Review Article

Advanced biotechnologies for wildlife fertility preservation

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Abstract

Reproductive biotechnologies are critical tools for saving and maintaining endangered species. Some successes have been reported with the use and integration of artificial insemination (with fresh or frozen-thawed semen) in conservation programs. However, not a single species is currently managed through oocyte freezing or embryo-based technologies. This is primarily due to the lack of knowledge of species biology, as well as inadequate facilities, space, expertise, and funding needed for their successful application. More fundamental studies of animal reproductive biology as well as more fertility preservation options are needed with all parties involved (reproductive technologists, zoo biologists and conservationists) adopting parallel efforts to sustain wild populations and habitats.

Keywords: wildlife, reproduction, conservation biology, biotechnologies, fertility preservation, cryopreservation

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Introduction

The International Union for Conservation of Nature (IUCN) estimates that 25% of mammals, 12% of birds, 20% of reptiles, 30% of amphibians, 20% of fishes, 30% of invertebrate and 55% of plant species are threatened with extinction (http://www.iucnredlist.org). Many of these wild species populations are small and fragmented in their habitat with little or no genetic exchange which increases homozygosity and inbreeding that, in turn, lead to a bad adaptive capacity for environmental changes and fertility problems (Wildt et al., 2010). In addition to protecting species in their natural habitat (*in situ* conservation), it is critical to maintain viable populations in captivity (*ex situ*) for eventual reintroductions. However, reproduction fitness may be impaired in captivity by small space, health and husbandry problems, non-adapted diets, modified sexual behavior or infertility (Wildt et al., 2010). Therefore, conservation breeding can be optimized with assisted reproductive techniques (ART) to overcome the issues listed above. These approaches have been widely promoted over the past decades for enhancing breeding management and sustaining small populations of rare species (Holt et al., 2014). Besides the techniques of artificial insemination (AI), embryo transfer (ET), and in vitro fertilization (IVF), a wide range of methods and tools have been developed (Comizzoli et al., 2000 and 2012). These include non-invasive hormonal assessment for accumulating fundamental knowledge in diverse species (e.g. ovulatory mechanisms, seasonality, pregnancy, infertility) and manipulating reproductive activities (e.g. superovulation, estrous synchronization). Among these critical tools, germplasm cryobiology has also played a key role in establishing biorepositories for capturing extant genomic diversity (Comizzoli et al., 2012). However, critical knowledge of reproductive traits is first needed before developing ARTs. Unfortunately, very little is known about species biology (reproduction in only 250 species has been properly described) with our efforts still remaining mainly concentrated on mammals and birds (Comizzoli and Holt, 2014).

The objective of the present article was to review (1) existing reproductive biotechnologies to preserve the fertility of wild species populations and (2) emerging technologies associated with the need to change the paradigm that are also critical to solve conservation issues.

Development and use of reproductive biotechnologies for wild species conservation (mammals and non-mammals): For the past 20 years, major progresses in wildlife reproductive science have been made with the help of non-invasive endocrine monitoring (measuring fecal or urine steroid metabolites) to either (1) study reproductive traits such as ovarian cyclicity or seasonality of testicular activity (2) monitor pregnancies (3) assess stress through cortisol level or (4) design the best protocols to enhance fertility or induce ovulation (Holt et al., 2014). Unfortunately, as in domestic species, ovarian response is highly variable and oocyte quality may be impaired by exogenous hormones.

AI is currently the most extensively applied ART. Initial successes were achieved in bovids because of the significant development of ARTs in cattle production. AI has been successfully applied to produce live offspring in 14 species of non-domestic bovids and seven cervid species (Comizzoli, 2015). However, AI has not been integrated in the routine management of endangered ungulates yet. In wild carnivore conservation, the progress in AI is best illustrated by the basic research on ferret reproduction seasonality, semen cryopreservation methods, and laparoscopic AI (Comizzoli et al., 2009). To date, more than 150 kits (60% success with fresh sperm) have been produced by AI, including multiple litters of kits that have been produced from frozen founder sperm stored for as long as 20 years. However, AI in other carnivore species (felids, canids) is far from being routinely used and still has a really poor success rate (Comizzoli et al., 2009).

Thirty years after the first successful interspecies embryo transfer in a wildlife species, there has not been a single example of genetic management based on that technique (Monfort et al., 2014). Success related to the transfer of embryos produced by IVF also remains limited even though this technique (from the oocyte recovery through the IVF with fresh or frozen-thawed semen to the in vitro culture of embryos) is in theory the fastest and most efficient way to propagate small populations. The technical complexity associated with the high procedural costs also limits the development and implementation in conservation programs. In addition, the scarce knowledge of the kinetics of embryo development and foeto-maternal recognition leads to many losses of pregnancies.

For all bird species, successful application of AI still requires pre-emptive research into semen collection and processing (much more complex than in mammals because of the fragile sperm cells), access to sufficient numbers of birds for basic and applied research, baseline knowledge of species biology, and appropriate facilities and expertise (Blanco et al., 2009).

Amphibian unique reproductive patterns and mechanisms, key to species propagation, have only been explored in a limited number of laboratory models. The development of applied reproductive technologies for amphibians has been useful for a few threatened species only. These include non-invasive fecal and urinary hormone assays, hormone treatments for induced breeding or gamete collection, and artificial fertilization (Kouba et al., 2013). The hormonal control of reproduction in amphibians has hardly been studied in comparison with fish and mammals.

Germplasm cryopreservation and genome resource banking efforts: Genome resource banking (GRB) refers to the collection, processing, storage and use of germplasms (sperm, eggs, embryos, ovarian and testicular tissues) and other biomaterials (blood products, DNA samples) that can be used for understanding and sustaining biodiversity. If used properly in association with ARTs, GRB has the potential to decelerate the loss of gene diversity in captive populations by reintroducing original genetic material (without removing genetically valuable individuals from the wild) and decrease the interval between generations (Comizzoli and Holt, 2014).

Semen cryopreservation represents the most extensive effort, with live births reported after AI. Recent progresses in vertebrates have recently been reviewed (Comizzoli and Holt, 2014) which include pioneering studies of endangered gazelles and Iberian lynx (Grade et al., 2003; Gañán et al., 2009). Sperm processing challenges are also illustrated from amphibian to fish studies (Kouba et al., 2013; Torres et al., 2016). Recently, there has been some success in cryopreserving sperm cells from a variety of coral species (*in vitro* production of larvae). Based on that, GRB has been established to help offset these threats to the Great Barrier Reef and other areas (Hagedorn et al., 2014).

Oocyte freezing remains challenging and unsuccessful in wild species and will require more research before becoming a standard procedure (Comizzoli and Holt, 2014). Despite extensive efforts conducted in different wild mammals, not a single individual has been produced from a frozen-thawed egg. In amphibian and fish, the potential for cryopreservation of the female is also challenging, with no offspring reported to date from cryopreserved oocytes. Egg size and structure, and yolk composition appear to create technical barriers to cryopreservation.

As an alternative to fully grown gametes, gonadal tissue preservation has become a promising option in vertebrates (Comizzoli et al., 2012). Ovarian and testicular tissues are systematically banked but the production of mature gametes (through xenografting or long-term in vitro culture) has not happened yet in wild species. In amphibians, the direct cryopreservation of immature ovarian follicles holds promise, but will need to be combined with procedures such as xeno-transplantation to generate mature, ovulated oocytes. Cryopreservation of primordial germ cells also holds promise, but will likely need to be combined with the generation of chimeras to obtain adults that can produce viable gametes (Clulow et al., 2014). This approach also seems to be the future for birds and fish ARTs (Comizzoli and Holt, 2014).

Need for more fertility preservation approaches: Even though the results are not satisfactory using classical ARTs, more fertility preservation options are necessary to save species. It is also worthwhile thinking beyond systematic characterizations and considering the application of cutting edge approaches to universally preserve the fertility of a vast array of species (Comizzoli and Wildt, 2013). Regardless of the specific technology to be explored, new tools will require the significant use of 'models' (usually domestic animals) for comparable wildlife species. This need has been recognized and adhered to for three decades (Wildt et al., 2010). It is essential to consider the practicality of initial testing and application, which will likely require exploration first in a taxonomically related 'model'. Even then, if a certain technique works efficiently in the model, it may require further modifications to be used effectively in the species of interest. Traditionally, close relatives have been selected; for example, domestic cat (for wild felids), domestic dog (for wild canids), red or white-tailed deer (for wild cervids), brushtail possum (for endangered marsupials) or common frogs or toads (for rare amphibians). Finally, there are species that are so specialized that models may be unavailable. Examples of these include elephant, rhinoceros, and giant panda (among hundreds of others), all of which will most likely require direct studies, although based on best available knowledge or predictions from work performed in other species (Wildt et al., 2010).

Filling the gap between technology and animal conservation: The barrier to 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 non-domestic species. Zoo community has been too slow to recognize that current management paradigms are insufficient for sustaining hundreds of species across diverse taxa (Monfort, 2014). Likewise, conservationists have often minimized the role of zoos and resisted biotechnology when their own efforts to stem the loss of biodiversity and wild places have fallen short. Reproductive technologists, zoo professionals, and conservation biologists all have the same goal, which is to save species and the ecosystems they require for survival. Success will require collective efforts to identify extant limitations and fundamental gaps in knowledge, both intellectual and practical, and joint efforts to secure long-overdue improvements.

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 (e.g. elephant AI already serves this purpose); and eventually to permit routine exchange of genetic material between zoo and wild populations (Holt et al., 2014). As Monfort (2014) clearly highlights: 'The justification for a return to building basic knowledge boils down to this: what is the ultimate value of using ARTs to produce endangered animals, or even resurrect extinct species, if we lack the capacity to manage and sustain these species in the first place? If we cannot now sustainably manage an oryx, Eld's deer or cheetah with or without ARTs, then what chance do we have of sustaining resurrected woolly mammoth, quagga or dodo in the future? Our strategy and focus must change or the true potential of ARTs for managing endangered species will never be fully realized'.

Conclusion

The application of reproductive biotechnologies for the preservation of endangered mammalian species is limited by several factors. Obtaining healthy and genetically valuable offspring after AI or IVF/ET depends on the existing knowledge of the reproductive physiology of each particular species; however, little is known about the physiology of most wild animals. Captivity and poorly available biological material (often in disparate locations) also increase obstacles for research progress. The role and relevance of ARTs in contributing to species conservation are inextricably linked to whether or not zoos and conservation centers invest in developing improved understanding of overall species' biology, and reproduction, in particular. Reproductive biotechnologies combined with sound husbandry and management, appropriate facilities, and parallel efforts to sustain wild populations and places, offer the best chance for conservation success. Zoos and conservation centers must adopt such holistic conservation strategies or they risk becoming living museums exhibiting relic species that no longer exist in nature.

References

Blanco JM, Wildt DE, Höfle U, Voelker W, Donoghue AM. 2009. Implementing artificial insemination as an effective tool for ex situ conservation of endangered avian species. Theriogenology. 71(1): 200-13.

Comizzoli P, Mermillod P, Mauget R. 2000. Reproductive biotechnologies for endangered mammalian species. Reprod Nutr Dev. 40(5): 493-504.

Comizzoli P, Crosier AE, Songsasen N, Gunther MS, Howard JG, Wildt DE. 2009. Advances in reproductive science for wild carnivore conservation. Reprod Domest Anim. 44 Suppl 2:47-52.

Comizzoli P, Songsasen N, Hagedorn M, Wildt DE. 2012. Comparative cryobiological traits and requirements for gametes and gonadal tissues collected from wildlife species. Theriogenology. 78(8): 1666-1681.

Comizzoli P, Wildt DE. 2013. Mammalian fertility preservation through cryobiology: value of classical comparative studies and the need for new preservation options. Reprod Fertil Dev. 26(1): 91-8.

Comizzoli P, Holt W V. 2014. Recent advances and prospects in germplasm preservation of rare and endangered species. Adv Exp Med Biol. 753:331-56.

Comizzoli P. 2015. Biotechnologies for wildlife fertility preservation. Anim Front. 5(1): 73-78.

Clulow J, Trudeau VL, Kouba AJ. 2014. Amphibian declines in the twenty-first century: why we need assisted reproductive technologies. Adv Exp Med Biol. 753:275-316.

Garde JJ, Soler AJ, Cassinello J, Crespo C, Malo AF, Espeso G, Gomendio M, Roldan ERS. 2003. Sperm cryopreservation in three species of endangered gazelles (Gazella cuvieri, G. dama mhorr, and G. dorcas neglecta). Biol Reprod. 69(2): 602-11.

Gañán N, González R, Garde JJ, Martínez F, Vargas A, Gomendio M, Roldan ERS. 2009. Assessment of semen quality, sperm cryopreservation and heterologous IVF in the critically endangered Iberian lynx (Lynx pardinus). Reprod Fertil Dev. 21(7): 848-59.

Hagedorn M, Spindler R. The reality, use and potential for cryopreservation of coral reefs. 2014. Adv Exp Med Biol. 753:317-29.

Holt W V, Brown JL, Comizzoli P. 2014. Reproductive science as an essential component of conservation biology. Adv Exp Med Biol. 753:3-14.

Kouba AJ, Lloyd RE, Houck ML, Silla AJ, Calatayud N, Trudeau VL, Clulow J, Molinia F, Langhorne C, Vance C, Arregui L, Germano J. 2013. Emerging trends for biobanking amphibian genetic resources: The hope, reality and challenges for the next decade. Biol Conserv. 164:10-21.

Monfort SL. 2014. Reproductive Sciences in Animal Conservation. 753: v-vii.

Torres L, Hu E, Tiersch TR. 2016. Cryopreservation in fish: current status and pathways to quality assurance and quality control in repository development. Reprod Fertil Dev. (article in press)

Wildt DE, Comizzoli P, Pukazhenthi B, Songsasen N. 2010. Lessons from biodiversity-the value of nontraditional species to advance reproductive science, conservation, and human health. Mol Reprod Dev. 77(5): 397-409.

บทคัดย่อ

ความก้าวหน้าทางด้านเทคโนโลยีชีวภาพสำหรับการเก็บรักษาความสมบูรณ์พันธุ์ของสัตว์ป่า

ปิแอร์ โคมิซซูลี่

เทคโนโลยีชีวภาพทางระบบสืบพันธุ์ เป็นเครื่องมือที่สำคัญในการอนุรักษ์และรักษาสัตว์ป่า ใกล้สูญพันธุ์ให้คงอยู่ต่อไป ถึงแม้จะมีการรายงานความสำเร็จมาบ้างแล้ว เช่น การใช้และการผสมผสานการผสมเทียม (ทั้งน้ำเชื้อสดและน้ำเชื้อที่ผ่านการแช่แข็งและทำละลาย) เข้าในโปรแกรมการอนุรักษ์พันธุ์สัตว์ป่า แต่ความสำเร็จในด้านอื่นๆ เช่น การแช่แข็งโอโอไซต์ และการเลี้ยงตัวอ่อนภายนอกร่างกายด้วยเทคนิคต่างๆ ยังไม่ประสบความสำเร็จในระดับ นำไปใช้งานได้ในสัตว์ชนิดใดเลยในปัจจุบัน สาเหตุเกิดจากการขาดองค์ความรู้พื้นฐาน ทางชีววิทยาของสัตว์แต่ละชนิด ตลอดจนเครื่องมือ การพัฒนาด้านเทคนิคต่างๆ พื้นที่ในการทำวิจัย ความเชี่ยวชาญ และทุนวิจัย ยังไม่เพียงพอและเหมาะสม ซึ่งเป็นปัจจัยสำคัญที่จะทำให้การนำไปใช้งาน ได้ประสบผลสำเร็จ การศึกษาพื้นฐานทางด้านชีววิทยาการสืบพันธุ์ในสัตว์เช่นเดียวกับการแสวงหาทางเลือกในการเก็บรักษาความสมบูรณ์พันธุ์ ยังคงเป็นปัจจัยที่จำเป็น ที่จะต้องเชื่อมโยงเครือข่ายต่างๆ (นักวิจัยทางด้านระบบสืบพันธุ์ นักชีววิทยาจากสวนสัตว์ และนักอนุรักษ์ธรรมชาติ) มาทำงานร่วมกันเพื่อให้ประชากรสัตว์ป่ายังคงอยู่พร้อมกันกับแหล่งที่อยู่อาศัย

คำสำคัญ: สัตว์ป่า ระบบสืบพันธุ์ การอนุรักษ์ธรรมชาติ เทคโนโลยีชีวภาพ การรักษาความสมบูรณ์พันธุ์ การแช่แข็ง

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