrine monitoring. *Zoo Biology* 20 463-486. doi:10.1002/zoo.10028.

Eltringham, S.K., 1990. R. N. Owen-Smith 1988. Megaherbivores. The influence of very large body size on ecology. Cambridge University Press, xiii + 369 pages. ISBN 0-521-36020-X. Journal of Tropical Ecology 6, 256–257. https://doi.org/10.1017/S0266467400004454

Hermes, R., Göritz, F., Portas, T.J., Bryant, B.R., Kelly, J.M., Maclellan, L.J., Keeley, T., Schwarzenberger, F., Walzer, C., Schnorrenberg, A., Spindler, R.E., Saragusty, J., Kaandorp, S., Hildebrandt, T.B., 2009a. Ovarian superstimulation, transrectal ultrasound-guided oocyte recovery, and IVF in rhinoceros. Theriogenology 72, 959–968. https://doi.org/10.1016/j.theriogenology.2009.06.014

Hermes, R., Göritz, F., Saragusty, J., Sós, E., Molnar, V., Reid, C.E., Schwarzenberger, F., Hildebrandt, T.B., 2009b. First successful artificial insemination with frozen-thawed semen in rhinoceros. Theriogenology 71, 393–399. https://doi.org/10.1016/j.theriogenology.2008.10.008

Hermes, R., Hildebrandt, T.B., Walzer, C., Göritz, F., Gray, C., Niemuller, C., Schwarzenberger, F., 2012. Estrus induction in white rhinoceros (Ceratotherium simum). Theriogenology 78, 1217-1223. https://doi.org/10.1016/j.theriogenology.2012.05.015

Hermes, R., Hildebrandt, T.B., Walzer, C., Göritz, F., Patton, M.L., Silinski, S., Anderson, M.J., Reid, C.E., Wibbelt, G., Tomasova, K., Schwarzenberger, F., 2006. The effect of long non-reproductive periods on the genital health in captive female white rhinoceroses (Ceratotherium simum simum, C.s. cottoni). Theriogenology 65, 1492–1515. https://doi.org/10.1016/j.theriogenology.2005.09.002

Hildebrandt, T.B., Hermes, R., Walzer, C., Sós, E., Molnar, V., Mezösi, L., Schnorrenberg, A., Silinski, S., Streich, J., Schwarzenberger, F., Göritz, F., 2007. Artificial insemination in the anoestrous and the postpartum white rhinoceros using GnRH analogue to induce ovulation. Theriogenology 67, 1473–1484. https://doi.org/10.1016/j.theriogenology.2007.03.005

Metrione, L.C., Penfold, L.M., Waring, G.H., 2007. Social and spatial relationships in captive southern white rhinoceros (Ceratotherium simum simum). Zoo Biology 26, 487–502. https://doi.org/10.1002/zoo.20143

Ogden, J., 2011. International Studbook for the White Rhinoceros volume 12 (Ceratotherium simum). Bay Lake: Disney's Animal Kingdom for WAZA

Patisaul, H.B., 2012. Infertility in the Southern White Rhino: Is diet the source of the problem? Endocrinology 153 (4), 1568-1571. https://doi.org/10.1210/en.2012-1008

Radcliffe, R.W., Czekala, N.M., Osofsky, S.A., 1997. Combined serial ultrasonography and fecal progestin analysis for reproductive evaluation of the female white rhinoceros (Ceratotherium simum simum): Preliminary results. Zoo Biology 16, 445–456. https://doi.org/10.1002/(SICI)1098-2361(1997)16:5<445::AID-ZOO7>3.0.CO;2-A

Reid, C.E., Versteege, L., Ludwig, A., Hermes, R., 2012. Analysis of the European captive Southern white rhinoceros Ceratotherium simum simum population using the International Studbook for the African White Rhinoceros (2001-2004) and integrating reproductive health findings. International Zoo Yearbook 46, 209–220. https://doi.org/10.1111/j.1748-1090.2011.00164.x

Rhinos of the World https://www.worldrhino.com/white-rhinos-european-zoo/ (Access 10.11.2021)

Rhinos in Europe https://www.rhinos-in-europe.net/breitmaulnashorner/ (Access 10.11.2021)

Roth, T.L., Schook, M.W., Stoops, M.A., 2018. Monitoring and controlling ovarian function in the rhinoceros. Theriogenology 109, 48–57. https://doi.org/10.1016/j.theriogenology.2017.12.007

Patton ML, Swaisgood RR, Czekala NM, White AM, Fetter GA, Montagne JP, Rieches RG, Lance VA (1999) Reproductive cycle length and pregnancy in the Southern White Rhinoceros (Ceratotherium simum simum) as determined by fecal pregnane analysis and observations of mating behaviour. Zoo Biology 18 (1) 35-52. doi:10.1002/(SICI)1098-2361(1999)

Schwarzenberger, B.F., Walzer, C., Silinski, S., Tomasova, K., Göritz, F., Hildebrandt, T., Hermes, R., 2003. Problems associated with the low reproductive rate in captive white rhinoceroses (Ceratotherium simum) an update, Verh.ber. Erkrg. Zootiere (2003) 41; pages 301 - 303.

Schwarzenberger, F., Brown, J.L., 2013. Hormone monitoring: An important tool for the breeding management of wildlife species, Wiener Tierärztliche Monatsschrift 100, 209-225.

Schwarzenberger, F., Möstl, E., Palme, R., Bamberg, E., 1996. Faecal steroid analysis for non-invasive monitoring of reproductive status in farm, wild and zoo animals. Animal Reproduction Science 42 (1-4), 515-526. https://doi.org/10.1016/0378-4320(96)01561-8

Schwarzenberger, F., Palme, R., Bamberg, E., Möstl, E., 1997. A Review of Faecal progesterone metabolite analysis for non-invasive monitoring of reproductive function in mammals. International Journal of Mammalian Biology 62, 214–221.

Schwarzenberger, F., Walzer, C., Tomasova, K., Vahala, J., Meister, J., Goodrowe, K.L., Zima, J., Strauß, G., Lynch, M., 1998. Faecal progesterone metabolite analysis for non-invasive monitoring of reproductive function in the white rhinoceros Ceratotherium simum. Animal Reproduction Science 53 (1-4), 173-190. https://doi.org/10.1016/S0378-4320(98)00112-2

Stoops, M.A., West, G.D., Roth, T.L., Lung, N.P., 2014. Use of urinary biomarkers of ovarian function and altrenogest supplementation to enhance captive breeding success in the Indian rhinoceros (Rhinoceros unicornis). Zoo Biology 33 (2), 83–88. https://doi.org/10.1002/zoo.21106

Swaisgood, R.R., Dickman, D.M., White, A.M., 2006. A captive population in crisis: Testing hypotheses for reproductive failure in captive-born southern white rhinoceros females. Biological Conservation 129, 468–476. https://doi.org/10.1016/j.biocon.2005.11.015

Tubbs, C., Hartig, P., Cardon, M., Varga, N., Milnes, M., 2012. Activation of southern white rhinoceros (Ceratotherium simum simum) estrogen receptors by phytoestrogens: Potential role in the reproductive failure of captive-born females? Endocrinology 153, 1444–1452. https://doi.org/10.1210/en.2011-1962

Van den Houten, T., 2013. International studbook for white rhinoceros 13th edition (Ceratotherium simum). Malton: Flamingo Land Resort for WAZA

Versteege, L., 2018a. European Studbook White Rhino, 2017 (Ceratotherium simum). Beekse Bergen: Safari Park Beekse Bergen for the EEP and EAZA Rhino TAG

Versteege, L., 2018b. EAZA White rhino EEP Best Practice Guideline (Ceratotherium simum). Safari Park Beekse Bergen July 2018

Versteege, L., 2015. European Studbook White Rhino, 2014 (Ceratotherium simum). Beekse Bergen: Safari Park Beekse Bergen for the EEP and EAZA Rhino TAG

Versteege, L., 2012. European Studbook White Rhino 2011 (Ceratotherium simum). Beekse Bergen: Safari Park Beekse Bergen for the EEP and EAZA Rhino TAG

Versteege, L., 2009. European White Rhino Studbook 2009 (Ceratotherium simum). Beekse Bergen: Safari Park Beekse Bergen for the EEP and EAZA Rhino TAG

Ververs, C., van Zijll Langhout, M., Hostens, M., Otto, M., Govaere, J., Durrant, B., van Soom, A., 2017. Reproductive performance parameters in a large population of game-ranched white rhinoceroses (Ceratotherium simum simum). PLoS ONE 12. https://doi.org/10.1371/journal.pone.0187751