Sustainable Reproductive Management of Southern White Rhinoceros (*Ceratotherium simum simum*) in Australasia into the Future

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Abstract

Despite the recent addition of wild caught founders to the captive population of white rhinoceroses (Ceratotherium simum simum) in Australasia and subsequent moderate reproductive success, sustainable long term reproductive management of this species in captivity remains problematic. Threats to sustainable regional management include insufficient captive spaces and the consequent need to curtail breeding in reproducing animals, reproductive failure in 38% of wild caught females, an apparent sex bias towards male calves and a poor understanding of the factors leading to extremely low levels of reproductive success in captive born animals. The integration of assisted reproductive technologies in to management strategies for this species will allow for enhanced natural breeding and enable the reproductive and genetic potential of non reproducing individuals to be realised.

Introduction

In 1999 the global captive population of southern white rhinoceros (*Ceratotherium simum simum*) was declining at annual rate of -3.5% per annum compared with a 5-6% annual increase in the wild population [Emslie and Brooks, 1999]. Since that time the captive population has been augmented with additional wild caught founders and this population is now marginally self-sustaining [Foose and Wiese, 2006]. This sustainability is threatened by the imminent loss of aged but reproductively viable original founders and extremely limited reproductive success in all captive born animals. The Australasian situation mirrors that of the global population with an initial importation of animals, some reproduction and then subsequent demographic collapse as the result of reproductive failure. Since 1999 two further imports of wild caught founders (and an additional captive born animal) have bolstered the population and additional moderate reproductive success has been obtained. Sustainability of this program is threatened principally by insufficient places to house offspring and the consequent need to limit breeding, reproductive failure in 38% of wild caught founder females to date, an apparent sex skew towards male offspring and a poor understanding of the factors contributing to low reproductive success in captive bred male and female white rhinoceros. Failure to address and manage these issues will have profound consequences for this population necessitating further imports in two to three decades time.

Reproductive failure in captive females

In the past 10-15 years numerous researchers have investigated aspects of reproductive physiology and pathology in female rhinoceroses in an attempt to elucidate causes of sub-fertility and infertility. These investigations have identified uterine and ovarian pathology, variable oestrus cycle length, acyclicity and various other physiological and pathological changes in female rhinoceroses [Hermes et al, 2004, Hermes et al, 2006, Brown et al, 2001, Schwarzenberger et al, 1999]. A variety of potential behavioural mechanisms have been postulated to account for low reproductive rates in both founders and in captive born individuals including reproductive suppression and intra-specific behavioural deficiencies in captive born females. Swaisgood et al [2006] utilising data obtained from a number of facilities holding white rhinoceroses failed to find statistically significant trends to support these theories. The authors of this study concluded that reproductive failure in captive born females occurs post copulation. Conflicting evidence has been obtained from animals managed in the European Endangered Species Programme where 76% of nulliparous females have an intact hymen indicating factors related to events prior to copulation are a significant cause of reproductive failure in these animals [Hermes et al, 2006]. Other potential factors include diet, pathogen exposure, social experience, permanent associations with individual males in captivity, enhanced growth and earlier puberty in captivity. The relationship between chronic stress experienced in captivity and reproductive failure requires further examination [Carlstead and Brown, 2005].

Recently Hermes at al [2004] postulated an asymmetric ageing process as a cause for premature reproductive senescence in non-reproducing females. Cystic uterine hyperplasia is a common finding in adult female white rhinoceroses. Less frequent pathological findings in female white rhinoceroses include intra-mural leiomyomas of the uterus and cervix, intra-luminal uterine adenomas, para-ovarian cysts and mesovarial leiomyomas. It is postulated that these pathological changes are the result of long term oestrus cycle activity and resultant long term exposure to sex steroids without pregnancy. It should be noted that managing animals in a way that promotes the development of disease constitutes an animal welfare problem. Despite these advances in understanding the pathological consequences of prolonged sex-steroid hormone exposure without pregnancy the root causes of reproductive failure in this species in captivity remain largely unknown.

Reproductive failure in captive males

Until recently little consideration has been given to any potential male contribution to infertility and sub fertility in captive rhinoceroses. Given that only 19% of male white rhinoceros managed as part of the global Species Survival Program and only 4% of captive born male white rhinoceroses in North America have sired offspring further investigation is warranted [Fouraker and Wagener, 1996, Hermes, 2005]. Further work is required to fully elucidate the effect of various exogenous influences on male fertility. Season appeared not to influence testosterone production in one study of captive male rhinoceroses [Brown et al, 2001] yet one of two studies targeting free-ranging animals demonstrated an association between faecal testosterone metabolite concentration and season [Kretzschmar et al, 2004]. Social situation (dominant versus subordinate) appears to influence testosterone in wild white rhinoceros bulls [Rachlow et al, 1999] and semen quality and accessory sex gland size in captive white rhinoceroses [Hermes et al, 2005]. Recent assessment of white rhinoceros males held in two Australian zoos has demonstrated higher faecal testosterone metabolite concentration from bulls that have sired offspring compared with unsuccessful sires and no apparent seasonal effect on testosterone levels [Portas unpublished]. Age related fibrosis of the testicular parenchyma is evident in male white rhinoceros over the age of fifteen years but does not appear to have an adverse effect on semen production with aged males frequently the most reliable semen donors [Hermes et al 2004]. An additional potential problem has been the recent documentation of testicular neoplasia in captive white and black rhinoceros bulls in Australia [Portas et al, 2005, Portas et al, 2006].

Captive rhinoceroses tend to be held at higher population densities than wild animals, have reduced opportunities for exhibiting some natural behaviours, exist in artificial social groupings, are subjected to stressors and pathogens not encountered in the wild and subsist on a diet that may vary significantly in terms of caloric value, mineral and vitamin composition and physical form when compared with that found in the wild. Any or all of these factors have the potential to affect the fertility of captive males. Further investigation into management related causes of infertility and sub-fertility in both male and female rhinoceroses is required.

Recommendations for assessment of non-breeding females

- Initiate non invasive endocrine monitoring. The presence or absence of cyclic activity, irregular oestrous cycle lengths and periods of anoestrous can be assessed by monitoring faecal progestogen metabolites. An enzyme immunoassay utilising the Quidel clone 425 antibody [Graham et al, 2001] is available in Australia through the reproductive laboratory at Western Plains Zoo.
- Perform transrectal ultrasonography and manual vaginal examination to assess reproductive tract health. When performed by an experienced operator ultrasonography can be used to demonstrate uterine and ovarian pathology (including cystic endometrial hyperplasia, endometritis, cystic ovarian changes and uterine and ovarian tumours) and assess ovarian activity. Where facilities and resources permit serial transrectal ultrasonography is a useful adjunct to non invasive endocrine monitoring.

Once the health status of an individual's reproductive tract has been assessed via ultrasonography and the animal's endocrine profile established informed management decisions can be made with respect to salvaging the animal's reproductive potential.

General recommendations for enhancing reproduction in (identified) healthy non breeding female white rhinoceros

- Ultimate goal: the inter calving interval for reproducing cows should be no more than 5 years (ideally 3.5-4 years)
- Ideally, females should have been pregnant once by the age of 15 years, since reproductive pathology starts to develop in non-reproducing females from this age on
- Females that are acyclic or that demonstrate normal or variable cyclic activity and minor reproductive tract pathology should be subject to intensive management for breeding. This should include exclusive access to a bull with proven breeding success.
- Female – female bonds such as long term mother /daughter relationship should be considered a possible cause for cessation of cyclic activity.
- Transfer of acyclic females to a breeding situation in another institution: Data from Europe demonstrates resumption of cyclic activity in approximately 50% of acyclic females transferred to a new institution [Schwarzenberger pers com].
- Transfer of non reproducing but cycling females to other institutions and the subsequent introduction to 'new' proven males may help overcome pair incompatibility.
- Artificial insemination: Healthy nulliparous females that demonstrate normal or variable cyclic activity with moderate degenerative reproductive tract changes ≤ 15 years should be prioritised

as candidates for artificial insemination [Hildebrandt et al, 2007]. Pregnancy, in addition to being the desired outcome for non reproducing females, is protective against further degenerative reproductive tract changes.
- Infertile females: Females with severe reproductive tract changes should be considered as candidates for oocyte rescue. The development of in vitro oocyte maturation (IVM), fertilisation (IVF) and intracytoplasmic sperm injection (ICSI) techniques for the production and subsequent cryopreservation of embryos offers the first step in preserving the reproductive and hence genetic potential of these females.
- Post mortem: Ovaries rescued immediately post mortem can still be used to harvest oocytes for IVM, IVF, ICSI and embryo production.

Captive born females should be given priority for achieving early reproduction. Given the extremely low rates of reproductive success in this class of animals initiating non invasive endocrine monitoring from two to three years of age is imperative. While the causes of the low rate of reproductive success seen in these animals are poorly understood general recommendations include housing these animals with companions of a similar age following weaning, avoiding permanent associations with a single bull and incorporating these animals in large multi female groups that contain, where possible, one or more breeding adult females. To achieve long term sustainability of the captive Australasian white rhinoceros population this class of animals must receive cooperative regional prioritisation for informed management from the time of birth.

Recommendations for assessment of non-breeding males

- Perform transrectal and transcutaneous ultrasound examination of accessory sex glands and gonads respectively. The results of this examination in conjunction with the results of semen analysis provide good predictive indicators of fertility and infertility in male white rhinoceroses.
- Perform electroejaculation under anaesthesia using appropriately designed equipment operated by experienced personnel. Semen collected during this procedure is then analysed using standard techniques and categorised on the basis of motility and morphology using recently established criteria [Hermes et al, 2004].
- Aspermic ejaculates or the presence of testicular parenchyma abnormalities are indications for ultrasound guided testicular biopsy. Tissue obtained by this technique can then be assessed by histopathology to further quantify spermatogenesis or to characterise neoplastic masses.
- Faecal testosterone metabolites can be monitored using an enzyme immuno assay to provide endocrine data complimentary to the above techniques. A gonadotrophin releasing hormone challenge provides a further tool for assessing testicular function and endocrine status of white rhinoceros bulls [Portas unpublished].

General recommendations for enhancing reproductive potential of non breeding males

- Non reproducing males housed in multi male/multi female groups should instead be housed as a single male in a multi female group. Manipulating herd structure and consequently social status of the animal may result in improved fertility.
- Non-reproducing males should have access to healthy females only. Female – female bonds between fertile and infertile females may be a factor preventing inexperienced, non-reproducing males from breeding.

- In general breeding access should be limited to one viable female when in oestrous. Non reproducing males housed with a multi female group should be considered for transfer to a new multi female group
- Non reproducing males housed with a single female should be transferred to a multi female group or the accompanying female should be exchanged.
- Long term beta-carotene supplementation may improve fertility in poorly performing males.
- All males should be considered candidates for electroejaculation and cryopreservation of semen such that their reproductive potential is preserved.
- Ensure that all young males are given the opportunity to develop appropriate social behaviours through access, during their adolescence to a range of other rhinoceros of varying age classes and sex.

Options for the future

If this program is to remain viable into the future consideration needs to be given to the allocation of additional spaces for this species in the region to allow for sustainable reproduction. Global population management should be considered to alleviate some of the problems associated with management of a long lived species in a region with a relatively small number of available spaces. Repatriation of offspring to stable African range states is a possibility that warrants further discussion. Management euthanasia of male offspring surplus to the breeding program, applied judiciously, has the potential to relieve some pressure on the limited number of spaces available for managing this species. Wider regional support for research into sperm sex sorting technologies would allow for the more rapid refinement and application of these techniques, offering a potential solution for managing the apparent sex skew towards male offspring. High priority must be accorded to managing captive born animals for early reproduction and investigating potential causes of reproductive failure in these animals.

Despite recent advances, the understanding of the reproductive biology of the white rhinoceros in captivity remains poorly understood. A clear role therefore exists for the integrated application of assisted reproductive technologies such as non invasive endocrine monitoring and ultrasonography to elucidate reproductive physiology in captive animals. This information can then be utilised to make informed management decisions to enhance natural breeding. Additionally the developing technologies of artificial insemination, sperm sex sorting, oocyte retrieval and in vitro fertilisation in rhinoceros species offer a means to salvage the genetic potential of unrepresented individuals and manage the apparent sex bias towards male calves.

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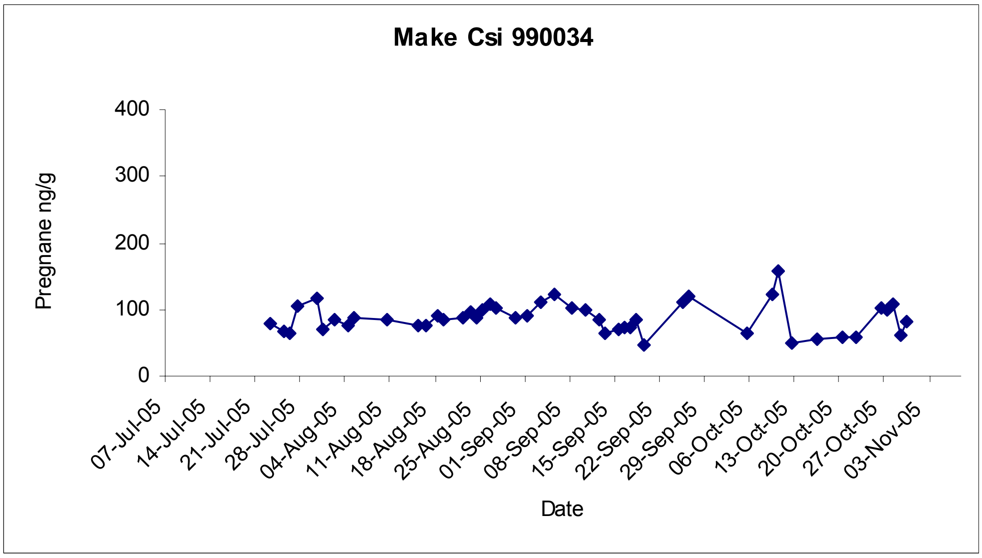

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