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Advances and Challenges in Understanding and Assisting Reproduction of Wild Animal Species

Avanços e desafios na compreensão e reprodução assistida de espécies selvagens

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Abstract

Studying reproduction in wild animals is complex as there are as many biological traits as there are species. What we currently know is minimal compared to the large number of species that remain unstudied. In addition to the impressive diversity of natural mechanisms, other complexities limit the progress of wildlife reproductive science - little interest in animal reproduction, difficult access to animals, lack of expertise, hard working conditions, and insufficient funding. Despite those challenges, some species are being saved from extinction with the help of a precise understanding of reproduction, development of assisted reproductive technologies, and creations of cryo-banks. Those advances originate from huge progresses in non-invasive measurements of steroid metabolites in urine or fecal samples to study and monitor reproductive functions and pregnancies. Progresses in cryobiology also have been impactful in animal conservation. Importantly, emerging technologies (transcriptomics, microfluidics) and additional research areas (reproductive aging, microbiomes) could lead to more successes and address current challenges in the reproduction of rare and endangered species. However, while some emerging approaches like stem cell technologies may sound promising, it is necessary to design holistic strategies considering all available tools to optimize investments, time, and efforts in conservation.

Keywords: Animal conservation, reproduction, biotechnologies, biobanking

Introduction – Why conserving wild animal species and the fundamental role of conservation breeding

Even though it is part of the evolution of life on Earth, extinction is now occurring at a much higher rate because of detrimental human activities (habitat destruction, over-hunting/fishing, and poaching). The International Union for Conservation of Nature (IUCN) estimates that 30% of invertebrates, 20% of fishes, 41% of amphibians, 20% of reptiles, 14% of birds, 25% of mammals, and 55% of plant species are threatened with extinction (IUCN 2020). Many of these wild animal populations are small and fragmented in their habitat with little or no opportunity for genetic exchange. This increases homozygosity and inbreeding that, in turn, leads to a bad adaptive capacity to environmental changes as well as fertility problems (Comizzoli, et al., 2019). Animal conservation aims at understanding and sustaining biodiversity because the disappearance of a single species can compromise the functioning of entire ecosystems (Comizzoli and Holt, 2019). Core activities in wildlife conservation revolve around the maintenance of genetic diversity to ensure sustainability and resilience of animal populations. This can be achieved by protecting species in their natural habitat (in situ conservation), which is one of the highest priorities and involves multiple disciplines (from ecology to social sciences). However, it also is critical to maintain viable populations in captivity (ex situ) for eventual reinforcements or reintroductions in the wild. This latter approach of conservation breeding includes several disciplines as well (from husbandry to cellular biology). Understanding the basic traits of reproductive biology is a non-negligible part of this effort. Currently, there is still little knowledge about reproduction in many animal species (for example, less than 5% in mammalian species; Comizzoli et al., 2019). However, once this knowledge is secured, conservation breeding can be optimized with assisted reproductive technologies (ART) associated with Genome Resource Banking (GRB). These approaches have been widely promoted over the past decades for enhancing breeding management and sustaining small populations of rare and endangered species (Comizzoli and Holt, 2019; Herrick, 2019). The objective of the present article is to review advances and challenges in understanding and assisting reproduction in wild animal species.

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Understanding, assisting, and monitoring reproduction in wild animal species

Studying reproduction in wild species is complex as there are as many biological mechanisms and traits as there are species. The same diversity is true when developing protocols to assist reproduction or freeze cells and tissues. For instance, in wild cat species, many features differ across that family (non-seasonal reproduction vs. breeding seasons, spontaneous vs. induced ovulations, different number of ovulations per estrus cycle, or different gestation lengths) (Thongphakdee, et al.,, 2020). As in other mammals, semen from different cat species displays a spectrum of tolerance to cold shock and freezing/thawing. Although successes in artificial insemination with fresh or frozen semen have been reported in many wild species, the number of births by assisted reproduction is still too low to significantly impact the genetic management of rare animal populations (Comizzoli and Holt, 2019; Herrick, 2019; Mastromonaco and Comizzoli, 2018). Regarding non-mammalian species, reproduction also is highly diverse and remain largely understudied, although there have been promising ART developments for amphibians (Byrne and Silla, 2020). In addition to the impressive diversity of natural reproductive mechanisms in wildlife, other complexities limit the progress of research - little interest in animal reproduction, difficult access to animals, lack of expertise, hard working conditions, and insufficient funding. Fortunately, despite the difficulties, some species are being saved from extinction with the help of a precise understanding of reproduction, development of ARTs, and creations of cryo-banks (best examples being the conservation efforts in giant pandas and black-footed ferrets) (Pierre Comizzoli and Holt, 2019).

Understanding how to monitor and control functions and reproductive stages is critical to breed species either naturally or through ARTs. In terms of monitoring functions of testes, ovaries, or reproductive tissues, non-invasive measurements of steroid metabolites in urine or fecal samples have been critical to understand or assist reproduction in wild species (Brown, 2018; Sontakke, 2018). However, there are marked differences in seasonal, environmental, and social influences on ovarian cycle dynamics, ovulatory mechanisms, testicular activities, and responses to ART, even in closely related species.

Pregnancies can be tracked by endocrine and ultrasound techniques. However, using ultrasonography to check conception rates, presence of embryo implantation sites, embryonic vesicles, and fetal development (after natural breeding, artificial insemination, or embryo transfer) is challenging in wild species. It often requires a sedation, which can be detrimental, especially at the early stage of pregnancy. The other option is to train animals to be safely restrained without sedation during transrectal or transabdominal examinations. This approach requires multiple years of animal training, specialized infrastructures, and a lot of expertise, which is not possible or practical for all species. Nevertheless, a recent report on early pregnancy detection in non-sedated cheetahs via transabdominal ultrasound shows that it is doable and ends up being highly informative (Crosier et al., 2020). Transrectal ultrasonography with proper restrain also has become a routine technique to monitor elephant pregnancies (Thongtip et al., 2009). Lastly, transabdominal ultrasonography in the last two weeks of gestation remains the only way, so far, to confirm a pregnancy in giant pandas (Martin-Wintle et al., 2019). For a vast majority of wild species, the most practical and common method to detect and monitor pregnancies remain the non-invasive assessment of steroid metabolite levels in urine or feces (blood collections are possible but still require a minimum of restrain and are not always feasible). A species by species, and sometimes hormone by hormone, approach is essential for developing effective pregnancy monitoring strategies (Brown, 2018). In some wild ungulate species, it has been possible to adapt pregnancy tests developed in domestic livestock. However, early pregnancy is difficult to detect through non-invasive technique in many wild species (for instance, clouded leopards, cheetahs, or giant pandas) as the progesterone elevation does not allow to distinguish a real pregnancy from a pseudopregnancy. Therefore, new non-steroid indicators have to be identified as it was recently reported in cheetahs (Koester, et al., 2017). For giant pandas, because of the delayed implantation and the absence of indicators, new strategies also are required (Comizzoli, 2020; Martin-Wintle et al., 2019). Importantly, non-invasive monitoring of hormones can help to determine the exact timing of birth (or the end of a pseudo-pregnancy), like in elephants or giant pandas, which is crucial for breeding management and preparation for neonatal care.

Additional areas of research and strategies to enhance wild animal reproduction

Although significant progress has been made in wildlife reproductive science and ART, there is an urgent need for more options for fertility preservation, including systematic collection and preservation of germplasm for the long-term (Comizzoli and Wildt, 2017). For example, ongoing efforts are gearing towards the development of germplasm storage at ambient temperatures, which would greatly reduce the costs and simplify storage as well as exchange of biomaterials (Comizzoli, 2020).

New tools like genomics and bioinformatics have progressively changed the concept of genetic value of individuals (as well as the genetic management of wild animal populations) by exploring gene sequences and alleles in a much deeper and precise way (Johnson and Koepfli, 2014). Transcriptomic/epigenetic analysis also have revealed transgenerational transmission of traits and the impact of environmental changes on wild species (Rey et al., 2020). Importantly, these changes include the impact of endocrine disrupting chemicals (Robaire et al., 2022). All these ‘omics’ approaches are now getting more and more integrated into animal management in the wild and in conservation breeding centers.

Other technologies of interest are microfluidic devices that were recently developed in human or domestic animal reproductive medicine. These tools could increase knowledge in fertility and contribute to the success of ART in wildlife as well. Yet, dedicated efforts are required to meet specific needs in animal conservation (cost-effectiveness, application to multiple species, and field-friendly use) (Le Gac et al.2020).

Studying and overcoming reproductive aging in wild species also is becoming increasingly important, especially in conservation breeding. Interestingly, multiple aging traits are conserved across very different species (depletion of the ovarian reserve or no decline in testicular functions), but unique features also exist (endless reproductive life or unaltered quality of germ cells) (Comizzoli and Ottinger, 2021; Holtze et al., 2021). So far, reproductive aging in wild animal species has been mainly documented at the level of overall reproductive senescence rather than at the germ cell development mechanisms. There is an urgent need to study cellular and molecular characterizations behind reproductive aging observations. There also is a need for more research in male aging across all species (Comizzoli and Ottinger, 2021).

Another emerging dimension in conservation biology is the study of reproductive microbiomes. Communities of microbes have coevolved in animal organisms and are found in almost every part of the body. This emerging research area is highly relevant to conservation biology from captive breeding management to successful reintroduction or maintenance of wild populations. There is a link between microbial communities (within female or male reproductive systems) and fertility, from conception to birth outcome. In addition, it is critical to understanding how reproductive microbiomes are affected by environmental factors (including captivity, contact with other individuals, or changes in the ecosystem) to optimize conservation efforts (Comizzoli and Power, 2019; Comizzoli et al., 2021).

Lastly, we also need to favor interdisciplinary approaches in all species (with bioengineering for instance) to find innovative solutions, develop new tools, and make faster progress in understanding and sustaining reproduction. Importantly, we need to create more exchanges with human reproductive medicine and take advantage of technologies that are now routine in fertility clinics (Comizzoli et al., 2018; Paulson and Comizzoli, 2018).

Conclusions and take-home messages

Although knowledge about reproduction is progressing and more tools are becoming available, there is still of lot of research to conduct to fully understand reproductive biology and physiology of many wild species. The barrier to the successful application of ARTs is not a shortage of new techniques, but rather a fundamental lack of ‘conservation capital’—trained scientists, sufficient numbers of research subjects, funding, and appropriate facilities designed specifically to study and manage nondomestic species.

Aligning technological capability with good animal management and sound conservation principles will make it increasingly possible to apply ARTs to increase reproductive efficiency; to readily transport gametes (sperm, eggs, embryos), raw DNA or genomes to overcome increasingly onerous international animal importation restrictions; to facilitate zoo-to-zoo animal exchanges; and eventually to permit the routine exchange of genetic material between zoo and wild populations. In addition, what is the ultimate value of using ARTs to produce endangered animals if we lack the capacity to manage and sustain these species in the first place? (Monfort, 2014).

While it is important to invest in promising approaches like stem cell technologies or the areas mentioned above, ART combined with sound husbandry and management, appropriate facilities, and parallel efforts to sustain wild populations and places, offers the best chance for conservation success. It also is crucial not to wait for a given species to be on the brink of extinction to start research programs in reproduction, use intensive management, and implement conservation efforts.

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