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Managed wildlife breeding-an undervalued conservation tool?

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ABSTRACT

Knowledge of and the technologies and resources applied to the ex situ care for wildlife have improved greatly in recent years. This has resulted in numerous successes bringing back populations from the brink of extinction by the reintroduction or restoration of animals from conservation breeding programmes. Controlled breeding of wildlife by humans is discussed controversially in society and in scientific circles and it faces a number of significant challenges. When natural breeding fails, Assisted Reproduction Technologies (ART) have been postulated to increase reproductive output and maintain genetic diversity. Furthermore, technical advances have improved the potential for successful collection and cryopreservation of gametes and embryos in many wildlife species.

With the aim of creating a better understanding of why ex situ and *in situ* conservation of threatened species must complement each other, and under which circumstances ART provide additional tools in the rescue of a threatened population, we elucidate the current situation here by using as examples three different megavertebrate families: elephantidae, rhinoceridae and giraffidae.

These mammal families consist of charismatic species, and most of their members are currently facing dramatic declines in population numbers. On the basis of these and other examples, we highlight the importance of captive zoo and other managed wildlife populations for species survival in a human dominated world. Without the possibility to study reproductive physiology in trained or habituated captive individuals, major advances made in wildlife ART during the past 20 years would not have been possible. This paper reviews the benefits and future challenges of large mammal conservation breeding and examines the role of assisted reproduction in such efforts.

1. Introduction

Scientists internationally argue that a 6th phase of mass extinction has already begun, threatening mammals, birds, fish, reptiles, amphibians, invertebrates, plants, and ultimately, humankind. Especially for our large terrestrial mammals in Africa and Asia, the future looks bleak [1]. For example, overall giraffe (*Giraffa camelopardalis* spp.) numbers plummeted by 40% during the past 30 years over the entire African range [2]. Elephants, both Asian (*Elephas maximus*) and African (*Loxodonta africana*) species, although admired for their great size and intelligence, are similarly under pressure. Recent studies on African savannah elephants [3] suggest that the real population size may only be one quarter of the 475,000 animals, originally anticipated by the African Elephant Database published in 2016. Southern White rhinos (*Ceratotherium simum simum*) were estimated to number 9000 individuals in the Kruger National Park of South Africa in 2015, but according to trends and predictions may to date have decreased to a third of the previous numbers, mainly due to the rhino horn poaching crisis in southern Africa [4].

The White rhino is nevertheless still the most numerous of all five rhino species. The Asian rhino species, especially, show significantly low numbers. There are probably only 80 Sumatran rhinos (*Dicerorhinus sumatrensis*), around 70 Javan rhinos (*Rhinoceros sondaicus*) and 3900 Indian rhinos (*Rhinoceros unicornis*) left in the wild [5].

Instead of mourning for the loss of the untouched wilderness and, therewith, our large vertebrate habitats, we must be prepared to manage the few remaining pockets as best we can in support of species conservation. This approach includes setting up conservation breeding programmes (CBP) and consideration of the value of breeding wildlife in confined reserves in or outside their natural

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habitat, as well as active human intervention into the process, such as additional feeding, watering holes, parasite control, but also culling, contraception and exchange of individuals.

Yet still today we define wildlife populations as „*in situ*", referring to free-roaming wild animals in their natural habitat, reproducing more or less undisturbed. And „ex *situ*", meaning wildlife held outside its natural habitats [6], typically in a restricted area, and usually with reproduction controlled by selection of breeding partners or implementation of population control. Since truly wild habitats are gradually disappearing, the healthy and independent *in situ* populations that we romantically imagine, are vanishing. Former natural environments become converted into managed reserves, thereby resulting inevitably in human influence on the animal populations, even in these natural surroundings.

The situation currently manifests in southern Africa more than anywhere else. We need to realize that the ability to keep megafauna like elephant, rhino and giraffe under managed conditions, whether in a smaller reserve within the natural range or in a modern zoo setting, is beneficial for the survival of these species. And these three mammal families stand exemplary for conservation breeding programs (CBP) with positive results. Therefore, the conventional vision of *in*/*ex situ* conservation will not appropriately denominate the many facets of wildlife CBPs. And these three mammal families stand exemplary for CBP's with positive results.

According to the International Union for Conservation of Nature (IUCN) guidelines, conservation in the wild is the ultimate objective [7]. Several examples exist, however, in which mammals became extinct in the wild, and could only be reestablished in their natural habitat after CBP's were established [8,9]. Instead of keeping wild and captive populations of a species as separate entities, all population managers, irrespective of being *in situ* or *ex situ*, must act together and manage all populations of a species under a common strategy, the so called "One Plan Approach" [9]. In situations of low individual numbers, each animal becomes valuable for genetic diversity, regardless of whether born in a zoo or roaming *in situ* in a protected area. The One Plan Approach postulates the joint development of conservation management strategies and actions for all individuals of a populations as an integrated approach to species conservation planning. Therefore, we also have to face the challenge of linking unconnected individuals and populations. Integrating and advancing proactive wildlife reproduction management, using scientific tools and technologies, is an essential step for sustainable wildlife breeding and conservation.

To overcome the logistical problem of segregated populations, assisted reproductive techniques (ART), such as artificial insemination (AI) or embryo transfer (ET), could help to exchange genetics, but also to have *ex situ* preserved genes transferred back into the natural environment, without the risks associated with the re-introduction of an individual animal raised under unnatural conditions.

Thorough knowledge of the animals' reproductive physiology and the needs for optimum reproductive output is essential before any ART is attempted. To gather such knowledge, study of individuals in captive breeding programmes in which they are accessible for observation, close handling and sample collection is indispensable [10,11].

2. Managed wildlife breeding programmes -successes and challenges

In general, long-term sustainability of a population is possible, if its reproduction at least equals mortality, it is demographically stable and a genetic diversity of at least 90% is maintained [12]. Ideally, it should maintain the potential to respond to environmental changes without becoming adapted to live only under captive conditions.

To keep a viable population of a species that is threatened in its natural habitat, permanent or temporarily managed and intensified CBP's to maintain or restore biodiversity in nature are considered an appropriate measure and these will become even more important in the future [13]. Unfortunately, all too often it is adopted as a final solution, when all other methods have failed.

A CPB can be accommodated within or outside the natural range of the species in question. Outside the natural range, CBP's are mainly established in zoological facilities and are needed in response to habitat loss, political and economical instability of a country, and to educate the general public; but they face considerable challenges. Costs, space, resources, lack of genetic diversity of founder populations and regulatory barriers, such as government restrictions or political discrepancies, are just some of the hurdles.

North American and European zoos have both organized their zoo breeding programmes through the Species Survival Plan® (SSP®) and the European Endangered Species Programmes (EEP) and European Studbooks (ESB). Individual animals within a species across accredited institutions are managed as a single population, coordinated by a studbook keeper [14].

Upon critical reflection, however, many captive species breeding programmes lack genetic diversity, and, in some instances, they simply fail to become self-sustaining [15]. In an analysis undertaken over a decade ago, it was shown already that out of 89 SSP programmes, fewer than 50% of those populations could be considered viable [13]. On top of this, zoological institutions house only a fraction of the animal diversity that exists worldwide. For example, for threatened vertebrates, 15% of the world' species are kept [13]. For most taxa, the management of a viable zoo metapopulation of >250 individuals would require the coordination of 11–24 accredited facilities within a radius ideally not exceeding 2000 km [9].

Another option is to establish intensively managed breeding stations within, or close, to the natural habitat site, with or without prerelease enclosures. Such set-ups have helped a number of conservation breeding and reintroduction programmes, such as these for the Iberian lynx (*Lynx pardinus*) in Portugal and Spain [16,17], the black footed ferret (*Mustela nigripes*) in the USA [18], the Giant panda (*Ailuropoda melanoleuca*) in China [19] and the Wood bison (*Bison bison athabascae*) in Canada [[20], this issue]. In the three latter cases, assisted reproduction technologies (ART) played, or still play, an indispensable role for the CBP [[18,20] this issue [21]].

For our largest terrestrial vertebrates, elephants, giraffes and rhinos, CBP's have been established with generally good success in range countries and zoos worldwide. Improved husbandry and stringent exchange of breeding bulls have resulted in a self-sustaining Asian elephant population in European zoos for the past 5 years with an average of 15 births annually [22]. Due to the longevity of up to 60 years and a slight sex ratio skew towards male calves, the main challenge here will be to breed enough females while adequately housing bull elephants [22]. Giraffes mostly breed without difficulties in zoos. However, due to recent developments in taxonomic classification, which points now towards four giraffe species instead of up to 10 subspecies, North American and European zoo associations need to adapt their breeding programmes [23]. In U.S. zoos, giraffes are bred in three different (sub)species, whereas in Europe currently six subspecies are still bred in separate studbooks [24]. Some of these subspecies, such as the Kordofan giraffe (*G.c. antiquorum*), exist in very small numbers, whereas others, like the Rothschild's giraffe (*G.c. rothschildi*) are even more numerous in zoos than in their African range countries. Thus, genetic and/or animal exchange between zoos and wild populations is desirable.

The Southern white rhino EEP is classified as self-sustaining to date with 318 (135 males, 183 females) individuals maintained in

>75 different participating zoological facilities and with an average growth rate of annually 5% since 2012 [25]. The genetic variability is sufficient, as is the current growth rate [25,26].

The Southern white rhino is also a perfect example for a successful extensive management approach in range countries. Since animals can be owned privately in South Africa, it is beholden upon large private land owners to protect and breed these pachyderms with, currently, more than one third of the white rhino and also black rhino (*Diceros bicornis*) populations being in private hands [27]; due to latest trends in rhino poaching, this figure may well have reached 50% at present. The largest privately owned white rhino population numbers more than 1300 animals, with >1000 calves bred over 20 years and an annual growth rate of 7% [[28], pers. comm., Cyrillus Ververs, November 2019]. The advantage of such a breeding operation is that all the animals are held in one 8000 ha area, comprising different, separately fenced, breeding groups, thereby making any exchange of breeding partners logistically easier and faster compared, for example, to zoological institutions. Additionally, the natural environment and climate lead to a more cost effective care for these animals compared to heated housing and supplemental feeding in a city zoo outside the natural range.

The ongoing research and subsequent improvements in terms of husbandry and reproductive management for the three megavertebrate examples show that it is very much possible to establish CBP's *ex situ*. At the same time, more extensive managed breeding in reserves within range countries need further consideration in the light of sustainability. The assisted reproduction technology advances may now connect the different populations and boost the idea of the One Plan Approach.

3. Improving conservation breeding programmes (CBP) through species-specific reproductive research and ART

Although not without challenges, in the absence of CBP's, for many species their conservation status would be worse. For example, despite further room for improvement, CBPs have played a major role in 13 of the 68 ungulate species that have shown an improved status in IUCN Red List reassessments [8,13]. To overcome the so-called "sustainability crisis" of CBP's, a number of strategies have been put forward, one of which is reproductive sciences [15]. Each species has evolved a distinct reproductive physiology and valid studies of these exist today for only about 250 species, mostly mammals and birds [29]. To improve reproductive output within CBP's, knowledge of the basic reproductive physiology of any species is inevitable. And talking further about any implementation of ART, very detailed information is mandatory and here, understanding the unique anatomy as well as ovarian function of each species, is one key point [30]. Fig. 1 lays out the basic pathway necessary to understand the reproductive traits of any particular species prior to implementing breeding programmes and ART (Fig. 1).

Because ART is applied with great success in humans and domestic animals, the community at large does not understand why it is not more easily transferred to wildlife. In reality, live offspring have been produced following AI or ET in only around 50–60 wildlife species [29,31]. When reviewing the literature, a first-time success is reported in many cases, but subsequent regular applications are not [31,32]. Few positive examples of repeatable protocols for AI have been presented in black footed ferrets [18,32], Asian and African elephants [33], the giant panda [21], the wood bison [20, this issue] or possibly the bottlenose dolphin *Tursiops truncatus* [34]. All these animals have been studied intensively in captivity and they are charismatic species, and mostly relatively easy to handle due to trainability or tractability.

To introduce a basic reproduction technique as straight forward as AI into the breeding programme of a particular species requires thorough groundwork. First and foremost, without fundamental knowledge of the species-specific reproductive physiology and anatomy, no ART protocol can be developed and, thus, any success may not be repeatable and sustainable, all as pointed out by Jewgenow et al. (2018) and Herrick (2019) [10,11].

The call for sophisticated biotechnology to be applied becomes strident at present, when a particular species is close to extinction or is extinct in the wild. At this stage, however, it is basically too late to establish reasonable and repeatable methods for assisted reproduction. Quite understandably, a holistic and science-based approach towards repeatable and sustainable ART applications requires a certain number of healthy individuals of a species and time. ART is only able to play a role in conservation of a particular species, if the technology has been studied in that or a closely related species. Therefore, reproduction specialists are facing a dilemma, besides the large burden of other constraints (Table 1.), when they are pushed into action at a very late stage of the process in a species nearing extinction.

The Rhinoceridae family is one of the latest and most valid examples of the last minute rescue approach. For example, the Northern white rhino (*Ceratotherium simum cottoni*) story has attracted considerable media attention recently. Only at the point when one geriatric male and two females of this subspecies still existed, both of which were declared to be suffering from uterine pathology, was action to work on advanced ART, including stem cell and cloning research, called for [35].

Given the fact that the simple procedure of embryo transfer has not yet been accomplished in any rhinoceros species, all of which are declared to be threatened, endangered or critically endangered at the present time (IUCN Red List, 2019), it is clear that any road to success will be a difficult one. The example of the now extinct Pyrenean ibex (*Capra pyrenaica pyrenaica*) gives a glimpse of what needs to be established in terms of embryo numbers to accomplish one single term birth: >400 cloned embryos of this species were produced, and from the 74 of those that could be transferred, only one recipient female remained pregnant to term, and the single newborn survived for only a few minutes [36]. Thus, in terms of rebuilding a genetically diverse and reproductively viable Northern white rhino population, such a goal seems completely out of reach, the more so when considering that the Pyrenean ibex had the enormous advantage of having a domestic goat hybrid, an abundant, well studied and easily handled recipient, as the surrogate mother. Whether produced by intracytoplasmatic sperm injection (ICSI) [37] or through induced pluripotent stem cells [38], the Northern white rhino would need several births of unrelated male and female offspring surviving to maturity and successfully reproducing on their own, while the only potential recipient would be the Southern white rhino. The minimum number of animals needed to re-establish a viable, genetically effective, population is often quoted to be > 50. For a realistic chance for long term survival in the wild, a population of >500 animals is needed [39]. The Southern white rhino grew back during the last century from 150 to 200 individuals to about 9000 individuals across South Africa beginning of the 21st century [40].

Numerous other factors put constraints on applying ART to wildlife (Table 1). Many populations that are reduced in size face similar fertility problems, whereby females are post reproductive and could not even conceive via natural mating [41,42]. Although such females may be used for oocyte collection and produce embryos via IVF techniques to be placed into surrogate mothers, such an undertaking would also require young females with good oocyte quality and, again, reproductively healthy recipients [43]. On top of this, most funding is available for *in situ* conservation compared to

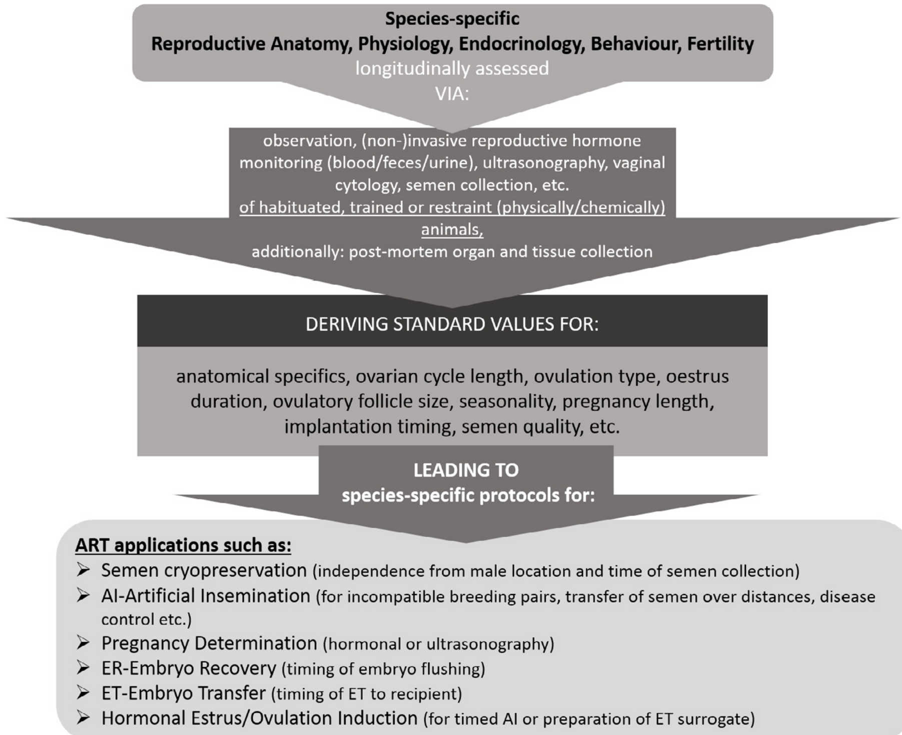

Fig. 1. Schematic of the research pathway to successful implementation of assisted reproductive technologies into a species breeding program.

ex situ breeding and ART research. But we, nevertheless, have to accept the paradigm shift towards managed wildlife breeding under the pressures of climate change and human population growth.

4. ART development and achievements in megavertebrates

Any detailed knowledge published to date in reproductive sciences concerning popular megavertebrate species, such as elephant, rhino or giraffe, have derived mainly from the study of specimens held under human care. Unrestricted access to study the captive animals is inevitable for any ART development and it has resulted in a wealth of knowledge following the approach depicted in Fig. 1. Several reviews summarize the current knowledge in reproductive sciences and the use of ART in elephants [33,44], giraffes [24] and rhinoceros species [45,46] and an overview of these is presented in Table 2.

Successful collection and cryopreservation of semen from Asian and African elephants [47,48], from all, but the Javan rhino species (currently none in captivity) [49–52] and in giraffes [24,53] has led to the successful AI using fresh and frozen-thawed semen in Asian [54] and African [55] elephants, in Rothschild's giraffes [24] and in Southern white [52] and Indian rhinoceros [59] (Table 2). To the authors' knowledge, to date only two giraffe calves have been born following the use of fresh semen and a single calf was produced

Table 1
Although proposed to be a valuable and important tool in species conservation breeding, assisted reproduction technologies (ART) development is still not as far advanced and regularily applied as in human medicine or domestic animal production. The most prevalent reasons and main constraints for the limited success of ART in endangered species are summarized in the table. CITES = Convention on International Trade in Endangered Species of Wild Fauna and Flora; Nagoya Protocol = Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity [60].

Central issue:	Main constraints in ART development for non-domestic species:
Population size	➢ Lack of detailed knowledge on reproduction, but time for long-term studies not available due to critically endangered status already ➢ Too few individuals hamper statistical evaluation of findings and thus recognition of trends ➢ Each individual becomes too valuable to be used in research experiments ➢ Accessibility and tractability of individuals is problematic in most species, posing a threat to the examiner or a risk for animal self-injury
Fertility	➢ Lack of fertile individuals in captive populations ➢ Asymmetric reproductive aging, if not bred at a young age and continuously ➢ Large individual variation in non-domestic species in contrast to domestic animals which were selected for fertility over a long time and bred to our needs, including adaptation to ART
Economy	➢ Domestic animal/human reproduction research: highly competitive and commercial field ➢ Low drive in research due to lack of funds and monetary revenue ➢ Competition for conservation funding (non-profit organizations need money for *in situ* conservation)
National and international politics	➢ Country boarders, rules and regulations when researching endangered species (CITES permits, Nagoya protocol, Government Veterinary Health Regulations) ➢ Government interest low/no priorities given to ART research compared to *in situ* conservation ➢ NGO competition around high profile species result in hampered new approaches rather the acknowledging the value of every strategy
Public acceptance	➢ Disconnection between humans and nature resulting in the believe that wildlife should best be left undisturbed ➢ In the public mind, ART application equals "playing god" and is ethically questionable ➢ New generation acceptance of captive breeding and wildlife kept in zoos is low

Table 2
Examples for known key reproductive features and achievements in assisted reproduction in three different large mammal families, elephant, rhino and giraffe. Abbreviations: EM: Asian elephant, LA: African elephant, WR: white rhino, BR: black rhino, IR: Indian rhino, SR: Sumatran rhino, LH: Luteinizing hormone, FSH: Follicle stimulating hormone, IV: in vitro, AI: Artificial insemination, IVM: in vitro maturation, IVF: in vitro fertilisation; ICSI: intracytoplasmatic sperm injection.

	Elephant	Rhino	Giraffe
Number of species	3	5	4–6
Reproduction Physiology Data:			
Female Maturity (wild)	(8–12)	(5–6)	(2.5)
Estrus Cycle length (days)	95–120	25-28 (BR)/30–65 (WR)	14
Gestation length (days)	600–680	450-540	430-490
Estrus cycle monitoring:			
Progesteron, Oestrogen measured	yes	yes	yes
LH peaks measured	yes	yes	yes
FSH, Inhibin measured	yes	no	yes
Ovarian ultrasonography (repeated)	yes	yes	yes
Ovulation type	spontaneous	intermediar: partially induced (SR), and spontaneous (WR, IR, BR)	spontaneous
Ovulatory follicle size (cm)	1.8–2.2	3.0 (WR, BR)-14.0 (IR)	2.0
Assisted Reproduction Techniques achieved			
Successful semen collection methods	transrectal massage, EE	EE	transrectal massage, EE
Cryopreservation of sperm	yes	yes	yes
Hormonal Estrus induction	no	yes (WR, IR)	yes
AI (fresh semen)	yes (EM, LA)	yes (WR, IR)	yes
AI (frozen semen)	yes (EM, LA)	yes (WR, IR)	yes
Sperm sex-sorting	yes (EM, LA)	yes (WR, BR, IR)	no
Ovum-Pick up	no	yes (WR, BR, SR)	no
IVM/IVF (ISCSI)	no	yes (WR, BR, SR)	no
IV Embryo production/transfer	no/no	yes (WR, BR)/no	no/no
REFERENCES	[33,44,47,48,54,55,58]	[33,37,45,46,49, 50,51,52,56,58]	[24,53]

after AI with frozen-thawed semen [24]. Probably a dozen calves derived from frozen and fresh semen AI in the Southern white rhino and the Indian rhino, in which four calves have been born so far [56]. However, AI has been most widely used in captive elephant breeding programmes. In both the elephant species housed in Western zoos, >40 calves haven been now been born from fresh semen insemination during natural estrus [55]. And although, due to anatomical constraints, semen is deposited endoscopically into only the vagina, rather than the uterus, fresh and frozen-thawed semen appears to work equally well in African elephants, since at least six pregnancies were established using semen cryopreserved previously in South Africa after collection from wild bulls [48,55]. Reproductive specialists have another advantage in correctly timing the insemination in elephants. Female elephants show two distinct LH peaks, usually 19–21 days apart, and only the second LH peak results in ovulation [44].

Through the ability to nowadays cryopreserve semen of elephant, rhino and giraffe species, and the potential for successful AI therewith, two other important conservation strategies can now be achieved: First, genetic exchange in support of isolated populations; and second Genetic Resource Biobanking [57]. By shipping cryopreserved semen samples, *in situ* and *ex situ* populations can be connected in both directions and separations by country and continents can be bridged. New founder animal genes can be introduced while the male itself remains in the original population. Semen collection has been performed successfully by our group in both, wild and zoo elephants, giraffes and rhinoceros species (Fig. 2). Currently, we are establishing for example a large biobank focused on African elephant and rhinoceros semen as a genetic resource back-up in South Africa (unpubl. data). Through sperm cryopreservation of as many different bulls of each species from various areas of the country, we hope to achieve a viable semen bank to enrich any isolated population in the future. Again, privately owned rhinos in smaller reserves are advantageous for this project and are the source of semen samples for the rhino sperm bank. These rhinos are more intensively managed, they undergo general anesthesia for various reasons in regular intervals and they require fewer permits by regulatory authorities compared to the animals maintained in South African National Parks. Over a period of eight months, we were already able to collect semen by electroejaculation from 21 Southern white rhino bulls in three different provinces in the country. Of the ejaculates collected, 12 were cryopreserved in 0.5 cc straws by a field friendly rack-freezing methods over liquid nitrogen vapor. The aim is to cryopreserve 100 high quality ejaculates over the next years. The resulting frozen semen maybe simply stored for future use or applied immediately to enrich any CBP.

The elephant is already an excellent example how to implement the One Plan Approach and how ART can connect the bespoke *in situ* and *ex situ* populations by obtaining sperm from wild African elephants for the use for AI in the European studbook [55].

Additional technologies, such as the ability sex-sort spermatozoa, will be needed in the future for efficient wildlife breeding. Sortability of spermatozoa for their X or Y-chromosomes has been demonstrated for Asian and African elephants, Southern white rhino, black and Indian rhino [58,59]. Achievement of higher numbers of female births after AI with sex-sorted semen would boost enormously the sustainability of breeding programmes with long-lived animals, such as elephant and rhino.

5. Conclusion

The use of ART makes the most and immediate sense in a combined approach, including natural breeding in zoological facilities and range country reserves. In the face of current human population growth and land exploitation, we must consider how to intensively manage the remaining habitats and their wildlife, especially in African countries. All aspects of reproduction management will play an important role for reintroduction and restoration of wild mammal populations. However, it will require a much better orchestrated collaboration between governmental and non-governmental organizations, zoo associations, individual zoological institutions and research facilities, with input from basic reproduction sciences.

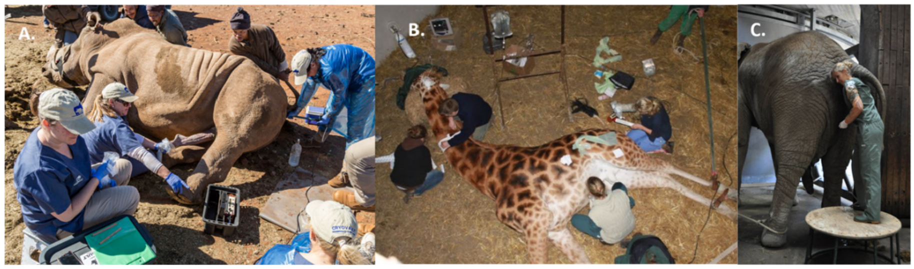

Fig. 2. Example for semen collection in three megavertebrate species under different conditions. A. Southern white rhino, chemically restraint, in a privately owned farm in South Africa, using a battery driven electro ejaculator with a specially designed probe, photo credit: Hemmersbach Rhino Force; B. Nigerian giraffe, general anesthesia, in a zoo, using the same electroejacultor with a different custome-made probe, note the additional leg restraint for security of operators; C. African elephant, behaviourally and physically restraint, in a zoo, semen collection is performed in the trained, conscious animal by transrectal prostate massage.

In the light of vast changes in climate and habitats, conservation breeding programmes should not be the last resort, but rather accepted as an integral part of any sustainable conservation strategy now, including funds directed towards further research. The increased knowledge and potential described in this review as having been gained in large vertebrate species has relied on the study of captive animals. Reproduction research and the adaptation of assisted reproduction protocols is dependent upon accessibility and handling of the focal species. It is also a question of initiating research early enough in time, to have ART solutions readily available when most needed. Without CBP's, we may have missed opportunities to acquire important information. And, without doubt, we would have lost even more species by now.

Declaration of competing interest

None.

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