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COMMENTARY

"Use it or Lose it": Characterization, Implications, and Mitigation of Female Infertility in Captive Wildlife

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Zoos and other ex situ wildlife institutions can play an important role in species conservation by maintaining populations for education and research, as sources for potential re-introduction or reinforcement, and as ambassadors for financial support of in situ conservation. However, many regional zoo associations are realizing that current captive populations are unsustainable, with many programs failing to meet demographic and genetic goals to ensure long-term viability. Constraints on population size due to limited space often mandate delayed and/or less frequent breeding, but for females of many species this can have profound effects on fertility. A retrospective analysis combined with published literature and reliable anecdotal reports reveals that, when females are housed in a non-breeding situation for extended periods of time, reproductive changes that negatively impact fertility have occurred in multiple species, including canids, elephants, white rhinoceros, Seba's bats, wildebeest, stingrays, and some felid species. Competing space needs and changing interest in taxa for exhibits over time compound the problem. Counter strategies to breed early and often have their own demographic and genetic consequences as well as logistical and political implications. Strategies to mitigate the sustainability crisis in these taxa might include a mixed strategy in which young, genetically valuable females are bred earlier and at more regular intervals to ensure reproductive success, in combination with the judicious use of available tools to manage the number of offspring produced, including contraception and culling. An understanding of the issues at stake is the first step towards developing management strategies for sustainable populations. Zoo Biol. 33:20–28, 2014.

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INTRODUCTION

Zoos and other ex situ wildlife institutions have played a critical role in the conservation of many species (Hoffmann et al., 2010). As the rate of species endangerment increases, zoos are likely to have an even greater role in the future by managing populations for a variety of conservation benefits, including as assurance populations, potential sources for reintroduction or reinforcement, education and research opportunities, and ambassadors for financial support of in situ conservation activities (Traylor-Holzer et al., 2013). The intensive population management required relies on periodic transfers of animals between institutions and reliable reproduction to promote population health. However, regional zoo associations globally are facing the unsustainability of many captive populations, with most breeding programs not reaching their demographic and genetic goals for long-term viability (Baker, 2007; Lees and Wilcken, 2009; Ballou and Traylor-Holzer, 2011; Leus et al., 2011; Long et al., 2011; Lacy, 2012). Efforts to address the problem have focused primarily on re-structuring regionally managed programs to prioritize resources and increase options for program participation and expansion,

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including inter-regional cooperation and management. Any resulting additional genetic founder lines and increased population size will benefit a managed species, however, only if reproductive success is reliable and high. For some species, poor reproductive success may be a contributing factor that requires attention if the population is to become sustainable.

The need to improve reproductive success is increasingly being recognized for many managed populations, leading to the exploration of alternative management strategies, such as centralized breeding centers and mate choice options (Asa et al., 2011; CBSG, 2011; Conway, 2011; Sawyer et al., 2011). The path from breeding recommendation to the successful production of offspring can be interrupted at various stages due to a variety of causes, from political constraints (e.g., shipping restrictions, space limitations, non-compliance) to biological ones (e.g., behavioral incompatibility, health issues, inbreeding effects). Historically, evaluation of program success has been primarily outcome-based (e.g., transfers completed, offspring produced) with little or no attention paid to points in the process where breakdown may have occurred. In the United States, the Association of Zoos and Aquariums (AZA) has developed PMCTrack, a web-based database, in an attempt to begin to collect at least some of this information for AZA programs.

Many factors, both managerial and biological, undoubtedly contribute to the failure of breeding recommendations, but the overall fertility potential of individual animals in these programs, key to reproductive success, is rarely a consideration, with a theoretical assumption that every animal has an equal likelihood of reproducing. Often the typical response to failed breeding attempts is to recommend a different breeding partner rather than to identify any biological causes of reproductive failure. Reproductive biologists can monitor reproductive processes, assess and even assist fertility, but most zoo managed programs do not incorporate these measures into their breeding recommendations. One approach to filling this niche within AZA has been the establishment of the South-East Zoo Alliance for Reproduction & Conservation, a consortium of seven United States zoos and wildlife centers (Birmingham Zoo, Dallas Zoo, Disney's Animal Kingdom, Jacksonville Zoo, North Carolina Zoo, Micanopy Zoological Preserve, and White Oak Holdings) working to address species' reproductive potentials by systematically investigating breeding failures across taxa using reproductive technology to maximize fertility. There is growing appreciation for and acceptance of a role for hormone monitoring in evaluating ovarian cycles, but causes of infertility are seldom systematically explored, yet merit attention.

Broadly speaking, females are more likely to experience reproductive failure, primarily because their reproductive systems and the processes involved are considerably more complex than those of males. The emergence of the population sustainability crisis, together with the realization that there appears to be a significant incidence of female infertility among captive wildlife populations, prompted this review of the literature to explore how broadly it may apply across species. Specifically, we looked for evidence of an association between female reproductive history, particularly prolonged periods without producing offspring, and uterine pathology or infertility. Furthermore, because successful reproduction is fundamental to managed zoo populations, we have examined the implications of current population management practices on the reproductive health of females. We then considered the changes required to plan a female's reproductive life to maintain her fertility and the impact of those changes on how populations might need to be managed from both demographic and genetic perspectives. These topics were first presented in a session "Use It or Lose It: How Reproductive Management Affects Population Sustainability" at the 2011 AZA Annual Conference.

REPRODUCTIVE CHANGES IN NON-BREEDING FEMALES

It would be difficult and costly to proactively study the effects of non-breeding on the reproductive health of diverse endangered species, but the following retrospective studies and preliminary data contribute to our hypothesis that nulliparity or long inter-birth intervals are detrimental to female reproductive fitness in wildlife species. Animal managers working with domestic hoofstock have long known that keeping females "open" (non-pregnant) for extended periods of time results in difficulty or failure to resume breeding, and that reproductive problems such as dystocia and stillbirths are more likely to occur in aged females that have not previously reproduced or have not reproduced for long periods. This particular problem was highlighted in a Zoo Biology editorial in 1998 (Lindburg and Durrant, 1998), although then, as now, scientific data with sufficient numbers were lacking, relegating this issue to an anecdotal report. It is now emerging that reproductive failure linked to extended periods of non-breeding is widespread among species, the effects of which are only now being realized as many zoo populations are proving to be non-sustainable. Data on the incidence of reproductive pathology in domestic species such as cattle, sheep, pigs, and goats are lacking because they are routinely bred, slaughtered before they are reproductively senescent, or culled when sub-fertile individuals are identified. Therefore, reproductive pathology rarely has a chance to develop.

The negative effects of excess estrogen on the female reproductive tract of many species has been well described: a study in 1957 proved that administration of exogenous estrogen in rabbits caused uterine enlargement, cervical and endometrial polyps, endometrial hyperplasia, pyometra, adenomysosis, leiomyomas, and metastatic endometriosis (Meissner et al., 1957). Similarly in humans, a study on 451 females who were administered exogenous estrogen, compared to 888 control women who were not, showed a six-fold increase in the risk of endometrial carcinoma and a 15-fold increase in risk in long-term users of <5 years, even when administered in a cyclic fashion to more closely mimic a natural cycle (Antunes et al., 1979). Estrogen can stimulate uterine pathology in all species studied, but there are profound species differences in response to progesterone or the synthetic progestins. Although progesterone or progestins can be used to counter the effects of estrogen in the primate uterus, they exacerbate the stimulatory effect in felids and canids and probably in other carnivore species as well (Asa and Porton, 1991).

Prospective studies in multiple species would be challenging to conduct to examine the effects of breeding versus non-breeding females and potential effects on fertility. However, numerous examples and anecdotal reports combined lend strong support to the "Use it or Lose it" hypothesis, as will be seen in the following examples.

Canids

The AZA Wildlife Contraception Center (WCC) recently completed a study of the factors associated with uterine pathology in the seven canid species in AZA-managed programs (Asa et al., this issue). The primary intent was to assess possible effects of contraceptives, but the use of contraceptives was compared to other variables of reproductive history, including number of litters produced and years neither producing litters nor contracepted. The results revealed that some contraceptive methods were associated with higher incidence of uterine pathology, but that was also true for the number of years separated from males instead of using contraception. That is, not allowing a female to reproduce, whether through separation or through contraception, could increase her risk of uterine pathology. That outcome is important, of course, in terms of a female's health, since uterine infection (pyometra) can be fatal if not diagnosed soon enough. However, other forms of uterine pathology, such as endometrial hyperplasia, may not have a direct effect on the female's health but can compromise fertility by interfering with implantation or pregnancy maintenance. In either case, the female may be lost to the breeding population, which can have demographic and genetic implications for the population.

Elephants

The incidence of cystic endometrial hyperplasia (CEH) in reproductively aged Asian and African elephants (*Elephas maximus indicus*; *Loxodonta africana*) shows that Asian elephants are particularly susceptible to CEH, with 67% of Asian elephants ($n = 18$) aged 26–57 years showing CEH, all but one of which was nulliparous while the remaining individual was primiparous (Agnew et al., 2004). An apparent reproductive protective effect of parity in elephants was mentioned in a recent survey that showed that overall for Asian and African elephants reproductive cyclicity was 24.1% higher in females that had one or more pregnancy within the last 15 years compared to cows that had not been bred (Dow et al., 2011). Placentation in the elephant is similar to that of carnivores in that it is endothelialchorial and zonary with a uniform number and thickness of endometrial glands, and it has been suggested that this may reflect similar hormonal control of endometrial growth and provide a rationale as to why elephants appear to be more susceptible to reproductive pathology like carnivore species versus other ungulate species that rarely show CEH (Agnew et al., 2004).

White Rhinoceros

A comprehensive paper on white rhino reproductive pathology demonstrates that a free-ranging wild female white rhino would typically experience only 30–90 estrous cycles in a lifetime, and the rest of the time would be pregnant or in lactational anestrus. In contrast, captive non-reproducing females might experience over 300 estrous cycles, exposing them to an estrogen-dominant physiological environment 10 times more frequently. This in turn is thought to result in a premature, progressive development of reproductive pathology and concomitant reduced fertility, generally described as asymmetric reproductive aging (Hermes et al., 2004). An increase in leiomyomas and/or CEH represents the most common reproductive lesion in female rhinoceros and elephants, yet their incidence is <10% in reproducing multiparous white rhinoceros (Hermes et al., 2006). Similar lesions have been reported in the Northern white rhino, with five of six observed lesions demonstrating leiomyomas, CEH, and chronic endometritus, rendering the population infertile and functionally extinct.

Seba's Bats

A retrospective survey of necropsy records from three populations of Seba's bats housed as all-female groups for 5 or more years, revealed a high incidence of reproductive disorders, particularly endometrial hyperplasia (Napier et al., 2009). Of 169 females, 27 (16%) were reported as having uterine abnormalities on necropsy, and further histopathology revealed abnormal uterine pathology in 27 of 39 uteri (though it is likely that gross abnormality would have prompted further histopathology of the uterus, potentially skewing the results). Prior to the introduction of a male to the group, the cases of uterine lesions found on histopathology examination were one case in 1997 and 2001, three cases in 2002, and six cases in 2003. Males were introduced in 2004, after which the cases were reported as three in 2004, four in 2005, and none in 2006, 2007, and 2008. The numbers are too low to confirm the protective effects of parity, but proliferative changes in uterine tissue have been shown to be a common manifestation of an estrogen-dominant physiological environment. Interestingly, another finding in this study suggested that an enlarged uterus may have been implicated in acute renal failure by causing pressure and hypoxic insult to the kidney, indicating for the first time that long-term effects of not breeding females may impact other endpoints that compromise health as well.

Wildebeest

Data analysis from one institution reported on a herd of four female wildebeest, which was maintained as an all-female group for 8 years before then adding a male to the group. One female had previously had one calf; the rest were nulliparous. Of the four, two became pregnant but suffered dystocia and had stillborn calves, and the other two showed udder development but failed to give birth to offspring. In this case, it is possible a uterine insufficiency contributed to the dystocia and what may possibly have been two resorptions or abortions (Metzler and Penfold, unpublished data).

Stingrays

Female rays housed in all-female groups in several aquaria have exhibited reproductive disorders, predominantly enlarged ovaries filled with cystic and/or caseous structures (hypothesized to be unovulated follicles), and exuberant uterine responses with over-production of histotroph (a nutritive milk produced by the uterus that is ingested by the embryonic stingray), resulting in loss of appetite, lethargy, and even sudden death. This reproductive disorder has been reported in stingray females of several species at multiple institutions, indicating that this problem may be widespread and presents a significant health problem. In the wild, females would normally experience regular pregnancy and concomitant elevated progestin and low estrogen circulating hormone concentrations. It is hypothesized that in all female groups in captivity, lack of mating and pregnancy results in chronically elevated estradiol. Histotroph and egg production in elasmobranchs are initiated by estradiol, and estradiol in turn is regulated by progesterone (Gelsleichter, 2004). Preliminary data from five female stingrays affected with the above reproductive disorder revealed circulating estradiol was 100-fold higher than in stingrays that did not show any reproductive problems, and progesterone was significantly lower in affected versus unaffected female stingrays (Mylniczenko and Penfold, unpublished data).

Felids

A higher incidence of endometrial hyperplasia in felids has been reported for older felid females with a history of nulliparity and/or progestin contraception (Munson et al., 2002). Detailed keeper notes maintained at White Oak Holdings from 1985 to 2011 on 19 different female cheetahs recorded the total number of observed matings, defined as observed intromission by the male, and the age of the females (Meeks and Penfold, unpublished data). The number of cases of pseudopregnancy and pregnancy were recorded, though it is possible that pseudopregnancies may have been under-reported. Females aged 3–4 years old had a 76% likelihood of producing a litter following breeding, compared to a 53% chance in females aged 5–6 and a mere 9% chance in females older than 7 years. Once a female cheetah started to reproduce, she was generally more likely to continue to reproduce, and a couple of individuals continued throughout their lives until about 11 years of age. One female aged 11 years, held at another institution, gave birth to offspring after a history of pseudopregnancies and the supposition that she was likely post-reproductive, demonstrating that there are always exceptions to accepted theories. Although uterine health status was unknown, these results clearly demonstrate the importance of starting to breed female cheetahs when young, in that animals are more likely to reproduce when young and then through their reproductive life than if bred at a more advanced age. This is further confirmed by a study that showed no reduction in fertilization and embryo development rates of oocytes collected from young or old cheetahs, but a significant increase in the incidence of endometrial hyperplasia with age showing 19% in cheetahs 2–5 years old, 50% in cheetahs 6–8 years old, and 87% in cheetahs >9 years (Crosier et al., 2011).

Tigers (*Panthera tigris*) are another species thought to be experiencing a challenge similar to the cheetah. An analysis of factors associated with the success of AZA Tiger Species Survival Plan breeding recommendations ($n = 495$) for three tiger subspecies over 22 years suggests the age and reproductive history of the female to be primary indicators of litter production (Saunders et al., In press). The probability of a female producing a litter within 1 year after being given a breeding recommendation declines after 5–6 years of age. Across all ages this probability is substantially lower for nulliparous females as compared to parous females (i.e., those that have produced offspring in the past). For example, when paired with a reproductively experienced male, an 11-year-old parous female has a 60% probability of success within 1 year, while a nulliparous female of the same age has an estimated probability of success of only 22%. Similar to the observations in cheetahs, the frequency of ovarian cyclicity in tigers was not found to differ with female age or parity. While the cause has not been confirmed and could be a combination of physiological, behavioral, and other factors, the net result is that previously parous females have substantially higher breeding success. Current data suggest that, for tigers, long inter-birth intervals do not appear to be a significant concern, and so attention should be focused on breeding nulliparous females before they become too old and their ability to ever reproduce is significantly jeopardized.

In contrast, the retrospective study of canids (Asa et al., in press) did not find age of first reproduction to increase the risk of uterine pathology in most species, but instead risk was associated with the number of reproductive events throughout a female's life. It is important to recognize potential species differences in response, but the overall observations suggest that pregnancy can be protective against infertility and uterine pathology.

Naked Mole Rats

Lastly, an unusual species whose reproductive strategy supports our hypothesis is the naked mole rat (*Heterocephalus glaber*). This eusocial species has only one breeding female in a colony and the remaining females do not breed and are effectively maintained non-pregnant, yet to the best of our knowledge there is no increased incidence in uterine cancers and pathology. It is admittedly difficult to obtain necropsy data on a small fossorial, colonial species that prefers warm temperatures, as rapid tissue decomposition reduces necropsy data, but endocrine data reveal that non-breeding females have reduced basal LH and a reduced response to a GnRH challenge compared to the breeding female who has higher circulating estradiol and progesterone concentrations (Faulkes et al., 1990). This indicates that non-breeding females may be reproductively quiescent, which may reduce their exposure to gonadal hormones and any associated risk of uterine pathology.

STRATEGIES TO MITIGATE EFFECTS OF "NON-BREEDING"

Contraception potentially can provide a management tool to help improve the sustainability of zoo populations. Progesterone, as experienced during pregnancy, can reverse the effects of endometrial hyperplasia and protect against endometrial cancers in humans (Gambrell et al., 1983). The mechanism behind how progesterone counters estrogen effects includes changes in enzyme activity as well as changes in steroid receptors in the endometrial tissue (Gambrell et al., 1983). So theoretically, administration of exogenous progesterone in some species might be one way to mitigate long-term exposure to estrogen.

However, in contrast to the protective effect of progesterone on the uterine endometrium of many species, in carnivores progesterone can exacerbate the deleterious effects of estrogen (Asa et al., this issue). Thus, treatment of these species with progestins would be counterproductive and actually increase the incidence of pathology. Instead, a complete suppression of both estrogen and progesterone is necessary, such as with the GnRH agonist Suprelorin, especially if the initial stimulation phase is avoided by treatment with a product such as Ovaban (Asa et al., this issue). Because repeated exposure to gonadal hormones without an intervening pregnancy is associated with reproductive pathology in so many species, we believe that extended periods without pregnancy in many wildlife species in captivity may be causing uterine changes that result in subfertility or infertility.

Until now, the fact that some previously contracepted females subsequently failed to reproduce has been underscored, but the same attention had not been paid to non-contracepted females that were not bred, giving a potentially misleading impression that only contraception can lead to subfertility or infertility. However, evidence is mounting that repeated non-conceptive cycles may carry as much risk as the use of some contraceptive products. An approach that could help maintain female fertility would be to alternate production of offspring with periods when conception was prevented either by separation or contraceptive treatment, rather than clustering breeding recommendations early or late in a female's life.

More routine monitoring and additional research on the effects of contraception in a broader range of species is needed if we are to continue to improve contraceptive options. As the canid reproductive pathology analysis demonstrates, the use of contraceptives must be compared to other approaches for controlling reproduction, especially separation. The AZA WCC and its partner, the Reproductive Health Surveillance Program based at Michigan State University, can serve as centers for compiling and analyzing data and reproductive tissue samples (see www.stlzoo.org/contraception for details of these programs).

Separation and contraception are two strategies to restrict breeding. Ultimately, if increased breeding is the best solution to promote fertility and reproductive success, then how animal managers deal with the resulting increase in offspring production poses another challenge.

BALANCING OFFSPRING PRODUCTION WITH DISPOSITION FROM A CURATORIAL PERSPECTIVE

Zoo curators have always known that they need to breed animals to fill exhibits and maintain genetically and demographically stable populations of animals for the long term. Breeding species also serves as a strong reminder of what zoos can do for conservation, be it through propagation of individuals for reintroduction, scientific study of animals, or supporting education efforts to increase biological and conservation literacy. Recent findings suggesting that interruption of breeding for extended periods of time can jeopardize future fertility should further reinforce our focus on breeding animals regularly.

If breeding animals has always been a focus in zoos, why do they find themselves in a sustainability crisis? Space is the primary reason. There are not enough spaces available to maintain populations large enough for long-term viability for all of the species currently in zoos. Related to this, space allocated to a given species is subject to a number of biological, management, and non-biological factors. Among mammals, ungulates, nocturnal species, and small mammals seem to be particularly vulnerable to changing priorities in space allocation in zoos. For example, in the United States over the last 10 years, the AZA Taxon Advisory Groups (TAG) for ungulates estimate that nearly 1,000 ungulate spaces have been lost in AZA institutions. Similarly, interest in creating nocturnal exhibits and working with small mammals has decreased significantly. What is also very worrisome is how fickle interest is in certain species that are powerful icons of what zoos can do for conservation. Both Arabian oryx (*Oryx leucoryx*) and Przewalski's horses (*Equus ferus przewalskii*) were only saved from extinction due to the work of zoos with their own and private collections, yet interest in these species has dropped significantly.

How then do curators balance the need for breeding species with the challenges of offspring disposition when space is limited? There are some biological realities to grapple with. Most species are reproductive throughout their adult lives in the wild, where survival is generally lower and lifespan shorter. Reproductive potential is generally higher in captivity than what is needed to maintain a population at a fixed size. Most mammals are polygynous, and typically at least 50% of the offspring born are male, often leading to a population of non-breeding males. Finally as discussed above, accumulating evidence suggests that preventing reproduction by any means carries some level of risk to future breeding.

Current options for managing the production and disposition of offspring can be distilled into five practices: (1) institutions can temporarily or permanently suspend or delay breeding animals; (2) they can breed and keep the offspring at their institution forever ("breed and keep"); (3) they can breed and try to place offspring in other regional association accredited facilities ("breed and place"); (4) they can work with non-accredited partners to increase space and keep more individuals breeding ("breed and send out"); or (5) they can opt to cull (humanely euthanize) animals. Each of these options has benefits and implications for the individual animals, the population, and the institution, which are discussed below.

A commonly used approach for managing production and disposition of offspring is to temporarily or permanently delay or suspend breeding of certain individuals with a long-term view of being able to breed the population at the facility in the future. This practice can buy an institution some time for determining the disposition of offspring already produced. This broad category of practices includes chemical, hormonal, or mechanical contraception, permanent sterilization, separation of the sexes, and removal of environmental conditions for breeding. All of these options can alter or in some cases eliminate natural physiological and behavioral processes or result in animals living in unnatural social groupings, which could be argued to negatively impact welfare. These practices also carry some risk to future reproduction or the manager's ability to predict when the animal will be able to breed again. The choice to sterilize must be very carefully thought out before it is implemented, as sterilized individuals cease to contribute to the population genetically and demographically yet continue to use institutional resources.

The alternative approach—production of additional offspring—leads to its own problems regarding the options for their disposition. There are not many situations where an institution can pursue a "breed and keep" strategy only. Eventually contraception, sterilization, placement of individuals, mixed-species exhibition, time-sharing in exhibits or formation of additional groups, which means creating space, is necessary to maintain a breeding population.

Breeding and placing animals at accredited institutions (within or between regions, e.g., AZA, EAZA, JAZA, etc.) is the default option in many cases. However, for many populations that are at or near carrying capacity in their region, these transfers may not happen in the timeframe that allows the institution to keep breeding. The institution may need to become the kind of facility that can hold offspring beyond sexual maturity, again suggesting that zoos need to create more space. Even if the zoo can house sexually mature offspring, it may have to suspend or delay breeding by some means.

With the new AZA animal management program designation system in North America, it now is easier to work with non-accredited facilities, but doing so does require more caution and responsibility on the part of the sending institution. Regardless, the private sector can be a very valuable resource in terms of space and in some cases could be the deciding factor in the sustainability of the population. Best practices for engaging with the private sector are beyond the scope of this commentary, but successful models do exist for a variety of species.

The last option, which is also considered by many to be the most difficult to pursue, is humanely euthanizing unneeded animals for management reasons. The level of complication involved in using this tool varies by taxon and by age of the animal being considered for euthanasia. Concern is also raised about the welfare of culled animals, but culled animals do not *experience* reduced welfare compared to living animals, unless they are culled inhumanely. Welfare reflects a combination of positive and negative mental, physical, and emotional states that are co-dependent and vary over time (AZA Animal Welfare Committee, 2012, http://www.aza.org/Membership/detail.aspx?id=378). Longevity of an animal does not translate into "better welfare," as welfare is not a cumulative characteristic for the individual. Thus, culled animals do not have "less welfare" than animals that are not culled. Some argue that there are welfare considerations for conspecifics and relatives of the culled individual. It is true that some animals express distress at the loss of offspring, but it is also true that this happens regularly in the wild, due for example to predation, dispersal, or disease. It also appears to be the case that animals experience only temporary distress when offspring are removed, die, or disperse. Whether to cull at birth, age of dispersal or weaning, or some other time is an institutional decision. Arguments could be made for culling neonates, as this is an age when mortality is often high in the wild. This would possibly prevent keepers or visitors from becoming attached to surplus individuals, although it would not give females any parental care experiences. Conversely, culling at the age of dispersal would mimic another critical event in a wild animal's life. Mortality tends to be common at dispersal, and in many cases parents are weaning their offspring, forcing them away from the natal territory or group, or severing the bond with offspring during this period. Thus, culling at this age would allow females to experience parental care, might minimize distress to conspecifics, but might also be more difficult for animal care staff and visitors. It is important to acknowledge that culling does allow individuals to express a wider range of natural behaviors (courtship, mating, parental care) than do non-reproducing animals. Culling keeps animals in a natural reproductive state longer, by permitting more regular use of reproductive organs and processes, and allows managers to control age structure and sex ratio in the population.

Culling is already in practice in many AZA institutions. A confidential phone survey of 33 AZA institutions was conducted to get a sense of current practices in the AZA community regarding culling. Those contacted included zoos in many regions of the United States, urban and rural zoos, large and small zoos, as well as private and city- or state-funded zoos. Fifty-two percent of the institutions contacted already had culling as part of their institutional disposition policy, while 33% did not. Other institutions had a policy for culling only certain taxa (e.g., fish or rodents) or developmental stages (e.g., eggs) or were planning to add it to their policy. Overall, 45% of institutions reported that culling was currently in use, and an additional 23% reported having used it in the past. In this sample, 79% of institutions were currently using culling for mammal populations.

Culling is also practiced in European and Australian zoos. In many European countries there is a strong belief in allowing zoo animals to live as natural a life as possible, and they believe that it is better for the welfare of an animal to express as much of its natural behavior as possible in captivity, including courtship, mating, birth, parental care, and living in appropriate social groups. They also believe that breeding maintains reproductive health. For these reasons, many institutions pursue a breed-and-cull strategy. Culling occurs across a variety of taxa including ungulates, carnivores, and primates, but there are country-by-country differences. The European zoo community feels this is an ethical issue, not an animal welfare issue and recently approved a euthanasia policy that clearly outlines their rationale for culling and principles for its use (http://www.eaza.net/about/Documents/EAZA%20Euthanasia%20statement.pdf).

They have ethical objections to sending animals into the private sector or limiting the kinds of behavior the animals are able to express when on contraception or separated by gender. AZA institutions have generally found these practices to be less objectionable.

One of us (D.P.) analyzed a number of ungulate populations in common between AZA and EAZA to determine whether culled populations appeared healthier demographically and whether culling affected sex ratio of populations. Comparing age pyramids from studbooks and published masterplans for populations that are over approximately 100 individuals, the age pyramids often appeared more stable in the EAZA populations. Additional space very likely helps; there are approximately 100 more EAZA member institutions than AZA institutions. In comparing 13 ungulate populations between the two regions, the AZA populations were more male-biased than the EAZA populations in 60% of species, and in these species, AZA was on average 10% more male-biased than EAZA. In those species where EAZA populations were more male-biased, the bias was on average only 4%. An analysis of the data showed that on average, an AZA population manager has to manage 11 more males per population than his or her EAZA counterpart in this sample of ungulates. Thus, culling could allow managers to better address gender imbalances in AZA populations in addition to promoting sustained fertility and better welfare.

It is clear that whatever tools an institution or manager decides to adopt, more time, resources, and energy need to be devoted to fulfilling our obligation to manage *populations* of animals for long-term sustainability. Maintaining fertility through regular reproduction can contribute greatly to sustainability. When deciding to interrupt breeding via contraception or separation for any prolonged period, consideration of future breeding needs in the larger population and a conservative approach are recommended. Regular reproduction likely will mean that more offspring could be produced than are needed for our populations. Zoos should find partners in the private sector and begin developing relationships with more of them now. Finally, zoos need to consider culling as part of their tool boxes for population management and prepare themselves to use it.

IMPLICATIONS FOR POPULATION VIABILITY AND MANAGEMENT

If reproductive success is indeed compromised in some species by delaying reproduction, what are the consequences for population viability? And what are the consequences of various population management strategies that might be considered to counteract this phenomenon?

Low reproductive success can have demographic and genetic effects in all phases of a species management program. Beginning with the initial founders, low reproductive success can cause poor and uneven genetic representation of wild-caught founders, forever limiting the potential gene diversity of the population. During the early years of a program low reproductive success can limit population growth, causing the population to remain small and therefore more vulnerable to stochastic effects, such as skewed sex ratio, uneven reproduction, and genetic drift, in turn leading to instability and more rapid loss of genetic diversity. Difficulty in breeding genetically valuable individuals limits the benefits that can be gained from genetic management, also increasing the rate of loss of gene diversity.

Low reproductive success during the founding and growth phases may be less likely due to the effects of delayed reproduction (provided that space is available to grow the population) and more likely due to other factors (e.g., husbandry, nutrition, social groupings, temperament). Managers are more likely to delay first reproduction and/or extend the inter-birth interval when the population has reached its current capacity or target population size and relatively little reproduction is needed to maintain the population. Fewer breeding recommendations are made and are often focused on individuals of genetic value. At capacity individuals have fewer breeding opportunities throughout their lifetime and may not be recommended to breed until well into their adult years. It is in these situations that the impacts of delaying or extending reproduction are most likely to occur.

Populations at capacity are, by necessity, managed tightly. Too many offspring, and zoos quickly run out of room, while too few offspring can lead to sparse or empty exhibits. Predictability of the expected number of offspring from a set of breeding recommendations becomes important. The effects of delayed reproduction can make reproductive output less predictable unless carefully understood, monitored, and taken into consideration when breeding recommendations are made. Lower success rates mean that more recommendations are needed to produce the desired number of offspring, introducing more stochastic variation in the number of actual offspring produced. There are genetic consequences as well—more breeding recommendations means that managers must select more breeders and use animals lower on the mean kinship list. This means that the average genetic value of the offspring is lower than if the same number of offspring was produced by fewer breeders higher on the mean kinship list. Genetic management is less effective, and gene diversity is lost more rapidly.

Depending upon the cause and species, a variety of management strategies can be effective in improving reproductive success, from providing different environmental conditions to offering a choice of mates. To counteract physiological (and possibly behavioral) consequences of delayed reproduction or extended periods without reproduction, however, the obvious strategy is to breed animals earlier after sexual maturity and/or more often. Potential benefits to this strategy include faster growth to the target population size, better retention of founder gene diversity, potentially more effective genetic management (due to fewer breeding recommendations), and increased demographic stability. Drawbacks include the shortening of generation time (the average age of reproduction), meaning that gene diversity will be lost more rapidly, and lack of space within the program for the additional offspring produced.

Removing these surplus offspring from the managed population, either by placement in institutions outside of the program and/or by culling, offers more benefits for population viability. The ability to select which individuals to remove allows managers to manipulate sex ratio and age structure and maintain the desired population size. It is also possible to reduce or even negate the effect on generation time by removing individuals from early litters and retaining offspring from subsequent litters to lengthen generation time. Essentially the net effect on population size and genetics is as if surplus offspring were not produced. This strategy also is less likely to impact other species programs as extra space is not needed within the regional association institutions. Although placement outside of the program and culling may be viable options for some species, their utility is limited for now for those management programs within AZA and many other regions facing this dilemma. For many species, there is an immediate challenge of how to manage within the allotted space for the species to counteract low reproductive success resulting from delayed or infrequent reproduction.

How can species managers balance the benefits of increased reproductive success by breeding young females with the effects of shorter generation time? At what point does early and/or frequent breeding lead to improved viability through increased demographic stability and growth and increased ability to breed genetically valuable individuals? Each species is different in terms of its reproductive biology, lifespan, and requirements, and each population is different in terms of its genetic characteristics and the relative spread of relatedness (variation in mean kinship values) among its members. Therefore, the most effective strategy for balancing these conflicting issues also will likely differ.

A mixed-breeding management strategy is being considered for tigers within AZA as a compromise between genetic management and reproductive success that proposes a two-step process for selecting breeding pairs. The first step would involve pairing all reproductively naïve females that meet specific age and genetic value criteria (as an example, those females under 7 years of age and ranked in the top 70% of the mean kinship list). This would place priority on attempts to produce offspring from inexperienced females before their probability of breeding success begins to decline, and hopefully would significantly increase the success of any future attempts to breed them. Additional breeding pairs needed to meet population goals could then be formed in the second step by applying stricter genetic management, working down from the top of the female mean kinship list regardless of the age or reproductive history of the female. This second step would by design focus on breeding genetically valuable females that are either older first-time breeders (lower success rate) or experienced breeders of any age (higher probability of success). Employing the first step will hopefully function to reduce the number of older first-time breeders in favor of increased proportion of experienced breeders and larger effective population size.

By varying the age and genetic criteria for females used in step one, the optimal balance between breeding early and applying genetic management can be investigated. These options are being explored through stochastic modeling of ex situ tiger populations for eventual potential application in a masterplan setting. A similar strategy might be considered for other taxa, especially felid species, to maintain breeding potential in females.

CONCLUSIONS

We recognize that the cases presented here do not represent large numbers of individuals nor were part of controlled scientific studies to directly examine the effects of gonadal hormones over extended periods of a female's reproductive lifespan. Nevertheless, the similarities in female reproductive problems observed across taxa form a compelling argument as to why wildlife species in zoos should be bred earlier and/or more frequently than is currently recommended.

Planning a female's reproductive life to maintain fertility should integrate her genetic value, the space available for offspring, and reproductive physiology of her species. Options for limiting the number of young she produces in a lifetime and managing the intervals between births include separation of the sexes and contraception. However, the choice is not a simple one. There are profound species differences in response to the various contraceptive products available for use in wildlife. For example, MGA implants and other synthetic progestins are effective in most species but are associated with serious side effects in carnivores but not in primates (Asa, 2005; Munson et al., 2005). In contrast, Surprelorin implants appear safer, but time to reversal is extremely variable. Separation may be easier for some species or individuals, but as discussed above, allowing females to experience repeated cycles without at least occasional pregnancies can compromise fertility. Data currently available to the AZA WCC and the results of their analysis of canids in particular (Asa et al., this issue) show one contraceptive regimen (Suprelorin plus Ovaban) to be the safest option for maintaining reproductive health.

The negative impacts of delayed reproduction or extended periods without reproduction may be threatening the viability of many zoo managed populations. Counter strategies to breed early and often have their own demographic and genetic consequences as well as logistical and political concerns. These challenges will likely intensify as more species are in need of intensive management and zoo populations reach their allotted carrying capacities and vie for limited space and resources. Understanding this phenomenon in our various managed populations and exploring management strategies to balance these issues will be important to the short- and long-term viability of these populations.

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