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Reproductive problems directly attributable to long-term captivity—asymmetric reproductive aging

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Abstract

Problems attributable to long-term captivity have been identified and are responsible for the difficulties in establishing successful reproduction in captive populations of wildlife, specifically, elephants and rhinoceroses. Historically, non-reproductive periods of 10–15 years in nulliparous female rhinoceroses and elephants have not been considered problematic. New evidence suggests that prolonged exposure to endogenous sex steroids and that long stretches of non-reproductive periods induce asymmetric reproductive aging in captive animals. The consequences are reduced fertility, shortened reproductive life-span and, eventually, irreversible acyclicity. Although age-related reproductive lesions have also been documented in male rhinoceroses, they continue to maintain a longer reproductive life-span than females. Since human and domestic animal models have already indicated that early pregnancy provides natural protective mechanism against asymmetric reproductive aging processes and premature senescence, it is imperative that appropriate counter measures such as assisted reproductive technologies (ART) be utilized to ensure early pregnancy in captive animals for their preservation and to ensure increased genetic diversity of the captive populations. © 2004 Elsevier B.V. All rights reserved.

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1. Introduction

Wild animals have been managed in captivity for more than 2000 years (Rookmaaker, 1998). Captive management has improved dramatically during this period. Improvements in the housing facilities, nutrition, husbandry, and medical care of modern zoos and regional and global management programs are responsible for the successful establishment of self-sustaining captive populations. Despite this long history of animal management in captivity and the abundant knowledge acquired about wild animal husbandry, reproduction

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remains limited or absent in many species. Captive populations of endangered species like the Asian and African elephants; the white, Sumatran and Indian rhinoceroses; or the giant panda are not self-sustaining. As a result, zoo conservationists and animal managers are confronted with geriatric and reproductively quiescent captive populations. Statistics predicts that 75% of the females among the African elephant populations will be at the post-reproductive phase in just 15 years (Wiese, 2000). It has only been during the last 20 years that advances in endocrinology, endoscopy, and ultrasonography have elucidated some of the underlying causes for poor reproductive success in captive megavertebrate species. This report will briefly discuss different aspects of reproductive problems attributable directly to the long-term captivity of exotic species. It primarily focuses on the knowledge gathered on aspects of reproduction in rhinoceroses and elephants and the difficulties involved in the successful reproduction of an aging captive population. However, the different effects of long-term captivity on reproduction discussed here may also apply in principle to other captive species, that are currently experience poor reproductive success.

2. Cycling, non-reproducing females

A reproducing female white rhinoceros in captivity may produce up to nine calves. Considering a pregnancy of 16 months and a lactational anestrus of approximately 12 months, this female exhibited only 90 estrous cycles during her reproductive life-span (Ochs and Frädrieh, 2001). With pregnancy and lactation dominating the endocrine status, the estrous cycle is a rare event during her reproductive life. Endocrine surveys and frequent observations of wild white rhinoceros females report even shorter intervals between successive births (1.6 years), indicating that pregnancy and lactation are the most common endocrine profile (Owen-Smith, 1973, 1975; Göritz et al., 1999; Mraz et al., 2001; Kretzschmar, 2001) with possibly as few as 30 estrous cycles per reproductive life-span.

These numbers in reproducing females compare to 310 estrous cycles in captive non-reproducing females. Non-reproducing females have displayed 90 estrous cycles by the age of 16 years, the same age at which the first signs of pathological alterations are detected in the genital tract (Hermes et al., 2001a). In the past, these non-reproductive periods of 10–15 years in nulliparous female rhinoceroses and elephants were not considered problematic. Captive breeding programs classified permanently cycling females who were 30 years of age and older as potential breeding candidates based on the long reproductive life-span of these animals in the wild. Despite an increased longevity of mammals in captivity (Jones, 1992), studies suggest that non-reproductive female elephants have a shortened reproductive life-span (Montali et al., 1997; Hildebrandt and Göritz, 1998; Hildebrandt et al., 2000b). Extreme cases demonstrate reproductive life-spans ending 15 years earlier (Fig. 1) compared to successfully reproducing females (Hildebrandt et al., 2000b).

The cause of this premature senescence is an asymmetric reproductive aging process. The reproductive organs of non-reproducing female rhinos and elephants are exposed to prolonged periods of sex steroids from continuous ovarian cycle activity. The central effects of this asymmetric aging process include progressive development of genital pathology with subsequent reduction of fertility (Montali et al., 1997) and, presumably, the utilization of the follicular stock at a higher rate (Sopelak and Butcher, 1982; Hinrichs, 1997).

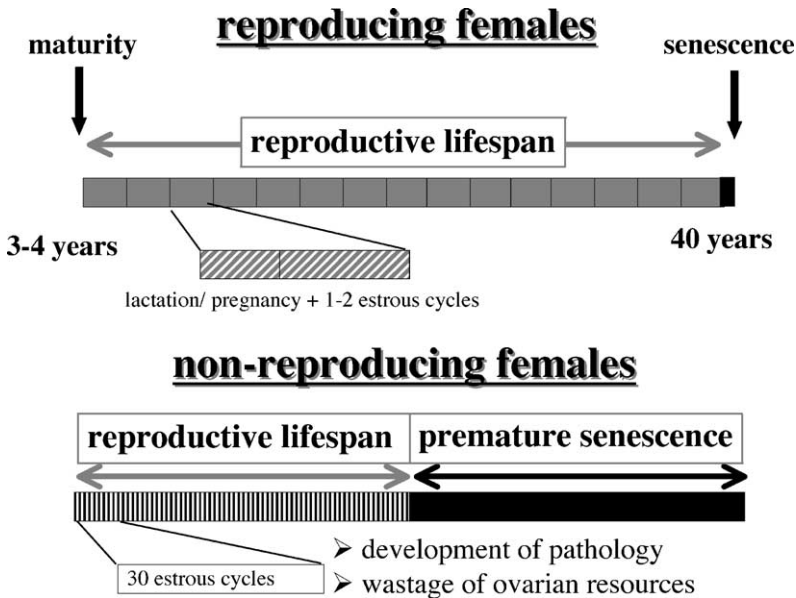


Fig. 1. Schematic graph of the reproductive aging process in reproducing and non-reproducing female rhinoceroes and elephants in captivity.

Steroidhormone-dependent tumor development and the promotion of tumor growth by estrogen and progesterone have been well established (Kawaguchi et al., 1989; Rein et al., 1995; Maruo et al., 2000; Walker, 2002) and explain some of the findings in captive, non-reproducing yet continually cycling megavertebrates.

Leiomyoma of the reproductive tract and/or cystic hyperplasia represent 80% of the most common lesions found in non-reproducing female rhinoceroes and elephants. This is in contrast with the incidence of <10% of genital pathology detected in reproducing, multiparous white rhinoceros (Hermes et al., 2004). In this regard, captive megavertebrates are equivalent to humans and animal models in which fibroids occur in 70% of patients in reproductive age (Cramer and Patel, 1990; Walker, 2002) whereas the risk of uterine leiomyomas in parous women is reduced by half and the incidence is further reduced significantly in multiparous women (Parazzini et al., 1988, 1996). Similar to the “human model”, the size and number of these lesions in rhinos and elephants increase with age, progressively reducing fertility. In Asian elephants and Asian rhinoceroes, uterine leiomyomas are the most frequently seen genital tumors associated with age (Montali and Citino, 1993; Montali et al., 1997; Hildebrandt et al., 2000b; Schaffer et al., 2001). The African rhinoceroes and African elephants show a greater affinity to develop uterine cystic hyperplasia, although uterine leiomyomas may also occur. It has been found that endometrial cysts first appear singly, then over time with advancing age, multiple cysts or clusters appear representing an accumulation of secretions within the endometrial glands (Hermes et al., 2004).

The devastating result of this asymmetric aging process and subsequent development of genital pathology has been demonstrated in the captive population of the northern white rhinoceros (*Ceratotherium simum cottoni*). The northern white rhinoceros is the most en-

dangered among the larger mammalian species of the world with a wild population of approximately 30 animals, closely followed by the Sumatran rhinoceros with a population of approximately 300 individuals. Poaching and political instability are the greatest threats to the long-term survival of these critically endangered species. As the genetic diversity is diminished by such low numbers, the success of captive breeding management becomes increasingly important. However, 53 years of captive management of the northern white rhinoceros has resulted in only four offspring (Ochs and Frädrieh, 2001). Reproductive assessment by transrectal ultrasound, performed in all non-reproducing captive northern white rhinoceroses revealed uterine leiomyomas, cystic hyperplasia and cystic ovaries as underlying causes for reproductive failure in captive females. Ultrasonographic identification and classification of lesions were based on data generated from 46 southern white rhinoceroses (Hermes et al., 2001c). This reference data demonstrated the variety and age-dependence of genital tract lesions occurring in this species.

Five out of six female captive northern white rhinoceroses examined were considered post-reproductive, 10–15 years earlier than their counterparts in the wild, due to the presence of severe cystic hyperplasia, chronic endometritis, uterine leiomyomas, polyps and para-ovarian cysts (Fig. 2). These five post-reproductive females represent 36% of the entire female population (Hermes et al., 2002). In the Sumatran rhinoceroses, the situation is equally serious with only one reproducing pair of animals left in captivity and the wild population being on the brink of extinction (Roth, 2002).

3. Acyclic, non-reproducing females

3.1. Acyclicity in rhinoceroses

In the white rhinoceroses, the absence of ovarian cyclicity marks the end point of the asymmetric aging process with follicular resources presumably exhausted. Starting at puberty (age of 4 years), rhinoceroses' ovaries develop waves of pre-ovulatory follicle development every 14 days on average (Fig. 3). The follicular activity occurs years before the first luteal activity is measured (Hermes et al., 2001a, c). Long-term, non-conceptive estrous cycles without periods of pregnancy and lactation continuously diminish the limited follicular stock. At the end of this asymmetric aging process, the ovary progresses towards irreversible acyclicity with the follicular stock nearly depleted as demonstrated in mares and the female rat (Sopelak and Butcher, 1982; Hinrichs, 1997). Also similar to the mare and rat, erratic luteal activity and acyclicity were reported in a large number of non-reproducing white rhinoceroses in the mid-reproductive age range (Schwarzenberger et al., 1998; Patton et al., 1999). Small, inactive ovaries of different forms dominate the clinical picture of these acyclic females (Hermes et al., 2001a,c, 2002) suggesting that these females have entered a premature senescence 15–20 years prior to the expected reproductive life-span, due to prolonged periods (decades) of follicle wastage. As the reproductive system is spent, the aging process becomes irreversible and it is, therefore, not surprising that treatments such as changes in the social environment or various hormonal regimens have failed to re-establish a normal ovarian activity in acyclic female white rhinoceroses (Schwarzenberger and Walzer, 1995; Czekala personal communication). Another important consideration is that exoge-

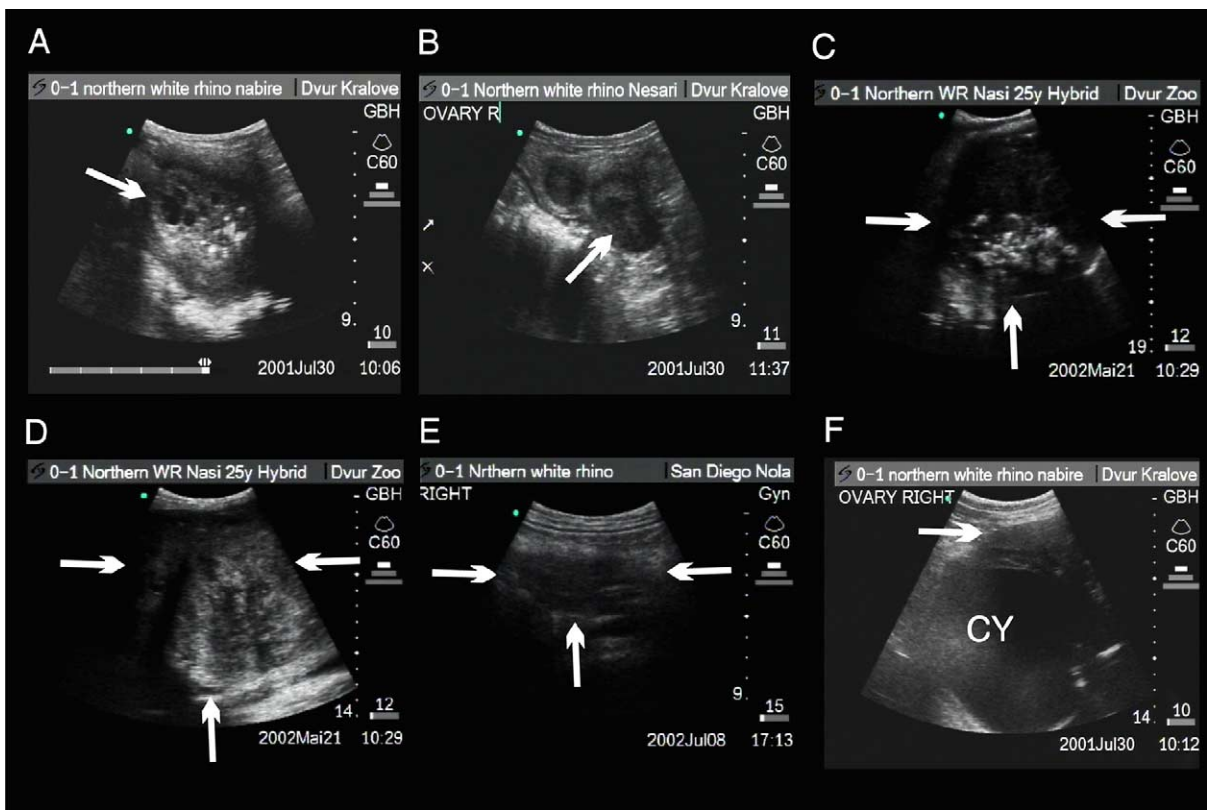


Fig. 2. Sonograms of genital lesions from five captive, non-reproducing female northern white rhinoceroses: (A) Advanced stage of cystic hyperplasia of the endometrium ($n = 3$). Cysts (arrow) and hyperechogenic fibrotic tissue dominate the endometrial appearance; (B) medium sized uterine tumor (arrow) situated in the wall of the right left uterine ($n = 2$). The leiomyoma shows a lower echogenic pattern when compared to the uterus ($n = 1$); (C) advanced stage of a uterine leiomyoma (arrows) with central hyperechogenic necrotic areas; (D) body of a cervical polyp situated in the vagina; (E) inactive ovary of female showing long term acyclicity ($n = 5$); and (F) ovarian cyst (cy) on the contra-lateral atrophic ovary ($n = 5$).

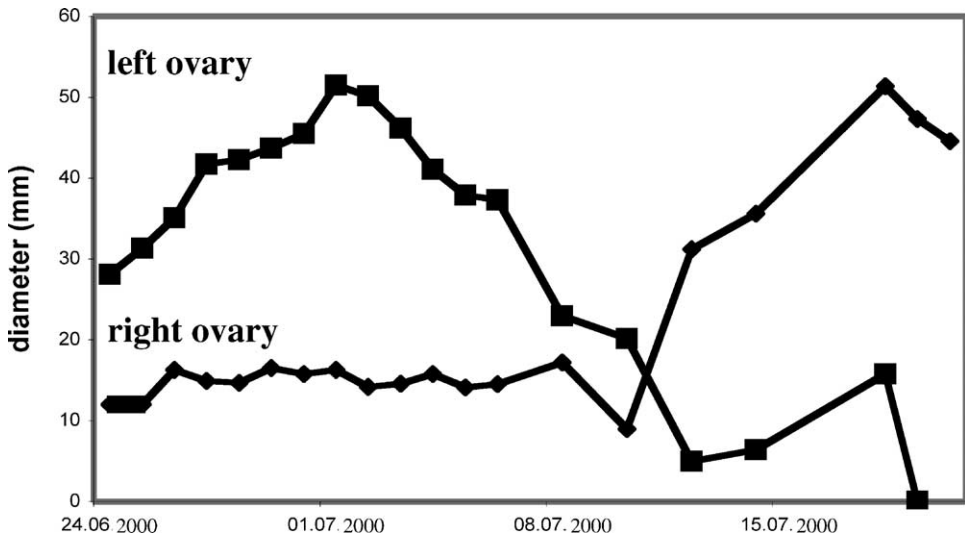


Fig. 3. Two week interval of a dominant follicle development in subadult, non-cycling white rhinoceroses 3–10 years old ($n=9$).

nous hormone treatments applied without prior assessment of reproductive anatomy and endocrinology, bears with it the risk of further progression and development of pre-existing reproductive lesions.

If, however, ovarian activity can be induced by hormone treatment (Schwarzenberger and Walzer, 1995), the chance for successful conception late in the reproductive life-span can have many potential challenges. For example, inexperienced or unproven rhinoceros bulls may not be able to detect induced estrous cycle activity in previously non-cycling females. Moreover, in females with advanced age, decreased oocyte viability and uterine factors contributing to reduced receptivity may occur. In women, laboratory and domestic animals, infertility and early pregnancy loss are largely attributed to reduced oocyte viability due to age-related changes within the aging oocyte. Less viable ova lead to a decrease in oocyte fertilization rate or polyspermic fertilization, as well as a reduced ability for embryo hatching, implantation, and embryogenesis. Aged uterine tissue has a decreased capacity to utilize steroids; therefore, less efficient uterine function can contribute to a failure to establish pregnancy. Accumulatively, these factors lead to a dramatic decrease in pregnancy potential demonstrated in a broad range of species with increasing age (Hinrichs, 1997; Sauer, 1997).

Another important factor for consideration is that the corpora lutea on ovaries of aged females are less likely to support and maintain pregnancy due to insufficient progesterone production. In black and Sumatran rhinoceroses, supplementation of progesterone has been reported to prevent recurring pregnancy losses (Schaffer and Bryant, 2001; Roth, 2002) thereby counteracting insufficient progesterone production by the corpora lutea or possibly by the placenta. In summary, natural breeding or assisted reproduction attempts are less likely to succeed in older female rhinoceroses with a history of long non-reproductive periods.

3.2. Acyclicity in elephants

Different from rhinoceroses, elephants have apparently developed a natural mechanism to prevent the asymmetric aging of reproductive organs. Low ranking females or the matriarch of an elephant herd may enter an acyclic period temporarily (Brown et al., 1999, 2000). As a response mechanism to changes or challenges in their social environment, captive female elephants on breeding loans to other zoos were reported to cease their reproductive cycle activity (Oehrke, personal communication). A long-term cessation of estrous cycles have also been associated with elephants who are subjected to intense physical activity, such as in circuses or work camps, or when social positions within the herd are challenged thereby creating undue stress. Although these females are temporarily non-reproductive, the positive aspect of this acyclicity is the termination of the asymmetric aging process. The temporary absence of circulating ovarian hormones prevents steroid-dependent tumor development or cystic hyperplasia thus pausing the reproductive aging process. The asymmetric reproductive aging process remains delayed until the estrous cycle activity is resumed and the aging clock is restarted, thus prolonging the reproductive life-span of these animals, compared to that of cycling non-reproductive female elephants. In elephants with a history of late puberty or long periods of temporary acyclicity, the incidence of genital pathology is reduced, as seen in cases of early pregnancy (Hildebrandt, personal communication). Comparably, induced suppression of hormone secretion by the hypothalamus–pituitary axis in rats early in their reproductive life inhibited the aging of tissues and delayed the development of pathology (e.g., tumors) and prolonged their life-span (Everitt and Meites, 1989).

4. Pregnancy as a prevention for reproductive disorders

To prevent this asymmetric reproductive aging process and the subsequent shortened reproductive life-spans, establishing pregnancies in young animals by either natural breeding or, if necessary, by means of assisted reproduction, seems imperative. Tribal women indigenous to South America have on average 110 estrous cycles during her reproductive lifetime with high parity lactation, late menarche and lower incidences of reproductive lesions. In comparison, today's women in developed countries have longer non-reproductive periods with an estimation of approximately 400 estrous cycles (Coutinho and Segal, 1999). It appears that one or multiple pregnancy and long periods of amenorrhea can be regarded as an effective physiological approach to tumor prevention. Pregnancy in younger women reduces significantly the risk of developing breast cancer by protective differentiation of ductal epithelial cells and by maternal stimulation of the immune system with the enhancement of the tumoricidal effect of cytotoxic T-cells (Russo and Russo, 1994). The risk of uterine leiomyomas is greatly reduced in multiparous women and rats from >70% to 10% (Walker, 2002).

The positive effects of pregnancy in humans seem similar to what occurs in captive megavertebrates. Considering that rhinoceroses and elephants in the wild are either pregnant or lactating during the greater part of their reproductive life, it is not surprising that reproductive tumors or cystic hyperplasia are relatively non-existent in the wild. In cap-

tive, parous elephants and rhinoceroses, the incidence of tumors and endometrial cysts is significantly smaller compared to similarly aged nulliparous females (Hildebrandt, unpublished data). Case reports of old, primiparous rhinoceroses and elephants show that these animals maintain their capability for conception until late in their reproductive life despite minor pathological lesions (Ochs and Frädrieh, 2001; Hermes et al., 2002). In summary, the human model suggests that pregnancy early in the reproductive life should be regarded as a valid physiological approach to the prevention of reproductive pathology in captive megavertebrates.

In order to obtain a self-sustaining captive population despite aging founders, breeding management might increasingly rely on the development of assisted reproduction technologies (Olson and Wiese, 2000). The successful reproduction of a few genetically valuable founders might further enhance the limited genetic pool of wild populations in future reintroduction programs (Hildebrandt et al., 2002), although habitat protection is the most important task for the conservation of any species in the wild. However, to obtain higher pregnancy rates in any non-domesticated species by assisted reproduction requires further development of species-specific technologies and protocols (Hildebrandt et al., 2000a, 2002).

5. Problems associated with assisted reproduction in aged animals

Even if proper management and medical intervention succeed in establishing pregnancies in older females for the first time in their lives, dystocia, maternal aggression and inexperience in maternal/infant care are problems of concern. In elephants, all cases of dystocia with medical intervention (with one exception) have been reported in females older than 24 years of age (Lange et al., 1999). Besides a prenatal herpes virus infection, the lack of physical fitness, excessive bodyweight and an inability to adapt to the birthing process have been reported to create dystocia in older, primiparous elephants (Richman et al., 1999). In order to counter the labor-induced risks in older females, pregnancies should be monitored carefully by hormonal analysis, and the animals themselves should be conditioned physically through training and diet by reducing their caloric intake to lose excess weight. Detailed birthing and medical intervention protocols have been developed and are available for older elephants (Flügger et al., 2001; Schaftenaar et al., 2001) to deal with the increasing problems associated with the reproduction of animals after prolonged non-reproductive periods in captivity.

6. Reproductive aging in non-reproducing males

Male rhinoceroses and elephants do not appear to experience the asymmetric reproductive aging and premature senescence as observed in females. More important influences on this matter are the long-term detrimental effects caused by unsuitable social environments, which impact the reproductive performance and fertility (Hildebrandt et al., 2000c; Hermes et al., 2001b,d; Seror et al., 2002, Hermes et al., 2004).

Progressive changes in testicular morphology attributable to ageing, including germ cell degeneration and increased collagen fiber development in the interstitial space have been documented in man, domestic animals, the Indian rhinoceroses and the white rhinoceroses (Gosden et al., 1982; Johnson and Thompson, 1983; Johnson et al., 1987; Endo et al., 1996). In the rhinoceroses bull, changes in testicular morphology can be first detected at 15 years of age using diagnostic ultrasound examinations (Hermes et al., 2001d; Hermes et al., 2004). Despite the age-related morphological changes of the testes, e.g., reduced testis volume, diminished sperm motility and increased percentages of structurally abnormal sperm during the transitional period to reproductive senescence, the reproductive life-span of males still exceeds that of females by years, as previously reported for other species such as the black-footed ferret (Wolf et al., 2000). Whether these age-related testicular alterations are reflected in sperm or ejaculate characteristics is dependent on the species (Plas et al., 2000; Wolf et al., 2000). Some aged male rhinoceroses proved to be the most reliable semen donors during artificial breeding attempts with few structurally abnormal sperm (Hermes et al., 2004). Nevertheless, negative effects of long-term captivity on male fertility are commonly found in rhinoceroses and elephants. This is presumed to be derived from social stress or intense physical activity (Hildebrandt et al., 2000c; Hermes et al., 2001d; Hermes et al., 2004). Further investigations into sub-fertility and infertility in males due to individual mate preferences, female dominance, multiple male or bachelor herd management, are needed.

7. Conclusions

Prolonged non-reproductive periods during long-term maintenance of female rhinoceroses and elephants in captivity, are associated with an asymmetric reproductive aging process and subsequent development of genital pathology and premature senescence. Historically, animal managers were not aware of the potential for genital pathologies or the accelerated aging process in captive females because of regular ovarian cycle activity and overtly absent clinical signs of reproductive disorders. However, captive management programs have now acknowledged the usefulness of new technologies including ultrasonography and endocrine monitoring to evaluate older, non-reproductive individuals before breeding recommendations are made and resource are spent (American Zoo and Aquarium Association, *Species Survival Plan*, 1997; European Association of Zoos and Aquaria, *Elephant Studbook*, 1999; Hildebrandt et al., 2000a). Reproductive aging associated with long-term captivity warrants further investigation and the development of an appropriate counter measures such as assisted reproduction technologies. It is recommended that pregnancies should be established early in the reproductive life of a female to provide natural protective mechanisms that operate to guard against the asymmetric reproductive aging processes and premature senescence in order to protect these endangered species.

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